UNIVERSITI PUTRA MALAYSIA

ASSOCIATION OF INDOOR AND OUTDOOR BACTERIAL AND FUNGAL BIOAEROSOLS WITH RESPIRATORY SYMPTOMS AMONG CHILDREN AGED 10-11 years IN SELECTED SCHOOLS IN HULU LANGAT, MALAYSIA

NOR HUSNA BT MAT HUSSIN

FPSK(p) 2011 63

ASSOCIATION OF INDOOR AND OUTDOOR BACTERIAL AND FUNGAL BIOAEROSOLS WITH RESPIRATORY SYMPTOMS AMONG CHILDREN AGED 10-11 years IN SELECTED SCHOOLS IN HULU LANGAT, MALAYSIA

NOR HUSNA BT MAT HUSSIN

MASTER OF SCIENCE
UNIVERSITI PUTRA MALAYSIA
2011

ASSOCIATION OF INDOOR AND OUTDOOR BACTERIAL AND FUNGAL BIOAEROSOLS WITH RESPIRATORY SYMPTOMS AMONG CHILDREN AGED 10-11 years IN SELECTED SCHOOLS IN HULU LANGAT, MALAYSIA

NOR HUSNA BT MAT HUSSIN

MASTER OF SCIENCE
UNIVERSITI PUTRA MALAYSIA
2011

ASSOCIATION OF INDOOR AND OUTDOOR BACTERIAL AND FUNGAL BIOAEROSOLS WITH RESPIRATORY SYMPTOMS AMONG CHILDREN AGED 10-11 years IN SELECTED SCHOOLS IN HULU LANGAT, MALAYSIA

By

NOR HUSNA BT MAT HUSSIN

Thesis submitted to the School Graduate Studies, Universiti Putra Malaysia, in fulfilment of the Requirement for the Degree of Master in Science

December 2011

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master in Science

ASSOCIATION OF INDOOR AND OUTDOOR BACTERIAL AND FUNGAL BIOAEROSOLS WITH RESPIRATORY SYMPTOMS AMONG CHILDREN AGED 10-11 years IN SELECTED SCHOOLS IN HULU LANGAT, MALAYSIA

By

NOR HUSNA MAT HUSSIN

December 2011

Chair: Zailina bt Hashim, PhD

Faculty: Faculty of Medicine and Health Science

The presence of bacterial and fungal bioaerosols is almost inevitable in most enclosed environment and they are not all pathogenic as some may exist as a normal flora while some are airborne pathogens. This study was designed to characterize the type and bacterial and fungal bioaerosol concentrations in primary schools and its association with respiratory symptoms among school children. Environmental monitoring and bioaerosol sampling were conducted in five randomly selected schools. The sampling device used for monitoring bacteria and fungal bioaerosol was the Duo SAS Super 360 microbiological air sampler and Q-Trak IAQ Monitor was used for monitoring the temperature and relative humidity. Identification of bacteria and fungi was done using both conventional and molecular methods. Modified ISAAC questionnaire was distributed and completed by 201 school children together with their parents for respiratory health assessment. Normal flora

bacteria was the most frequently isolated bacteria including *Staphylococcus* spp., *Pseudomonas* spp. and *Bacillus* spp. *Terribacillus* spp. found in this study has never been reported from any other studies. The most frequently isolated fungal genera were *Aspergillus, Penicillium, Fusarium, Rhizopus* and *Zygomycetes*. The average bacterial concentration in indoor and outdoor air were 1025(±612) CFU/m^3 and 1473(±1261) CFU/m^3, respectively while the average fungal bioaerosol concentration in indoor and outdoor air were 292(±83) CFU/m^3 and 401(±235) CFU/m^3, respectively. The percentages of bacterial and fungal samples that were within the ACGIH recommended levels were 44% and 33.8% respectively. Ratio of indoor to outdoor fungi concentration was below 1.0 suggesting minimal inside generative source for fungal bioaerosols. The ratio of indoor to outdoor bacteria concentration was approaching 1.0 suggesting the presence of potential internal generative source of microorganisms and inadequate ventilation. Building occupants might be one of the potential sources of microorganisms in indoor air as bacteria concentration without occupants was significantly lower than with occupants ($p<0.05$). School children commonly reported chronic cough with phlegm (29.9%), itchy watery eyes and nose (28.4%) and wheezing or whistling in chest (20.9%). Allergy to dust recorded the highest prevalence (35.8%) compared to the other allergies. The most frequently isolated bacteria *Staphylococcus* spp. and *Bacillus* spp. showed no association with respiratory symptoms except with sore throat. *Aspergillus* spp. showed association with common reported respiratory symptoms among school children compared to *Penicillium* and

Rhizopus spp. exposure. Asthma and allergy showed association with parental history of asthma and allergy but no association with bioaerosol exposure. Exposure to high fungi concentration that is more than 500CFU/m^3 in indoor air of school environment was the prominent risk factors associated with respiratory symptoms reported by school children as compared to bacteria concentration and types of bioaerosols isolated.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

PERKAITAN DI ANTARA BAKTERIA DAN KULAT 'BIOAEROSOL' DALAM PERSEKITARAN SEKOLAH DENGAN GEJALA PERNAFASAN DI KALANGAN KANAK- KANAK 10-11 tahun di SEKOLAH DALAM DAERAH HULU LANGAT, MALAYSIA

Oleh

NOR HUSNA MAT HUSSIN

Disember 2011

Pengerusi: Zailina bt Hashim, PhD

Fakulti: Fakulti Perubatan dan Sains Kesihatan

Kehadiran bakteria dan kulat bioaerosol hampir tidak dapat dielakkan dalam persekitaran yang tertutup dan bukan semua jenis mikroorganisma ini patogenik kerana ada yang mungkin wujud sebagai flora normal di dalam udara. Kajian ini adalah direkabentuk untuk mengenalpasti jenis dan kepekatan bakteria dan kulat di sekolah rendah dan kaitannya dengan gejala pernafasan di kalangan kanak- kanak sekolah. Pemantauan alam sekitar dan persampelan 'bioaerosol' telah dijalankan di lima buah sekolah yang dipilih secara rawak. Peranti persampelan yang digunakan untuk pemantauan bakteria dan kulat bioaerