# CONDITIONS ASSOCIATED WITH LEVELS OF ALLERGENS AND FUNGAL AEROSOLS IN SELECTED HOMES OF SELECTED PRIMARY SCHOOL CHLIDREN IN DURBAN

**By**

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## ABSTRACT

This indoor environment study formed part of the South Durban Health Study (SDHS) that investigated the health effects of exposure to ambient air pollution. Homes of children from seven communities corresponding schools were recruited to participate. This study was designed to determine characteristics in the homes that are associated with higher or lower levels of allergens and fungal aerosols.

Homes were inspected using a field tested walkthrough checklist to collect data on home characteristics associated to adverse health effects. The characteristics include dampness, visible mould, type of flooring, type of bedding, type of heating systems, and building type and age. Dust samples for allergen analysis were collected from the bedding and the floor of the sleep area used by the children. Air samples from all rooms in the house were collected on malt extract agar, the media used for identifying and quantifying airborne fungal aerosols.

More than 70% of the homes were single units standing on their own, 20% were attached houses (flats or apartments) and the rest (10%) were informal houses. Construction material of the homes comprised of bricks (93%), wood (5%) and other material (2%) such as corrugated iron of which 94% were formally constructed. Dampness signs were observed in 51% of the homes and visible mould growth 13% of them. In all them, at least one characteristic that is hypothetically associated to elevated house dust mite allergens was found. Levels of mould (*Asp f 1*) allergen and house dust mite (*Der p 1* and *Der f 1*) allergen were comparable to levels found in other parts of the world. *Asp f 1* allergen levels ranged between 0.32-1.37µg/g and *Der p 1* and *Der f 1* allergen levels ranged from undetectable to 49.61 and from undetectable to 39.31µg/g of

dust respectively. Some home characteristics from walkthrough checklist were associated with *Asp f 1*, *Der p1* and *Der f 1* allergen levels when simple regression analysis was performed. *Asp f 1* was significantly associated with single family home [OR= 0.004 (95%CI 0.004–0.35)] and polyester filled pillows [OR= 0.07 (95%CI 0.01–0.61)] in logistic regression models. *Der p 1* allergen was associated with observed extent of roof dampness [OR= 0.33 (95%CI 0.13–0.81)].

Fungal aerosol mixture consisted of *Cladosporium spp.* as the predominant genus together with other genera such as *Aspergillus, Penicillium* and *Fusarium* were, to a lesser extent, identified in the samples from the homes. Mean concentration of total indoor fungal aerosol of indoor and outdoor were 1108 CFU/$m^3$ and 1298 CFU/$m^3$ respectively. Individual genera of fungi in the childrens sleep area had mean levels of 783 CFU/ $m^3$, 30CFU/ $m^3$, 64CFU/ $m^3$, 48CFU/ $m^3$ and 43 CFU $m^3$ for *Cladosporium spp., Aspergillus spp., Penicillium, spp., Fusarium spp.* and *Rhizopus spp.* respectively. Simple regression showed some conditions in the homes to be predictors of higher levels of total fungal aerosols. In a linear regression models, total outdoor fungal levels were a protective effect on total indoor fungal levels [β= 0.542 (95%CI 0.437–0.647)] whilst homes with hard floors had about 25 CFU/$m^3$ [β= 5.235 (95%CI 0.557–9.913)] in the homes were significantly associated.

This study showed the need to adapt observational instrument/ checklist/ questionnaire to suit the environment or the study area of interest. As other studies and findings indicated, the best way to assess exposure to biological pollutants indoors needs a combination of two or more methods, i.e. direct and indirect methods.

## DECLARATION

I declare that this is the original work of Mr. Nkosana Jafta, except where specific indication is given to the contrary. This research project makes use of indoor monitoring data (walkthrough data, allergen levels and fungal aerosol levels) collected in homes of the South Durban Health Study participants. This work has not been submitted previously to this or any other University.

_____ _____ _____

Mr. Nkosana Jafta Date Place

## PRESENTATIONS

**Jafta N, Batterman S, Gqaleni N, Naidoo R & Robins T. 2007.**" Characterisation of biological pollutants (allergens and fungi) in low-to-medium income households in South Africa". *Presented at the19th ISEE Conference, 04–09 September 2008, Mexico City, Mexico.*

**Jafta N, Gqaleni N, Naidoo R, Batterman S & Robins T**. "Conditions associated with allergen levels in the bedrooms of primary school children in Durban". *Presented at the SATS-ALLSA-CF Conference, 2–5 March 2007, Cape Town, South Africa.*

**Jafta N, Gqaleni N, Naidoo R, Batterman S & Robins T**. "Allergen levels in the residences and schools of primary school children in Durban". *Presented at the 3rd Public Health Association of South Africa Conference, 15–17 May 2006, Midrand, Johannesburg, South Africa.*

**Jafta N, Gqaleni N, Naidoo R, Batterman S & Robins T**. "Fungal aerosols and allergens in the seven Durban communities". *Presented in the AstraZeneca College of Health Sciences Research Symposium, 28–29 September 2005, University of KwaZulu-Natal, Durban, South Africa.*

**Jafta N, Gqaleni N, Naidoo R, Batterman S & Robins T.** "Indoor air quality of selected residences of in Durban communities". *Presented at the 17th Conference of the International Society Environmental Epidemiology, 13–16 September 2005, Sandton, Johannesburg, South Africa.*

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## ACRONYMYS AND ABBREVIATIONS

| | |
|---|---|
| ASHRAE | The American Society of Heating, Refrigerating and Air-Conditioning Engineers |
| ARI | acute respiratory infections |
| BSA | bovine serum albumin |
| C | Celsius |
| CFU | colony forming units |
| DG18 | dichloran 18% glycerol agar base |
| CI | confidence interval |
| DEAT | Department of Environmental Affairs and Tourism |
| *Der* | *Dermatophagoides* |
| *Der p* | *Dermatophagoides pteronyssinus,* |
| *Der f* | *Dermatophagoides farinae* |
| DUT | Durban University of Technology |
| DOH | Department of Health |
| EPA | United State Environmental Protection Agency |
| ELISA | enzyme-linked immunosorbent assays |
| UKZN | University of KwaZulu-Natal |
| UM | University of Michigan |
| VOC | volatile organic compounds |
| HVAC | Heating, Ventilation and Cooling Air Conditioner |
| HDM | house dust mite |
| HWTC | home walkthrough checklist |
| IAP | indoor air pollutants |
| IAQ | indoor air quality |
| IEQ | indoor environmental quality |
| IgG | immunoglobulin G |

| | |
|---|---|
| IOM | Institute of Medicine |
| MCS | multiple chemical sensitivity |
| MEA | malt extract agar |
| NIOSH | National Institute of Occupational and Safety Health |
| NRC | United States National Research Council |
| OR | odds ratio |
| PBS-T | phosphate buffered saline-0.5% tween |
| PM | particulate matter |
| RAST | radioallergosorbent tests |
| RH | relative humidity |
| RIA | radioimmunoassay |
| SBS | sick building syndrome |
| SD | standard deviation |
| SDHS | South Durban Health Study |
| sqrt | square root |
| USA | United States of America |
| WHO | World Health Organisation |
| WTC | walkthrough checklist |

# CHAPTER 1

# BACKGROUND AND IAMS OF THE STUDY

## 1.1 BACKGROUND

Monitoring and assessment of pollutant levels indoors is an expensive exercise as it needs skilled personnel, expensive equipment and advanced laboratories for sampling as well as analysis of different pollutants. Biological pollutant (fungal aerosol and allergen) levels have been associated with poor indoor conditions of the homes by studies in other parts of the world. In this dissertation, the question of using walkthrough checklist (questionnaire) as an instrument of detecting biological pollutant levels in the homes of children in Durban was tackled.

This study was carried out in Durban, one of the four largest cities in South Africa and situated in east coast of the country. The South Durban region (also known as South Durban Basin) situated in the south east of the city, is the hub of many large and small industrial activities which amongst them include two large petro-chemical refineries, paper mill, tank washer, water works and other small industries (Robins *et al*. 2002). Communities of Merebank, Austerville/Wentworth and Bluff live around these industries and are daily exposed to their emissions. Environmental accidents from explosions, spills and leaks of underground pipelines caused by these industries had been reported, but one of the main concerns was industrial emissions (Friends of the earth 2002). For many years, south Durban community groups, and others, have raised concerns about potential

health effects of ambient air pollution caused by emissions from these industries. Researchers and investigators had also pointed out that there was industrial, regional and national need to develop and implement scientifically rigorous studies to assess any impacts of air pollutants on health of the communities living in close proximity to these industries (Groundwork 2003; Robins *et al*. 2002).

In 2003, with South Africa still in the process of drafting an Air Quality Regulations and Standards, two national governmental departments; Department of Environmental Affairs and Tourism (DEAT), and Department of Health (DOH), together with eThekwini Municipality (Durban Metro) commissioned a study on health effects of air pollution in children and adults in the Durban area. University of KwaZulu-Natal (UKZN) and its collaborators namely Durban University of Technology (DUT) and University of Michigan (UM), USA undertook this study. The study known as South Durban Health Study (SDHS) looked at health effects of air pollution on communities in south Durban region compared to communities in north Durban which is not highly industrialised region (Figure 1).

School children and their families from these two regions were recruited to participate in the study (SDHS) that had a number of objectives, including air monitoring of different ambient pollutants, health assessments on children and adults, epidemiology study on participants and their families; and indoor environment assessment of the homes of participating children in the study. Seven schools were involved in the project with 3 schools participating from north Durban communities namely Ferndale Primary in Newlands East, Briardale Primary in Newlands West and Ngazana Primary in KwaMashu. From south Durban communities, four schools which took part in the study

were Nizam Road Primary in Merebank, Assegai Primary in Austerville, Entuthukweni Primary in Lamontville, and Dirkie Uys Primary in Bluff.

Indoor environment has become important as ambient environment, over the years as many researchers started associating it to respiratory and other morbidities. Most investigators had linked some pollutants found indoors to respiratory illnesses such as asthma, dermal disorders and chronic illnesses like cancer, heart and vascular diseases (IOM 2000; Sundell 2000). Within the SDHS, the indoor environment monitoring was done to assess at its effects as a confounder to ambient air pollution. Different pollutants, i.e. dust allergens, fungal aerosols, particulate matter (PM 10) and volatile organic compounds (VOC) (Figure 2).

## 1.2 AIM

To determine association between home characteristics determined by home walkthrough checklist (WTC) with levels of indoor biological pollutants collected from the homes of primary school children.

**Specific Aims:**

1. To use a WTC to investigate the extent of mould contamination and dampness in homes of primary school children
2. To identify and quantify fungal aerosols and house dust mite allergens in dust collected from homes.
3. To assess the relationship between home characteristics (include dampness, mould growth, etc.) determined by the home WTC and concentration of allergens and fungal aerosols collected.

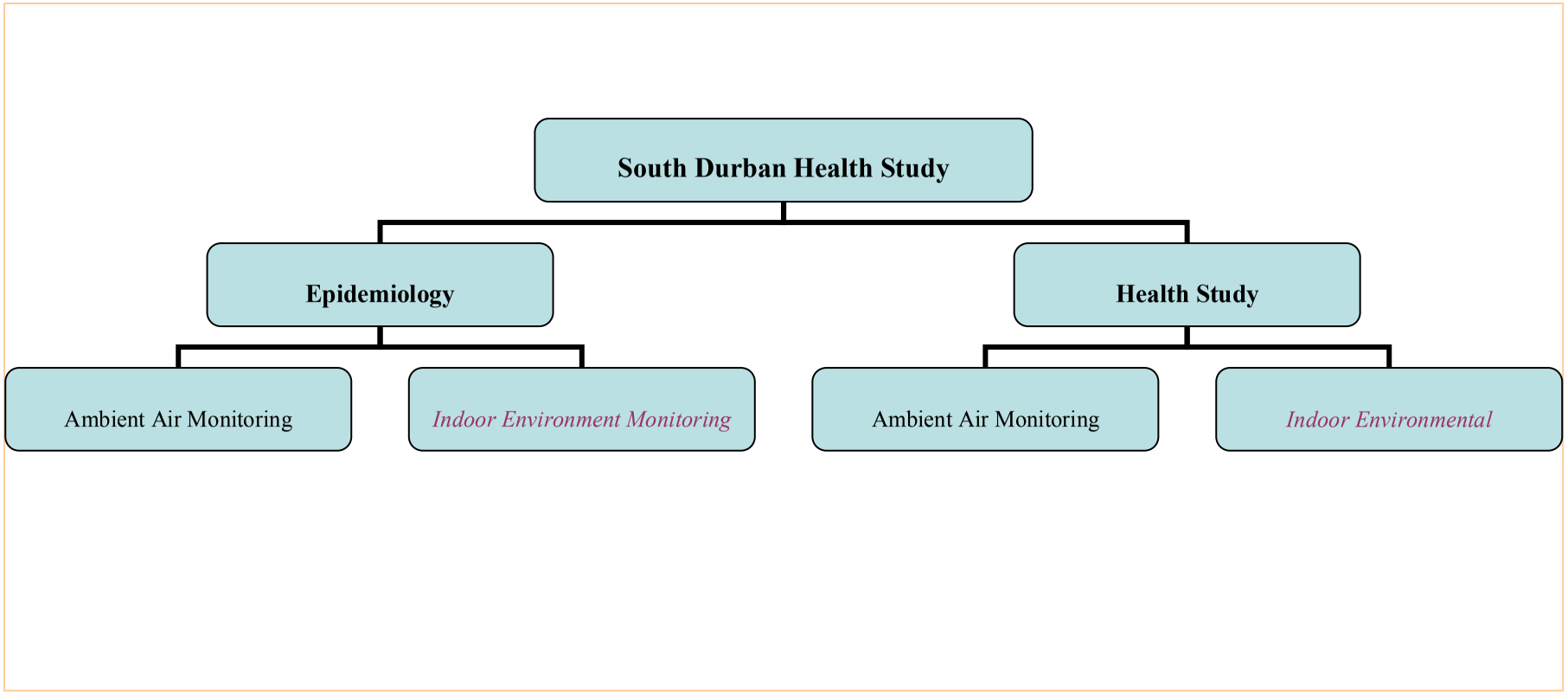



**Figure 1:** Diagram showing South Durban Health Study with indoor air and ambient assessment as part of both epidemiology and health studies

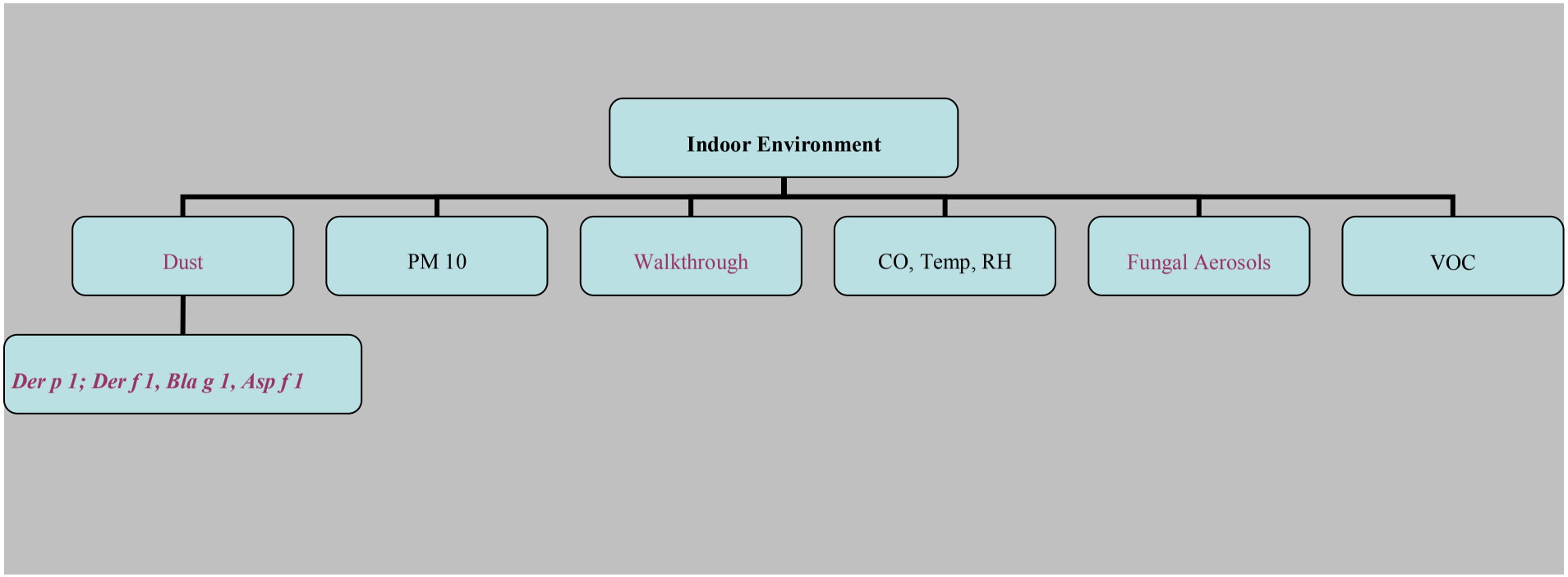



**Figure 2:** Diagram showing indoor air pollutants that were assessed during the SDHS project

## 1.3 ETHICAL APPROVAL

Ethical approval for the study was obtained from the University of KwaZulu-Natal's Bioethics committee as part of the SDHS (E117/03) and as indoor environment study on its own before it progressed (Ref no: H206/03).

## 1.4 STRUCTURE OF THIS DISSERTATION

This dissertation has 5 additional chapters that are separated according to subject investigated. These chapters are as follows:

Chapter 1 (this chapter) introduces the study, with reference to the background of the study, research site, sample selection of participants, ethical application and structure of the dissertation.

Chapter 2 is an overview literature review on indoor environment, its health implications, and methods used in indoor environment monitoring.

Chapter 3 looks at home walkthrough checklist (questionnaire) as an instrument for investigating indoor environment and how it was used in this study.

Chapter 4 investigates biological (mould and dust mite) allergens found in homes that participated in the study and home characteristics that are predictors of higher levels of allergens.

In Chapter 5 fungal aerosol levels associated with home characteristics in children's home are investigated.

Chapter 6 is a discussion on overall findings of the study and conclusion in relation to the objectives as well as recommendations for the homes and research in general.

Chapters 3 – 5 are structured as follows: 1) introduction to the subject at hand and reference to publications on the issue investigated, 2) methodology used for data collection and statistical analysis, 3) results and 4) discussion of the findings on the subject.

# CHAPTER 2

# LITERATURE REVIEW

The World Health Organisation's (WHO) definition of health in its constitution states that, "health is a complete physical, mental and social well being and not merely the absence of disease or infirmity" (WHO 1985).

Air pollution is a major environmental health problem affecting developed and developing countries around the world. Increasing amounts of potentially harmful gases and particles are being emitted into the atmosphere on a global scale, resulting in damage to human health and the environment (WHO 1999). Indoor air quality [a component of indoor environmental quality or (IEQ)] is as important as an outdoor air quality when addressing risks from air pollution because of the time people spend indoors and infiltration of outdoor air into indoor environment (EPA 1999; Sundell 2000; WHO 1999). Jantunen (2000) states that one should expect rather high correlation between personal exposures and indoor concentration exposure because of time people spend indoors. In 1999, WHO estimated that amongst the leading causes of the global burden of disease are acute respiratory infections (ARI) that accounted for 6% of the total global burden of disease and mortality and these are mostly found in developing countries.

## 2.1 INDOOR AIR QUALITY

Indoor air quality (IAQ) is the description of chemical, physical and biological characteristics of air in an indoor (residential or occupational) environment. In residential settings there are many contributors to indoor pollution from sources such as human activities, biological sources and outdoor air. People in indoor environment are exposed to some of the anthropogenic (man-made) products that include environmental tobacco smoke, gaseous products [such as nitrogen oxides, sulphur oxides, ozone and other gases like volatile organic compounds (VOC)] and airborne particles in form of particulate matter ($PM_{10}$ and $PM_{2.5}$). Biological agents such as house dust mite, cockroach and mould allergens in air and dust are also found in high levels indoors and are implicated in adverse health effects that are associated with exposure to indoor environment (IOM 1993; IOM 2000).

## 2.2 HEALTH EFFECTS ASSOCIATED WITH INDOOR ENVIRONMENT

Exposure to indoor pollutants is mainly through inhalation than dermal contact or ingestion. Indoor environmental factors are thought to play part in three different levels when an individual is exposed to the pollutants. The pollutant can get (1) the immune system to react unfavourably to some factor in the environment (sensitization) or (2) the pollutant can trigger symptoms in those already sensitized and/ or (3) then maintaining the inflammation in the mucosa of the respiratory passages (Sundell 2000).

Molhave (2000) suggested three classes of health effects associated with indoor environment, namely (1) priority effects, (2) secondary effects and (3) hypothetical or potential effects. Priority effects are described as health effects for which the causality is

well established. Usually there are official guidelines or recommendations set for indoor air pollutants because there is a clear relationship with exposure to these indoor air pollutants (IAP) and adverse health effects. This association had been reported in literature worldwide (WHO 1982; Gold *et al*.1992). Secondary IAQ effects are less adverse effects with a well-known and documented association to IAQ. The causal or agent may not be exactly known to cause the illness therefore in some cases recommendations may be a preferred tool for giving guidance on IAQ. Hypothetical or potential IAQ effects are effects that are postulated but not yet proven to be related to IAQ. Some of the effects may be considered adverse but present levels of knowledge prohibit an official rational preventative action (Mølhave 2000).

Institutions or health organisations involved in occupational and environmental health like WHO, EPA, NIOSH, ASHRAE, government departments of different countries as well as other professional organizations in consultancy and academic fields are involved in setting guidelines, standards or recommendations for indoor environment (EPA 1998; WHO 1999). Between these institutions, pollutants might have different guideline levels for occupational or environment settings depending on the data available or data each organisation used when setting guidelines or standards or recommending acceptable pollutant levels for indoors (IEE 1996; EPA 1998; WHO 1999). Occupational environments have well defined standards or guidelines compared to residential settings because of the well defined exposure level, duration and frequency of IAP.

Some health effects have been well studied in the indoor air environment namely respiratory effects, immune and hypersensitivity or cellular effects that include cancer and genotoxic; neurogenic and sensory effects; and cardiovascular system effects

(Mølhave 2000; Sundell 2000). Health effects associated with indoor air quality well defined in the occupational settings are summed up as sick building syndrome. These effects are also associated with noise, light and indoor climate i.e. temperature and relative humidity (WHO 2000).

Sick building syndrome (SBS), which is not a well-defined entity, is referred to when people complain of related or many different symptoms after spending a lot time in a certain building (Mølhave 2000). Multiple Chemical Sensitivity (MCS) is when a chemical is involved and symptoms are usually more severe than SBS. Respiratory symptoms that include airway infections, Legionnaires' disease and rhinitic asthma are some of IAQ related health effects. Other adverse health effects are immune effects and other hypersensitivity that include allergic disease, which are asthma, allergic rhinitis and atopic eczema (Bakke and Nordman 1997; Mølhave 2000).

Association between respiratory diseases and indoor environment pollutants have been well studied, and most associations made with asthma. In reports of literature and data from Europe, USA and other parts of the world, allergies and other biological pollutant related illnesses were found to be among the most important illnesses related to indoor environment (IOM 1993; Sundell 2000). In the South African context, Gqaleni (1999) indicated that in an indoor dust there is a range of allergens that are implicated with allergic levels found in that particular environment.

## 2.3 BIOLOGICAL AGENTS FOUND INDOORS

The presence of biological pollutant or allergen sources namely fungi, cockroaches, house dust mites, bacteria, slime moulds, algae, protozoa, and viruses on

building and building material has been recognised as one of the important risk factors for indoor air contamination. Domestic animals, rodents, insects and plants are as well important sources of allergens indoors (Mage 1984; IOM 1993). Exposure to indoor biological pollutants or allergens is through inhalation as pollutants are airborne or contact with the person through inhalation from reservoirs (e.g. settled dust) or other sources. IOM (1993) highlighted that allergic constituents of indoor air are predominantly of biologic in origin. Chemically, these major allergens are protein molecules that had been identified and characterised in the case of house dust mites, cockroaches, cats, dogs, and certain fungi (IOM 1993).

Indoor house dust is heterogeneous containing inorganic and organic substances in it and is as important as indoor air because its constituents may aggravate respiratory symptoms (Matthews 1998).

### 2.3.1 Dampness, Fungi and Fungal Allergens

#### *2.3.1.1 Occurrence of dampness, fungi and fungal propagules*

Home dampness may reflect poor overall ventilation and increased concentrations of indoor pollutants or growth of fungi that is pervasive throughout the outdoor environment. Fungal spores are always present in dust and on horizontal surfaces but require a relative humidity of 65% or greater to exist to grow (Gravesen 1979).

There are more than 200 genera and 1 000 000 species of fungi that human beings are constantly exposed to (IOM 2000). Fungi are eukaryotic organisms characterised primarily by their filamentous morphology and saprobic lifestyle. To digest food, fungi excrete enzymes into the environment initially as probes to evaluate food availability, then to digest complex carbon compounds. Some fungi change their pattern of enzyme

excretion with the change in food source or with changing environmental conditions. Excreted enzymes are some of the major fungal allergens. During digestion and processing of food, fungi produce many secondary metabolites such as mycotoxins and antibiotics that are highly toxic. The primary reproduction of fungi is by production of airborne spores, which form a major fraction of both the outdoor and the indoor large-particle aerosol (IOM 2000).

Fungal growth and spore dissemination depends on available substrates, season, climate and human activity. Human activities like not fixing leaks or generating excessive moisture, affect the number and types of fungal sources in buildings. Food materials (building surfaces) and temperature affect the amount of water required, as does the strain of fungus. Fungal spores become airborne indoors when disturbed by air movement and normal human activities (O'Rourke *et al*. 1990; IOM 2000). Contaminated air conditioners and humidifiers can actively spray spores, fragments, and dissolved fungal allergens into the air (Burge *et al*. 1980; Braur *et al*. 1988; Kumar *et al*. 1990). Composition of airborne fungal aerosols depends on the strength of the sources, as well as on dissemination factors, mixing, dilution, and particle removal. Natural aerosols are almost always composed of mixed species although in some situations indoor environments with actively disseminating reservoirs, aerosols may contain particles derived from a single fungus (IOM 1993).

#### *2.3.1.2 Health effects associated with fungi and its propagules*

Exposure to fungi might mean exposure to fungal derived allergens, irritants, toxins and sometimes potentially infectious units (IOM 2000). In a study by Dales *et al.* (1991), symptoms of respiratory health effects increase with increased numbers of damp

and mould sites in Canadian homes. Some studies suggested that varieties of fungal spores are allergic and give rise to respiratory disorders (Holmberg 1987). Other symptoms associated with fungi and spore exposure include breathlessness, aching joints, backache, blocked nose, eye and skin irritation, cough, wheezing, phlegm and common colds (Mage 1984; Holmberg 1987; Dales *et al.* 1991). Certain fungi had been reported to grow saprophytically in the mucous lining of the lungs of patients with allergic bronchopulmonary fungosis or aspergillosis and in the sinuses of people with allergic fungal sinusitis (IOM 1993). Allergic fungal constituents and their health effects are documented in literature as some had been identified (Pearce *et al.* 2000; Chapman 2003).

#### *2.3.1.3 Types of fungi and fungal allergens found in indoor environments*

Fungal allergens are a range of proteins of different potency that are produced by fungi of different strains, species, and genera. They are contained within spores, mycelium or culture medium (Burge *et al*. 1989; Fadel *et al*. 1992; Cruz *et al*. 1997; IOM 2000). Different defined allergens have been isolated from fungi, namely, *Asp f* 1 and *Asp f* 3 from *Aspergillus fumigatis* with molecular weight of 18kD, *Alt a 1* and *Alt a 2* from *Alternaria alternata, Cla h* 1 produced by *Cladosporium herbarum* with molecular weight of 13kD, *Penicillium citrinum* and *P. herbarum* species produce a 33kD and 68kD protein respectively (IOM 2000). Most of these allergens are found in dust as they are not suspended in the air for long because of their weight or mass.

Sensitisation to fungi is one of major indicators of adverse health effect findings in the health literature from all over the world (Pearce *et al.* 2000). Species of *Alternaria, Aspergillus, Cladosporium* and *Penicillium* have been implicated in sensitisation in

different groups of society from children to elderly people and workers exposed to the fungi. When testing individual's sensitisation to fungi, a mixture of fungal allergens is often used. This is the approach because fungal occurrence in the air is usually in a mixture form; therefore an individual can sometimes be sensitive to one fungal species or more fungal species at a time (Nordvall *et al*. 1990).

### 2.3.2 House Dust Mites and Mite Allergens

#### *2.3.2.1 Occurrence of house dust mite and its allergens*

House dust mites have been shown to be contributors to allergens found in house dust, and studies associate exacerbation and causal of adverse respiratory conditions to these allergens. This observation was first reported by Dekker (1928) in Germany when he identified mites in bedding. There are many different species of dust mites, but the predominant ones in most parts of the world belong to the family of *Pyroglyphidae*: *Dermatophagoides pteronyssinus, D. farinae* and *Euroglyphus maynei*. In the Netherlands, United Kingdom, Scandinavia, India and South Africa, *D. pteronyssinus* are the dominant species (Mathews 1988).

House dust mites are approximately 0.3 mm in length, eight-legged and sightless; and they live on skin scales and other debris. They absorb water through a hygroscopic substance extruded from their leg joints. If the humidity falls dust mites withdraw from the surfaces, but it can take months for mites in sofas, carpets, or mattresses to die (Arlian *et al*. 1982; Platts-Mills and Chapman 1987). Moving mites can be seen by light microscopy but a great majority of mites found in dust are dead, so it becomes difficult to separate them from other dust particles. Mites excrete partially digested food and digestive enzymes as feacal particles surrounded by a peritrophic membrane which keep

them intact (Tovey *et al*. 1981a). The feacal particle has a size of 10-25 um in diameter and an allergen content of approximate 0.2ng. It is estimated that 100,000 mite fecal particles may exist per gram of house dust (Jones 1998). Exposure to these allergens is thought to be through active disturbance of the allergen reservoirs in bed, soft furniture, and carpets. Because of the large size, house dust mites' allergens remain airborne for relatively short time periods (on the order of minutes) (Tovey *et al*. 1981b). Exposure to house dust mite reservoirs happens when an individual is asleep or in close contact with the bedding (pillow or mattress which contain high concentrations of mite allergens).

Some of the allergens isolated and identified from mite extracts are *Der p 1, Der p 2* and *Der f 1* and are implicated in the etiology of asthma. Other allergens isolated and identified from dust mite cultures are *Der p 3*, *Der f 2* and 3, and *Eur m 1*. Molecular weights for these allergens range from 14kDa for *Der p 2* and *Der f 2* to 29kDa for *Der p 3* and *Der f 3*. Group 1 dust mites allergens *Der p 1, Der f 1* and *Eur m 1* have molecular weight of about 25kDa (IOM 2000).

Most houses contain at least 3 of the 4 requirements for house mite growth which are: (1) a site that can provide nest for mites (e.g. bedding, carpets, sofas etc.); (2) presence of humans which guarantees an abundance of food in the form of human scales; (3) favourable temperature for mite optimal growth; and (4) humidity. Dust mites have a range optimal growth temperature between $18^0$C and $27^0$C and ambient humidity is one factor that determine whether the house have high concentration of mites (IOM 1993; IOM 2000). In cold, dry climate and high-altitude areas, mite growth is poor unlike warm, humid and low altitude areas where mites thrive. This observation is explained by Custovic *et al*. (1998) in an occasional review of literature stating that areas of altitude

higher than 1500m above sea level have significant lower mite levels compared to areas at sea level.

#### *2.3.2.2 Health implications associated with exposure to dust mites and its allergens*

Many studies found that house dust mite sensitivity is strongly associated with asthma. In a review report of different studies by IOM (1993), house dust mite exposure had a strong association as a causal agent as well as exacerbating agent of asthma in children. Sneezing, runny/stuffy nose, watery/itchy eyes are some of the symptoms that might result from exposure to house dust mites. Dust mites are the main contributor of half of the asthma cases in USA (IOM 2000).

Atopy or sensitisation to house dust mite had also been documented in studies in South Africa. These studies show an interesting difference in urban and rural levels of sensitisation to HDM. In Durban, an urban area, 95.0% of 29 children in the study showed positive sensitisation to dust mite antigen tested by skin prick test and only 16.0% of the 190 farm workers in Western Cape were positive to skin prick test (Manjra *et al*. 1995; Jeebhay *et al*. 2002). In rural schools of Eastern Cape, South Africa, 25.5% of rural children had positive reaction to aeroallergen skin prick test compared to urban schools in the Western Cape, South Africa, with 29.8.0%, 42.5% and 45.4% of Marconi Beam, Kirstenhof and Table View respectively (Steinman 2004). Other studies from other parts of the world support this finding of high susceptible or prevalence of illnesses associated with biological indoor air pollutants in children living in urban areas compared to rural areas.

## 2.4 EXPOSURE ASSESSMENT

Assessing exposure involves numerous techniques to identify contaminants, contaminant sources, environmental exposure media, transport through each medium, chemical and physical transformations of the contaminant, routes of entry to the body, intensity and frequency of contact, and spatial and concentration patterns of the contaminant (Gravesen 1979; Saad and el-Grindy 1990). The NRC (1991) report defines exposure to a contaminant as an event that occurs when there is contact at a boundary between a human and the environment with a contaminant of a specific concentration for an interval of time. Exposure measures can be classified as objective or subjective depending on the method used. For assessment of environment, methods such as questionnaires and walkthrough checklists are used whereas for objective assessment, sample collection from environment or personal exposure monitoring of subject is used as measure of exposure. The approaches can also be grouped as direct or indirect measures of exposure to the pollutants. Figure 3 shows classification of surrogate exposure assessment approaches to indoor pollutants.

Direct exposure measures give proximal measures of individual exposure than do the indirect approaches. Direct measures include personal monitoring –involving the measurement of concentrations using monitors carried by individual subject, and biomarkers involves measurement of the agent or its metabolite in biological samples such as urine and blood. Personal monitoring is ideal when done in occupational settings, although in some environmental studies, this type of monitoring has been carried out in residential and other microenvironments; such as the EXPOLIS study in Europe (Jurvelin *et al*. 1997; Koistinen *et al*. 2004).

Indirect exposure refers to environmental area monitoring (e.g., room sampling), models (e.g. micro-environmental modelling), real-time questionnaires (e.g. walkthrough checklist), recall questionnaires and real-time diaries. Indirect approaches are more practical in large-scale studies and better suited to long-term exposure characterisation (IOM 2000). Most studies that look at environmental exposure and its association with health of the exposed population use this type of assessment.

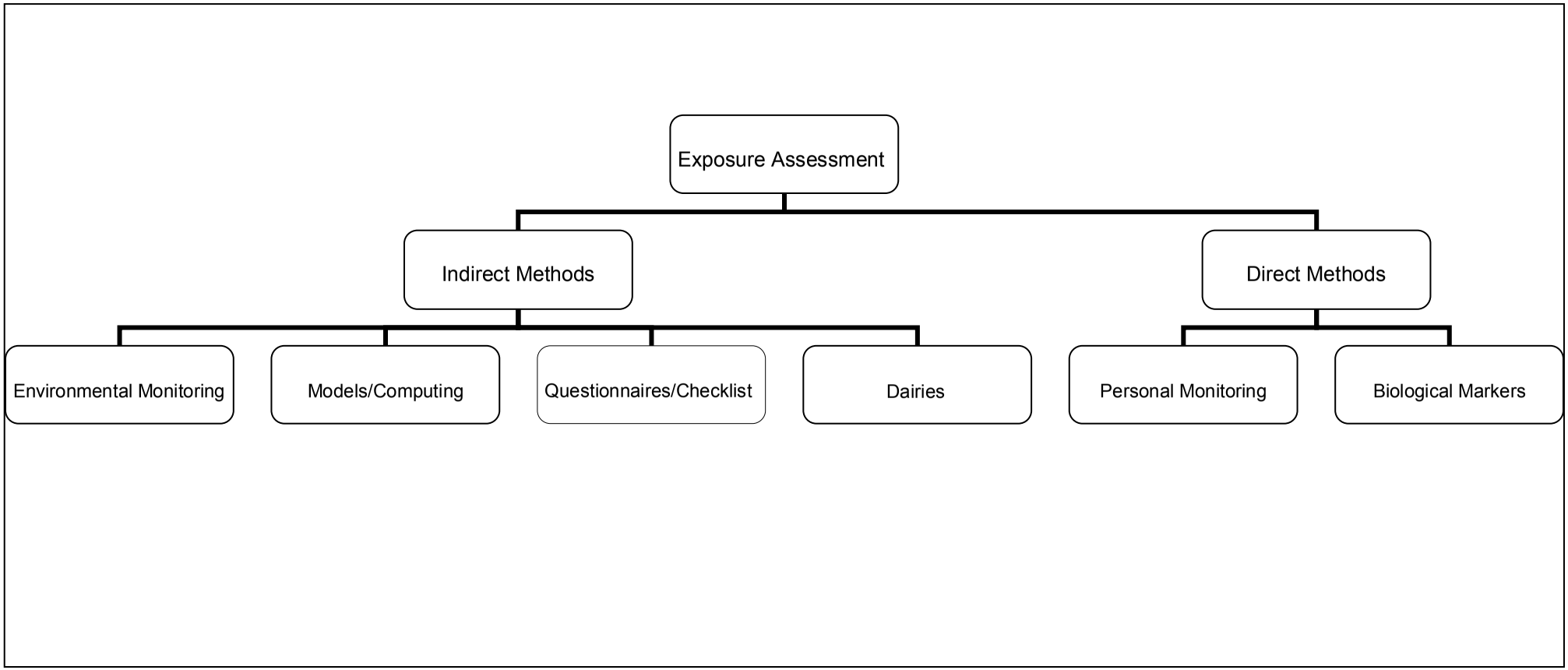



**Figure 3:** Classification of surrogate assessment approaches. *Source*: IOM (2000) adapted from NRC (1991)

### 2.4.1 Approaches Employed in Biological Exposure Assessment

No single method is sufficient to detect and monitor all different pollutants and/ or allergens that can be present in the indoor environments (Samson 1985). Designing a sampling strategy requires consideration of the nature of the pollutant source, the nature (including the size and expected concentrations) of the pollutant particles, and the parameters that influence the choice method (i.e. observation or reservoir or air sampling) and analytical approach, the sampling plan (amounts of sample to be collected, times and locations to be sampled) and approaches of analysis and interpretation of the data (NRC 1985).

Assessment of exposure to indoor biological pollutants involves multiple steps. Monitoring biological pollutants takes time, methods of sampling, and analysis into consideration, as these are factors that are very important. The site at which people spend majority of their time (such as living room or bedroom) in residences is usually chosen for environmental sampling. Material or sample is collected from reservoir from which exposure can be assumed or extrapolated using mathematical models (Swanson *et al.* 1990). Sample collection methods in indoor environment include observation, bulk or reservoir sampling, and air sampling.

#### *2.4.1.1 Direct Assessment*

Direct exposure measures are the most appropriate methods when investigating exposure and health effects. Biological monitoring has the potential to assess worker exposure to industrial chemicals by all routes, including inhalation, skin absorption, and ingestion. Selection of an appropriate biomarker for an exposure requires knowledge of

the distribution, metabolism, and excretion of the toxicant sufficient for selection of the proper compound to be determined, biological medium to be sampled, and time for obtaining a specimen (NRC 1991). Often, most of the toxicological and pharmacological information available is from experimental animals and, thus, not always directly applicable to humans.

#### *2.4.1.2 Indirect Assessment*

##### *2.4.1.2a Questionnaires/ Walkthrough Checklists*

On the other hand most environmental epidemiology studies use questionnaire as one of their most important instruments when investigating exposure to dampness, moulds and other biological agents. In these questionnaires the investigator/s conducts a walkthrough inspection observing conditions of the environment and activities of the occupants and/or they ask questions to the occupants about the environment. Potential pollutant reservoirs/sources such as visible fungal growth, dampness, water damage, presence of pet etc. have to be observed and noted as important characteristics when investigating biological pollutants (Dales *et al*. 1991; Chew *et al*. 1998; Garrett *et al*. 1998; Gqaleni *et al*. 2000; Sekhotha *et al*. 2000).

This way of collecting data is known as observational sampling. Observational sampling includes sensory perceptions of an indicator (e.g. odours, visible fungal growth, dampness etc.) and observation of factors known to be related to specific kinds of sources (type of floor, type of bedding etc.). Observations can be formalised or casualised, as is often the case in environmental investigations (IOM 1993). The questionnaire may include some sections of questions directed to occupants, about their observations of the conditions such as heating system, water leaks, dampness and visible mould growth in

the recent past. Some answers to the questions can be subjective as the investigator or respondent might give answers that are his or her personal observation (bias). This means that different people responding to the questions might give different answers to the same observation therefore it is important for the investigators to be trained as to standardise this method of data collection (minimise bias).

Some researchers had identified this possible bias of reported house conditions by occupants and evaluated the use of questionnaire as an appropriate tool for investigating indoor environment conditions. Although conditions had been associated with levels of indoor pollutants in the homes it had been found that in different regions they differ (Kuehr *et al.* 1994; Van Strien *et al.* 1995; Chew *et al.* 1998). Association of house conditions with health effects is one of the most investigated subjects on indoor environment quality.

#### *2.4.1.2b Micro-environmental Monitoring*

Micro environmental assessments involve sampling and analysis of pollutant on the indoor environment of concern. In the microenvironment, sources of pollutants have been strongly associated with exposure of occupants to the pollutants. Reservoirs of potential pollutant like visible mould growth, dampness, presence of pets etc. had been observed and recognised as important factors when investigating indoor environment for biological pollutants.

*(i) Sample Collection*

Source reservoir samples have been used as indicators of exposure to most indoor biological pollutant studies. In many investigations air and dust samples had been collected for analysis of micro-organisms (Gravesen 1979; Saad and el-Grindy 1990;

Dales *et al.*1991; Flannigan *et al.* 1994; Garrett *et al*. 1998; Sekhotha *et al*. 2000). Because of high variability in counts of micro-organisms in air samples even when samples are taken in rapid succession, some researchers have suggested that indoor microbiological determination should concentrate on indoor dust (Flannigan *et al*. 1994).

Dust can be collected using a vacuum dust collector that maintains constant flow and / or vacuuming a given surface for a standard length of time and / or vacuuming carefully measured sections of a surface, or by using a combination of these methods. Surface samples are prepared by swabbing or pressing a plate of culture medium or a sticky tape against a surface. These samples are then inoculated onto a growth medium for colony identification before isolation. The samples are useful in identification of fungus where there is an obvious microbial contamination (Macher and First 1984).

Volumetric air samples are taken in the microenvironment (room or building) of interest usually near the breathing zone of an individual to measure human respiratory exposure to bioaerosols. These samples are usually collected using suction devices. Small filter cassette samplers and low flow rate suction impactors are ideal for use in indoor environments (Macher and First 1984).

*(ii) Analysis of Aerosols and Reservoir Samples*

Bioaerosols can be analysed from collected air and reservoir samples. Analysis of pollutants of biological origin usually use of culture or cultural assays, microscopy, immunoassays, biochemical assays, and bioassays. Of these, the most commonly used methods are cultural assay, microscopy, and immunoassay (IOM 2000).

Cultural assays evaluate viable particles (viable spores) as indicators of concentration of airborne bioaerosols or reservoir samples. A choice of one or more

sampling medium is important for identification of a wide range of different microbial organisms. Sub-culturing of colonies using different media can be used as another method for identification of organisms on a genius level. Morphologically distinct cultures can be studied by naked eye. Microscopy is also used for identification of morphologically different cultures and colonies (IOM 2000; Samson *et al*. 2000). Compound microscopy can be used to identify biological aerosols that include fungal cultures, bacteria, yeast and other microbes, from generic to species level. If more detail is required, samples can also be viewed under a scanning electron microscope (SEM) or a direct epifluorescence microscope. This method of analysis can be used for samples collected through suction impaction and rotating impactors. House dust mites prevalence can be determined in settled house dust by counting under a microscope after separation from the dust sample by flotation or suspension (IOM 2000).

Immunoassays measure the pollutant or allergen or antigen (e.g. proteins) rather than an indicator and are essential for most amorphous allergens including those from mites, mould, cats, and cockroaches. Immunochemical assays for allergens are available for only few fungi, primarily because fungal allergens are poorly characterised and purified. House dust mite allergen concentrations in dust or other reservoirs are studied mainly by use of immunochemical assays. Total mite allergen content can be assessed by radioallergosorbent tests (RAST). Individual mite allergens can be measured with enzyme-linked immunosorbent assays (ELISA) or radioimmunoassay (RIA) (Seltzer 1996). Furthermore, ELISA assays have been shown to be highly reproducible and can quantify antigen levels to less than 1.0ng/mg dust (Munir *et al*. 1993; van Strien *et al*. 1994).

An indirect method for assessing mite allergen levels in house dust is by measurement of guanine, which is a nitrogenous excretory product of arachnids (Le Mao *et al*. 1989; IOM 2000).

## 2.5 GUIDELINES FOR RESIDENTIAL BIOLOGICAL INDOOR AIR POLLUTANTS

World Health Organisation (WHO) has set guidelines for exposure to some biological pollutants. In a workshop held at Minster Lovell, Oxfordshire, England, Sept. 19–21, 1990, under the auspices of the International Association of Allergology and Clinical Immunology and the World Health Organisation supported by other research institutions from across Europe, recommendations of threshold limits for allergen levels considered risky to sensitisation and development asthma were proposed (Platt-Mills *et al*. 1992). Table 1 show different allergens and their proposed threshold limits. Because from one geographical region to another, one dwelling to another, conditions are very different, it is difficult to determine baseline levels of fungal aerosols in general. Thus no suggested limits or guidelines are set for fungal exposure that means each dataset is considered on its own. Comparison of fungal aerosol levels is done within the dataset which is an internal comparison.

**Table 1**: Threshold limits for house dust mite, cockroach and cat allergens recommended by WHO.

| **Allergen Source** | **House dust mite** | **Cockroach** | **Cat** |
|---|---|---|---|
| *Allergen type* | *Der p ; Der f* | *Bla g* | *Fel d I* |
| Lower threshold limit | 2.0 - 10µg/g dust | >/= 2.0µg/g dust | 8µg/g dust |
| Upper threshold limit | >10µg/g dust | | |

# CHAPTER 3

# CONDUCIVE CONDITIONS FOR BIOLOGICAL POLLUTANTS

## 3.1 INTRODUCTION

Exposure of occupants to indoor pollutants in residences had been associated with respiratory morbidity in many studies across geographical regions including those with tropical climates (Dales *et al*. 1991; Husman *et al*. 1996; Chew *et al*. 1998; Sekhotha *et al*. 2000; Gent *et al*. 2002; El Sharif *et al.* 2004; Spengler *et al.* 2004). Many studies associated morbidity with house conditions/characteristics, not level of known allergens in the air or dust. House conditions such as dampness, humidity and visible fungal growth is considered a risk factor to development of respiratory illnesses and/or exacerbation of respiratory symptoms such as wheeze, persistent cough, breathlessness etc. (Austin and Russel 1997; Sundell 2000; Gent *et al*. 2002; Zock *et. al*. 2002; Belanger *et al*. 2003; Spengler *et al*. 2004; Simoni *et al*. 2005). Presence of some of these conditions have been linked to proliferation of biological pollutants such as house dust mite, cockroach and fungal allergens, but this association is not well studied except for some few (Van Strien *et al*. 1994; Dales *et al*. 1997; Chew *et al*. 1998; Howden-Chapman *et al*. 2005). Hypothetically, Durban's temperate climate is favourable for indoor biological pollutant proliferation with high humidity ranging between 75% and 90% and warm temperature range between $18^0$C and $30^0$C throughout the year.

In environmental and occupational settings, most investigators in indoor environment had used questionnaire or walkthrough checklist as an investigative instrument in identifying conditions or possible sources of pollutants. This makes the use walkthrough checklist or questionnaire important, if mitigation or correction steps are to follow investigation. Trained personnel are needed to conduct this type of investigation as use of checklist or questionnaire needs constancy. Data from different studies show that questionnaires and walkthrough checklists can be used for assessing households for exposure indicators such as dampness, visible fungal growth, ventilation, flooring type and presence of other characteristics that are associated with proliferation of biological pollutant sources. These studies also came to different conclusions on home characteristics that can be used as indicators of risk factors of exposure in home (Van Strien *et al*. 1994; Dales *et al*. 1997; Williamson *et al*. 1997; Chew *et al*. 1998; Danaviah *et al*. 1998; Garrett *et al*. 1998; Nicholai *et al*. 1998; Ross *et al*. 2000; Ren *et al*. 2001; Van Strien *et al*. 2004).

Some studies that looked at association between housing conditions and health effects are discussed below. These conditions differ from house to house even in the same community because of activities and socioeconomic factors of occupants. In Glasgow, Williamson *et al*. (1997) investigated whether house characteristics including dampness and presence of fungal growth were associated with asthma in subjects living in those houses. 42% of the subjects reported that the houses they lived in had dampness. Dampness was underestimated by occupants in this study with reported dampness and observed dampness by the investigator having 63% agreement. Visible fungal growth was observed by investigators in 26% (57/222), with 33 (15%) of those homes

categorised as having significant fungal growth meaning fungal growth is obvious and widespread. There was significant correlation between dampness and fungal growth scores of a dwellings (r= 0.51, p= 0.005). The study also found a negative correlation between dampness and predicted $FEV_1$ of the subjects (r= -0.30, p= 0.006) and positive correlation between asthma severity and severity of dampness in the house.

A cross-sectional study, on 4$^{th}$ grade children in Munich, was undertaken to assess the relationship between dampness and other factors considered as risk in asthmatic children and adolescents with persistent respiratory symptoms and bronchial hyper-reactivity. Again in this study, questionnaires were used to collect data on house conditions and respiratory symptoms. Nicolai *et al*. (1998) used direct questions such as "do you have a damp spot in your home?" to find out about dampness conditions in children's homes. 17% of homes in this study had dampness in the homes and was associated with respiratory illnesses of children living in these homes.

Dales *et al*. (1997) evaluated the questions commonly used in questionnaires to indicate the presence of fungi in homes on 403 families in Ontario, Canada. Living areas in these homes had total viable fungi geometric mean concentrations of 50% greater in the dust when visible fungi, water damage, or fungal or musty odours were reported. Visible mould growth was the best indicator of predominant indoor fungi which is *Aspergillus* and *Penicillium species* in these houses. Bias on reporting dampness by occupants in this study was noted by the authors as smokers tend to be less likely to report visible fungal growth, and those that report allergies were more likely to report visible fungal growth. These are some of the problems or bias responses likely to surface

when occupants self report home conditions. Investigators found that questionnaire reports of conditions were associated with the levels of viable indoor fungi found in dust.

1000 homes of infants in Northeastern USA were used to evaluate questionnaires commonly used in epidemiologic studies to obtain house characteristics or activities as predictors of fungal aerosol exposure. In this study, Ren *et al.* (2001) found that presence of a cat in the home was also consistently and positively related to concentrations of culturable fungi in indoor air. Presence of some conditions, namely humidifier, air conditioning, wood-burning stove, presence of dog, drapes or curtains, observation of fungi and mildew, and building age; that are usually associated with elevated levels of fungal propagules in an indoor environment were not related. Some house conditions were predictors of certain variables like presence and absence of certain airborne fungal species and levels of these airborne fungal species.

Van Strien *et al*. (1994) used a checklist to collect information about home characteristics and occupant exposure to dust mite allergen (*Der p 1*) in 516 houses in the Netherlands. The checklist used was completed by a trained investigator and it assessed likelihood of the house being infested by house dust mites. House and occupant characteristics included type and age of house, materials used in construction, insulation, ventilation system, heating system, type of floor cover, presence of upholstery furniture, indoor cloth drying, frequency of vacuum cleaning, presence of curtains, fungal stains, dampness stains, etc. House characteristics that were significantly associated with higher *Der p 1* level in dust were wall to wall carpet, upholstered furniture, wall insulation, number of occupants and indoor clothes drying.

Van Strien *et al*. (2004) in other study on home and occupant characteristics that could be determinants of elevated house dust mite allergens. Season, temperature, age of the building, presence of drapes/curtains, heating system, type of housing, mattress cover, stuffed toys on bed, washing temperature of bedding, etc. were some of the characteristics considered in this study. Results showed that *Der f 1* and *Der p 1* levels were higher in living areas of homes with electric heating, single family houses, houses without air conditioner, in homes with upholstered furniture, houses with reported fungi or mildew, older homes, homes with more than seven rooms, lower bedroom temperatures and ethnic group of the family. Between these characteristics significant association with higher *Der p 1* and *Der f 1* levels was observed with absence of air conditioner, lower indoor temperatures and reported fungi or mildew.

Home condition that El Sharif *et al*. (2004), found in their study significantly associated with allergen levels in Palestinian homes was ground floor dwelling in the building. Location of settlement (i.e. region where the home is situated) was also significantly associated with high *Der p 1* and low *Der f 1* levels in refugee homes.

In Boston, Chew *et al*. (1998) evaluated a questionnaire as an instrument to be used in predicting levels of indoor allergens in a cross sectional study. In this study, researchers investigated positive and negative predictive power of home characteristics for cat, house dust mite and cockroach allergen exposure. Type of building, thickness of carpet and mattresses were significant predictors of dust allergen levels in Boston homes, but other house characteristics were not significantly predictive of levels of allergens.

Some studies had investigated association between levels of indoor biological pollutants and house conditions as indicated above. But there is still gap in understanding

this association because of regional difference in climate and different practices of the occupants that might affect pollutant levels in the homes (Figure 4).

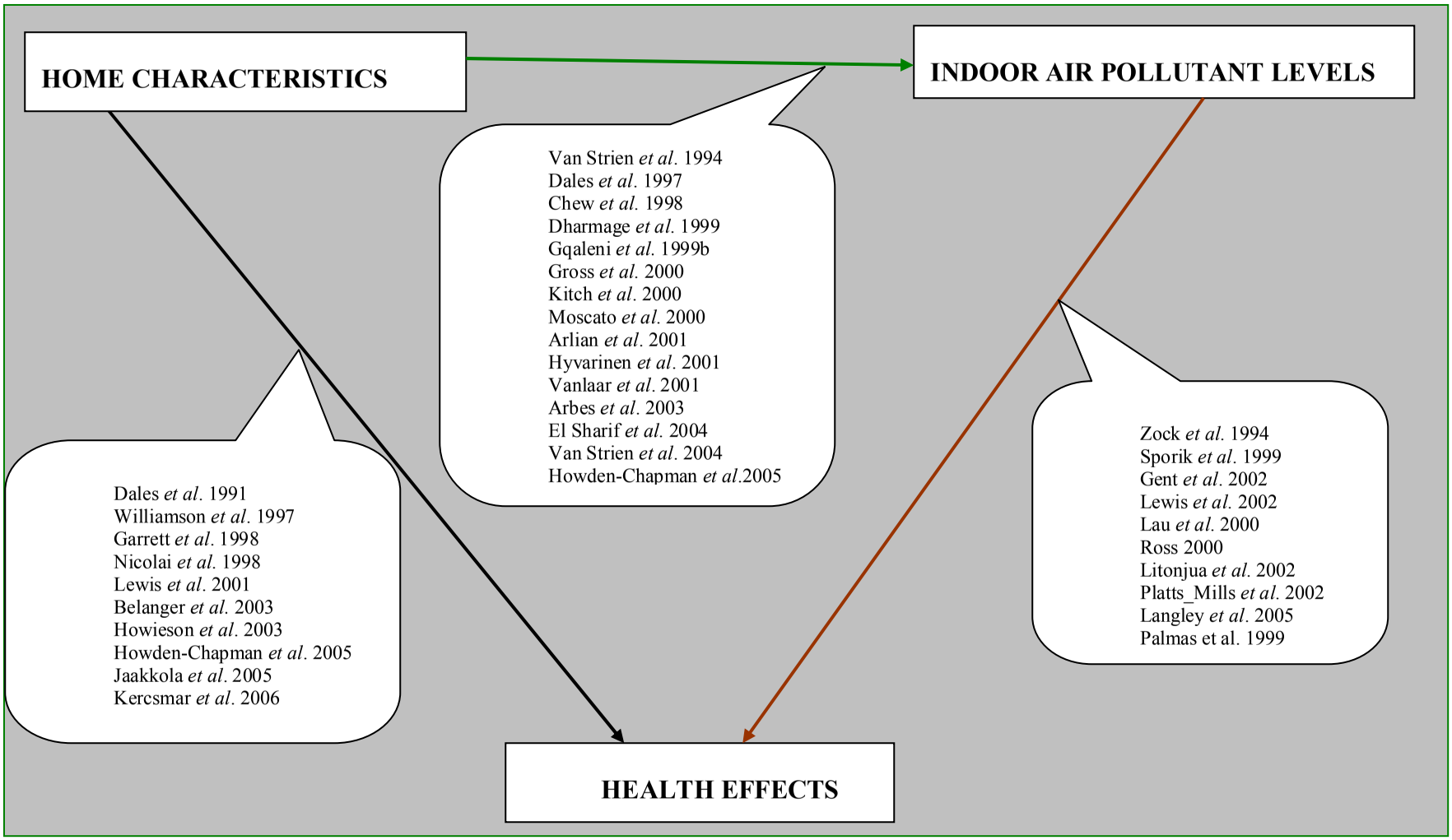



**Figure 4:** Diagram showing studies that looked at relationship of health affects as a result of exposure to home characteristics and indoor air pollutant levels, and effects of home characteristics on allergen levels.

## 3.2 METHODOLOGY

### 3.2.1 Sample Selection

A sample of households of about 20 school children from each school in the South Durban Health Study list was selected for the household indoor environment assessment. Initially in a SDHS, a list was compiled from the screening questionnaire that identified schoolchildren with persistent asthma, mild asthma, and non-asthmatics. Persistent asthmatics' households were targeted for recruitment first and if at least 20 households per neighbourhood could not be recruited, then other learners' households (with mild or no asthma) were recruited. The selected school children from the households participating in the indoor assessment also participated in other aspects of the SDHS, e.g., bi-hourly lung function tests, baseline pulmonary tests, and allergenic tests.

Potential households were recruited by telephone and by visits to their homes. If the household selected for recruitment was not available for sampling on a preferred date, investigators move on and recruited other potential participants. Most of the participating homes were part of the study because of convenience for both the investigator and the participant. The indoor assessment was scheduled with the child caregiver or other adult person living with the child. A written or oral consent (Appendix 1) was obtained from the participating households before and assessment was done in the households. Assessment included a walkthrough investigation, fungal aerosol sampling and dust sampling.

### 3.2.2 Walkthrough Inspection/ Data Collection

The walkthrough evaluation of the homes was based on a standardised instrument that was previously field tested and used in a Community Action Against Asthma (CAAA) project in Detroit, USA (Baldwin 2003). This CAAA walkthrough instrument was modified to reflect South African conditions, with questions directed at informal homes included (e.g., roofing made of tarpaulin (sail), or asbestos, or corrugated iron, or presence and absence of ceiling; and walls made of materials such as corrugated iron sheets, or cardboard, etc.) (Appendix 2). Because this checklist investigated exposure of children to indoor pollutants, sleep area, play area instead of bedroom and / or living room when assessing children's homes were used. Room-by-room in the homes, observational data was collected by investigators together with questions directed to a child caregiver or an adult respondent. Observational questions were about type of house, building age; conditions of the resident i.e. water damaged surfaces, moist and damp floors, moisture problems (sources, indicators), ventilation, heating) and fungal growth in different areas of the house. Other indoor environment information collected on conditions that are considered favourable for allergen proliferation such as type of floors, type of furnishing, type of ventilation, type of bedding used by the child, presence of stuffed toys in the area etc. Data about extent of damage by water or fungal growth was collected using surface area in square metres ($m^2$) covered by the stains or fungal growth as a measure. To explain the extent of damage, covered surface area was assigned as follows "<0.3 $m^2$ = mild", "0.3 to 1.0 $m^2$ = moderate" and ">1.0 $m^2$ = high". Other questions were directed to child caregiver and they include questions about child's activity while indoors such as "where did the child sleep?", "where did the child spend

most of his time if not in the sleep area?", "where does the child take his or her bath?" etc.

The data was captured as coded in the WTC with "1=Yes" and "2=No". Before working with it, the data was recoded as to harmonise it for further analysis. Coded responses for a "2=No" response were changed to "0=No". A response of "9=Don't know" were excluded from the dataset when doing statistical analysis of the data.

WTC identified variables as direct or indirect exposure variables on surface of each room and this was used when creating new composite variables. Dampness and fungal exposure were classified as direct exposure because their presence could be identified, while dust mite exposure was indirect exposure as only conditions associated with it were identified.

The idea of creating new variables was to quantify the variables about conditions in the homes. Some responses in the checklist were dichotomous (Yes / No) and new continuous variables were created to quantify the extent of exposure to conditions in the homes. In each room, variables associated with exposure to dampness were averaged by taking weighted average of all associated dichotomous variables and a new continuous composite score was created (Appendix 3). For example, if responses to questions about indicators of dampness on the surfaces are all (weighted mean) "No=0" then composed dampness score would be equal to "0". If responses were mixed, then the new created variable would be between "0.10" and "0.99". A dampness composite score for entire home (weighted mean for all rooms) was created as well. The similar process was repeated when creating new composed variables for exposure to visible fungal growth and dust mite in each room and entire home. If it were not for this, a room with dampness

problem on the wall only and a room with dampness on all surfaces (i.e. ceiling, wall and floor) would have the same score.

Total weighted exposure variable that includes dust mite, fungi and dampness composite scores was created for the whole house with direct exposure scores having twice the weight than indirect exposure scores. In all, 22 new composite variables that sum or explain house conditions for exposure to dampness, dust mite, fungal growth and the combination of these exposure composite variables were created.

New composed variables assumed scores of between 0.00 and 1.00. The new composite scores were further categorised according to extent of dampness, fungal growth and dust mite exposure. The categories were arbitrarily chosen with "1= the exposure from all possible sources in the room or home" and "0=no possible exposure in the room or home" to biological pollutants of interest. A score of "0.5" considered moderate because it is half way between extreme and none. These scores were assigned as 1.00 = extreme, 0.51 - 0.99 = high, 0.50 = moderate, 0.01 – 0.49 = mild and 0.00 = no damage.

### 3.2.3 Statistical Analysis

All data collected was captured on SPSS version 13.0 for Windows (SPSS Inc., Chicago) database and cleaned before transferring it to STATA 9.0 for Windows (StataCorp LP, Texas) statistical package for analysis. A STATA 9.0 statistical package was used when analysing original home walkthrough checklist variables and newly created composite variables. Frequencies of different WTC variables and frequency distribution of the new composed variables were analysed. Correlations between original WTC variables were analysed as well as between new composed variables and original

WTC variables. Certain variables were given priority when relationships were explored namely surface type and characteristics such as fungal growth e.g. ceiling presence versus water stains, roof type versus visible mould growth; water stains versus fungal growth etc.

## 3.3 RESULTS

Walkthrough inspection data was collected from 136 of the 137 homes visited. One house which a walkthrough checklist administered to was not excluded from the dataset meaning it was not part of any analysis in this dissertation. Inspection or observational samplings of the homes as well as interviews were done by trained investigators, for each home and each room in the house. Inter-observer variability between was not tested with assumption that it is not that significant because there were 128 house versus 8 homes inspected between the two investigators. In the dataset invalid data points were omitted thus some associations in the analysis reflected number not adding up to the expected number.

More than 70% of the houses visited were single family houses (stand alone houses/not attached for a single family) whilst others were duplexes or flats or apartments and shacks (informal houses).Table 2 shows home baseline characteristics from all the homes visited for indoor investigation. Of the 136 caregivers or parents interviewed in children's homes, 14 (10.3%) did not know when their homes were built and 69 (50.7%) indicated that their homes were built before 1978, that is more than 25 years ago. Most houses visited (35.8%) had 4 rooms, followed by houses with 5 rooms (20.9%). A majority (92.6%) of the homes was made of bricks and other 5.1% were constructed of

wood. The 5.9% homes that were informally constructed were made from different materials like bricks, wood, corrugated iron, etc.

Most children, 86.8%, involved in this indoor study used bedroom as their sleep area while 5.9% used living room and other 6.6% used other rooms or areas in the house including kitchen and outside building rooms. Children preferred using living room in their homes as a play area while others (21) used their sleep area and few used the study room (Table 3). In the end 113 children use different room as play area different from the room they use as sleep area. The number of kitchens and bathrooms separate from sleep area and play area that were investigated are 123 and 124 respectively.

**Table 2:** Basic demographic house characteristics data of children's homes

| **House characteristics** | | **Number of homes n=136 (%)** |
|---|---|---|
| ***Type of house*** | single family | 96 (70.6) |
| | flat / duplex | 27 (19.8) |
| | other | 13 (9.6) |
| ***House built*** | before 1978 | 69 (50.7) |
| | after 1978 | 53 (39.0) |
| | don't know | 14 (10.3) |
| ***House made of*** | bricks | 126 (92.6) |
| | wood | 7 (5.1) |
| | other material | 3 (2.2) |
| ***Number of rooms*** | 1 | 5 (3.7) |
| | 2 | 14 (10.4) |
| | 3 | 13 (9.7) |
| | 4 | 48 (35.8) |
| | 5 | 28 (20.9) |
| | 6 | 19 (14.2) |
| | 7 | 4 (3.0) |
| | 8 | 2 (1.5) |
| | 10 | 1 (0.7) |
| ***Type of construction*** | formal | 128 (94.1) |
| | informal | 8 (5.9) |
| ***Heating system*** | electric heater | 18 (13.24) |
| | paraffin heater | 2 (1.5) |
| | open wood/coal stove | 1 (0.7) |

**Table 3**: Different rooms used as sleep area and play area by children in the homes (n=136)

| Room used as | Room used | Number of homes (%) |
|---|---|---|
| Sleep area | bedroom | 118 (86.8) |
| | living room | 8 (5.9) |
| | other room | 9 (6.6) |
| Play area | same bedroom as sleep area | 21 (15.4) |
| | living room | 109 (80.1) |
| | other room | 6 (4.4) |

Home characteristics data collected from homes showed differences between rooms (Table 4). Most children's sleep area had a ceiling underneath the roof and a majority of sleep areas without ceiling were asbestos roofed. In the play areas, less number of homes did not have ceiling and again majority of them were asbestos roofed. This trend was observed in the kitchen and bathroom as well. Hard floors without rugs in the homes were the predominant type of flooring found in most play and sleep areas whilst carpeting, cement and earth were found as well. The room that had a wall-to-wall carpeting (25%) compared to other rooms was the sleep areas whilst other rooms house it was found in less ratio ranging from 0.0% to 12.6%. Heating of the homes was not high in the visited homes only 24 (18%) homes using heater or some kind of heating system in their homes. The use of a fan or Heating, Ventilation, Cooling Air Conditioner (HVAC) for cooling the indoor space was high at 62.5% of homes.

Water stains on building surfaces are indicators of present or previous water damage or dampness. Of 136 homes, a high number of homes had visible signs of water stains on child's sleep area roof or ceiling and with most of those homes covering a surface area of more than 1.0 $m^2$. Most play areas (64.6%) in homes did not have any signs of water damage or dampness whilst a large number of kitchens (67.5%) had no dampness signs.

Table 5 shows the presence and extent of water damage or mould growth on surface in different rooms. In all rooms, surface area covered by water stains and mould growth were mostly greater than 1.0 $m^2$ (moderate damage), ranging from 50% to 100% number of rooms with moderate damage. Also noted in the table is that bedroom and bathroom had the highest number than other rooms for both water stains and mould growth.

Composed variables for dampness and fungal growth showed that 48.5% and 64.0% of the homes had no dampness or moisture damage signs and no visible fungal growth respectively in child's sleep area (Table 6). The exposures were low (composite scores less than 0.5) to dampness and visible fungal growth in sleep area respectively. Of all sleep areas of the children that had water stains or dampness as indicators at the time of visitation few had visible mould growth. Figure 5 shows frequency distribution of composed variable scores of the three housing conditions (dust mite, dampness and mould growth) for entire home across study homes. Composed dampness and fungal growth scores showed positively skewed distributions for entire house. House dust mite composite scores for each room and entire house for the homes studied showed bathroom to be the only room to have highest scores compared to other rooms. The frequency distribution for house dust mite in different rooms shows a close to normal distribution.

**Table 4:** House conditions that when present or absent they hypothetically influence biological allergen levels in different rooms of the homes

| Home indicators or conditions | | Number of sleep areas n=136 (%) | Number of play areas n=113 (%) | Number of kitchens n=123 (%) | Number of bathrooms n=124 (%) |
|---|---|---|---|---|---|
| ***Ceiling*** | ceiling present | 86 (63.2) | 81 (71.7) | 87 (64.0) | 85 (68.5) |
| | ceiling absent | 50 (36.8) | 32 (28.3) | 37 (27.2) | 39 (31.5) |
| ***Roof made of*** | asbestos | 39 (78.0) | 25 (78.1) | 30 (81.1) | 32 (86.5) |
| ***Floor covering*** | hard floor | 83 (61.0) | 93 (83.8) | 113 (91.9) | 83 (69.2) |
| | Carpet | 34 (25.0) | 14 (12.6) | 0 (0.0) | 0 (0.0) |
| | Cement | 18 (13.2) | 4 (3.6) | 10 (8.1) | 37 (30.8) |
| | Other | 5 (3.7) | 0 (0.0) | 0 (0.0) | 0 (0.0) |
| ***Hard floor*** | with rugs | 34 (41.0) | 34 (36.6) | 32 (28.3) | 29 (34.9) |
| ***Fan /air con present*** | | 36 (26.5) | 49 (43.4) | 6 (4.9) | |
| ***Peeling paint*** | ceiling / roof | 13 (9.6) | 8 (7.1) | 11 (8.9) | 19 (15.3) |
| | Wall | 15 (11.0) | 11 (9.7) | 12 (9.8) | 29 (23.4) |
| ***Plaster falling*** | ceiling / roof | 0 (0.0) | 0 (0.0) | 2 (1.6) | 1 (0.8) |
| | Wall | 6 (4.4) | 3 (2.7) | 4 (3.3) | 12 (9.7) |
| ***Water stains/Moisture*** | ceiling / roof | 48 (35.3) | 34 (30.1) | 35 (28.5) | 42 (33.9) |
| | Wall | 30 (22.1) | 14 (12.4) | 14 (11.4) | 33 (26.8) |
| | Floor | 9 (6.6) | 7 (6.3) | 4 (3.3) | 20 (16.5) |
| ***Leaking plumbing*** | | | | 22 (18.3) | 33 (27.5) |
| ***Visible mould growth*** | ceiling / roof | 36 (26.7) | 18 (15.9) | 14 (11.4) | 34 (27.4) |
| | Wall | 16 (11.8) | 4 (3.5) | 3 (2.4) | 26 (21.1) |
| ***Musty smell, mildew or visible mould*** | | 22 (16.2) | 11 (9.8) | 6 (4.9) | 24 (19.4) |

**Table 4:** ***(continued)***

| | | | | | |
|---|---|---|---|---|---|
| ***Stuffed toys present*** | | 47 (34.6) | 8 (7.1) | 2 (91.6) | |
| ***Cloth covered chairs present*** | | 32 (23.5) | 32 (23.5) | 16 (13.0) | |
| ***Heater used*** | Electrical | 13(9.6) | | | |
| ***Curtain present*** | | | 109 (99.1) | 108 (89.3) | 50 (46.7) |
| ***Filling in the pillow*** | foam | 68 (50.0) | | | |
| | feathers | 4 (2.9) | | | |
| | polyester | 58 (42.6) | | | |
| | other material | 2 (1.4) | | | |
| ***Child sleeps on*** | bed with mattress | 127 (93.4) | | | |
| | sofa | 2 (1.5) | | | |
| | mattress on floor | 5 (3.4) | | | |
| | other | 2 (1.5) | | | |
| ***Type of blankets used*** | wool | 65 (47.8) | | | |
| | cotton | 40 (29.4) | | | |
| | acrylic | 4 (2.9) | | | |
| | blend | 1 (0.7) | | | |
| | comfort | 67 (49.3) | | | |

**Table 5:** Distribution of water stains or visible mould growth on the roofs/ceilings and walls of different rooms of visited homes determined by surface area covered ("<0.3 $m^2$ = mild", "0.3 to 1.0 $m^2$ = moderate" and ">1.0 $m^2$ = high")

| Dampness or mould growth | Room | Surface covered | *Mild damage* No. of homes (%) | *Moderate damage* No. of homes (%) | *High damage* No. of homes (%) | TOTAL (%) |
|---|---|---|---|---|---|---|
| **Water stains** | Sleep area | ceiling / roof | 10 (20.8) | 9 (18.8) | 29 (60.4) | 48(35.3) |
| | | wall | 1 (3.3) | 6 (20.0) | 23 (76.7) | 30 (22.1 |
| | Play area | ceiling / roof | 5 (14.3) | 6 (17.1) | 24 (68.6) | 35 (25.7 |
| | | wall | 0 (0.0) | 3 (21.4) | 11 (78.6) | 14(10.3) |
| | Kitchen | ceiling / roof | 5 (14.3) | 12 (34.3) | 18 (51.4) | 35 (25.7 |
| | | wall | 0 (0.0) | 1 (7.10 | 13 (92.7) | 14(10.3) |
| | Bathroom | ceiling / roof | 9 (21.4) | 10 (23.8) | 23 (54.8) | 42 (30.9 |
| | | wall | 1 (3.0) | 5 (15.2) | 27 (81.8) | 33(24.3) |
| **Mould growth** | Sleep area | ceiling / roof | 5 (13.5) | 6 (16.2) | 26 (70.3) | 37 (27.2 |
| | | wall | 3 (18.8) | 2 (12.5) | 11(68.8) | 16(11.8) |
| | Play area | ceiling / roof | 1 (5.60) | 2 (11.1) | 15 (83.3) | 18 (13.2 |
| | | wall | 1 (25) | 0 (0.0) | 3 (75) | 4 (2.9 |
| | Kitchen | ceiling / roof | 0 (0.0) | 1 (7.1) | 13 (92.7) | 14 (10.3 |
| | | wall | 0 (0.0) | 0 (0.0) | 3 (100) | 3 (2.2 |
| | Bathroom | ceiling / roof | 2 (5.9) | 13 (38.2) | 19 (55.9) | 34 (25.0) |
| | | wall | 2 (7.7) | 11 (42.3) | 13 (50.0) | 26 (19.1) |

**Table 6:** Frequency distribution of homes with direct and indirect composed scores for dampness, mould growth and house dust mites (indicator of possible exposure to sources)

| Variable | Composed variable score | No. of sleep areas n=136 (%) | No. of play areas n=113 (%) | No. of kitchens n=123 (%) | No. of bathrooms n=124 (%) | Indicator level |
|---|---|---|---|---|---|---|
| ***Dampness*** | 1.0 | 1 (0.7) | 5 (4.4) | 1 (0.8) | 9 (7.3) | Extreme |
| | 0.51-0.99 | 8 (6.6) | 4 (3.5) | 8 (6.5) | 18 (14.5) | High |
| | 0.50 | 17 (12.5) | 2 (1.8) | 5 (4.1) | 2 (1.6) | Moderate |
| | 0.01-0.49 | 42 (30.8) | 29 (25.7) | 26 (21.1) | 36 (6.5) | Mild |
| | 0.00 | 67 (49.3) | 73 (64.6) | 83 (67.5) | 59 (46.6) | None |
| ***Mould*** | 1.0 | 7 (5.1) | 1 (0.9) | 0 (0.0) | 4 (3.2) | Extreme |
| | 0.51-0.99 | 11 (8.1) | 4 (3.5) | 5 (4.1) | 23 (18.5) | High |
| | 0.50 | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | Moderate |
| | 0.01-0.49 | 31 (22.8) | 22 (19.5) | 13 (10.6) | 26 (21.0) | Mild |
| | 0.00 | 87 (64.0) | 86 (76.1) | 105 (85.4) | 71 (57.3) | None |
| ***Dust mite*** | 1.0 | 0 (0.0) | 0 (0.0) | 0 (0.0) | 1 (0.8) | Extreme |
| | 0.51-0.99 | 1 (0.7) | 6 (5.3) | 29 (30.6) | 16 (12.9) | High |
| | 0.50 | 10 (7.4) | 45 (39.8) | 1 (0.8) | 0 (0.0) | Moderate |
| | 0.01-0.49 | 125 (91.9) | 59 (52.2) | 81 (65.9) | 46 (37.0) | Mild |
| | 0.00 | 0 (0.0) | 3 (2.7) | 12 (9.8) | 61 (49.2) | None |

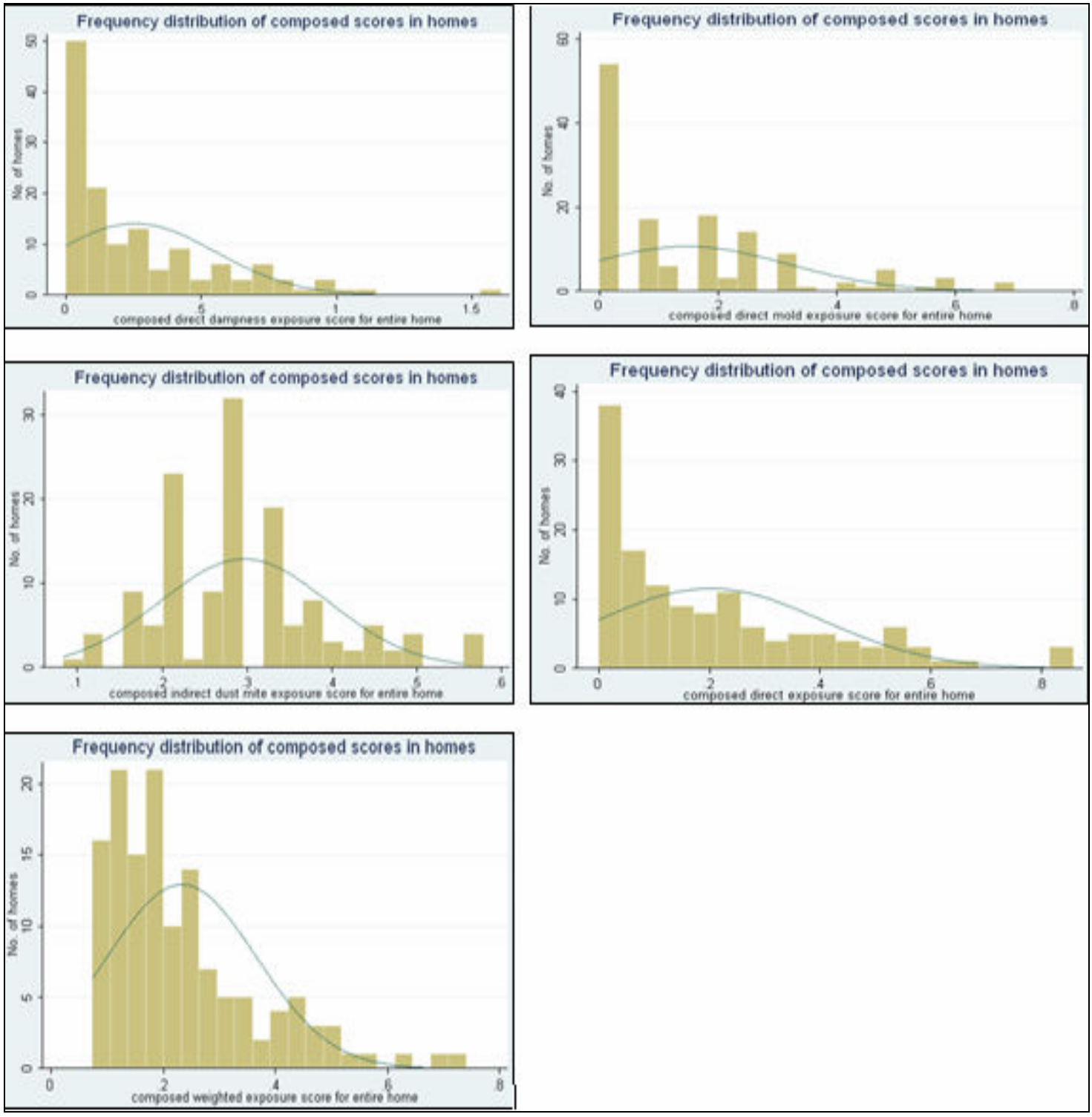



**Figure 5:** Frequency distribution of walkthrough checklist composed scores for dampness, fungal allergens and dust mite allergens for entire home

Some variables in each room and entire house had statistically significant correlation (Table 7). Strong negative correlation varied from r= -0.249 (p = 0.005) in the bathroom to r = -0.528 (p<0.001) in the kitchen between when relationship between the presence of a ceiling with water stains and fungal growth on the roof/ceiling was explored. The relationship between asbestos roofing in homes without ceiling was strongly correlated to water stains on the roof in all the rooms with correlations ranging from r= 0.385 (p = 0.019) in bathroom and r= 0.579 (p<0.001) in sleep area. Presence of water stains on roof/ceiling was statistically significant when correlated with fungal growth on the roof for all rooms. Presence and extent of wall water stains was as well correlated with fungal growth and high fungal contamination on the wall. Use heating system, use of fan, type of flooring, type of bedding, etc. which are conditions that can be attributed to occupant behaviours or activities, showed no significant correlation with any other conditions such as fungal growth, dampness, water damage, etc.

**Table 7:** Pearson correlation coefficients of home walkthrough checklist variables in different rooms of the house

| Room | variable | roof $H_2O$ stains | extent of roof stains | roof mould | extent of roof mould | wall $H_2O$ stains | extent of wall stains | wall mould | extent of wall mould | floor $H_2O$ damage |
|---|---|---|---|---|---|---|---|---|---|---|
| Sleep | presence of ceiling | **-0.426 | **-0.639 | **-0.408 | -0.267 | **-0.404 | **-0.569 | -0.100 | -0.316 | *0.226 |
| | asbestos roofing | **0.579 | -0.075 | **0.531 | | -0.113 | 0.172 | -0.316 | 0.655 | -0.203 |
| | roof water stains | | | **0.427 | -0.062 | **0.312 | 0.144 | 0.160 | **0.558 | |
| | roof mould | **0.427 | **0.469 | | | | | | | |
| | wall water stains | | | | | | | **0.411 | 0.126 | |
| Play | presence of ceiling | **-0.487 | **-0.367 | **-0.478 | **0.467 | -0.181 | -0.174 | 0.0141 | -0.333 | |
| | asbestos roofing | **0.572 | -0.125 | *0.467 | | -0.269 | -0.354 | 0.095 | | -0.269 |
| | roof water stains | | -0.107 | **0.611 | -0.101 | *0.222 | -0.101 | 0.083 | 0.577 | **0.305 |
| | roof mould | **0.611 | *0.373 | | | -0.218 | 0.522 | 0.154 | | |
| | wall water stains | *0.222 | 0.100 | | | | | **0.509 | | |
| Kitchen | presence of ceiling | **-0.528 | *-0.349 | **-0.322 | 0.175 | -0.154 | -0.277 | -0.126 | | -0.178 |
| | asbestos roofing | **0.512 | -0.166 | 0.294 | | -0.119 | | 0.116 | | 0.144 |
| | roof water stains | | | **0.398 | -0.145 | *286 | -0.207 | 0.017 | | **0.296 |
| | roof mould | **0.398 | **0.591 | | | 0.033 | 0.113 | 0.109 | | **0.222 |
| | wall water stains | *0.285 | -0.122 | 0.033 | 0.113 | | | **0.441 | | 0.078 |
| Bathroom | presence of ceiling | *-0.249 | *-0.493 | 0.144 | 0.060 | **-0.573 | -0.341 | *-0.257 | **-0.621 | **-0.402 |
| | asbestos roofing | *0.385 | | 0.191 | | *0.507 | | 0.320 | | 0.304 |
| | roof water stains | | | *0.210 | *0.340 | *0.261 | 0.232 | **0.299 | *0.405 | 0.105 |
| | roof mould | *0.210 | 0.140 | | | -0.087 | 0.075 | *0.214 | -0.376 | -0.062 |
| | Wall water stains | *0.261 | *0.358 | -0.087 | 0.180 | | | **0.418 | *0.530 | **0.539 |

Significant correlations at
** alpha = 0.001 *alpha = 0.05

## 3.4 DISCUSSION

This study was able to describe characteristics and conditions considered risk factors of exposure to indoor environment of the children's homes. Walkthrough investigations to the homes were done by trained investigators, who were able to estimate the extent of dampness or visible fungal growth in the homes using surface area covered. Using some home characteristics, composed indirect and direct exposure of children to conditions were quantified for different rooms and for entire home. Home characteristics that were of interest are described in this dissertation and some of them had been associated with proliferation of indoor biological pollutant levels.

Type of housing structure was an important factor in this study when using home walkthrough checklist in assessing conditions in the homes of the learners. Some homes were formal low cost houses, publicly built as part of government's housing programme for communities with low economic income or replacement of informal homes/shacks in urban areas. The small sample size could not allow stratification analysis of homes for meaningful analysis of differences between types of housing structures namely informal houses and formal, and type of housing i.e. single family unit versus flat, etc.

The number of homes affected by fungal growth or dampness was comparable to what other studies conducted in Durban found. Homes with visible fungal growth or dampness problems investigated by Gqaleni *et al*. (199b) and Sekhotha *et al*. (2000) found that 42% and 30% of the homes in Durban respectively had fungal problems. Gqaleni *et al*. (1999b) investigated homes in informal settlements whereas Sekhotha *et al*. (2000) investigated only formally constructed homes part of the study. In our study

different types of buildings were part of the study and they included single family homes, apartments or flats and homes constructed informally or situated in an informal settlement. In a European cities survey of fungal growth problems in homes showed that cities incidence of homes with visible fungal growth range from 2.9 % in Norway to 36% in Italy (Andriessen *et al*. 1998; Boquette *et al*. 2006). Although not investigated in this study warmer humid climate of Durban might to be significant contributor to surface fungal growth when compared to colder dry climates.

Fungal growth was visible in 26% of the homes and 15% of the homes classified as 3 (cover area greater than 1.0 $m^2$) in this study which is something that not many studies did. Sekhotha *et al*. (2000) also categorised extent of mould in Durban homes and found that most homes with fungal growth had bad or moderate contamination (20% and 16% respectively). In Glasgow homes Williamson *et al*. (1997) investigated and graded both dampness and visible fungal growth observed on building surfaces. 51% of the homes had moisture detected in them with 19% having one area graded as 3 (51-75% relative humidity) or 4 (>76% relative humidity).

Our study confirmed a finding by Sekhotha *et al*. (2000) that the worst affected rooms with fungal growth in Durban homes were bathrooms. Dampness and fungal growth in the bathroom show occupants' lack of moisture control in the homes. Sleep areas, which were bedrooms, living rooms and outbuilding rooms, of the children were the second mostly affected rooms with dampness and visible fungal growth in the homes. This finding is in contrast to a study by Sekhotha *et al*. (2000) that kitchens were second most affected rooms in the homes. The correlation results in Table 7 were showed consistence in absence of water stains and visible fungal growth on the roof/ceiling in

rooms with ceiling in them. This is an important finding as exposure to fungi can be controlled by installation of ceiling underneath the roof. Dampness (water stains) both on the roof/ceiling and on the wall were significantly associated with visible fungal growth which is an indication that moisture or water damage results in fungal growth in these homes. Severity of dampness or fungal growth on the surfaces in most homes showed that these conditions were not necessary a once-off damage but were almost permanent conditions in many households.

Type of floor had been found to be one of the characteristics that have impact on amount of dust mite allergens (Van Strien *et al*. 1994; Chew *et al*. 1999; Mihrshahi *et al*. 2002). Carpeting or soft flooring in sleep area is considered a predictor of exposure for occupants to elevated dust mite allergens. Carpeted floors in the sleep area floor were found in 25% of the homes in our study which is lower than what other studies show. In different studies such as in study 51% of the homes, in Netherlands 66% of the homes, in Spain 68.6% of the homes and in Sydney 81.5% of the homes had carpeted floors in the bedrooms (Van Strien *et al*. 1994; Chew *et al*. 1999; Mihrshahi *et al*. 2002; Boquette *et al*. 2006).

Heating of indoor environment using heaters at any time of the year was low in these study homes. This observation was expected as Durban has a fairly warm climate throughout the year instead the use of fans or air conditioners in the homes was high. In Spain, a region with Mediterranean climate, use of heaters and air conditioners was found in 67.9% of the homes (Boquette *et al*. 2006).

Walkthrough investigations in an indoor assessment procedure is essential tool or instrument as it does not only points or indicate sources and possible sources of indoor

pollution but is helpful in mitigation and control of indoor environment pollutants when pollutants are identified. The associations made about extent of moisture or dampness damage with visible fungal growth is important. In some cases dampness can be observed but no visible fungal growth identified.

# CHAPTER 4

# HOME CHARACTERISTICS AND INDOOR ALLERGENS

## 4.1 INTRODUCTION

Indoor biological allergens are a major contributor to the quality of indoor environment. These allergens are a result of proliferation of microorganisms such as mould growth on the building surfaces, house dust mites on carpets, upholstery and bedding as well cockroaches in the kitchen. Animal dander from domestic animals like cats, dogs, genie pigs, etc. had proved to be another important source of allergen exposure of individuals in the homes.

Some studies had investigated association of indoor allergen levels with adverse health effects. Lau *et al.* (2000) found no association between exposure to indoor allergens and asthma at age 7 year children (β-coefficient 0.0177, SE 0.0204; p=0.38) whereas Lewis *et al.* (2002) found that cockroach and cat allergens contributed to asthma morbidity in sensitised women who used steroid (model adjusted for race and area poverty (OR 6.2 [95%CI 2.7-14.1], $p \leq 0.05$); whilst a majority of the studies had associated home characteristics or conditions with adverse health effects (Yang *et al*. 1998; Arlian *et al*. 2001; Litonjua *et al*. 2002; Howieson *et al*. 2003). The relationship between home characteristics and levels of indoor allergens had also been studied by some investigators but their findings are not consistent (van Strein *et al*. 1994; Chew *et*

*al*. 1998; Dharmage *et al*. 1999; Gross *et al*. 2000; Vanlaar *et al*. 2001; Arbes *et al*. 2003; van Strien *et al*. 2004).

Vanlaar *et al*. (2001) studied relationship between home conditions and allergen levels in two inland towns in Australia. Only two features in the homes were associated with high allergen levels. Homes with evaporative coolers had 3.34 (95%CI 1.39 – 8.04), (p=0.007) times higher levels of *Der p 1* from bed and 3.94 (95%CI 1.58 – 9.84), (p=0.003) times higher levels from floor dust compared to homes that don't have them. Beds or mattresses that were older than 5 years had higher levels of *Der p 1* than newer beds OR=3.0, (95%CI 1.21 – 7.46), (p=0.017). For duvets made of synthetic material there was strong association with higher *Der p 1* levels but not statistically significant.

In Netherlands, an intervention study, participants were instructed to make some changes in their homes as a measure of controlling asthma incidence. *Der p 1* allergen levels in samples collected from carpeted floors were higher than levels from smooth floor. Presence of smooth floors was a house condition that had a positive effect on the children's health (Zock *et al*. 1994). Moscato *et al*. (2000) found that *Der p 1* allergen levels were 12.22 higher (95%CI, 7.34 – 14.63) in beds with pillows filled with synthetic material and when pillows were filled with wool or feather levels were lower. Home conditions or activities such as washing bedding at temperatures above $60^0$C and others did not show any statistical significance. Bedrooms had higher *Der p 1* and *Der f 1* levels than living rooms. No other associations were found between home characteristics and allergens levels in these Italian homes.

In USA, van Strien and colleagues (2004) studied influence of air conditioning, humidity, temperature and other household conditions on dust mite allergen levels.

Levels of *Der f 1* and *Der p 1* allergens in dust from bedrooms and living rooms were significantly higher in homes with high relative humidity, lower temperatures, clothed upholstered furnisher, no air conditioner, older homes, using electric heating, single family homes, homes with reported mould and mildew bedrooms with carpets or rugs.

Age of the home, presence of musty or mildew smell and humidifier were associated with higher levels of dust mite allergens in a study of US homes. Predictors of higher dust mite allergens in the multivariate analysis were higher humidity ($p<0.001$), older homes ($p=0.002$), frequent musty or mildew odour ($p=0.01$), single family home ($p=0.09$) and homes with out forced air heater ($p=0.09$) (Arbes *et al*. 2003).

In a children's case-control Netherlands study of home dampness and respiratory symptoms, Van Strien and colleagues (1994) looked for a relationship between home characteristics and dust mite allergens in the residences. *Der p 1* concentrations in the bedroom were seven fold higher in dust collected from smooth floors with rugs compared to that dust collected from smooth floors ($p<0.001$). Dust from wall to wall carpeted floors had 6 fold higher *Der p 1* levels than that from smooth surfaces ($p<0.001$). Living room dust mite allergen samples that were grouped according to type of flooring showed significant relationships some home characteristics. Smooth floor had significant association with upholstered furnisher (OR=1.6 95%CI 1.10 – 2.33); smooth floor with rug had association with indoor clothes drying [OR=1.77, (95%CI 1.05 – 2.99)], non-insulated walled home [OR=3.00 (95%CI 1.78 – 5.05)] and wall-to-wall carpet also had association with insulted walls (OR=1.72 [95%CI 1.02 – 2.90), wooden floor [OR=1.83 (95%CI 1.25 – 2.68)], floor cover aged 4 to 7 years [OR=2.07 (95%CI 1.30 – 3.28)] and floor cover aged >8 years [OR=2.28 (95%CI 1.43 – 3.66)].

High *Der p 1* levels were associated with high relative humidity and low room temperature in German homes. Age of the carpets was also significantly associated to higher levels of *Der p 1* allergen. Ground floor dwellings in this study had 4 times higher levels of *Der f 1* allergens compared to 4$^{th}$ floor and above dwellings. Also *Der p 1* allergen levels were higher for ground floor compared to higher levels. Mattresses aged older than 10 years contained 10 times higher *Der f 1* levels than new mattresses (Gross *et al*. 2000).

Dharmage *et al*. (1999) also find that Der *p 1* levels were 1.9 times higher in bedrooms on the ground floor compared to bedrooms on upper floors. Age and type of the house were also conditions that affected higher levels of bed *Der p 1* allergens in homes. Higher floor levels of *Der p 1* were associated with heavy drapes / curtains, extractor fan, central heating and walls made of weather board. Predictors of higher levels of bedroom HDM were sampling season, age of the house, type of wall, type of heating used, floor covering and dampness in the bedroom.

Other studies had also found other home characteristics as predictors of HDM higher levels of allergens in the homes (Couper *et al*. 1998; Cunningham 1998; Leung *et al*. 1998; Luczynska *et al*. 1998).

In a study on non industrial occupational settings, investigators studied association of relative humidity, moisture and temperature to levels of mould allergens. These environmental factors were not associated with concentration of *Asp f 1* allergen in collected air and dust. Findings of this study also showed no significant association of *Asp f 1* allergen with any other building characteristic as well and there was no association of allergen levels with airborne and settled fungal levels (Ryan *et al*. 2001).

Chew *et al*. (1998) investigated the power of home characteristics to predict allergen exposures at high levels associated with sensitisation or exacerbation of asthma in sensitised asthmatics. The findings had that single family home or duplex and type of carpeting were predictive characteristics of higher dust mite allergens. Relative humidity and temperature were not predictors of dust allergens. In this study, Chew and colleagues (1998) concluded that home characteristics are not reliable predictors of allergen levels in the homes.

Findings of studies that investigated home characteristics association with levels of indoor allergens are not consistent; they found different predictors of allergen level. The reason of this inconsistency of characteristics could be the occupant activities, geographical location and meteorological differences between regions where these studies were conducted.

## 4.2 METHODOLOGY

### 4.2.1 Dust Sampling

After administering a walkthrough checklist (see section 3.2.1) to the caregiver of the participating child, the sleep area of the child was identified. A sample of dust was collected from the bedding and soft floor (carpet or rug) of the sleep area of the child using a vacuum cleaner (Wap Vs300s Combi Cleaner, Bellenberg ) equipped with a specialised nozzle and a 40 mm paper filter (Macherey-Nagel, Germany ). Collected dust sample on filter paper was folded and covered with aluminium foil before being placed into a ziplock bag for transportation and storage. The samples were stored at $4^0$C until they were sieved and extracted for allergens.

### 4.2.2 Extraction of Dust Samples

Dust samples were sieved through a 355μm diameter sieve to remove large particles and fibres from the fine dust. A subsample of mass of 100mg of each sieved dust sample was weighed into a 75mm x 12 mm plastic centrifuge tube and 2.0 ml of 0.05% Tween 20 in phosphate buffered saline, pH 7.4 (PBS-T) was added before the samples were resuspended using a vortex mixer. Then the samples were mixed end over end for 2 hours on a laboratory rocker (Stuart, Staffordshire) at room temperature. After 2 hours the samples were separated at 2,500 rpm with a centrifuge (Hermle Labortechnik, Wehingen) at $4^0$C for 20 minutes, then the supernatant which had extracts in it was removed with Pasteur pipette for antigen measurement and dust pellets discarded. Extracts were stored in a labelled freezer vial with clear coding for future analysis (see Appendix 4 for preparation of reagents for extraction and analysis of allergens).

### 4.2.3 Analysis of Allergens

#### *4.2.3.1 Mould allergen (Asp f 1) analysis*

A*sp f 1* monoclonal antibody for ELISA assay supplied by Indoor Biotechnologies (Wiltshire, UK) was used for mould allergen analysis. The supplied monoclonal antibody (mAb 4A6) was diluted 1000 times in 50mM carbonate-bicarbonate buffer. Microtiter plates (96 well) were incubated with 0.1ml of the diluted monoclonal antibody in each well over a 24 hour period double dilution from 40ng/ml to 0.04ng/ml were used to make standard control curve. A 10 times dilution of the sample was made and placed in the wells before serial dilutions of 1/20, 1/40, and 1/80 were made from the diluted sample. Samples and standards were incubated in the plate at room temperature

for 1 hour. Then the wells were washed 3x with PBS-T solution and plates incubated for 1 hour with 0.1 ml of 1000 times diluted Peroxidase conjugated Goat anti Rabbit IgG (Sigma, Saint Louis, USA). Again after incubation the wells were washed 3x and assays developed by adding 0.1ml of 1mM ABTS in 70mM citrate phosphate buffer, pH 4.2 and 1/1000 dilution of $H_2O_2$. The plates were incubated overnight at room temperature and absorbance read using plate reader at 405nm. Standard curve was used for calculating *Asp f 1* concentrations and they were expressed as µg/g of collected dust (µg/g dust).

#### *4.2.3.2 House dust mite (Der f 1 and Der p 1) allergen analysis*

ELISA assay supplied by Indoor Biotechnologies (Wiltshire, UK) was used for analysis of house dust mite antigen in dust samples. Supplied monoclonal antibody (mA-6A8) was diluted 1/1000 in 50mM carbonate-bicarbonate buffer, pH 9.6. A 96 well microtitre plates was incubated with 0.1ml of diluted monoclonal antibody in each well to make a 200ng/well mA-6A8 over a 36 hour period. After incubation wells were washed 3x with PBS-T before standards and samples were added and incubated for 1 hour. A standard control curve dilutions were prepared by making double dilutions from 250.0ng/ml to 0.5ng/ml (i.e. 250ng/ml, 125.0ng/ml, 75.0ng/ml, 32.0ng/ml, 16.0ng/ml, 8.0ng/ml 4.0ng/ml, 2.0ng/ml, 1.0ng/ml, and 0.5ng/ml). For the sample, a 10 times dilution was made and there after serial dilutions of 1/20, 1/40, and 1/80 were made. After 1 hour incubation the wells were washed 3x with PBS-T and plate incubated for another 1 hour with 0.1ml of 1/1000 diluted Streptavidin-Peroxidase (Sigma, Steinhem) (0.25µg Streptavidin-Peroxide reconstituted in 1 ml distilled water) in 1% BSA PBS-T. Again after incubation time the wells were washed 3x and assays developed by adding 0.1ml of 1.0mM ABTS in 70mM citrate phosphate buffer, pH 4.2 and 1000x diluted

$H_2O_2$. The plate was incubated overnight at room temperature and read using plate reader at 405nm. Concentration of HDM allergens in samples were calculated from standard curve and expressed as μg per gram of dust (μg/g dust).

### 4.2.4 Statistical Analysis

STATA 9.0 statistical package (StataCorp, College Station, TX, USA) was used for all statistical analysis of the data. Allergen data levels were skewed to the right for all the allergens analysed. For allergen concentrations, no transformation was able to normalise data and sample size was not big enough to perform linear regression on skewed data. Therefore threshold levels considered risky to sensitisation were used to categorise HDM allergen levels for further analysis. Because no documented level of fungal allergen in dust that is considered a risk when an individual is exposed to, *Asp f 1* allergen concentration median value was used to categorise *Asp f 1* levels to two categories: 1) 0=levels lower than median value and 2) 1=levels equal or higher than median value. HDM allergen levels were also categorised using threshold limit risk to sensitisation of 2.0μg/g of dust. These categorise were: 1) 0 = levels than 2.0μg/g of dust and 2) 1 = levels equal or greater than 2.0μg/g of dust.

For univariate analysis, descriptive and distribution statistics for all allergens were generated. Bivariate analysis was performed by comparing allergen levels according to home characteristics using Mann-Whitney and Kruskal-Wallis test as well as Chi-square test. The levels of allergens were considered to significantly different if the p-value was less than 0.05.

Assessment of association between categorised *Asp f 1* or *Der p 1* or *Der f 1* allergen levels and home characteristics was performed using simple and stepwise

logistic regression. Before analysis, WTC variables dummy variables were created for variables with more than two categories. Simple logistic regression between allergen levels and WTC variables was performed. Statistically significant variables in simple logistic regression were included in the stepwise multiple logistic regression. The point of entry (pe) to the model was set at 0.001 and point of rejection (pr) was set at 0.05. Association between allergens and home WTC was considered statistically significant at $\alpha$=0.05. Only sleep area WTC variables and composed variables were included in these analyses because dust for allergen analysis was only collected from children's bedding.

## 4.3 RESULTS

136 homes were visited but dust samples were collected from 126 homes. Absence of electricity and people asleep in the house during our visit were the some of reasons dust samples were not collected from other households.

### 4.3.1 Allergen Prevalence in Homes

#### *4.3.1.1 Mould allergen (Asp f 1)*

Table 8 shows descriptive statistics of the three allergens (*Asp f 1, Der p 1* and *Der f 1*) analysed in dust. *Asp f 1* allergen was detected in all 126 homes from which samples were analysed for mould allergen. 5 samples were censored and excluded from the dataset as outliers because they were very high compared to the rest of other data points therefore 121 *Asp f 1* allergen results were part of the dataset analysed in this dissertation. The concentration ranged from 0.32 to 1.37µg/g of *Asp f 1* allergen per gram of collected dust and a mean of 0.59µg/g with standard deviation of +/-0.25. Median

concentration for *Asp f 1* allergen was lower than the mean at 0.47µg/g of dust and frequency distribution of the levels in homes was positively skewed.

#### *4.3.1.2 House dust mite allergens (Der p 1 & Der f 1)*

All bed dust samples were analysed for both HDM allergens. Table 8 also shows descriptive statistics for the two dust mite allergens analysed. 3 high concentrations of *Der p 1* allergen were censored from the dataset and not included in further analysis. In 13 (10.3%) and 8 (6.3%) homes, *Der p 1* and *Der f 1* concentrations were below lower detection limit respectively and they were as well excluded for descriptive analysis. That means 110 homes and 118 homes were statistically analysed for *Der p 1* and *Der f 1* allergens respectively. In HDM allergen detected homes, mean concentration of *Der p 1* and *Der f 1* allergen in dust were higher than their medians. Although *Der f 1* allergen was detect in most samples compared to *Der p 1* allergen, *Der p 1* had higher levels of up to 49.61µg/g of dust. Concentration range and variance in allergen detected homes for *Der p 1* allergen was higher than that of *Der f 1* allergen. Both HDM allergens had a positively skewed distribution.

**Table 8:** Descriptive statistics for house dust mite (*Der p 1 & Der f 1*) allergen and mould (*Asp f 1*) allergen levels in dust samples from children's homes.

| **Descriptive Statistics** | | ***Der p 1* (µg/g)[#]** | ***Der f 1* (µg/g)[#]** | ***Asp f 1* (µg/g)** |
|---|---|---|---|---|
| n | | 110 | 118 | 121 |
| Mean (SD) | | 6.09 (10.42) | 2.09 (4.52) | 0.59 (0.25) |
| Median | | 2.26 | 1.00 | 0.47 |
| Variance | | 108.48 | 20.40 | 0.06 |
| Skewness | | 3.11 | 6.29 | 1.48 |
| Minimum | | 0.31 | 0.40 | 0.32 |
| Maximum | | 49.92 | 39.71 | 1.37 |
| Percentiles | $10^{th}$ | 0.70 | 0.40 | 0.40 |
| | $25^{th}$ | 1.15 | 0.60 | 0.43 |
| | $75^{th}$ | 5.53 | 1.61 | 0.68 |
| | $90^{th}$ | 14.54 | 2.74 | 1.02 |
| | $95^{th}$ | 36.35 | 6.58 | 1.15 |
| | $99^{th}$ | 49.74 | 36.52 | 1.37 |

[#]concentrations below detection limit were omitted for statistical analysis

### 4.3.2 Allergen Levels Differ by Presence or Absence of Home Characteristics

#### *4.3.2.1 Mould allergen (Asp f 1)*

Kruskal-Wallis test on untransformed data showed presence and absence some home characteristics having significant different levels of allergens between the homes. Single family homes (85 of 121) had higher *Asp f 1* allergen concentrations lower compared to other types of homes. Apartment or flat (24 of 121) homes had 0.12µg/g mould levels higher than other types of building structures. Sleep areas (8 of 36) with asbestos roofing without separate ceiling had 0.17µg/g lower *Asp f 1* levels than homes with other types of roofing. Homes with damp walls (93 of 121) in the sleep area had 0.12µg/g of *Asp f 1* allergen levels higher than those with dry walls and homes with opening windows in sleep area (34 of 121) had 0.21µg/g lower levels compared to homes

with no windows. Hard floors with rugs (77 of 121) in sleep area had levels of *Asp f 1* allergen higher than those with other types of floors including carpet and smooth floors. Polyester filled pillows (54 of 67) had lower *Asp f 1* levels than pillows filled with other material.

#### *4.3.2.2 House dust mite allergens (Der p 1 & Der f 1)*

House dust mite (*Der f 1*) allergen concentrations were lower in homes without ceiling (5 of 126) compared to homes with ceiling underneath the roof. Absolute levels of *Der f 1* allergens were 1.45µg/g lower in homes with hard floors than in carpeted floors or hard floors with rugs. Sleep areas with damp floors (8 of 125) had 1.08µg/g levels of *Der f 1* allergen in dust lower than sleep areas with dry floors. Use of heaters (12 of 126) and use of comforter (4 of 126) had lower levels of 1.09µg/g and 1.12µg/g of *Asp f 1* allergen respectively.

Type of bedding used by the child was the only characteristics significantly different for *Der p 1* allergen with polyester filled pillow (54 of 123) having and cotton blankets (39 of 123) had higher levels of *Der p 1*. Acrylic blankets (3 of 123) had lower allergen levels compared to other types of blankets. Comparing absolute averages there were some difference in averages of the allergens. *Der p 1* allergen concentrations in the dust were 1.87µg/g higher in apartment buildings compared to other types of dwellings. Comparing average levels, homes built before 1978 (75 of 123) and those which the caregivers did not know when they were built (12 of 123) had 2.80µg/g and 7.24µg/g levels higher of *Der p 1* allergens respectively. Wood built houses (5 of 123) had 2.24µg/g lower levels of *Der p 1* allergen compared to other types of housing in the study. Asbestos roofing in sleep area (35 of 44) had 1.02µg/g , damp walls (15 of 123)

had 1.65µg/g higher, presence of windows (118 of 123) had 4.47µg/g lower, sleep areas with cement floors (16 of 123) had 2.08µg/g higher, carpeted floor in sleep area (30 of 123) had 1.61µg/g of dust lower, hard floors with rugs (28 of 123) had 2.17µg/g higher than other floors, use heater in their sleep area (10 of 123) had 1.39µg/g lower, polyester pillows (54 of 123) had 2.48µg/g higher levels, foam filled pillows (60 of 123) had 2.07µg/g lower levels, use of wool blankets have (57 of 123) had 1.22 lower, use of cotton blankets (39 of 123) had 4.26µg/g higher.

Chi-square test was used to look at differences in the allergen levels in homes with different home characteristics as shown in Table 9. Different home characteristics showed high significant difference in *Der p 1* and *Asp f 1* allergen levels between homes with or without some home characteristics. For *Der f 1* allergens, the levels were only marginally significant for homes that use fan/ air conditioner and space heater.

**Table 9**: Comparison of allergen levels between homes with and without some home characteristics in the bedroom using chi square test

| Allergen | Home characteristics | $X^2$ | p-value |
|---|---|---|---|
| *Asp f 1* | wall water stains | 4.31 | 0.038 |
| | opening windows | 6.82 | 0.009 |
| | type of flooring | 7.92 | 0.048 |
| | filling in the pillow | 15.48 | 0.008 |
| | type of blanket | 10.28 | 0.036 |
| *Der p 1* | informal house | 4.14 | 0.042 |
| | ceiling water stains | 6.86 | 0.032 |
| | window present | 5.30 | 0.021 |
| | type of carpeting | 6.21 | 0.013 |
| *Der f 1* | use of fan/ air con | 3.67 | 0.055 |
| | use of a space heater | 3.28 | 0.070 |

### 4.3.3 Home Conditions that are Predictors of Higher Allergen Levels

#### *4.3.3.1 Mould allergen (Asp f 1)*

Performing simple logistic regression with allergen levels as dependent variables and home walkthrough variables as independent variables. Responses to approximately 70 questions were investigated for association. Predictors of *Asp f 1* allergen levels higher than the median value of 0.47μg/g in homes were single family home, flat/apartment, extent of roof and wall dampness, hard floor and type of bedding used (Table 10). Composed sleep area scores for dampness, house dust mite and mould were not significant predictors of level of mould allergen dust samples collected from the sleep area, but there was association of *Asp f 1* allergen with kitchen and entire home composed scores. Performing multivariate logistic regression to look at association between *Asp f 1* allergen levels and WTC variables, most variables were not statistically significant at p=0.05. In this analysis none of the possible confounders such as socioeconomic factors, humidity, house temperature, light, etc. were controlled for in the models because data for these variables were not collected. Other covariates such as season were not controlled for because of less number or no samples were colleted in other season compared to others.

**Table 10**: Crude univariate house conditions as predictors of *Asp f 1* allergen levels in dust collected from bedding and floor in children's sleep area (only significant predictors are shown in the table when alpha = 0.05)

| Allergen | Home characteristics | N | OR | 95% CI | p-value |
|---|---|---|---|---|---|
| ***Asp f 1*** | single family home | 85 | 0.41 | 0.18 - 1.93 | 0.034 |
| | flat/apartment | 24 | 2.88 | 1.15 - 7.23 | 0.024 |
| | extent of roof water stains | 34 | 3.02 | 1.02 - 8.95 | 0.047 |
| | wall water stains | 28 | 2.49 | 1.04 - 5.98 | 0.041 |
| | hard floor | 77 | 3.37 | 1.33 - 8.54 | 0.010 |
| | polyester filled pillow | 54 | 0.23 | 0.09 - 0.56 | <0.001 |

Multivariate logistic regression analysis was performed between allergen levels and home characteristics that were significant in simple logistic analysis at alpha= 0.05. Stepwise multivariate logistic regression analysis of categorised *Asp f 1* had single family home and use of polyester filled pillow [OR= 0.038 (95%CI 0.004 – 0.35) p=0.004] & [OR= 0.065 (95%CI 0.007 – 0.61) p=0.017 respectively] were significant.

#### *4.3.3.2 House dust mite allergens (Der p 1 & Der f 1)*

When performing simple (univariate) logistic regression, high statistically significant ($p<0.05$) associations were observed between some home characteristics in sleep area and HDM allergen levels and these were type of bedding and extent of roof dampness (Table 11). All home walkthrough composed scores of both mould and house dust mite exposure were not significant predictors of allergen levels.

**Table 11**: Crude univariate predictors of house dust mite (*Der p 1 & Der f 1*) allergens collected from children's bedding and sleep area floor (n=123)

| Allergen | Home characteristics | n | OR | 95% CI | p-value |
|---|---|---|---|---|---|
| ***Der p 1*** | unknown age of the house | 12 | 3.28 | 0.84 - 12.77 | 0.086 |
| | extent of roof water stains | 35 | 0.33 | 0.13 - 0.81 | 0.016* |
| | polyester filled pillow | 54 | 2.49 | 1.20 - 5.176 | 0.015* |
| | foam filled pillow | 60 | 0.44 | 0.21 - 0.90 | 0.025* |
| | comforter | 61 | 0.537 | 0.26 - 1.10 | 0.088 |
| | cotton blanket | 39 | 1.94 | 0.89 - 4.20 | 0.095 |
| ***Der f 1***[#] | single family home | 91 | 3.40 | 0.95 - 12.20 | 0.06 |
| | flat/apartment | 23 | 0.15 | 0.02 - 1.17 | 0.07 |
| | building material either than brick/wood | 3 | 8.70 | 0.76 - 100.05 | 0.083 |
| | wool blanket | 58 | 3.11 | 1.23 - 7.88 | 0.017* |

[#]n=126 *statistical significant at p=0.05

Performing stepwise multiple logistic regressions for both *Der p 1* and *Der f 1* allergen as dependent variables, all WTC variables statistically significant (alpha=0.05) for univariate analysis were included in the model. Elevated *Der p 1* allergen levels (equal or greater than 2.0μg/g dust) association was observed with extent of water stains on the roof [OR= 0.33 (95%CI 0.13 – 0.81) p=0.016]. In a stepwise logistic regression analysis *Der f 1* level had no significant association with any univariate predictors that were significant.

## 4.4 DISCUSSION

In recent years exposure to indoor allergens had been given attention when investigators are studying respiratory illness because of the role they play in etiologic

pathway of some diseases. This is the first study that looked at exposure to house dust mite and fungal allergens in Durban residential homes of different socioeconomic status especially South Durban Basin, a region known for high respiratory illnesses. This study was able to quantify allergen levels in Durban homes using objective measurements and was also able to identify some home characteristics that are predictors of higher allergen levels in the homes.

### 4.4.1 Levels and Predictors of Mould Allergen (*Asp f 1*)

Detection of *Asp f 1* allergens in all analysed dust samples indicate that fungal propagules can be distributed throughout the environment and surfaces easily. Homes with windows present in sleep area had lower absolute levels of *Asp f 1* in the dust; this could be an indicator of the effect of ventilation on fungal allergen levels indoors. In a study of environmental levels of *Asp f 1* allergen in office environment, Ryan *et al*. (2001) did not detect any traceable levels of *Asp f 1* in collected dust but very low levels were detected in air samples. Office environments have a controlled air movement it makes infiltration of these allergens from outdoors not as easy as compared to residential buildings and regular maintenance and cleaning.

Type of housing was a significant univariate predictor of levels of *Asp f 1* allergen indoors (Table 9), with single family home less than half times likely to have higher *Asp f 1* allergen whilst apartments were close to 3 times more likely to have higher levels.

Damp surfaces in the house were also indicators of higher fungal allergens, 2.5 times to 3.5 times likely for a house to have higher fungal allergens when there is dampness present than when there is not. Some studies associated dampness with

proliferation of fungal growth in the house (Garret *et al*. 1998) which would imply increased chances of fungal allergens.

Fungi is known to grow on organic material where it can get food (Gravesen *et al*. 1979); a finding that pillows filled with synthetic material being predictor of higher *Asp f 1* allergen in this study was not surprising. With synthetic materials having poor food source for microbial organisms, for mitigation or control measures avoidance of natural material is encouraged.

In multivariate logistic regression with categorised *Asp f 1* allergen levels as a dependent variable and WTC variables as independent variables. Again single family home was a significant predictor of *Asp f 1* allergen levels and other significant association was observed for synthetic filled pillows. In a multiple logistic regression, the significant variables were negative predictors, that is, they were protective of the negative outcome (higher *Asp f 1* allergen level).

### 4.4.2 Levels and Predictors of House dust mite Allergens (*Der p 1 & Der f 1*)

The average levels of HDM in the homes were not higher than other parts of the world. HDM levels were comparable with levels found else where in the world (Dharmage *et al*. 2001; Mihrshahi *et al*. 2003) with the number of homes with *Der* concentrations (40% & 29% of *Der f 1* & *Der p 1* respectively) 80% of homes in north eastern USA and 65% in Boston  equal and above 2.0 µg/g dust. (Chew *et al*. 1998; Leaderer *et al*. 2002).

In our study, strong univariate predictor of HDM allergen levels equal or greater than 2.0µg/g dust were the presence of polyester filled pillow by the child (Table 11). Beds with polyester filled pillow were almost 2.5 times likely to have higher *Der p 1*

allergens than those without. The finding is in line with what other studies found (Kemp *et al*. 1996; Hallam *et al*. 1999; Moscato *et al*. 2000). Pillows filled with synthetic material were likely to have lower levels of *Der p 1* allergens and this is the hypothesis because synthetic material does not have abundance of protein as would be in the case of natural material. Synthetic material is considered as a site less likely to be infested with HDM.

HDM thrive in humid conditions and climatic conditions as those in this part of the world are more likely to have a strong effect on HDM levels (Godish 2001). In this study, bedrooms with humidity or dampness signs were less likely (about one third times) to have higher levels of *Der p 1* allergens. Other studies in other parts of the world found opposing results to the one found by this study. For example Boquete *et al*. (2006) in a study of 4 provinces in Spain identified high humidity as an important condition associated high dust mite count in homes. In a national US study, some home characteristics were positive associated with higher HDM allergen levels and they include frequent musty or mildew odour and higher humidity. In a multiple logistic regression again extent of dampness was a negative predictor of higher allergen levels (Arbes *et al*. 2003).

*Der f 1* allergen had single family home and use of wool blankets as the WTC variables predictors of levels higher than lower threshold limit. With a single family house having 3.40 times the chance of having higher levels than other housing types, a finding by Matheson *et al*. (2003) is supported by this one. In their study, Matheson and colleagues (2003) suggested that the difference between single family homes and apartment or flat is ventilation practices. They pointed out that single standing unit are

well ventilated compared to multifamily units and this leads to drier and less humid multi family units. Wool blanket was a characteristic that showed 3.11 likelihood for higher allergens compared to other materials. In this instant wool blankets which are made from natural material were indicators of higher levels of *Der f 1* allergens (Moscato *et al*. 2000). Custovic *et al*. (1998) in review of the literature identified some characteristics that are associated to elevated levels. Humidity and altitude were conditions that they found strongly associated to allergen levels or adverse effects on exposed individuals.

# CHAPTER 5

# PREDICTORS OF LEVELS OF FUNGAL AEROSOLS

## 5.1 INTRODUCTION

Reservoir (surface and dust) and airborne fungi is considered risk to development of respiratory symptoms and sensitisation to fungi in exposed individuals especially children and elders. Literature has a strong evidence of dampness, humidity and visible fingi association with respiratory illnesses (Dales *et al*. 1991 Garrett *et al*. 1998; Douwes and Pearce 2003). Not many studies had investigated indoor levels of fungal aerosols and home characteristics (Dales *et al*. 1997; Garrett *et al*. 1998; Gqaleni *et al*. 1999b; Gent *et al*. 2002; Shelton *et al*. 2002). Below are some of the studies that examined association of some home characteristics with levels of fungal aerosols in the homes.

Garrett and colleagues (1998) investigated homes in Canada for fungal concentration levels and its association to home characteristics. Levels of outdoor fungal spores were significantly correlated with indoor levels of viable (r=0.41) and total (r=0.52) fungal spore concentrations at alpha=0.001. Humidity was significantly weakly correlated with both viable (r=0.22) and total (r=0.28) spore concentration. This showed strong contribution of outdoor sources to levels of fungal spores found indoors.

In Durban, South Africa, Gqaleni *et al*. (1999b) found that shacks; that were made of material such as aluminum sheet, cardboard and wood; had higher indoor fungal levels than outdoors (difference in absolute mean levels =147 CFU/$m^3$) compared to formal

brick constructed homes. These informal homes (shacks) had higher extent humidity and visible fungal growth presence in their surfaces as well compared to formal houses. The investigators concluded that some of the material used for building in these homes might contribute to higher levels of fungal spores.

In a study profiling airborne fungi in North America by Shelton *et al*. (2002), the questionnaire used enquired about reasons of fungal sampling of air by persons submitting the samples. Majority of them had health complaints, followed by visible fungal growth and then water damage as primary reason for conducting fungal air sampling. Buildings with visible mould growth had the highest mean (379 CFU/$m^3$) or median (141 CFU/$m^3$) level compared to buildings sampled for health complaints (mean = 101 CFU/$m^3$; median = 71 CFU/$m^3$) and other reasons. The ratio of indoor/outdoor levels was higher as well for these buildings with reported visible fungal growth than the other buildings. Buildings that reported hypersensitivity pneumonitis also had elevated indoor levels compared to buildings with no reported hypersensitivity pneumonitis. In this study the investigators also noted that 38% of the samples from buildings with visible fungi and 25% of the samples from water damaged buildings had *Stachybotrys* present.

Levels of indoor fungi in dust were associated with some reported home characteristics in a Canadian study (Dales *et al*. 1997). Geometric mean levels were higher for homes with reported fungal and mildew than when these were not reported. Mean levels of some fungal genera namely *Aspergillus* and *Penicillium* were best explained by indoor visible fungal growth. In this study, authors pointed reporting bias when respondents were reporting allergic symptoms and when they smoke cigarettes.

Prospective study in Connecticut and Massachusetts, USA, found an association between sampling season or home characteristics with higher levels of *Cladosporium*, *Penicillium* or "other fungi". There was a significant association between higher levels of *Cladosporium* and reported fungal growth ($p=0.012$) and water leaks ($p=0.003$). For higher levels of "other fungi" there was a significant association with heating system ($p=0.05$), and sampling season ($P<0.001$) (Gent *et al*. 2002).

Ren *et al*. (2001) investigated high fungal levels (>/= 1000 CFU//$m^3$) in infants' bedrooms and their relationship with home characteristics. Two types of media, MEA and DG18, were used as sampling media in this study. Significant positive associations of high levels vs. low levels using logistic regression were found in the homes in North eastern USA. Significant higher levels of fungal spores on DG18 were associated with every 10% increase of relative humidity [OR= 1.59 (1.374 – 1.840) $p<0.001$] and temperature increase of every $5^0$C [OR=2.252 (1.656–3.061) $p<0.001$]. On an MEA relative humidity increase of every 10% [OR=1.367 (1.190–1.571) $p<0.001$]; temperature increase of every $5^0$C [OR=1.992 (1.494–2.658) $p<0.001$] and use of air conditioner [OR= 0.607 (0.435–0.845) $p=0.003$] were positively associated with higher airborne fungi concentrations. The season in which the assessment was done is another factor influencing fungal concentrations with summer/autumn being a significant predictor of higher concentration (Ren *et al*. 2001).

A study by Chew and colleagues (2003) on Boston homes, found that relative humidity [OR=1.08 (1.05–1.11)], apartment building [OR=0.37 (0.19–0.71)] and outdoor fungi levels [OR=4.70 (2.57–8.57)] were predictors of higher indoor levels in these homes. Use of humidifier in summer was also an indicator of higher levels of airborne

indoor fungi. No other home characteristics were found to be associated with higher levels of airborne fungi in these homes.

A multi-centre study called "The Inner-city Asthma Study" in the USA; investigator found that some indoor characteristics are predictors of high airborne fungal levels. Higher total fungal concentrations in the homes were associated with forced air heating [OR=0.4 (0.3 - 0.7)], reported water dampness [OR=1.8 (1.2 - 2.9)], moisture signs [OR=3.4 (1.8 - 6.5)], musty smell [OR=2.5 (1.3 - 4.5)], temperature increase of $5^0$C [OR=0.8 (0.6 - 0.9)]. Other fungal genera such as *Alternaria, Aspergillus, Penicillium* and *Cladosporium* were also explained by some home characteristics in the homes (O'Connor *et al.*, 2004).

Between these studies there is a variation of home characteristics that are indicators of higher indoor fungal concentrations. Humidity, moisture and dampness seem to be constantly significant indicators of higher levels in many studies (Garrett *et al*. 1998; Ren *et al*. 2001; Chew *et al*. 2003; O'Connor *et al*. 2004). Visible fungal growth in homes were also indicators of higher levels of fungal aerosols as studies by Gqaleni *et al*. (1999b); Dales *et al*. (2000); Shelton *et al*. (2000) and Gent *et al*. (2002) indicated. Other characteristics such as season, heating the home, temperature increase, type of building, use of humidifier and use of air conditioner were shown to be indicators of higher levels of fungal aerosols indoors (Gqaleni *et al*. 1999b; Ren *et al*. 2001; Chew *et al*. 2003; O'Connor *et al*. 2004;). None of the home characteristics studied that had been constantly found to be associated with levels of fungal aerosols.

## 5.2 METHODOLODY

### 5.2.1 Fungal Aerosol Sampling and Analysis

#### *5.2.1.1 Fungal aerosol sampling*

In each of the homes visited (as cited in chapter 3.2), child's sleeping and/or room mostly used by the child was identified and fungal aerosol sample or samples collected from the room or rooms. All the windows and doors to the outside were closed before the samples were taken using impact sampler coupled with pump and agar plates. A calibrated two-stage Andersen sampler (Tish Environmental, Ohio) was wiped with 70% (v/v) alcohol:$H_2O$ and air-dried before and after samples were collected. Samplers were placed at the centre of the room 0.5 meters above the ground and away from the obstacle that could hinder airflow (wall, cupboards, wardrobes etc.). A sample of air was collected for 5 minutes at a flow rate of 15L/min on malt extract agar (MEA) plates (Oxoid Ltd, Hampshire) and dichloran 18% glycerol base agar (DG18) plates (Oxoid Ltd., Hampshire) (For composition and preparation of media refer to Appendix 5). Then agar plates were sealed, marked and transported to the laboratory where they were incubated in a temperature regulated microbial cabinet between $25^0$C and 28 $^0$C for a period of 3 to 5 days. After incubation period colonies were counted and identified and identified by a naked eye. The number of fungal colonies was expressed as colony forming units per cubic meter (CFU/ $m^3$). Each colony type was isolated from original culture plate and inoculated into other culture media for further identification.

#### *5.2.1.2 Further isolation and identification of fungal organisms*

Different colonies of fungi that were found in the samples were isolated by culturing each fungal colony onto agar plates of MEA and DG 18. Compound microscope was used to identify colonies by their colonial morphology. Visual observation of cultured fungi was made on cellular morphology by observing hyphal and spore morphology. Using reference to the book by Samson *et al*. (2000), identification of the organisms by their morphology was done by light microscope. Each colony was identified to its genera and confirmation test were done by the Medical Microbiology Laboratory at the Inkosi Albert Luthuli Central Hospital, Durban.

### 5.2.2 Statistical Analysis

The two media (MEA & DG18) used showed similar total fungal concentrations when colonies were counted. Only data of fungal concentrations sampled on MEA that had better detection of *Rhizopus spp*. was used in statistical analysis. STATA 9.0 statistical package (StataCorp, College Station, TX, USA) was used for analysis of the collected data. Descriptive statistical analysis of variables was performed on sleep area, play area, kitchen, bathroom, entire house/indoor and outdoor concentrations as part of univariate analysis. Only data from the sleep area which is the area the child spends most of the time, and the entire house used in further analysis.

Pearson correlation was performed between indoor levels and outdoor levels of fungal species, and between sleep area levels and outdoor levels. Fungal aerosol concentrations of each room, entire home (average between all the rooms in the homes) and outdoor were transformed using different functions (square root and log

transformation) to normalise distribution for regression analysis. All variables showed close to normal distribution after transformation as seen in Figure 6 to Figure 19.

Simple linear regression analysis was performed with transformed fungal aerosol concentrations as a dependent variable (entire home and sleep area) and WTC variables, including new composed variables, as independent variables. A stepwise multivariate analysis of the transformed total fungal aerosol levels (sleep area & entire home) as dependant variable was performed WTC variables significant in univariate analysis as independent variables with point of rejection =0.05 and point of entry =0.001. No attempt was made to analyse WTC indicators of different types of fungal genre. For both simple and multivariate regression analysis, statistical significance was considered at alpha <0.05.

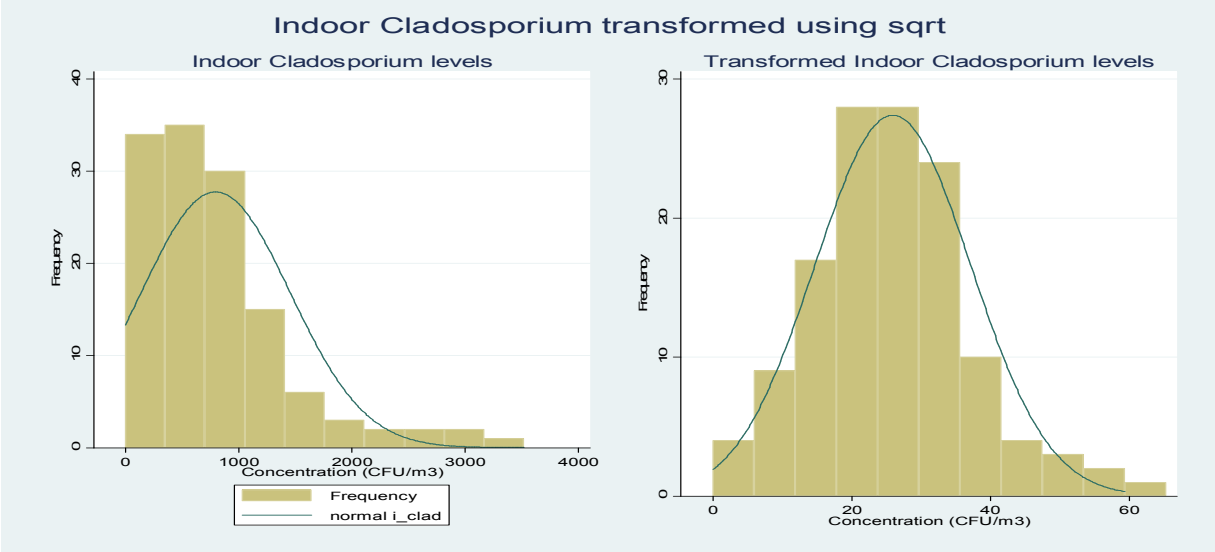



**Figure 6:** Frequency distribution histogram of indoor *Cladosporium* levels and sqrt transformed frequency

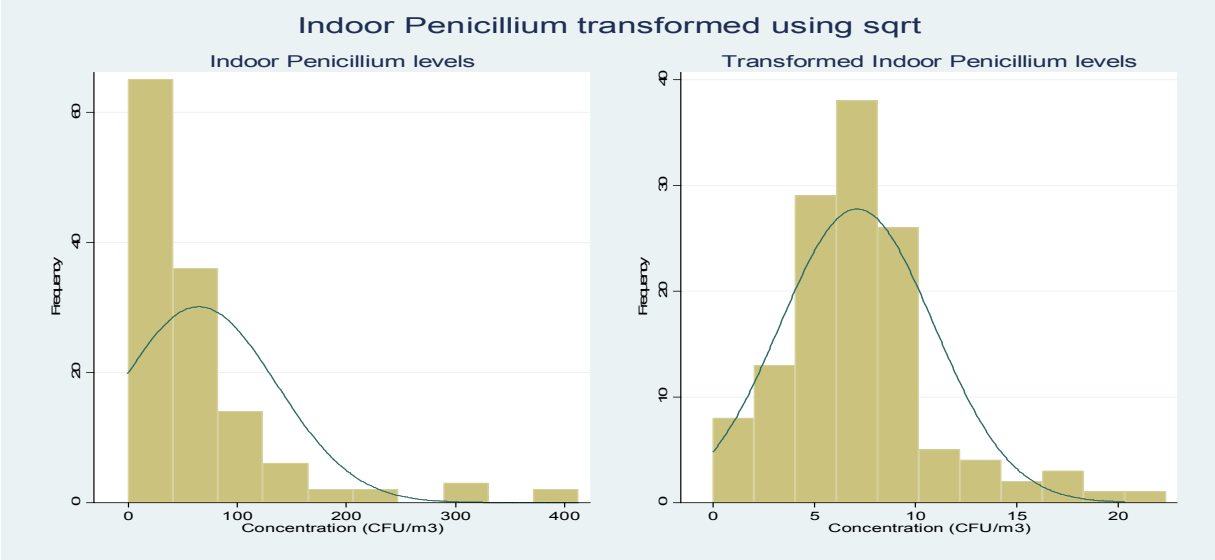



**Figure 7:** Frequency distribution histogram of indoor *Penicillium* levels and sqrt transformed frequency

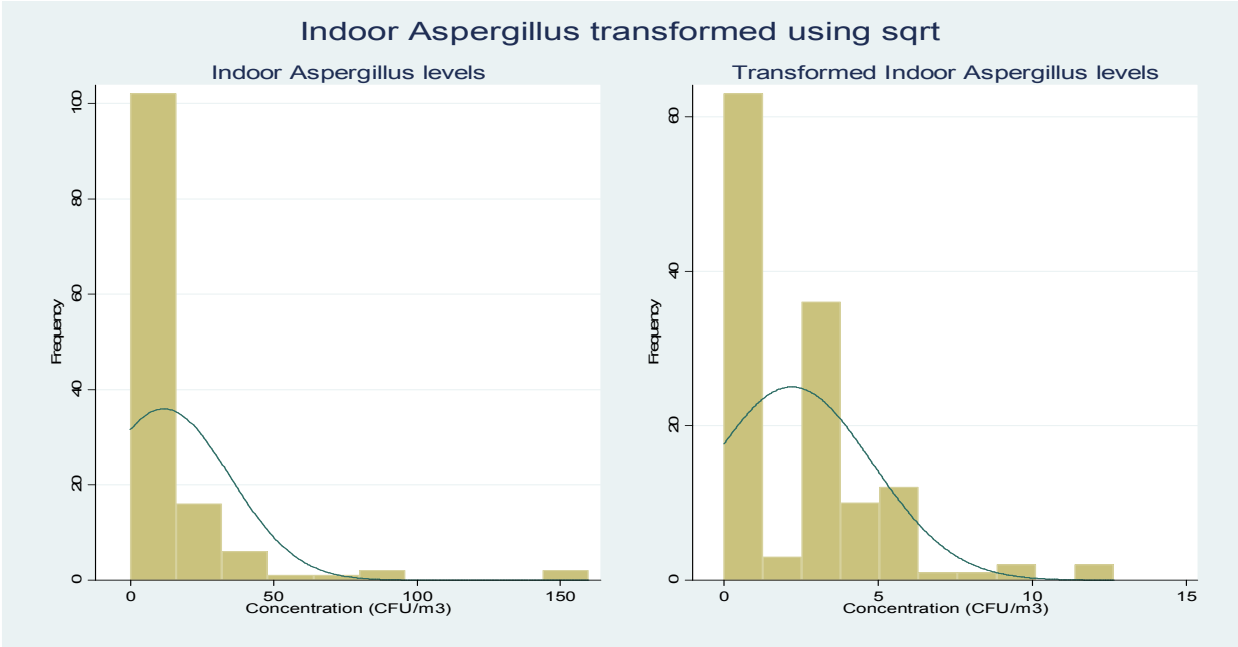



**Figure 8:** Frequency distribution histogram of indoor *Aspergillus* levels and sqrt transformed frequency

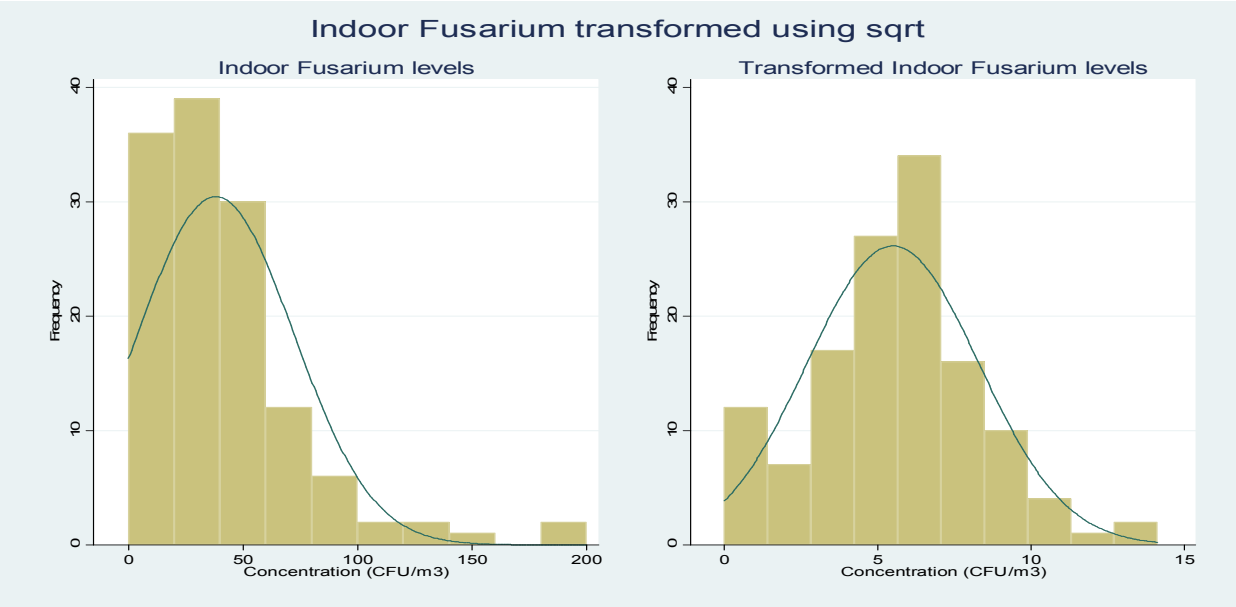



**Figure 9:** Frequency distribution histogram of indoor *Fusarium* levels and sqrt transformed frequency

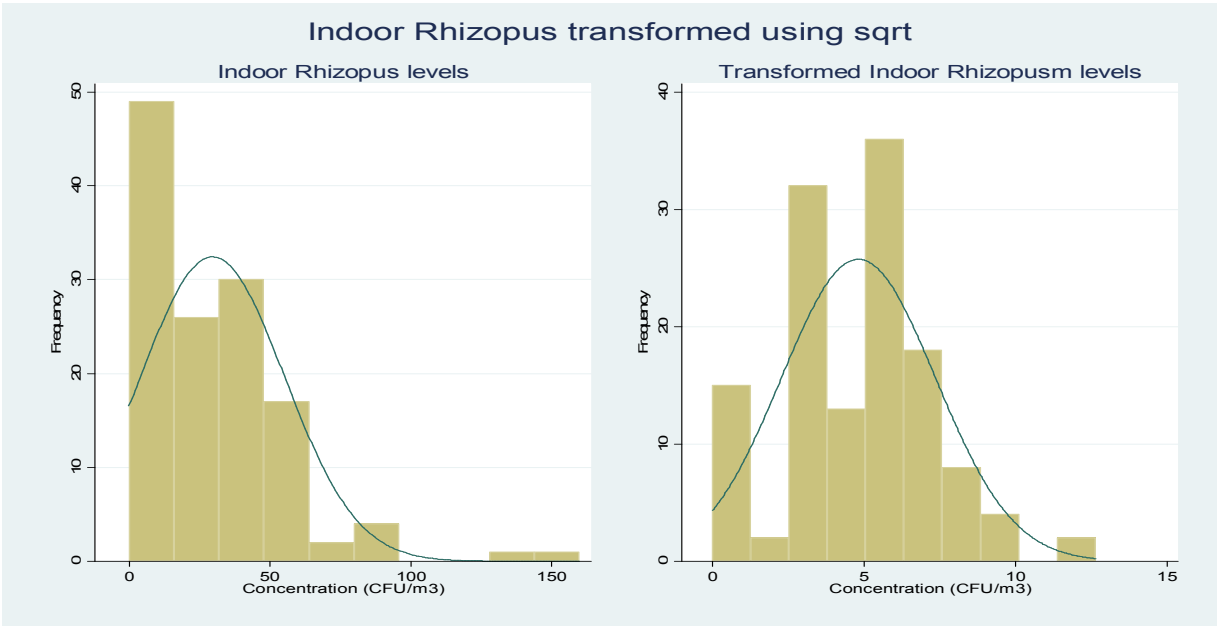



**Figure 10:** Frequency distribution histogram of indoor *Rhizopus* levels and sqrt transformed frequency

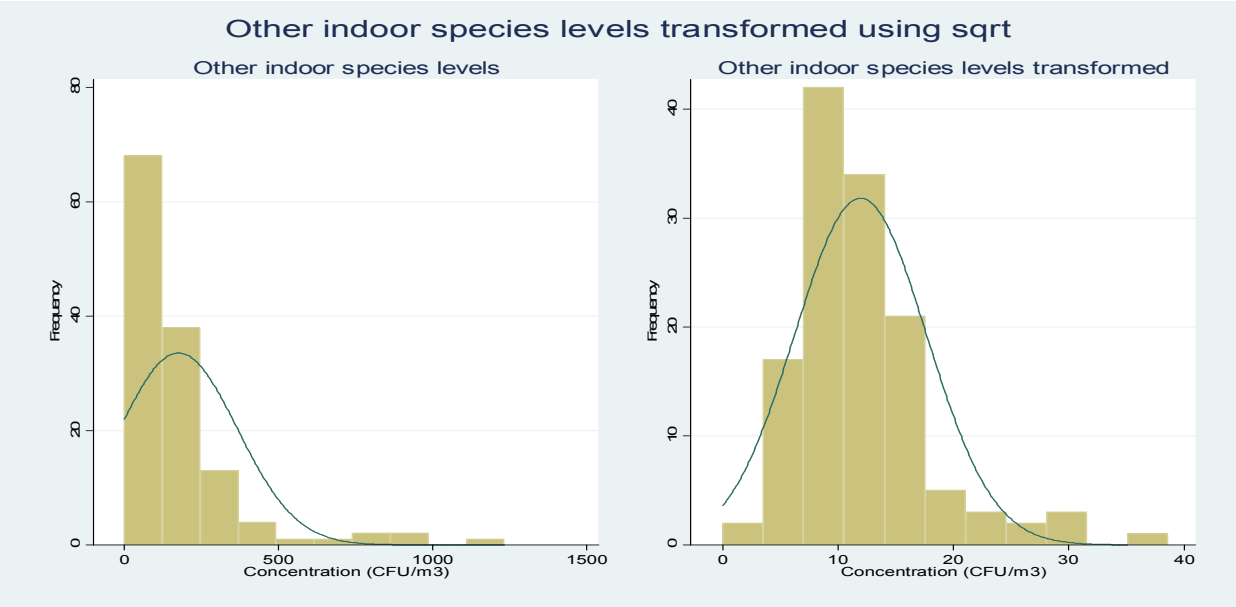



**Figure 11:** Frequency distribution histogram of indoor *unknown species* levels and sqrt transformed frequency

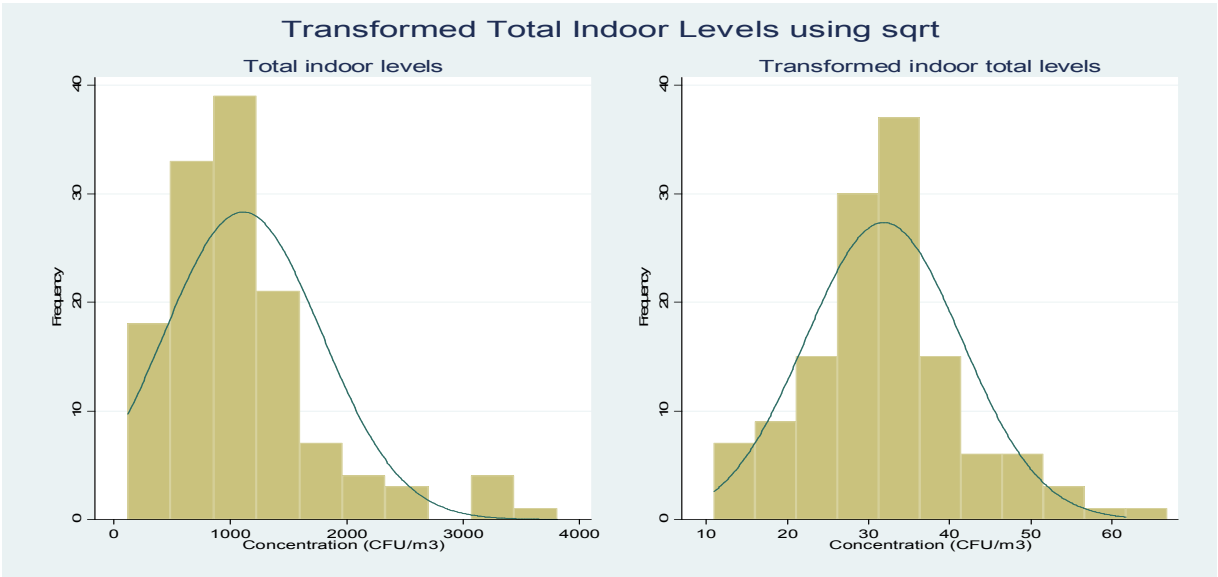



**Figure 12:** Frequency distribution histogram of indoor *Total species* levels and sqrt transformed frequency

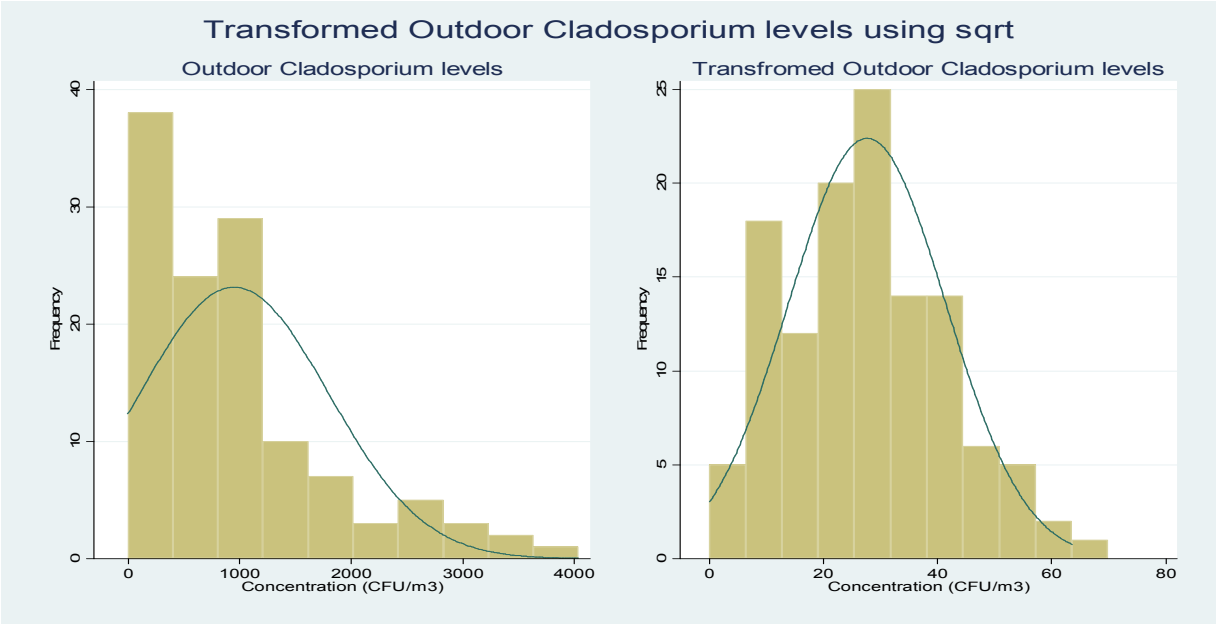



**Figure 13:** Frequency distribution histogram of outdoor *Cladosporium* levels and sqrt transformed frequency

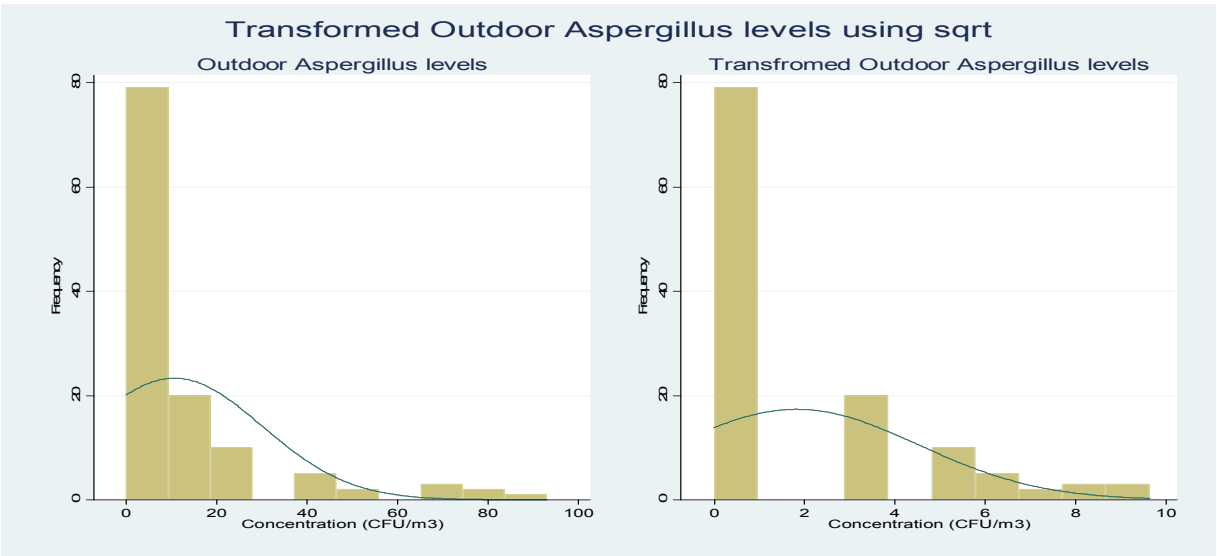



**Figure 14:** Frequency distribution histogram of outdoor *Aspergillus* levels and sqrt transformed frequency

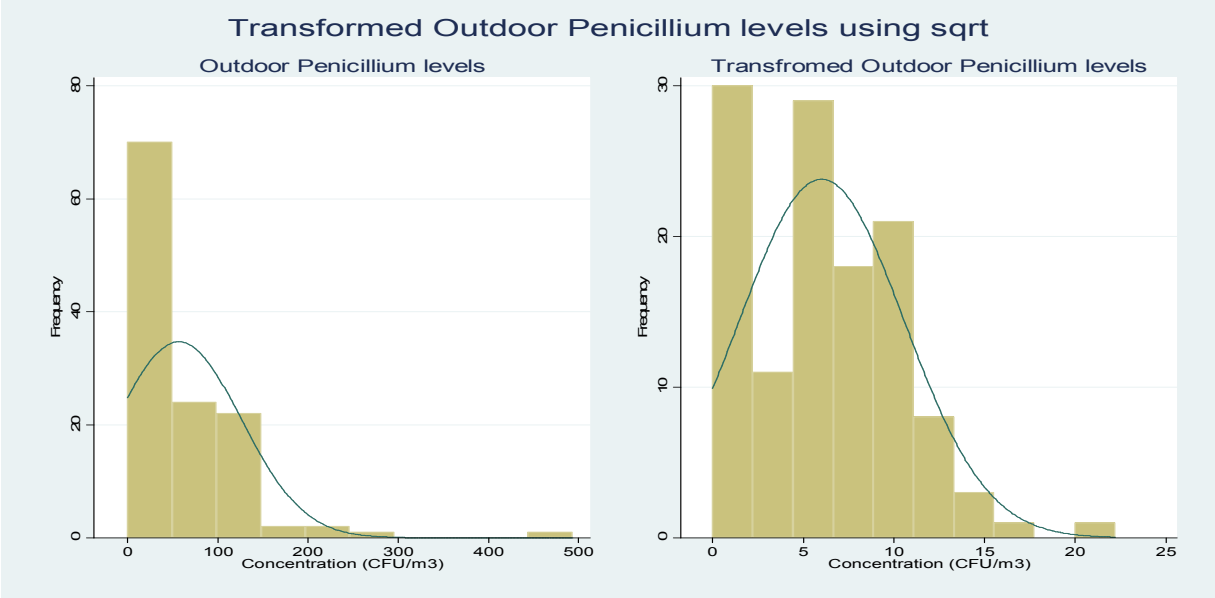



**Figure 15:** Frequency distribution histogram of outdoor *Penicillium* levels and sqrt transformed frequency

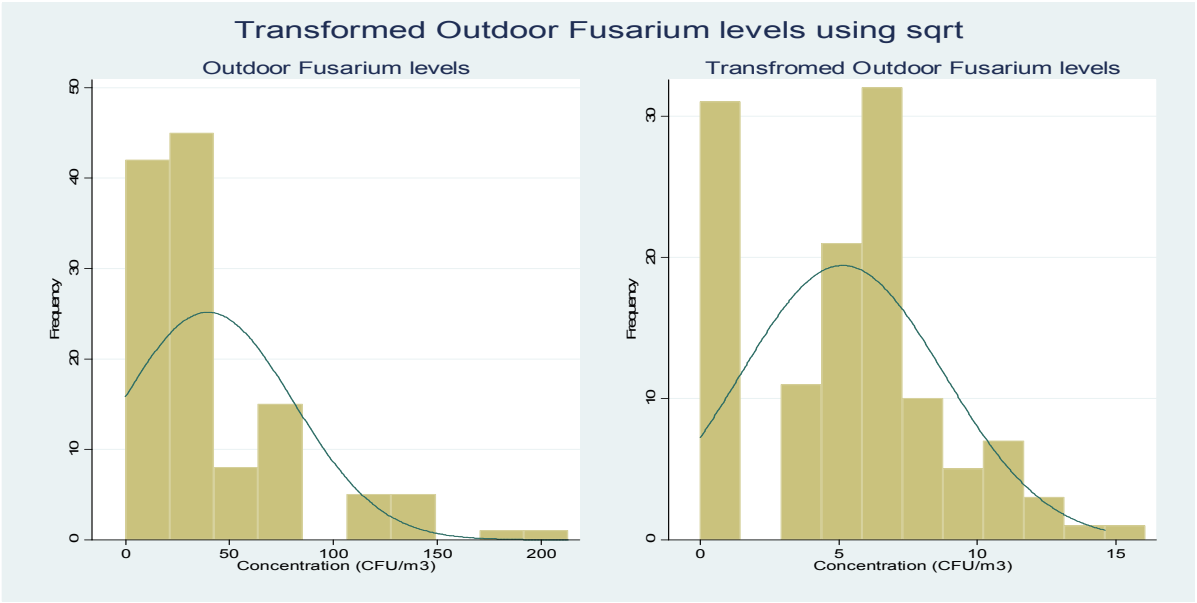



**Figure 16:** Frequency distribution histogram of outdoor *Fusarium* levels and sqrt transformed frequency

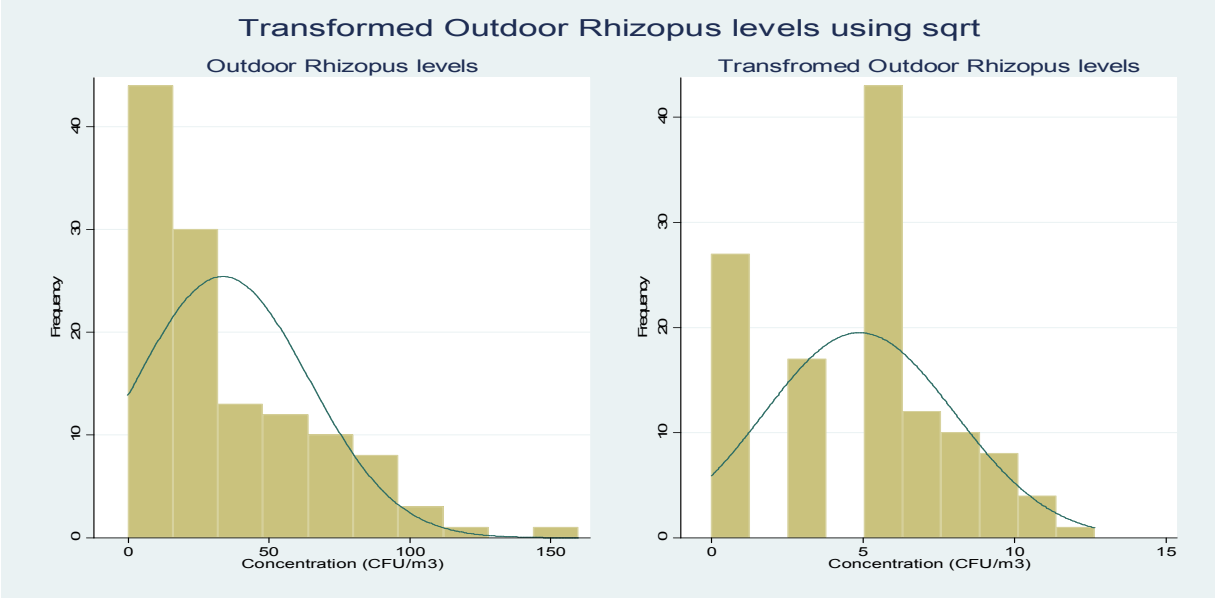



**Figure 17:** Frequency distribution histogram of outdoor *Rhizopus* levels and sqrt transformed frequency

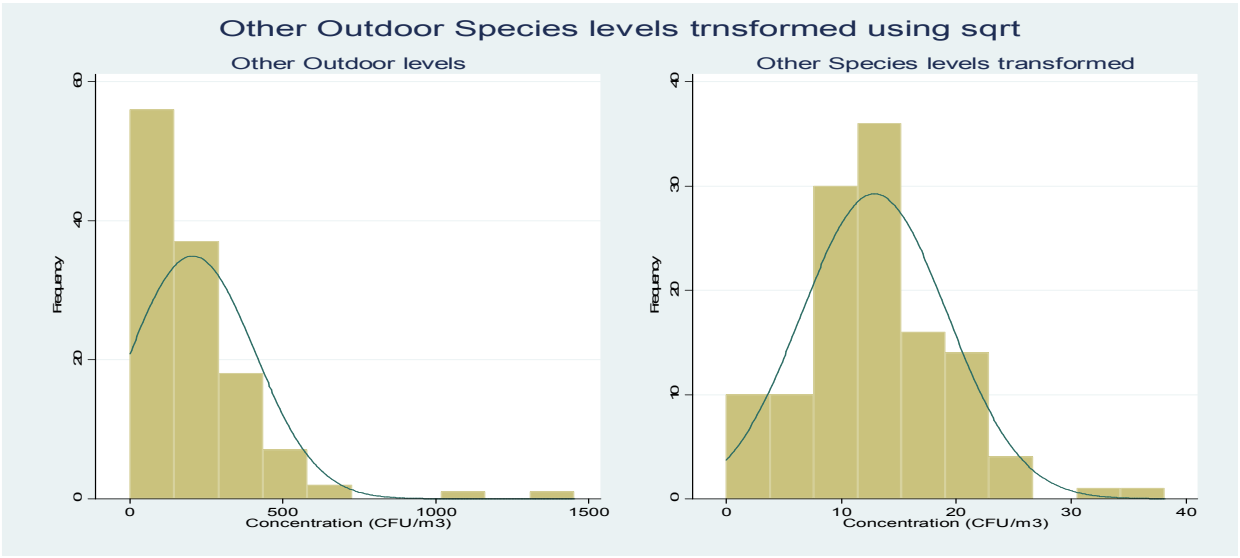



**Figure 18:** Frequency distribution histogram of outdoor *unknown species* levels and sqrt transformed frequency

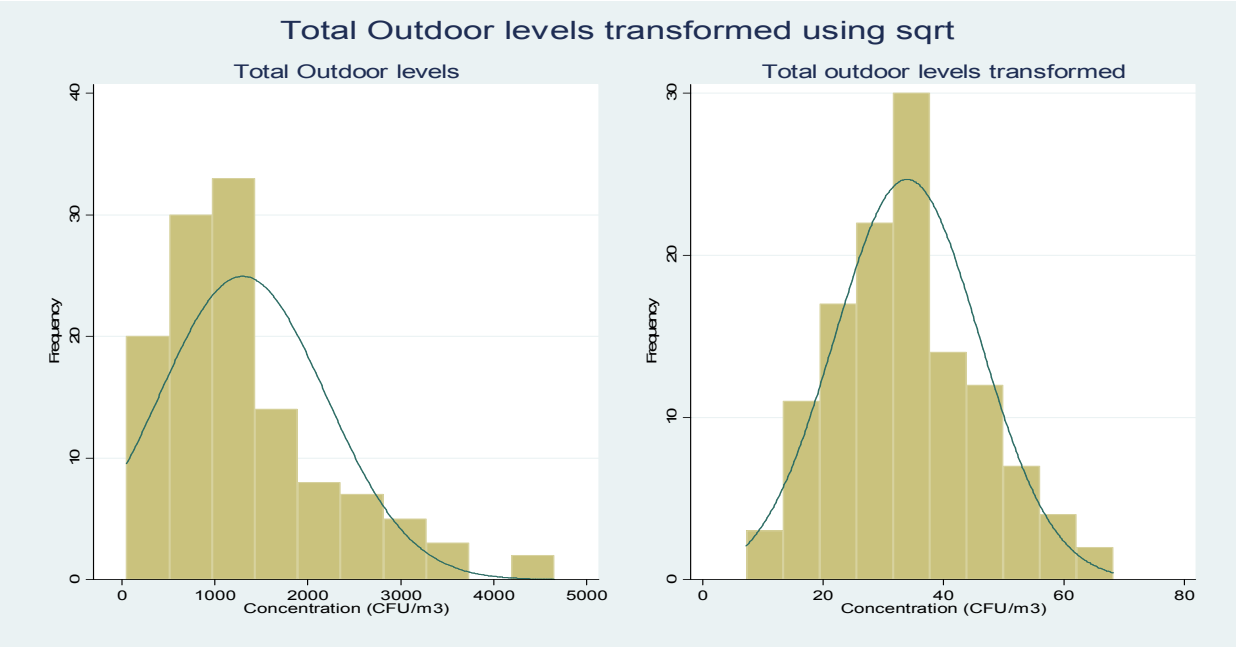



**Figure 19:** Frequency distribution histogram of outdoor *Total species* levels and sqrt transformed frequency

## 5.3 RESULTS

### 5.3.1 Prevalence of Fungal Aerosol Concentration in Homes

130 homes had at least a sample collected from the house and 126 had samples collected from child's sleep area. Some samples were excluded because some species over grew on MEA and that made colony counting impossible. Table 12 and Table 13 show descriptive statistics for viable fungal aerosols of different rooms and species of combined indoor concentrations and individual fungal species. Outdoor CFU/$m^3$ mean levels were 109 CFU/$m^3$ higher when compared to the indoor (entire home) total mean concentrations except for kitchen mean level which was equal. Only 22 bathroom samples were collected from the homes and their mean levels were lower than other rooms. Play area concentrations had the widest range of between 93-5253 CFU/$m^3$ and

bathroom concentrations with a range of between 93–1600 CFU/$m^3$. In the samples Spearman's partial correlation between indoor and outdoor total; and individual species showed different levels of relationships. For indoor total vs. outdoor total and indoor *Cladosporium* vs outdoor *Cladosporium* there were strong correlations ($r=0.684$, $p<0.001$) and $r=0.684$, $p<0.001$ respectively). *Penicillium* levels were moderately correlated with indoor and outdoor levels ($r=0.460$, $p< 0.001$) whereas *Aspergillus* and *Rhizopus* indoor vs. outdoor levels were weakly correlated ($r=0.391$, $p< 0.001$ and $r=0.396$, $p< 0.001$ respectively). *Fusarium* was very weakly correlated between indoor and outdoor levels at $r=0.223$ ($p=0.014$). Correlations were considered significant at $p=0.05$.

**Table 12:** Descriptive statistics of total CFU concentrations of fungal aerosols in different rooms, entire home (indoor) and outdoors

| | | Indoor | Sleep area | Play area | Kitchen | Bathroom | Outdoor |
|---|---|---|---|---|---|---|---|
| n | | 130 | 126 | 103 | 16 | 22 | 122 |
| Mean (SD) | | 1108 (675) | 1081 (609) | 1108 (905) | 1298 (889) | 810 (334) | 1298 (898) |
| Median | | 1010 | 1000 | 960 | 1160 | 840 | 1080 |
| Skewness | | 1.56 | 1.07 | 2.95 | 0.45 | 0.11 | 1.28 |
| Minimum | | 133 | 120 | 93 | 80 | 93 | 120 |
| Maximum | | 3807 | 3200 | 5880 | 2920 | 1600 | 4653 |
| Percentiles | $10^{th}$ | 414 | 373 | 280 | 107 | 413 | 347 |
| | $25^{th}$ | 700 | 693 | 613 | 660 | 587 | 640 |
| | $75^{th}$ | 1280 | 1293 | 1387 | 1893 | 973 | 1613 |
| | $90^{th}$ | 1947 | 1933 | 1760 | 2707 | 1107 | 2533 |
| | $95^{th}$ | 2400 | 2187 | 2200 | 2920 | 1267 | 3120 |
| | $99^{th}$ | 3433 | 3120 | 5253 | 2920 | 1600 | 4213 |

**Table 13:** Descriptive statistics of different fungal genera in children's sleep areas

| | | *Cladosporium*± | *Aspergillus*± | *Penicillium*± | *Fusarium* | *Rhizopus* |
|---|---|---|---|---|---|---|
| n | | 122 | 47 | 105 | 100 | 92 |
| Mean (SD) | | 783 (568) | 30 (44) | 64 (73) | 48 (32) | 43 (39) |
| Median | | 653 | 13 | 40 | 40 | 40 |
| Minimum | | 13 | 13 | 13 | 13 | 13 |
| Maximum | | 2947 | 147 | 520 | 173 | 320 |
| Percentiles | $10^{th}$ | 160 | 13 | 13 | 13 | 13 |
| | $25^{th}$ | 413 | 13 | 27 | 13 | 27 |
| | $75^{th}$ | 1013 | 27 | 80 | 53 | 53 |
| | $90^{th}$ | 1560 | 80 | 120 | 80 | 80 |
| | $95^{th}$ | 1933 | 120 | 147 | 173 | 107 |
| | $99^{th}$ | 2707 | 147 | 307 | 173 | 320 |

± undetected levels excluded from the analysis

*Cladosporium, Penicillium, Aspergillus, Fusarium* and *Rhizopus* were detected in 98.5%, 93.8%, 51.5%, 90.8% and 88.5% of homes respectively. Indoor, outdoor and individual room total concentrations were positively skewed except for bathroom level which was close to normal distribution. In both indoor and outdoor samples the proportion of concentration of fungal species was similar with *Cladosporium* being the predominant fungi followed by *Penicillium* and then *Fusarium, Aspergillus* and *Rhizopus* (Figure 20).

Sleep area concentrations of individual fungal species showed *Cladosporium* as a predominant species in most samples with an average of 802 (SD=572) CFU/$m^3$ and *Rhizopus* with the lowest concentrations of 32 (SD=39) CFU/$m^3$. In sleep area samples *Cladosporium, Penicillium, Aspergillus, Fusarium* and *Rhizopus* were detected in 96.8%, 83.3%, 37.3%, 79.4% and 73.0% of collected samples respectively. For proportion of fungal species levels in samples from sleep area, a similar pattern as indoor and outdoor was observed (Figure 21). As it was with indoor samples, sleep area levels of CFU showed strong correlation between sleep area and outdoor total ($r=0.544$, $p< 0.001$) plus sleep area and outdoor *Cladosporium* ($r=0.573$, $p< 0.001$). Weak correlations were observed with sleep area vs. outdoor *Fusarium* ($r=0.269$, $p=0.003$), sleep area vs. outdoor *Aspergillus* ($r=0.396$, $p<0.001$) and sleep area vs. outdoor *Rhizopus* levels ($r=0.303$, $<0.001$). Correlation between *Penicillium* sleep area vs. outdoor levels was very weak with $r=0.091$ ($p=0.336$).

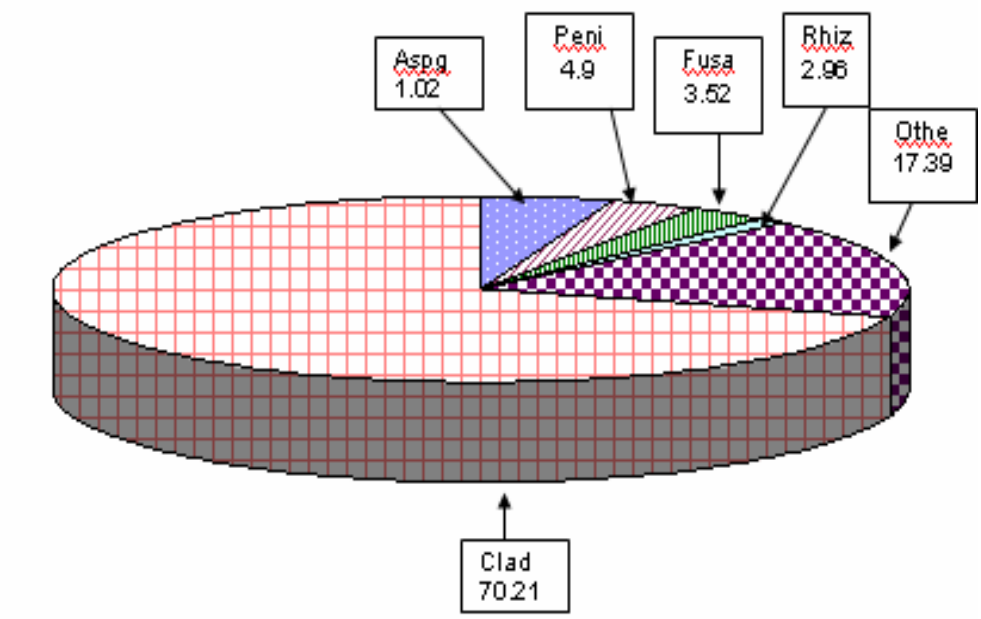
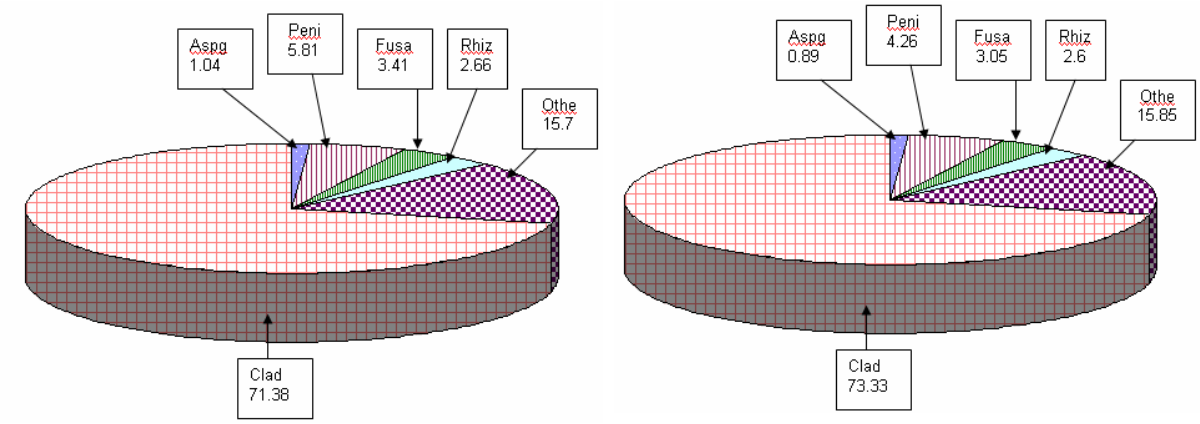



**Figure 20:** Pie diagram showing percentage proportions of total levels of different species of fungi from homes




(a) (b)

**Figure 21**: Pie diagrams showing proportions of (a) indoor and (b) outdoor levels of different species of fungi

Kruskal-Wallis test was performed on untransformed fungal aerosol data with presence and absence of home characteristics as grouping variable. Table 14 and Table 15 show fungal aerosol levels for total indoor and sleep area that are significantly different respectively, classified by selected home characteristics in the homes visited.

Homes with or without some housing conditions such as presence of ceiling, house built before 1978, type of roofing and use of air conditioner/fan were found to have significant different levels of fungal aerosols. Homes with damp or moist carpets and roof/ceiling water stains had higher levels than those without these conditions.

### 5.3.2 Home Characteristics as Predictors of Higher Fungal Aerosol Levels

Simple linear regression performed on transformed fungal aerosol concentration data showed a number of characteristics associated with levels of fungal aerosols in sleep area and entire home (p=0.05) (Table 16 and Table 17). For both entire home and sleep area presence of ceiling had significant association with indoor levels of *Cladosporium* and total concentrations. Age of the home (built after 1978) was only a predictor of higher *Fusarium spp*. levels in sleep area (β=1.52, p=0.011) and entire house (β=1.53, p=0.002). This means that house built before 1978 had significant 2.25 CFU/$m^3$ [(1.5 CFU$m^3$)$^2$] *Fusarium spp.* levels higher in sleep area and entire house.

In an all-inclusive multivariate regression analysis significant positive associations with total indoor fungal levels were noted with outdoor total fungal levels showed [β= 0.54 (95%CI 0.44 to 0.65), p<0.001] and hard floors [β=5.23 (95%CI 0.56 to 9.913), p=0.029]. Total outdoor fungal levels were on the protective side whilst homes with hard floors throughout the house were about 25 CFUm3 higher compared to those with other types of floors. Both single family home [β= -8.94 (95%CI -16.12 to -1.76), p=0.016] and attached housing [β= -10.90 (95%CI -19.24 to -2.56), p=0.011] were negative predictors of total fungal aerosols in the homes [ranging between 64 and 100 CFU/$m^3$ decrease for single family and attached housing respectively]. In sleep area the total fungal levels were significantly associated with 140 CF/$m^3$ increase when the house

had damp or wet carpet /rug [β= 12.1 (95%CI 1.71 to 22.52) p=0.023] was an indicator of higher total fungal levels in sleep area.

Some of the variables were dropped from the model because of collinearity. None of the composed walkthrough variables were significant (at alpha = 0.05) for both simple and multivariate regression models. Covariates that might have significant influence on fungal aerosol levels such as season, socioeconomic status, etc. were not controlled for in the models as some of the information was either not sufficient or not collected during information gathering.

**Table 14:** Comparison of median indoor fungal species levels by presence and absence of home characteristics

| fungal species | home characteristics | n | number of -ve responses | number of +ve responses | p-value |
|---|---|---|---|---|---|
| indoor total | presence of a ceiling | 130 | 47* | 83 | <0.001 |
| | presence of roof/ceiling water stains | 130 | 85 | 45* | <0.001 |
| | smooth hard floors | 130 | 51 | 79* | 0.031 |
| | moist/damp rug or carpet | 59 | 56 | 3* | 0.021 |
| indoor *Cladosporium* | presence of fan/air conditioner | 130 | 46* | 84 | 0.048 |
| | presence of a ceiling | 130 | 47* | 83 | 0.003 |
| | presence of roof/ceiling water stains | 130 | 85 | 45* | <0.001 |
| | presence of roof/ceiling visible mould growth | 129 | 94 | 35* | 0.033 |
| | moist/damp rug or carpet | 59 | 56 | 3* | 0.013 |
| indoor *Aspergillus* | single family home | 130 | 38* | 92 | 0.038 |
| | moist/damp rug or carpet | 59 | 56 | 3* | 0.015 |
| | water damaged floors | 129 | 120 | 9* | 0.048 |
| indoor *Penicillium* | wall water stains | 130 | 100 | 30* | 0.020 |
| indoor *Fusarium* | home built before 1978 | 130 | 63* | 67 | 0.011 |
| | home built after 1978 | 130 | 80 | 50* | 0.004 |
| | presence of a ceiling | 130 | 47 | 80* | 0.033 |
| | wall water stains | 130 | 100* | 30 | 0.004 |
| indoor *Rhizopus* | wall water stains | 130 | 100 | 30* | 0.010 |

§ all dependent variables transformed using square root [sqrt (variable)]
*median response higher than alternate response in sleep area levels

**Table 15:** Comparison of sleep area fungal aerosol species levels by presence and absence of home characteristics

| fungal species | sleep area characteristics | n | number of -ve responses | number of +ve responses | p-value |
|---|---|---|---|---|---|
| sleep area Total | presence of a ceiling | 126 | 46* | 80 | 0.039 |
| | presence of roof/ceiling water stains | 126 | 81 | 45* | 0.004 |
| | smooth hard floors | 126 | 50 | 76* | 0.0267 |
| | moist/damp rug or carpet | 57 | 54 | 3* | 0.018 |
| sleep area *Cladosporium* | presence of a ceiling | 126 | 46* | 80 | 0.032 |
| | presence of roof/ceiling water stains | 126 | 81 | 45* | <0.001 |
| | smooth hard floors | 126 | 50 | 76* | 0.047 |
| | level looped rug/carpet | 10 | 10 | 49* | 0.027 |
| | moist/damp rug or carpet | 57 | 54 | 3* | 0.010 |
| sleep area *Aspergillus* | moist/damp rug or carpet | 57 | 54 | 3* | 0.004 |
| sleep area *Penicillium* | informal built home | 126 | 117 | 9* | 0.025 |
| | asbestos roofing | 46 | 11 | 35* | 0.010 |
| sleep area *Fusarium* | home built before 1978 | 126 | 61* | 65 | 0.018 |
| | home built after 1978 | 126 | 78 | 48* | 0.003 |
| | wall water stains | 126 | 97* | 29 | 0.015 |
| | wall mould growth | 126 | 110* | 16 | 0.015 |
| sleep area *Rhizopus* | corrugated iron roofing | 46 | 39* | 7 | 0.038 |
| | presence of roof/ceiling water stains | 126 | 81 | 45* | 0.036 |

*median response higher than alternate response in sleep area levels
§ all dependent variables transformed using square root [sqrt (variable)]

**Table 16:** Univariate analysis by simple linear regression of transformed fungal concentrations with home characteristics

| dependent variables[§] | home characteristics | β-coefficient | constant | p-value |
|---|---|---|---|---|
| indoor bioaerosol total | presence of a ceiling | -6.35 | 35.93 | <0.001 |
| | presence of roof/ ceiling water stains | 6.80 | 29.52 | <0.001 |
| | ceiling damage | 5.58 | 31.23 | 0.034 |
| | wall damage | 5.11 | 30.92 | 0.023 |
| | smooth hard floor | 3.79 | 29.57 | 0.028 |
| | moist carpet / rug | 10.69 | 30.42 | 0.036 |
| indoor *Cladosporium* | presence of a ceiling | -7.28 | 30.56 | <0.001 |
| | presence of roof/ ceiling water stains | 8.31 | 23.03 | <0.001 |
| | presence of roof/ ceiling mould growth | 5.81 | 24.36 | <0.001 |
| | wall damage | 5.95 | 24.76 | 0.023 |
| | moist carpet / rug | 13.17 | 23.74 | 0.020 |
| indoor *Penicillium* | presence of wall water stains | 1.87 | 6.68 | 0.017 |
| indoor *Aspergillus* | single family house | -1.19 | 3.03 | 0.018 |
| indoor *Fusarium* | homes built before 1978 | -1.28 | 6.16 | 0.009 |
| | homes built after 1978 | 1.53 | 4.91 | 0.002 |
| | presence of wall water stains | -1.51 | 5.85 | 0.009 |
| | extent of wall mould growth | -2.57 | 11.90 | 0.002 |
| indoor *Rhizopus* | presence of wall water stains | 1.34 | 4.51 | 0.011 |
| | cement floor | -1.40 | 5.01 | 0.030 |

§ All dependent variables transformed using square root [sqrt (variable)]

p-value considered significant at alpha = 0.05

**Table 17:** Univariate linear regression analysis of transformed levels of fungal aerosols in children's sleep area with home characteristics

| dependent variables[§] | home characteristics | β-coefficient | Constant | p-value |
|---|---|---|---|---|
| sleep area total | informal built homes | 6.44 | 31.10 | 0.044 |
| | presence of a ceiling | -4.51 | 34.42 | 0.008 |
| | presence of roof/ ceiling water stains | 5.85 | 29.47 | <0.001 |
| | ceiling damage | 5.47 | 30.91 | 0.031 |
| | smooth hard floor | 4.14 | 29.06 | 0.014 |
| | thick carpet floor | 6.02 | 26.07 | 0.047 |
| | moist carpet / rug | 11.98 | 30.34 | 0.022 |
| sleep area *Cladosporium* | presence of a ceiling | -5.80 | 28.99 | 0.004 |
| | roof/ ceiling water stains | 7.57 | 22.60 | <0.001 |
| | ceiling damage | 6.65 | 24.51 | 0.026 |
| | presence of wall water stains | 4.72 | 24.22 | 0.041 |
| | smooth hard floor | 4.57 | 22.55 | 0.021 |
| | thick carpet floor | 8.30 | 18.29 | 0.017 |
| | moist carpet / rug | 15.23 | 23.33 | 0.011 |
| sleep area *Aspergillus* | moist carpet / rug | 4.33 | 1.05 | 0.010 |
| sleep area *Penicillium* | roof/ ceiling mould growth | 3.91 | 5.83 | 0.004 |
| | asbestos roofing | -4.23 | 10.08 | 0.010 |
| | corrugated iron roofing | 4.12 | 6.24 | 0.037 |
| | extent of wall mould growth | -2.16 | 10.71 | 0.033 |
| sleep area *Fusarium* | homes built before 1978 | -1.16 | 5.85 | 0.046 |
| | homes built after 1978 | 1.52 | 4.67 | 0.011 |
| | presence of wall water stains | -1.78 | 5.66 | 0.010 |
| | wall mould growth | -2.30 | 5.54 | 0.008 |

§ all dependent variables transformed using square root [sqrt (variable)]

## 5.4 DISCUSSION

In this study we were able to identify and quantify most common airborne fungal species in Durban homes and outdoors. Home characteristics, identified using a walkthrough checklist, that have an association with fungal aerosol levels were determined from children's sleep area.

### 5.4.1 Levels of fungal aerosol found in homes

Concentration of viable airborne fungal spores inside homes (average=1298 CFU/$m^3$ and max= 5253 CFU/$m^3$) were comparable to those of other studies conducted in America and Europe but lower when compared to levels of studies conducted in some countries in Eastern Europe (Garrett *et al*. 1998; Ross *et al*. 2000; Hyvarinen *et al*. 2001; Gorny and Dutkiewicz 2002;). Total indoor levels of fungal aerosols were strongly correlated to total outdoor levels ($r=0.684$, $p< 0.001$) therefore suggesting a contribution of outdoor to indoor levels in these homes. Constituents of the fungal spectrum in the homes was similar to that one found by other researchers in Durban with *Cladosporium spp*. and Eastern European studies (Gqaleni *et al*. 2000; Sekhotha *et al*. 2000; Gorny *et al*. 2002). Danaviah and colleagues (1998) had a different finding from their study with *Penicillium spp.* and *Aspergillus spp.* being the predominant species in Durban informal houses. The difference in fungal species in findings from Danaviah *et al*.'s (1998) study would be mostly accounted by difference in types of homes investigated. In a review of work done in Eastern Europe Gorny and Dutkiewicz (2002) found different results from the studies. In Poland the level of fungal concentrations were above $10^4$ in homes with visible fungal growth but less than 200 CFU/$m^3$ in control houses whilst the Russian study found levels of microbial concentration ranging from $2.7x10^4$ CFU/m3 in the

central part of the building in wool weaving mills. In studies conducted in Eastern Europe, Europe, North America and Australia had *Penicillium, Aspergillus, Alternaria* and *Cladosporium* species occurring repeatedly in the dwellings (Palmas *et al*. 1999; Ross *et al*. 2000; Gent *et al*. 2002).

### 5.4.2 Fungal aerosol levels differed by presence or absence of home characteristics

Kruskal-Wallis test was used to test fungal aerosol level difference between homes with some characteristics and those without. Homes with characteristics that are indicators of dampness had significant higher levels of total indoor fungal aerosols than those without. This finding supports the hypothesis that "*mould growth thrive on humid and damp surfaces.*" Homes with carpeted floors and those built after 1978 also had higher level of indoor total and other species compared to homes without or with different characteristics both in entire home and in the sleep area. Presence of ceiling underneath the roof and iron roofing in homes without ceiling had significant lower levels of bioaerosols compared to those without these characteristics. Corrugated iron roof does not allow water to sip through it unless it is damaged, but with asbestos and tarpaulin which are porous material water sitting on them for sometime do sip through. Therefore presenting a moisture damaged surface conducive for fungal growth after sometime. Ceiling presence in the house yielding significant lower levels is a positive result because most house with roof water stains or visible mould growth did not have ceiling. *Fusarium* levels being lower in homes with damp and/or mouldy surfaces indicate what some studies found, that is some *Fusarium spp*. such as *F. moniliforme* and *F. proliferatum* grow optimally in hot and dry conditions (Doohan *et al*. 2003).

### 5.4.3 Predictors of fungal aerosol levels

Results from simple regression showed that one of the indicators of level of total fungal aerosols indoors and *Cladosporium* was the presence of the ceiling in the house. This characteristic had a significant negative association with total CFU in entire home and with other fungal levels. Damp and mouldy surfaces show positive association with levels of fungal aerosols both in sleep area and entire house and this is consistent with the findings by Garrett *et al*. (1998) and Gent *et al*. (2002). A predictor of highest change in total indoor fungal aerosols and *Cladosporium* was the presence of moist carpet/rug in the house. For *Fusarium spp*. this trend was not true, damp conditions were negative indicators of indoor levels in sleep area and entire home. *Fusarium* levels results were not as expected with older homes. The older the home becomes, the more likely degradation and damages would occur on it therefore dampness or leaks occur leading to mould growth and increased fungal aerosol levels.

In a multivariate analysis outdoor fungal aerosol levels were predictor of elevated higher indoor fungal aerosol levels. According to Verhoeff and colleagues (1992), levels of indoor in a German study were influenced by outdoor levels in period from June to October (which Summer to Autumn/Fall). In our study we did not have enough data to look for times when outdoor levels contribute significantly to indoor levels. Assuming an all-year round contribution of outdoor levels to indoor levels, is more realistic because of sub-tropical climate in the region therefore windows are open most of the time, Therefore, practises such opening windows and doors during warm periods for cooling purposes would be an all-year round practise with contribution of outdoor levels to indoor levels  all-year round.

In entire house, hard floors as positive predictors of higher fungal aerosols are a finding that may be explained by comparing hard floor characteristics to carpeted floors. In an environment with tropical climate ventilation residences by opening windows and doors is a common practice. Carpeted floors will trap fungal particles in them where as smooth floors disperse the propagules all over the area and are picked up during sampling. This finding is in contrast to what other studies found (Chew *et al*. 2003).

In sleep areas, moist or wet carpets were the only indicators of higher levels of total fungal aerosol in the sleep area and this might suggest the role of carpet on levels of airborne aerosols not just on settled dust. During regression analysis some variables were dropped from the model due to collinearity. None of the composed WTC variables were significantly associated either during simple or multivariate analysis.

# CHAPTER 6

# INDICATORS OF BIOLOGICAL POLLUTANT LEVELS IN DURBAN HOMES

## 6.1 DISCUSSION

This study's objective was to characterise biological pollutants and determine their association to home characteristics. A walkthrough checklist was the primary instrument used to assess the homes in this study. Some additional home characteristics were included in the walkthrough checklist to adapt it for different homes in the communities. The results of this exercise improved the instrument/checklist because some of the characteristics added were significantly associated with levels of indoor pollutants.

Given the climatic conditions in Durban, the hypothesis was that "*the levels of indoor biological pollutants will be higher compared to other parts of the world with colder or varying temperature and relative humidity climate*". Fungal aerosol and dust allergen levels determined in the homes were comparable to other parts and subtropical regions of the world. In the homes there were no characteristics of that could be associated consistently higher pollutant levels but some characteristics were associated with more than one type of pollutant.

Regression analysis did not indicate many home characteristics associated with levels of fungal aerosols or allergens in the homes, and this is consistent with the published literature.

## 6.2 LIMITATIONS

The study had some limitations that recognised by the investigator which are:

- The small sample size limited the findings of the study from being extrapolated to the whole population although these findings give a basic profile of some allergen levels and estimates for further studies in the region.
- Levels of biological pollutants such as bioaerosols change over seasons and this study did not probe that question as this was cross-sectional study with only one visit to each home.
- Resources and time did not allow collection of some data and information, such as humidity and temperature changes, on the homes that might have been an indicator of biological pollutant levels.
- Limitation of availability of expertise on the field of mycology directly involved with the study made it not possible to isolate organisms that were familiar to the investigator therefore missing identification of some fungal genera and species that have important impact on health of the people.
- Collecting dust from the houses was not conducive for timed sampling. This would have given the study means to comparing amount of dust between homes.

## 6.3 RECOMMENDATIONS

The findings of the study suggest that the recommendations be directed to the government, educators, community organisations and parents of the children or communities who participated in this study on how to mitigate or control the indoor environment for minimal exposure to biological pollutants.

**Educators, community organisations and local government authorities**

- Educators and community organisations should educate the communities about implications of indoor practices and its impact on health of the people.
- Government's process of mass building of low cost houses should be done with a view of avoiding future problems that might result in conditions such as ineffective building ventilation and building structure with moisture and dampness problems. Countries such as UK, Canada, France and Sweden building regulations suggest a minimal continuous air exchange rate of 0.5 ac/h (litres air change/hour) which is very important in minimising moisture and dampness problems.

**Parents and caregivers**

- Repairing leaks and moisture sources as soon as they occur to avoid high humidity and dampness is one of most important and easy measure in controlling dampness and excessive moisture.
- Ventilating the house or reducing humidity below 45% also reduce moisture and dampness on surfaces and should be a regular practice in the homes especially in the bathrooms.
- Cleaning and vacuuming the house weekly or removing carpets, stuffed toys and clutter from the bedroom helps reduce exposure to accumulation of house dust mite allergens.
- Minimising carpet and upholstered furniture in the house reduce exposure to HDM allergens

In a region like south Durban, where ambient air pollution is implicated to exacerbation of respiratory illnesses, high indoor pollutant concentrations can also

contribute to the problem therefore it is important for the communities to know about indoor air pollution effects on health.

## 6.4 CONCLUSION

Both subjective (home walkthrough) and objective (sampling and analysis) methods of monitoring are essential when investigating indoor environment. This study showed the need to adapt observational instrument/ checklist/ questionnaire to suite the environment or study area of interest. As other studies and findings indicated, the best way to assess exposure to biological pollutants indoors needs a combination of two or more methods (that is direct and indirect method). Home walkthrough checklist is a very useful instrument in identifying home characteristics that previous investigations implicate in adverse health outcomes.

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# Appendix 1

**INDOOR AIR QUALITY OF SELECTED RESIDENCES AND SCHOOLS IN THE SOUTH AND NORTH DURBAN REGION, SOUTH AFRICA-2004**

1. **Title of research project**

Indoor air quality of residences in the South Durban Basin

2. **Name of the researchers**

Nkosana Jafta – B Med Sc (Honours)
Nceba Gqaleni – Ph D

3. **Purpose of the research**

This study is intended to describe the quality of indoor air in South Durban residences and schools in comparison with North Durban residences and schools. We will focus on fourth grade pupils using a walkthrough instrument and analysis of some of the known indoor pollutants and allergens. South Durban is a region with reported high emissions due to the local industries and for this reason it was chosen as a focus area in this research study.

Pollutants and indoor climate will be assessed and analysed using standardized protocols. Some of the pollutants that will be monitored and analysed are volatile organic compounds, allergens, fungal bioaerosols and carbon monoxide.

4. **Description of the research project**

If you agree to participate, the research will be carried out in your house. The research is about indoor air as well as other issues in the home or classroom of the children. A visit to the home will include the following.

**Walkthrough inspection**. A walkthrough inspection will be conducted by the researcher in the house. A child caregiver or an adult person who lives with the child will be asked some questions about the house and the child's activity in the house.

**Air samples**. Air samples will be taken from different rooms of the house which will include the room where the child sleeps as well as where he/she plays.

**Dust samples**. Samples of dust will be collected from the child's bed and the carpet or any other surfaces in rooms frequented by the child.

**Monitoring equipment**. Other equipment will be left for 24 hours in your house to monitor and collect dust.

5. **Duration of participation of the subject in the study**

There will be three visits to your house, the first session will include a walkthrough inspection, taking of air and dust samples as well as deploying the equipment in the house which will take a total of up to three (3) hours.

The second session will be for the researcher to check up and change the equipment that is left in the house which will take ten minutes.

The last session will last about twenty minutes and will be for the researcher to collect the equipment from the house.

6. **Risks and discomforts of the research**

The equipment that will be left in your house will produce a low noise which might take time for the people in the house to get used to.

7. **Measures to be taken to minimize risks and discomforts:**
N/A

8. **Expected benefits to subjects or to others**
The status of your indoor air environment will be identified and appropriate advice will be given as needed.
You will be provided with results and explanation about the state of allergens and air pollutants analysed from their homes unless you indicate that you do not wish to receive this information.
Participants will be informed about the importance of the indoor air environment and health effects associated with it.
Health officers in the area will have an idea about the indoor environment in the area.

9. **Costs to subject resulting from participation in the study**
Of all of the equipment used in the study, only a vacuum cleaner uses electricity during sampling while others use batteries and will be charged in the laboratory before being used or deployed in the house. Sampling using a vacuum will take five minutes at a cost of about R1-00 per house.

10. **Payments to subject for participating in the study**
The participants will receive no financial benefits from participating in this study.

11. **Confidentiality of information collected**
You will not be identified in any reports on this study. The records will be kept confidential to the extent provided by law. Only researchers on this study may have access to your results.

12. **Management of Physical Injury**
N/A

13. **Availability of further information**
If significant new knowledge is obtained during the course of this research which may relate to your willingness to continue participating, you will be informed of this knowledge. Also, you may contact the following office for answers to further questions about the research, your rights, or any injury you may feel is related to the study. You can contact Medical Research Administration at: Telephone # (031) 260 4495, Fax # (031) 260 4410, and email: ethicsmed@ukzn.ac.za.

14. **Voluntary nature of participation**
Your participation in this project is voluntary. Subsequent to your consent, you 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.

15. **Documentation of the consent**
One copy of this document will be kept together with our research records on this study. A second copy will be given to you to keep.

I have read [or been informed] of the information given above. I understand the meaning of this information. Dr./Mr./Ms. _____ has offered to answer any questions I may have concerning the study. I hereby consent to participate in the study.

**ADULT SUBJECT OF RESEARCH**

_____

Printed Name Consenting signature

**PERSON OBTAINING CONSENT**

_____

Printed Name Consenting signature

**DATE:** _____

# Appendix 2

August 12, 2004

**HOME WALKTHROUGH CHECKLIST**

PARTICIPANT'S I.D. # _____

Interviewer's Name _____

Caretaker's Name _____

Child's Name _____

DATE (DD/MM/YY)_____/_____/_____

Time: _____ AM/PM

[Please note: Some questions in this survey are to be read and others are for you to just observe. In situations where the respondent says something different than what you observe, ask the respondent to explain. "I noticed there are rugs on the floor, and I thought you said before that there are not usually rugs on this floor. Is something unusual going on right now?"]

[Read to caregiver] At this time I would like to walk through several rooms in the house with you. I will be writing down information about these rooms. I will also be asking you questions related to specific items in some of the rooms we will be looking at. This is an important part of the study that will help us give better advice about the indoor environment of your house.

[obs] 1. Type of home:

☐$_1$ A single family house
☐$_2$ A duplex or flat
☐$_3$ An apartment building
☐$_4$ Other (explain:_____)

[ask] Total number of rooms in the house [excluding toilets & b/rooms] _____

Approximate estimation of ground floor of entire house _____m x _____m

Is there an additional floor: ☐$_1$ Yes
☐$_2$ No

(iv) If yes, approximate dimension of the entire floor _____m x _____m _____)

[ask] 2. When was this home built?

☐$_1$ Before 1978
☐$_2$ 1978 or later
☐$_9$ Don't know

[obs] 3. Home is constructed mostly of:

☐$_1$ Wood
☐$_2$ Brick
☐$_3$ Other (explain: _____)

[ask] 4. Do you have central air conditioning/fan in your home?

☐$_1$ Yes
☐$_2$ No

[ask] 5. Did you do anything to prepare for this visit, such as cleaning the house?

☐$_1$ Yes
☐$_2$ No [SKIP TO 7]

[ask] 6. What did you do? _____

***Type of a Home***

| | |
|---|---|
| [obs] A. Is the home in an informal settlement? | ☐$_1$ Yes ☐$_2$ No |
| [obs] B. Is the home of informal construction? | ☐$_1$ Yes<br>☐$_2$ No [Skip to 7] |
| [obs] C. What are outside walls made of? CHECK ALL THAT APPLY | ☐$_1$ Corrugated iron sheets<br>☐$_2$ Wood<br>☐$_3$ Earth<br>☐$_4$ Cardboard<br>☐$_5$ Cement<br>☐$_6$ Other (specify: _____) |
| [obs] D. What is the roof made of? | ☐$_1$ Roof tiles<br>☐$_2$ Asbestos<br>☐$_3$ Corrugated iron sheets<br>☐$_4$ Tarplin (sail)<br>☐$_5$ Other (specify: _____) |
| [obs] C. What is the floor made of? | ☐$_1$ Earth<br>☐$_2$ Cement<br>☐$_3$ Tiling<br>☐$_4$ Wood<br>☐$_5$ Other (specify: _____) |
| [obs] E. Is there a ceiling in the house (that is separate from the underside of the roof)? | ☐$_1$ Yes<br>☐$_2$ No |
| [obs] F. Are there visible spaces or gaps between (that is, can sky or obvious light from outside be seen through a gap in):<br>F1. upper part of the wall and the roof or ceiling? | ☐$_1$ Yes<br>**☐$_2$ No** |
| **[obs] F2….the closed door and the door frame?** | ☐$_1$ Yes<br>☐$_2$ No<br>☐$_8$ Not applicable |
| [obs] F3. …door frames and outside walls? | ☐$_1$ Yes ☐$_2$ No |
| [obs] F4. … closed windows and the window frames? | ☐$_1$ Yes<br>☐$_2$ No<br>☐$_8$ Not applicable |
| [obs] F5. …window frames and wall? | ☐$_1$ Yes<br>☐$_2$ No<br>☐$_8$ Not applicable |
| [obs] F6. …in the outside walls themselves? | ☐$_1$ Yes ☐$_2$ No |
| [obs & ask] G. Roof/ceiling leaks during rain storms? | ☐$_1$ Not at all<br>☐$_2$ In one or two spots<br>☐$_3$ Extensively |
| [obs & ask] H. House floods (standing water covering the floor) during rain storms from water on the ground outside entering the house? | ☐$_1$ Not at all<br>☐$_2$ Occasionally<br>☐$_3$ Frequently |
| [obs & ask] I. Cooking is done inside of the house…. | ☐$_1$ Never …GO TO J<br>☐$_2$ Less than once a week<br>☐$_3$ A few times each week<br>☐$_4$ Every day |
| [obs & ask] I1. The usual fuel or energy source for cooking is… | ☐$_1$ Electricity<br>☐$_2$ Propane<br>☐$_3$ Paraffin (kerosine)<br>☐$_4$ Wood<br>☐$_5$ Coal stove<br>☐$_6$ Other (specify: _____) |
| [obs & ask] I2. Type of ventilation used for cooking... | ☐$_1$ None whatsoever<br>☐$_2$ Only opening of doors and windows |

| | |
|---|---|
| | ☐3 Hole in the roof or ceiling above cooking area<br>☐4 Pipe to the outside<br>☐5 Other (specify: _____) |
| [obs & ask] J. During cold weather, the house is heated by... | ☐1 Not heated<br>☐2 Electric heater<br>☐3 Paraffin (kerosine) heater<br>☐4 Wood stove<br>☐5 Coal stove<br>☐6 Other (specify: _____) |
| [obs] K. Evidence of smoke deposits from cooking or heating on walls, ceiling or underside of roof? | ☐1 None<br>☐2 Modest<br>☐3 Heavy |

***Child's Sleeping Area***

| | |
|---|---|
| [ask] 7. Where does [child] usually sleep?<br><br>[Say: "Let's start in that room."] | ☐1 Bedroom<br>☐2 Living Room/Family Room<br>☐3 Other (specify: _____) |
| [obs] 8. Approximate dimensions of room (estimate, do not measure) | _____ m × _____ m |
| [obs] 8.1. Is there a ceiling in the house (that is separate from the underside of the roof)? | ☐1 Yes [SKIP TO 9]<br>☐2 No |
| [obs] 8.2. What is the roof made of? | ☐1 Roof tiles<br>☐2 Asbestos<br>☐3 Corrugated iron sheets<br>☐4 Tarp (sail)<br>☐5 Other (specify: _____) |
| **Ceilings/Roof** | |
| 9. Peeling paint | ☐1 Yes ☐2 No |
| 10. Plaster falling | ☐1 Yes ☐2 No |
| 11. Tiles broken or missing | ☐1 Yes ☐2 No |
| 12. Water stains | ☐1 Yes ☐2 No |
| 12.1 | If yes, quantify: ☐1 < 0.3 $m^2$<br>☐2 0.3 to 1.0 $m^2$<br>☐3 >1.0 $m^2$ |
| 13. Visible mold or mildew | ☐1 Yes ☐2 No |
| 13.1 | (If yes, quantify: ☐1 < 0.3 $m^2$<br>☐2 0.3 to 1.0 $m^2$<br>☐3 >1.0 $m^2$ |
| 14. Other ceiling damage | ☐1 Yes ☐2 No<br>(If yes, specify: _____) |
| Walls | |
| 15. Peeling paint | ☐1 Yes ☐2 No |
| 16. Plaster falling | ☐1 Yes ☐2 No |
| 17. Tiles broken or missing | ☐1 Yes ☐2 No |
| 18. Water stains | ☐1 Yes ☐2 No |
| 18.1 | (If yes, quantify: ☐1 < 0.3 $m^2$<br>☐2 0.3 to 1.0 $m^2$<br>☐3 >1.0 $m^2$ |
| 19. Visible mold or mildew | ☐1 Yes ☐2 No |
| 19.1 | (If yes, quantify: ☐1 < 0.3 $m^2$<br>☐2 0.3 to 1.0 $m^2$<br>☐3 >1.0 $m^2$ |

| | |
|---|---|
| 20. Other wall damage | ☐1 Yes ☐2 No<br>(If yes, specify: _____) |
| **Windows** | |
| [obs] 21.1 Is there a window present in the room?<br><br>[obs] 21.2 Are any windows broken or cracked? | ☐1 Yes ☐2 No [SKIP TO 27.1]<br><br>☐1 Yes ☐2 No |
| 22. Window unit air conditioner/fan<br><br>[obs] 22.1 Present?<br><br>[ask] 22.2 Do you use it for cooling this room?<br><br>[ask] 22.3 Is the air conditioner/fan working?<br><br>[ask] 22.4 Do you usually use it on hot days?<br><br>[ask] 22.5 Do you use it at other times?<br><br>[ask] 22.6. Have you or anyone else changed the filter in the last year? | <br><br>☐1 Yes ☐2 No<br><br>☐1 Yes ☐2 No [SKIP TO 23]<br><br>☐1 Yes ☐2 No [SKIP TO 23]<br><br>☐1 Yes ☐2 No<br><br>☐1 Yes ☐2 No<br>(If yes, specify: _____)<br><br>☐1 Yes ☐2 No |
| [obs or ask] 23. Can at least one window in room be opened? | ☐1 Yes ☐2 No [SKIP TO 26]<br>(If no, why? _____) |
| [ask] 24. This time of year, is window open at night when [child] is sleeping? | ☐1 Yes ☐2 No |
| [ask] 25. Is the window open in spring, summer, or winter? | [CHECK ALL THAT APPLY]<br>☐1 Spring/Autumn<br>☐2 Summer<br>☐3 Winter |
| [obs/ask] 26. Do all the windows in the room appear to have tight seal? [Caulking missing, rattle test] | ☐1 Yes ☐2 No |
| **Floors** | |
| [obs] 28. Type of floor covering | [CHECK ALL THAT APPLY]<br>☐1 Wood floor, tile, or linoleum [SKIP TO 31]<br>☐2 Cement [SKIP TO 31]<br>☐3 Carpeting [if yes, see 29–30]<br>☐4 Rugs<br>% of floor covered with rugs _____<br>☐5 Other (specify: _____) [SKIP TO 31] |
| [obs] 29. If carpeting, what type? | ☐1 Level loop (flat)<br>☐2 Shag or plush<br>☐9 Don't know |
| [obs] 30. If carpeting or rugs, is any of it damp or moist to touch? | ☐1 Yes<br>☐2 No<br>☐9 Can't tell |
| [obs] 31. Signs of water damage, moisture, or leaks on floors? | ☐1 Yes (describe: _____)<br>☐2 No |
| **Other** | |
| [obs] 32.1 Is there currently a space heater in the sleeping area?<br><br>[ask] 32.2 Do you ever use a space heater in this room? | ☐1 Yes [SKIP TO 32.3] ☐2 No<br><br>☐1 Yes ☐2 No [SKIP TO 33] |

| | |
|---|---|
| [obs/ask] 32.3 If so, what kind of space heater do you use here?<br><br>[ask] 32.4 During which seasons do you use a/this space heater? | ☐$_1$ Parraffin<br>☐$_2$ Electric<br>☐$_3$ LP Gas<br><br>[CHECK ALL THAT APPLY]<br>☐$_1$ Winter<br>☐$_2$ Fall<br>☐$_3$ Spring<br>☐$_4$ Summer<br>☐$_5$ None |
| [obs] 33. How many chairs and couches are present? | _____ chairs & couches |
| [obs] 34. How many of these chairs and couches are cloth-covered? | _____ cloth-covered chairs & couches |
| [ask] 35. What does [child] sleep on? | ☐$_1$ Bed with mattress<br>☐$_2$ Mattress on floor<br>☐$_3$ Sofa<br>☐$_4$ Sofa bed<br>☐$_5$ Cot (no mattress)<br>☐$_6$ Futon<br>☐$_7$ Other (specify: _____) |
| [obs] 36. What is the pillow filled with? [LOOK AT LABEL ON PILLOW] | ☐$_1$ Feather<br>☐$_2$ Polyester<br>☐$_3$ Foam<br>☐$_4$ Other (specify: _____)<br>☐$_5$ No pillow<br>☐$_9$ Can't tell |
| [ask/obs] 37. What types of blankets/bedcovers do you use on [child's] bed? | [CHECK ALL THAT APPLY]<br>☐$_1$ Comforter<br>☐$_2$ Wool blanket<br>☐$_3$ Cotton blanket<br>☐$_4$ Acrylic blanket<br>☐$_5$ Blend (specify: _____)<br>☐$_9$ Don't know |
| [obs] 38. Stuffed toys visible in room | ☐$_1$ Yes ☐$_2$ No |
| [obs/ask] 39. Is there a forced air heating vent in the room? | ☐$_1$ Yes ☐$_2$ No [SKIP TO 41] |
| [obs] 40. If yes, is vent covered with a filter? | ☐$_1$ Yes ☐$_2$ No |

| | |
|---|---|
| [obs or ask] 41. Is a HEPA (High Efficiency Particulant Arrestor) air filter used in the room? | ☐$_1$ Yes<br>☐$_2$ No<br>☐$_9$ Don't know |
| [obs or ask] 42. Do you have problems with/Is there evidence (either you see or smell) of any of the following: | |
| 42.1 Cockroaches | ☐$_1$ Yes ☐$_2$ No |
| 42.2 Rodents (droppings?) | ☐$_1$ Yes ☐$_2$ No |
| 42.3 Food crumbs or open food on counters or floor? | ☐$_1$ Yes ☐$_2$ No |
| 42.4 Food stored unsealed? | ☐$_1$ Yes ☐$_2$ No |
| 42.5 Clutter such as unwashed dishes, papers, toys, food containers on counters or floors? | ☐$_1$ Yes ☐$_2$ No |
| 42.6 Mold or mildew? (visible signs or musty or mildewy smell) | ☐$_1$ Yes ☐$_2$ No |
| 42.7 Overflowing trashcan? | ☐$_1$ Yes ☐$_2$ No |
| 42.8 Tobacco smoke? (cigarette butts etc.) | ☐$_1$ Yes ☐$_2$ No |
| 42.9 Strong smelling cleaners (Ajax, 409 ...) | ☐$_1$ Yes ☐$_2$ No |

| | |
|---|---|
| 42.10 Scented candles? | ☐1 Yes ☐2 No<br>(if yes, how many are burning _____) |
| 42.11 Burning of incense? | ☐1 Yes ☐2 No |
| 42.12 Air freshener (such as plug-in)? | ☐1 Yes ☐2 No |

***Child's Playing Area***

| | |
|---|---|
| [ask] 43. In what area does [child] spend most of her/his time playing? | ☐1 Bedroom<br>☐2 Living Room/Family Room<br>☐3 Other (specify: _____) |
| [obs] 44. If this area is same as sleeping area, check box 1, skip this section, and go to the questions about the kitchen. | [CHECK ONE]<br>☐1 Place where child usually plays is same as sleeping area [IF SO, SKIP THIS SECTION AND GO TO THE TOP OF PAGE 10]<br>[Say: "Let's move on to the kitchen now."]<br><br>☐2 Place where child usually plays is different than sleeping area<br>[Say: "Let's go to that room now."] |
| [obs] 45. Approximate dimensions of room (estimate, do not measure) | ____ m × _____ m |
| [obs] 45.1. Is there a ceiling in the house (that is separate from the underside of the roof)? | ☐1 Yes [SKIP TO 46..1]<br>☐2 No |
| [obs] 45.2. What is the roof made of? | ☐1 Roof tiles<br>☐2 Asbestos<br>☐3 Corrugated iron sheets<br>☐4 Tarpaulin (sail)<br>☐5 Other (specify:_____) |
| **Ceilings/Roof** | |
| 46.1 Peeling paint | ☐1 Yes ☐2 No |
| 46.2 Plaster falling | ☐1 Yes ☐2 No |
| 46.3 Tiles broken or missing | ☐1 Yes ☐2 No |
| 46.4 Water stains | ☐1 Yes ☐2 No |
| 46.4.1 | (If yes, quantify: ☐1 < 0.3 $m^2$<br>☐2 0.3 to 1.0 $m^2$<br>☐3 >1.0 $m^2$ |
| 46.5 Visible mold or mildew | ☐1 Yes ☐2 No |
| 46.5.1 | (If yes, quantify: ☐1 < 0.3 $m^2$<br>☐2 0.3 to 1.0 $m^2$<br>☐3 >1.0 $m^2$ |
| 46.6 Other ceiling damage | ☐1 Yes ☐2 No<br>(If yes, specify: _____) |
| **Walls** | |
| 47.1 Peeling paint | ☐1 Yes ☐2 No |
| 47.2 Plaster falling | ☐1 Yes ☐2 No |
| 47.3 Tiles broken or missing | ☐1 Yes ☐2 No |
| 47.4 Water stains | ☐1 Yes ☐2 No |
| 47.4.1 | (If yes, quantify: ☐1 < 0.3 $m^2$<br>☐2 0.3 to 1.0 $m^2$<br>☐3 >1.0 $m^2$ |
| 47.5 Visible mold or mildew | ☐1 Yes ☐2 No |
| 47.5.1 | (If yes, quantify: ☐1 < 0.3 $m^2$<br>☐2 0.3 to 1.0 $m^2$<br>☐3 >1.0 $m^2$ |

| | |
|---|---|
| 47.6 Other wall damage | ☐1 Yes ☐2 No<br>(If yes, specify: _____) |
| **Windows** | |
| [obs] 48.1 Is there a window present? | ☐1 Yes ☐2 No [SKIP TO 55.1] |
| [obs] 48.2 Are any windows broken or cracked? | ☐1 Yes ☐2 No |
| [obs] 49. Covering on window | [CHECK ALL THAT APPLY]<br>☐1 Curtain/drapes<br>☐2 Blinds<br>☐3 Shades<br>☐4 None |
| 50. Window unit air conditioner/fan<br><br>[obs] 50.1 Present?<br><br>[ask] 50.2 Do you ever use a/the window unit air conditioner/fan in this room?<br><br>[ask] 50.3 Is the air conditioner/fan working?<br><br>[ask] 50.4 Do you usually use it on hot days?<br><br>[ask] 50.5 Do you use it at other times?<br><br>[ask] 50.6 Have you or anyone else changed the filter in the last year? | <br><br>☐1 Yes ☐2 No<br><br>☐1 Yes ☐2 No [SKIP TO 51]<br><br>☐1 Yes ☐2 No [SKIP TO 51]<br><br>☐1 Yes ☐2 No<br><br>☐1 Yes ☐2 No<br>(If yes, specify: _____)<br><br>☐1 Yes ☐2 No |
| [obs or ask] 51. Can at least one window in roombe opened? | ☐1 Yes ☐2 No [SKIP TO 54] |
| [ask] 52. This time of year, is window open when [child] is playing? | ☐1 Yes ☐2 No |
| [ask] 53. Is the window open in spring, summer, or winter? | [CHECK ALL THAT APPLY]<br>☐1 Spring<br>☐2 Summer<br>☐3 Winter<br>☐4 None |
| [obs/ask] 54. Do all the windows in the room appear to have tight seal? [Caulking missing, rattle test] | ☐1 Yes ☐2 No |
| **Floors** | |
| [obs] 56. Type of floor covering | [CHECK ALL THAT APPLY]<br>☐1 Wood floor, tile, or linoleum [SKIP TO 59]<br>☐2 Cement [SKIP TO 59]<br>☐3 Carpeting [if yes, see 57–58]<br>☐4 Rugs<br>% of floor covered with rugs _____<br>☐5 Other (specify: _____) [SKIP TO 59] |
| [obs] 57. If carpeting, what type? | ☐1 Level loop (flat)<br>☐2 Shag or plush<br>☐9 Don't know |
| [obs] 58. If carpeting or rugs, is any of it damp or moist to touch? | ☐1 Yes<br>☐2 No<br>☐9 Can't tell |
| [obs] 59. Signs of water damage, moisture, or leaks on floors? | ☐1 Yes (describe: _____)<br>☐2 No |
| **Other** | |

| | |
|---|---|
| [obs] 60. How many chairs and couches are present? | _____ chairs & couches |
| [obs] 61. How many of these chairs and couches are cloth-covered? | _____ cloth-covered chairs & couches |
| [obs] 62. Stuffed toys visible in room | ☐$_1$ Yes ☐$_2$ No |
| [obs/ask] 63. Is there a forced air heating vent in the room? | ☐$_1$ Yes ☐$_2$ No [SKIP TO 65] |
| [obs] 64. If yes, is vent covered with a filter? | ☐$_1$ Yes ☐$_2$ No |
| [obs or ask] 65. Is a HEPA (High Efficiency Particulant Arrestor) air filter used in the room? | ☐$_1$ Yes<br>☐$_2$ No<br>☐$_9$ Don't know |
| [obs/ask] 66. Do you have a problem /Is there evidence (either you see or smell) of any of the following: | |
| 66.1 Cockroaches | ☐$_1$ Yes ☐$_2$ No |
| 66.2 Rodents (droppings?) | ☐$_1$ Yes ☐$_2$ No |
| 66.3 Food crumbs or open food on counters or floor? | ☐$_1$ Yes ☐$_2$ No |
| 66.4 Food stored unsealed? | ☐$_1$ Yes ☐$_2$ No |
| 66.5 Clutters such as unwashed dishes, papers, toys, food containers on counters or floors? | ☐$_1$ Yes ☐$_2$ No |
| 66.6 Mold or mildew (visible signs, or musty or mildewy smell) | ☐$_1$ Yes ☐$_2$ No |
| 66.7 Overflowing trashcan? | ☐$_1$ Yes ☐$_2$ No |
| 66.8 Tobacco smoke? (cigarette butts etc.) | ☐$_1$ Yes ☐$_2$ No |
| 66.9 Strong smelling cleaners (Ajax, 409, etc.) | ☐$_1$ Yes ☐$_2$ No |
| 66.10 Scented candles? | ☐$_1$ Yes ☐$_2$ No<br>(if yes, how many are burning _____) |
| 66.11 Burning of incense? | ☐$_1$ Yes ☐$_2$ No |
| 66.12 Air freshener (such as plug-in)? | ☐$_1$ Yes ☐$_2$ No |

***Kitchen***

[Transition: "Now I'd like to look at the kitchen, please."]

| | |
|---|---|
| [obs] 67. Approximate dimensions of room (estimate, do not measure) | _____ m × _____ m |
| [obs] 67.1. Is there a ceiling in the house (that is separate from the underside of the roof)? | ☐$_1$ Yes [SKIP TO 68]<br>☐$_2$ No |
| [obs] 67.2. What is the roof made of? | ☐$_1$ Roof tiles<br>☐$_2$ Asbestos<br>☐$_3$ Corrugated iron sheets<br>☐$_4$ Tauplin (sail)<br>☐$_5$ Other (specify: _____) |
| **Ceiling/Roof** | |
| 68. Peeling paint | ☐$_1$ Yes ☐$_2$ No |
| 69. Plaster falling | ☐$_1$ Yes ☐$_2$ No |
| 70. Tiles broken or missing | ☐$_1$ Yes ☐$_2$ No |
| 71. Water stains | ☐$_1$ Yes ☐$_2$ No |
| 71.1 | (If yes, quantify: ☐$_1$ < 0.3 $m^2$<br>☐$_2$ 0.3 to 1.0 $m^2$<br>☐$_3$ >1.0 $m^2$ |
| 72. Visible mold or mildew | ☐$_1$ Yes ☐$_2$ No |
| 72.1 | (If yes, quantify: ☐$_1$ < 0.3 $m^2$<br>☐$_2$ 0.3 to 1.0 $m^2$<br>☐$_3$ >1.0 $m^2$ |
| 73. Other ceiling damage | ☐$_1$ Yes ☐$_2$ No |

| | |
|---|---|
| | (If yes, specify: _____) |
| **Walls** | |
| 74. Peeling paint | ☐1 Yes ☐2 No |
| 75. Plaster falling | ☐1 Yes ☐2 No |
| 76. Tiles broken or missing | ☐1 Yes ☐2 No |
| 77. Water stains | ☐1 Yes ☐2 No |
| 77.1 | (If yes, quantify: ☐1 < 0.3 $m^2$<br>☐2 0.3 to 1.0 $m^2$<br>☐3 >1.0 $m^2$ |
| 78. Visible mold or mildew | ☐1 Yes ☐2 No |
| 78.1 | (If yes, quantify: ☐1 < 0.3 $m^2$<br>☐2 0.3 to 1.0 $m^2$<br>☐3 >1.0 $m^2$ |
| 79. Other wall damage | ☐1 Yes ☐2 No<br>(If yes, specify: _____) |
| **Windows** | |
| [obs] 80.1 Is there a window present?<br><br>[obs] 80.2 Are any windows broken or cracked? | ☐1 Yes ☐2 No [SKIP TO 87.1]<br><br>☐1 Yes ☐2 No |
| 81. Covering on window | [CHECK ALL THAT APPLY]<br>☐1 Curtain/drapes<br>☐2 Blinds<br>☐3 Shades<br>☐4 None |
| 82. Window unit air conditioner/fan<br><br>[obs] 82.1 Present?<br><br>[ask] 82.2 Do you ever use a/the window unit air conditioner/fan in this room?<br><br>[ask] 82.3 Is the air conditioner/fan working?<br><br>[ask] 82.4 Do you usually use it on hot days?<br><br>[ask] 82.5 Do you use it at other times?<br><br>[ask] 82.6 Have you or anyone else changed the filter in the last year? | <br><br>☐1 Yes ☐2 No<br><br>☐1 Yes ☐2 No [SKIP TO 83]<br><br>☐1 Yes ☐2 No [SKIP TO 83]<br><br>☐1 Yes ☐2 No<br><br>☐1 Yes ☐2 No<br>(If yes, specify: _____)<br><br>☐1 Yes ☐2 No |
| [obs or ask] 83. Can at least one window in room be opened? | ☐1 Yes ☐2 No [SKIP TO 86] |
| [ask] 84. This time of year, is the window open when [child] is in the kitchen? | ☐1 Yes ☐2 No |
| [ask] 85. Is the window open in spring, summer, or winter? | [CHECK ALL THAT APPLY]<br>☐1 Spring/ Autumn<br>☐2 Summer<br>☐3 Winter<br>☐4 None |
| [obs/ask] 86. Do all the windows in the room appear to have tight seal? [Caulking missing, rattle test] | ☐1 Yes ☐2 No |
| **Floors** | |
| [obs] 88. Type of floor covering | [CHECK ALL THAT APPLY] |

| | |
|---|---|
| | ☐1 Wood floor, tile, or linoleum [SKIP TO 91]<br>☐2 Cement [SKIP TO 91]<br>☐3 Carpeting [if yes, see 89–90]<br>☐4 Rugs<br>% of floor covered with rugs _____<br>☐5 Other (specify: _____) |
| [obs] 89. If carpeting, what type? | ☐1 Level loop (flat)<br>☐2 Shag or plush<br>☐9 Don't know |
| [obs] 90. If carpeting or rugs, is any of it damp or moist to touch? | ☐1 Yes<br>☐2 No<br>☐9 Can't tell |
| [obs] 91. Signs of water damage, moisture, or leaks on floors? | ☐1 Yes (describe: _____)<br>☐2 No |

| **Other** | |
|---|---|
| [obs] 92. How many chairs and couches are present? | _____ chairs & couches |
| [obs] 93. How many of these chairs and couches are cloth-covered? | _____ cloth-covered chairs & couches |
| [obs] 94. Stuffed toys visible in room | ☐1 Yes ☐2 No |
| [obs/ask] 95. Is there a forced air heating vent in the room? | ☐1 Yes ☐2 No [SKIP TO 97] |
| [obs] 96. If yes, is vent covered with a filter? | ☐1 Yes ☐2 No |
| [obs or ask] 97. Is a HEPA (High Efficiency Particulant Arrestor) air filter used in the room? | ☐1 Yes ☐2 No<br>☐9 Don't know |
| [obs or ask] 98. Do you ever have any trouble with leaking plumbing in this room? | ☐1 Yes ☐2 No |
| [obs] 99. Do you have a problem/Is there evidence (either you see or smell) of any of the following: | |
| 99.1 Cockroaches | ☐1 Yes ☐2 No |
| 99.2 Rodents (droppings?) | ☐1 Yes ☐2 No |
| 99.3 Food crumbs or open food on counters or floor? | ☐1 Yes ☐2 No |
| 99.4 Food stored unsealed? | ☐1 Yes ☐2 No |
| 99.5 Clutters such as unwashed dishes, papers, toys, food containers on counters or floors? | ☐1 Yes ☐2 No |
| 99.6 Mold or mildew (visible signs or musty or mildewy smell) | ☐1 Yes ☐2 No |
| 99.7 Overflowing trashcan? | ☐1 Yes ☐2 No |
| 99.8 Tobacco smoke? (cigarette butts etc.) | ☐1 Yes ☐2 No |
| 99.9 Strong smelling cleaners (Ajax, 409, etc.) | ☐1 Yes ☐2 No |
| 99.10 Scented candles? | ☐1 Yes ☐2 No<br>(if yes, how many are burning _____) |
| 99.11 Burning of incense? | ☐1 Yes ☐2 No |
| 99.12 Air freshener (such as plug-in)? | ☐1 Yes ☐2 No |
| ***Kitchen-specific questions*** | |
| [obs or ask] 100. Is there a hood or vent with a working fan present over the stove/oven or as part of the stove/oven? | ☐1 Yes ☐2 No [SKIP TO 105]<br>☐9 Don't know [SKIP TO 105] |
| [obs] 101. Turn on the fan to test if it works | ☐1 Fan works<br>☐2 Fan doesn't work |
| [ask] 102. Is this hood or vent ventilated to the outside? | ☐1 Yes ☐2 No [SKIP TO 104]<br>☐9 Don't know |
| [ask/obs] 103. Can you show me where the vent is outside? | ☐1 Vent observed on outside wall<br>☐2 Vent not observed on outside wall |

| | |
|---|---|
| | ☐9 Can't tell |
| [ask] 104. How often is the fan or vent used when the stove is in use? | ☐1 Most of the time<br>☐2 Occasionally<br>☐3 Rarely<br>☐4 Never |

***Bathroom***

[Transition: "Now I'd like to see the bathroom, please."]

[If there are two bathrooms ask to see the one where the child usually takes his or her shower or bath.]

| | |
|---|---|
| [obs] 105. Approximate dimensions of room (estimate, do not measure) | _____ m × _____ m |
| [obs] 105.1. Is there a ceiling in the house (that is separate from the underside of the roof)? | ☐1 Yes [SKIP TO 106]<br>☐2 No |
| [obs] 105.2. What is the roof made of? | ☐1 Roof tiles<br>☐2 Asbestos<br>☐3 Corrugated iron sheets<br>☐4 Tarpaulin (sail)<br>☐5 Other (specify: _____) |
| **Ceilings/Roof** | |
| 106. Peeling paint | ☐1 Yes ☐2 No |
| 107. Plaster falling | ☐1 Yes ☐2 No |
| 108. Tiles broken or missing | ☐1 Yes ☐2 No |
| 109. Water stains | ☐1 Yes ☐2 No |
| 109.1 | (If yes, quantify: ☐1 < 0.3 $m^2$<br>☐2 0.3 to 1.0 $m^2$<br>☐3 >1.0 $m^2$ |
| 110. Visible mold or mildew | ☐1 Yes ☐2 No |
| 110.1 | (If yes, quantify: ☐1 < 0.3 $m^2$<br>☐2 0.3 to 1.0 $m^2$<br>☐3 >1.0 $m^2$ |
| 111. Other ceiling damage | ☐1 Yes ☐2 No<br>(If yes, specify: _____) |
| **Walls** | |
| 112. Peeling paint | ☐1 Yes ☐2 No |
| 113. Plaster falling | ☐1 Yes ☐2 No |
| 114. Tiles broken or missing | ☐1 Yes ☐2 No |
| 115. Water stains | ☐1 Yes ☐2 No |
| 115.1 | (If yes, quantify: ☐1 < 0.3 $m^2$<br>☐2 0.3 to 1.0 $m^2$<br>☐3 >1.0 $m^2$ |
| 116. Visible mold or mildew | ☐1 Yes ☐2 No |
| 116.1 | (If yes, quantify: ☐1 < 0.3 $m^2$<br>☐2 0.3 to 1.0 $m^2$<br>☐3 >1.0 $m^2$ |
| 117. Other wall damage | ☐1 Yes ☐2 No<br>(If yes, specify: _____) |
| **Windows** | |
| [obs] 118.1 Window present?<br><br>[obs] 118.2 Are any windows broken or cracked? | ☐1 Yes ☐2 No [SKIP TO 125]<br><br>☐1 Yes ☐2 No |
| 119. Covering on window | [CHECK ALL THAT APPLY] |

| | |
|---|---|
| | ☐1 Curtain/drapes<br>☐2 Blinds<br>☐3 Shades<br>☐4 None |
| [obs or ask] 120. Can at least one window in room be opened? | ☐1 Yes ☐2 No [SKIP TO 122] |
| [ask] 121. Is the window open in spring, summer, or winter? | [CHECK ALL THAT APPLY]<br>☐1 Spring/Autumn<br>☐2 Summer<br>☐3 Winter<br>☐4 None |
| [obs/ask] 122. Do all the windows in the room appear to have tight seal? [Caulking missing, rattle test] | ☐1 Yes ☐2 No |
| [obs] 123. Is an exhaust fan present? | ☐1 Yes ☐2 No |
| 124. Window unit air conditioner/fan<br><br>[obs] 124.1 Present?<br><br>[ask] 124.2 Do you ever use a/the window unit air conditioner/fan in this room?<br><br>[ask] 124.3 Is the air conditioner/fan working?<br><br>[ask] 124.4 Do you usually use it on hot days?<br><br>[ask] 124.5 Do you use it at other times?<br><br>[ask] 124.6 Have you or anyone else changed the filter in the last year? | <br><br>☐1 Yes ☐2 No<br><br>☐1 Yes ☐2 No [SKIP TO 125]<br><br>☐1 Yes ☐2 No [SKIP TO 125]<br><br>☐1 Yes ☐2 No<br><br>☐1 Yes ☐2 No<br>(If yes, specify: _____)<br><br>☐1 Yes ☐2 No |
| **Floors** | |
| [obs] 125. Type of floor covering | [CHECK ALL THAT APPLY]<br>☐1 Wood floor, tile, or linoleum [SKIP TO 128]<br>☐2 Cement [SKIP TO 128]<br>☐3 Carpeting [if yes, see 126–127]<br>☐4 Rugs<br>% of floor covered with rugs _____<br>☐5 Other (specify: _____) [SKIP TO 128] |
| [obs] 126. If carpeting, what type? | ☐1 Level loop (flat)<br>☐2 Shag or plush<br>☐9 Don't know |
| [obs] 127. If carpeting or rugs, is any of it damp or moist to touch? | ☐1 Yes<br>☐2 No<br>☐9 Can't tell |
| [obs] 128. Signs of water damage, moisture, or leaks on floors? | ☐1 Yes (describe: _____)<br>☐2 No |
| **Other** | |
| [obs/ask] 129. Is there a forced air heating vent in the room? | ☐1 Yes ☐2 No [SKIP TO 131] |
| [obs] 130. If yes, is vent covered with a filter? | ☐1 Yes ☐2 No |
| [obs or ask] 131. Is a HEPA (High Efficiency Particulant Arrestor) air filter used in the room? | ☐1 Yes ☐2 No<br>☐9 Don't know |
| [obs or ask] 132. Do you ever have any trouble with | ☐1 Yes ☐2 No |

| | |
|---|---|
| leaking plumbing in this room? | |
| [obs/ask] 133. Do you have problem (Is there evidence) (either you see or smell) of any of the following: | |
| 133.1 Cockroaches | ☐1 Yes ☐2 No |
| 133.2 Rodents (droppings?) | ☐1 Yes ☐2 No |
| 133.3 Food crumbs or open food on counters or floor? | ☐1 Yes ☐2 No |
| 133.4 Food stored unsealed? | ☐1 Yes ☐2 No |
| 133.5 Clutters such as unwashed dishes, papers, toys, food containers on counters or floors? | ☐1 Yes ☐2 No |
| 133.6 Mold or mildew? (visible signs or musty or mildewy smell) | ☐1 Yes ☐2 No |
| 133.7 Overflowing trashcan? | ☐1 Yes ☐2 No |
| 133.8 Tobacco smoke? (cigarette butts etc.) | ☐1 Yes ☐2 No |
| 133.9 Strong smelling cleaners (Ajax, 409, etc.)? | ☐1 Yes ☐2 No |
| 133.10 Scented candles? | ☐1 Yes ☐2 No (if yes, how many are burning _____) |
| 133.11 Burning of incense? | ☐1 Yes ☐2 No |
| 133.12 Air freshener (such as plug-in)? | ☐1 Yes ☐2 No |

***Basement***

| | |
|---|---|
| [ask] 134. Do you have access to a basement or crawl space? | ☐1 Yes [SAY: "Let's go there next."]<br>☐2 No [SKIP TO THE NEXT SECTION] |
| [obs] 135. Approximate dimensions of room (estimate, do not measure) | _____ m × _____ m |
| [ask] 135.1 Do you use the basement as a living space or a storage space or both? | ☐1 living space<br>☐2 storage space<br>☐3 both<br>☐4 other (specify:_____) |
| [obs] 135.2 Is there a ceiling in the house (that is separate from the underside of the roof)? | ☐1 Yes [SKIP TO 135]<br>☐2 No |
| [obs] 135..3 What is the roof made of? | ☐1 Roof tiles<br>☐2 Asbestos<br>☐3 Corrugated iron sheets<br>☐4 Tarpaulin (sail)<br>☐5 Other (specify: _____) |
| **Ceilings** | |
| 136. Peeling paint | ☐1 Yes ☐2 No |
| 137. Plaster falling | ☐1 Yes ☐2 No |
| 138. Tiles broken or missing | ☐1 Yes ☐2 No |
| 139. Water stains | ☐1 Yes ☐2 No |
| 139.1 | (If yes, quantify: ☐1 < 0.3 $m^2$<br>☐2 0.3 to 1.0 $m^2$<br>☐3 >1.0 $m^2$ |
| 140. Visible mold or mildew | ☐1 Yes ☐2 No |
| 140.1 | (If yes, quantify: ☐1 < 0.3 $m^2$<br>☐2 0.3 to 1.0 $m^2$<br>☐3 >1.0 $m^2$ |
| 141. Other ceiling damage | ☐1 Yes ☐2 No |
| **Walls** | |
| 142. Peeling paint | ☐1 Yes ☐2 No |
| 143. Plaster falling | ☐1 Yes ☐2 No |

| | |
|---|---|
| 144. Tiles broken or missing | ☐1 Yes ☐2 No |
| 145. Water stains | ☐1 Yes ☐2 No |
| 145.1 | (If yes, quantify: ☐1 < 0.3 $m^2$<br>☐2 0.3 to 1.0 $m^2$<br>☐3 >1.0 $m^2$ |
| 146. Visible mold or mildew | ☐1 Yes ☐2 No |
| 146.1 | (If yes, quantify: ☐1 < 0.3 $m^2$<br>☐2 0.3 to 1.0 $m^2$<br>☐3 >1.0 $m^2$ |
| Other wall damage | ☐1 Yes ☐2 No<br>(If yes, specify & quantify: _____) |
| **Windows** | |
| [obs] 148.1. Window present? | ☐1 Yes ☐2 No [SKIP TO 155] |
| [obs] 148.2. Are any windows broken or racked? | ☐1 Yes ☐2 No |
| [obs] 149. Covering on window | [CHECK ALL THAT APPLY]<br>☐1 Curtain/drapes<br>☐2 Blinds<br>☐3 Shades<br>☐4 None |
| 150. Window unit air conditioner/fan<br><br>[obs] 150.1 Present?<br><br>[ask] 150.2 Do you ever use a/the window unit air conditioner/fan in this room?<br><br>[ask] 150.3 Is the air conditioner/fan working?<br><br>[ask] 150.4 Do you usually use it on hot days?<br><br>[ask] 150.5 Do you use it at other times?<br><br>[ask] 150.6 Have you or anyone else changed the filter in the last year? | <br><br>☐1 Yes ☐2 No [SKIP TO 151]<br><br>☐1 Yes ☐2 No [SKIP TO 151]<br><br>☐1 Yes ☐2 No<br><br>☐1 Yes ☐2 No<br><br>☐1 Yes ☐2 No<br>(If yes, specify: _____)<br><br>☐1 Yes ☐2 No |
| [obs, or ask] 151. Can at least one window in room be opened? | ☐1 Yes ☐2 No [SKIP TO 154] |
| [ask] 152. This time of year, is window open when [child] is in basement? | ☐1 Yes ☐2 No |
| [ask] 153. Is the window open in spring, summer, or winter? | [CHECK ALL THAT APPLY]<br>☐1 Spring<br>☐2 Summer<br>☐3 Winter<br>☐4 None |
| [obs/ask] 154. Do all the windows in the room appear to have tight seal? [Caulking missing, rattle test] | ☐1 Yes ☐2 No |
| [obs/ask] 155. Is there a dehumidifier in the basement? If so, does it work? Is it currently turned on? | ☐1 None<br>☐2 Present, turned on<br>☐3 Present, not turned on but works<br>☐4 Present, but broken |
| **Floors** | |
| [obs] 156. Type of floor covering | [CHECK ALL THAT APPLY]<br>☐1 Wood floor, tile, or linoleum [SKIP TO 159]<br>☐2 Cement [SKIP TO 159] |

| | |
|---|---|
| | ☐3 Carpeting [if yes, see 157–158]<br>☐4 Rugs<br>% of floor covered with rugs _____<br>☐5 Other (specify: _____) [SKIP TO 159] |
| [obs] 157. If carpeting, what type? | ☐1 Level loop (flat)<br>☐2 Shag or plush<br>☐9 Don't know |
| [obs] 158. If carpeting or rugs, is any of it damp or moist to touch? | ☐1 Yes<br>☐2 No<br>☐9 Can't tell |
| [obs] 159. Signs of water damage, moisture, or leaks on floors? | ☐1 Yes (describe: _____)<br>☐2 No |

**Other**

| | |
|---|---|
| [obs] 160. How many chairs and couches are present? | _____ chairs & couches |
| [obs] 161. How many of these chairs and couches are cloth-covered? | _____ cloth-covered chairs & couches |
| [obs] 162. Stuffed toys visible in room | ☐1 Yes ☐2 No |
| [obs/ask] 163. Is there a forced air heating vent in the room? | ☐1 Yes ☐2 No [SKIP TO 165] |
| [obs] 164. If yes, is vent covered with a filter? | ☐1 Yes ☐2 No |
| [obs or ask] 165. Is a HEPA (High Efficiency Particulant Arrestor) air filter used in the room? | ☐1 Yes<br>☐2 No<br>☐9 Don't know |
| [obs or ask] 166. Do you ever have any trouble with leaking plumbing in this room? | ☐1 Yes ☐2 No |
| [obs] 167. Is there evidence (either you see or smell) of any of the following: | |
| 167.1 Cockroaches | ☐1 Yes ☐2 No |
| 167.2 Rodents (droppings?) | ☐1 Yes ☐2 No |
| 167.3 Food crumbs or open food on counters or floor? | ☐1 Yes ☐2 No |
| 167.4 Food stored unsealed? | ☐1 Yes ☐2 No |
| 167.5 Clutters such as unwashed dishes, papers, toys, food containers on counters or floors? | ☐1 Yes ☐2 No |
| 167.6 Mold or mildew?<br>(visible signs or musty or mildewy smell) | ☐1 Yes ☐2 No |
| 167.7 Overflowing trashcan? | ☐1 Yes ☐2 No |
| 167.8 Tobacco smoke? (cigarette butts etc.) | ☐1 Yes ☐2 No |
| 167.9 Strong smelling cleaners (Ajax, 409, etc.) | ☐1 Yes ☐2 No |
| 167.10 Scented candles? | ☐1 Yes ☐2 No<br>(if yes, how many are burning _____) |
| 167.11 Burning of incense? | ☐1 Yes ☐2 No |
| 167.12 Air freshener (such as plug-in)? | ☐1 Yes ☐2 No |

***General Questions***

[Transition: "Now, I'd like to ask you just a few more questions about your home."]

| | |
|---|---|
| [ask] 169. Do you have a garage? | ☐1 Yes ☐2 No [SKIP TO 173] |
| [ask] 170.1 Is the garage attached to the home? | ☐1 Yes ☐2 No [SKIP TO 173] |
| [ask/obs] 170.2 Is there a room or living space directly above the garage? | ☐1 Yes ☐2 No |
| [ask] 171. Do you keep a working car in the garage? | ☐1 Yes ☐2 No [SKIP TO 173] |
| [ask] 172. How long do you let car idle in garage before driving off? | ☐1 Less than 15 seconds<br>☐2 15–30 seconds |

| | |
|---|---|
| | ☐3 More than 30 seconds<br>☐9 Don't know |
| [ask] 173. Do you have a working clothes dryer in the home? | ☐1 Yes ☐2 No [SKIP TO 176] |
| [ask/obs] 174. Is it vented to the outside | ☐1 Yes ☐2 No |
| [ask] 175. Does it have a working lint filter? | ☐1 Yes ☐2 No |
| [ask] 176. Does your home have any of the following? | [CHECK ALL THAT APPLY]<br>☐1 Weather stripped windows<br>☐2 Weather stripped doors<br>☐3 Double paned windows<br>☐4 Storm windows<br>☐5 Storm doors |
| **Type of heating source** | |
| [ask] 177. What is the <u>main</u> heating source in your home? | [CHECK ONE]<br>☐1 Radiators (steam or hot water)<br>☐2 Forced air vents (furnace)<br>☐3 Electric baseboard heaters<br>☐4 Wood burning stove/fireplace<br>☐5 Electric Space Heater<br>☐6 Paraffin space heater<br>☐7 LP Gas Space Heater<br>☐8 Open stove<br>☐10 Other (specify:_____)<br>☐11 NOT APPLICABLE<br>☐12 NONE |
| [ask] 178. What <u>other</u> sources do you use for heat? | [CHECK ALL THAT APPLY]<br>☐1 Radiators (steam or hot water)<br>☐2 Forced air vents (furnace)<br>☐3 Electric baseboard heaters<br>☐4 Wood burning stove/fireplace<br>☐5 Electric Space Heater<br>☐6 Paraffin Space heater<br>☐7 Gas Space Heater<br>☐8 Open stove<br>☐10 Other (specify: _____)<br>☐11 NOT APPLICABLE<br>☐12 NONE |
| 179. What daytime indoor temperature do you maintain in your home during heating season? | ☐1 Less than 13<br>☐2 13–16<br>☐3 16–20<br>☐4 20–24<br>☐5 More than 24<br>☐9 Don't know |
| [ask] 180. May I see the furnace?<br><br>[obs/ask] 181. What is the energy source for the furnace/boiler?<br><br>[obs/ask] 182. What type of furnace is it?<br><br>[obs/ask] 183. Is there an air filter on or in furnace? | ☐1 No furnace [SKIP TO 187]<br><br>☐1 Gas<br>☐2 Fuel / oil<br>☐3 Electric<br><br>☐1 Forced air<br>☐2 Steam or water (radiator or boiler)<br>☐3 Other (specify: _____)<br><br>☐1 Yes ☐2 No [SKIP TO 187]<br>☐9 Can't tell [SKIP TO 187] |

| | |
|---|---|
| [obs] 184. Is the filter a HEPA filter? | ☐$_1$ Yes<br>☐$_2$ No<br>☐$_9$ Can't tell |
| [obs] 185. If the filter is easily removed, please look to see if it is clean, partially dirty, or very dirty. | ☐$_1$ Clean<br>☐$_2$ Partially dirty (screen is partially visible)<br>☐$_3$ Dirty (screen is completely dark)<br>☐$_9$ Can't tell |
| [ask] 186. How often do you clean or remove dust on your fan? | ☐$_1$ Once a week<br>☐$_2$ Monthly<br>☐$_3$ After 2 months<br>☐$_9$ Can't tell |

***Outside building***

| | |
|---|---|
| [ask] 187. Do you have/access to an outside building? | ☐$_1$ Yes [SAY: "Let's go there next."]<br>☐$_2$ No [ END HERE] |
| [obs] 188. Approximate dimensions of room (estimate, do not measure) | _____ m × _____ m |
| [ask] 189. Do you use the outside building as a living space or a storage space or both? | ☐$_1$ living space<br>☐$_2$ storage space<br>☐$_3$ both<br>☐$_4$ other (specify:_____) |
| [obs] 189.1. Is there a ceiling in the house (that is separate from the underside of the roof)? | ☐$_1$ Yes [SKIP TO 190]<br>☐$_2$ No |
| [obs] 189.2. What is the roof made of? | ☐$_1$ Roof tiles<br>☐$_2$ Asbestos<br>☐$_3$ Corrugated iron sheets<br>☐$_4$ Tarpaulin (sail)<br>☐$_5$ Other (specify: _____) |
| **Ceilings/Roof** | |
| 190. Peeling paint | ☐$_1$ Yes ☐$_2$ No |
| 191. Plaster falling | ☐$_1$ Yes ☐$_2$ No |
| 192. Tiles broken or missing | ☐$_1$ Yes ☐$_2$ No |
| 193. Water stains | ☐$_1$ Yes ☐$_2$ No |
| 193.1 | (If yes, quantify: ☐$_1$ < 0.3 $m^2$<br>☐$_2$ 0.3 to 1.0 $m^2$<br>☐$_3$ >1.0 $m^2$ |
| 194. Visible mold or mildew | ☐$_1$ Yes ☐$_2$ No |
| 194.1 | (If yes, quantify: ☐$_1$ < 0.3 $m^2$<br>☐$_2$ 0.3 to 1.0 $m^2$<br>☐$_3$ >1.0 $m^2$ |
| 195. Other ceiling damage | ☐$_1$ Yes ☐$_2$ No |
| **Walls** | |
| 196. Peeling paint | ☐$_1$ Yes ☐$_2$ No |
| 197. Plaster falling | ☐$_1$ Yes ☐$_2$ No |
| 198. Tiles broken or missing | ☐$_1$ Yes ☐$_2$ No |
| 199. Water stains | ☐$_1$ Yes ☐$_2$ No |
| 199.1 | (If yes, quantify: ☐$_1$ < 0.3 $m^2$<br>☐$_2$ 0.3 to 1.0 $m^2$<br>☐$_3$ >1.0 $m^2$ |

| | |
|---|---|
| 200. Visible mold or mildew | ☐1 Yes ☐2 No |
| 200.1 | (If yes, quantify: ☐1 < 0.3 $m^2$<br>☐2 0.3 to 1.0 $m^2$<br>☐3 >1.0 $m^2$ |
| Other wall damage | ☐1 Yes ☐2 No<br>(If yes, specify: _____) |
| **Windows** | |
| [obs] 202.1. Window present? | ☐1 Yes ☐2 No [SKIP TO 204] |
| [obs] 202.2. Are any windows broken or cracked? | ☐1 Yes ☐2 No |
| [obs] 203. Covering on window | [CHECK ALL THAT APPLY]<br>☐1 Curtain/drapes<br>☐2 Blinds<br>☐3 Shades<br>☐4 None |
| 204. Window unit air conditioner/fan<br><br>[obs] 204.1 Present?<br><br>[ask] 204.2 Do you ever use a/the window unit air conditioner/fan in this room?<br><br>[ask] 204.3 Is the air conditioner/fan working?<br><br>[ask] 204.4 Do you usually use it on hot days?<br><br>[ask] 204.5 Do you use it at other times?<br><br>[ask] 204.6 Have you or anyone else changed the filter in the last year? | <br><br>☐1 Yes ☐2 No [SKIP TO 210]<br><br>☐1 Yes ☐2 No [SKIP TO 210]<br><br>☐1 Yes ☐2 No<br><br>☐1 Yes ☐2 No<br><br>☐1 Yes ☐2 No<br>(If yes, specify: _____)<br><br>☐1 Yes ☐2 No |
| [obs, or ask] 205. Can at least one window in room be opened? | ☐1 Yes ☐2 No [SKIP TO 210] |
| [ask] 206. This time of year, is window open when [child] is in basement? | ☐1 Yes ☐2 No |
| [ask] 207. Is the window open in spring, summer, or winter? | [CHECK ALL THAT APPLY]<br>☐1 Spring<br>☐2 Summer<br>☐3 Winter<br>☐4 None |
| [obs/ask] 208. Do all the windows in the room appear to have tight seal? [Caulking missing, rattle test] | ☐1 Yes ☐2 No |
| **Floors** | |
| [obs] 210. Type of floor covering | [CHECK ALL THAT APPLY]<br>☐1 Wood floor, tile, or linoleum [SKIP TO 214]<br>☐2 Cement [SKIP TO 214]<br>☐3 Carpeting [if yes, see 211–213]<br>☐4 Rugs<br>% of floor covered with rugs _____<br>☐5 Other (specify: _____) [SKIP TO 214] |
| [obs] 211. If carpeting, what type? | ☐1 Level loop (flat)<br>☐2 Shag or plush<br>☐9 Don't know |

| | |
|---|---|
| [obs] 212. If carpeting or rugs, is any of it damp or moist to touch? | ☐1 Yes<br>☐2 No<br>☐9 Can't tell |
| [obs] 213. Signs of water damage, moisture, or leaks on floors? | ☐1 Yes (describe: _____)<br>☐2 No |
| **Other** | |
| [obs] 214. How many chairs and couches are present? | _____ chairs & couches |
| [obs] 215 How many of these chairs and couches are cloth-covered? | _____ cloth-covered chairs & couches |
| [obs] 216. Stuffed toys visible in room | ☐1 Yes ☐2 No |
| [obs/ask] 217. Is there a forced air heating vent in the room? | ☐1 Yes ☐2 No [SKIP TO 221] |
| [obs] 218. If yes, is vent covered with a filter? | ☐1 Yes ☐2 No |
| [obs or ask] 219. Is a HEPA (High Efficiency Particulant Arrestor) air filter used in the room? | ☐1 Yes<br>☐2 No<br>☐9 Don't know |
| [obs or ask] 220. Do you ever have any trouble with leaking plumbing in this room? | ☐1 Yes ☐2 No |
| [obs/ask] 221. Do you have a problem with /Is there evidence (either you see or smell) of any of the following: | |
| 221.1 Cockroaches | ☐1 Yes ☐2 No |
| 221.2 Rodents (droppings?) | ☐1 Yes ☐2 No |
| 221.3 Food crumbs or open food on counters or floor? | ☐1 Yes ☐2 No |
| 221.4 Food stored unsealed? | ☐1 Yes ☐2 No |
| 221.5 Clutters such as unwashed dishes, papers, toys, food containers on counters or floors? | ☐1 Yes ☐2 No |
| 221.6 Mold or mildew?<br>(visible signs or musty or mildewy smell) | ☐1 Yes ☐2 No |
| 221.7 Overflowing trashcan? | ☐1 Yes ☐2 No |
| 221.8 Tobacco smoke? (cigarette butts etc.) | ☐1 Yes ☐2 No |
| 221.9 Strong smelling cleaners (Ajax, 409, etc.) | ☐1 Yes ☐2 No |
| 221.10 Scented candles? | ☐1 Yes ☐2 No<br>(if yes, how many are burning_____) |
| 221.11 Burning of incense? | ☐1 Yes ☐2 No |
| 221.12 Air freshener (such as plug-in)? | ☐1 Yes ☐2 No |
| 222.1 What type of fuel/energy used to cook? | [CHECK ONE]<br>☐1 Electric<br>☐2 Wood<br>☐3 Coal<br>☐4 Paraffin<br>☐5 Propane (LP Gas)<br>☐6 Other (specify:_____) |
| 222.2 How many times a day do you use the fuel/energy? | ☐1 Once<br>☐2 Two times<br>☐3 Three times<br>☐4 More than three times<br>☐5 Other (specify:_____) |

Thank you very much!

End Time _____AM/PM

# Appendix 3

| Variable labels | Questions used to compose new variable |
|---|---|
| *cdhdampqg.1,qg.2,qg.3,qh.1,qh.2,qh.3,q12,q18,q20,q30,q31,q46.4,q47.4,q58,q59, q71,q77,q90,q91,q109,q115,q127,q128,* | |
| ***cdsdamp*** | ***qg.1,qg.2,qg.3,qh.1,qh.2,qh.3,q12,q18,q20,q30,q31*** |
| *cdpdamp* | *q46.4,q47.4,q58,q59* |
| ***cdkdamp*** | ***q71,q77,q90,q91*** |
| *cdbdamp* | *q109,q115,q127,q128* |
| ********** | |
| ***Cdhmold*** | ***q13,q19,q42.6,q46.5,q47.5,q66.6,q72,q78,q99.6,q110,q116,q133.6,q140,q146,q167.6,q194,q200,q221.6*** |
| *cdsmold* | *q13,q19,q42.6* |
| ***cdpmold*** | ***q46.5,q47.5,q66.6*** |
| *cdkmold* | *q72,q78,q99.6* |
| ***cdbmold*** | ***q110,q116,q133.6*** |
| ********** | |
| ***cihdust*** | ***q28.3,q28.4,q37.1,q37.2,q37.3,q37.4,q37.5,q38, q49.1,q56.3,q56.4,q62,q81.1,q88.3,q88.4,q119.1,q125.3, q125.4,*** |
| *cisdust* | *q28.3,q28.4,q37.1,q37.2,q37.3,q37.4,q37.5,q38* |
| ***cipdust*** | ***q49.1,q56.3,q56.4,q62*** |
| *cikdust* | *q81.1,q88.3,q88.4,q94* |
| ***cibdust*** | ***q119.1,q125.3,q125.4*** |
| ********** | |
| ***cdhtotal*** | ***cdhdamp,cdhmold*** |
| *cdstotal* | *cdsmold,cdsdamp* |
| ***cdptotal*** | ***cdpmold,cdpdamp.*** |
| *cdktotal* | *cdkmold,cdkdamp* |
| ***cdbtotal*** | ***cdbmold,cdbdamp*** |
| *cbhtotal* | *cdhtotal,cihdust* |
| ***cwhtotal*** | ***cbhtota cbhtota cihdust*** |

**CODING EXPLAINED**

| | |
|---|---|
| First letter | c – composite |
| Second letter | i/d/b – indirect or direct or both |
| Third letter | h/s/p/k/b – house or sleep area or play area or kitchen or bathroom |
| Last 4 letters | mold/damp/mite/total – mould or dampness or mite or total |

# Appendix 4

## SOLUTIONS AND REAGENTS FOR ELISA ASSAYS

**1. Preparation of 50mM carbonate/bicarbonate buffer, pH 9.6**

- Add 1.59g of Na2CO3
- Add 2.93g of NaHCO3
- Dissolve in 1 liter deionised water
- Add 0.10g of Thimerosal in 1 liter (can be added as preservative if necessary).

**2. Preparation of Phosphate buffered saline, pH 7.4 (PBS), containing 0.05% Tween 20 (PBS-T)**

- Add 8.00g of NaCl
- Add 0.20g of $KH_2PO_4$
- 1.15g of $Na_2HPO_4$
- 0.20g of KCl
- 0.10g of Thimerosal (optional)
- 0.5ml of Tween 20

All made up to 1 liter in deionised water.

**3. Preparation of 1% BSA PBS-T**

- Add 1g of bovine serum albumin (BSA) to 100ml PBS-T

**4. Streptavidin-Peroxidase**

- Reconstitute 0.25mg of Streptavidin-Peroxidase (Sigma S5512) in 1ml distilled water and store at -20°C in 50µl aliquots.
- Dilute 1/1000 in 1% BSA PBS-T for use in the assay.

**4. Substrate solution, 1mM ABTS in 70mM citrate-phosphate buffer, pH4.2**

To prepare 70mM citrate-phosphate buffer, pH 4.2

- Add 19.21g/L to prepare 0.1M anhydrous citric acid = Solution A,
- Add 053.65g/L to prepare 2M Dibasic Na Phosphate.7 $H_2O$ = Solution B

For 500ml buffer, mix 147ml of solution A + 103ml of solution B and make up to 500ml with deionised $H_2O$.
Add 274mg ABTS to 500ml buffer to make the substrate solution (contains 1mM ABTS).

ABTS = 2,2'-azino-di-(3 ethylbenzthiazoline sulphonic acid). The substrate solution is stable at 4°C in the dark.
Immediately prior to adding to assay plates, add 1µl 30% $H_2O_2$ solution/ml ABTS. The assay will not work if you do not add the $H_2O_2$.

# Appendix 5

## Preparation of malt extract agar (MEA)

- 50 g of malt extract powder (Oxoid Ltd, city) was suspended in 1 litre of distilled water
- To dissolved, the contents were boiled
- Then 1 vial of rehydrated chroramphanicol supplement was added
- The solution was autoclaved at $115^0$C for 10 minutes
- Agar was cooled to 45 – $50^0$C
- Then mixed well and poured to plates

## Preparation of dichloran 18% glycerol agar base (DG18)

- 15.75g of DG18 (oxoid ltd, city) powder was suspend in distilled water
- Contents were heated until they were completely dissolved
- 110g (90 ml) of glycerol (AR grade) was added to the solution
- 1 vial of rehydrated chroramphanicol supplement was added to the solution
- The contents were sterilized at $121^0$C for 15 minutes
- After autoclaving the solution was cooled to $50^0$C mixed well and poured into Petri dishes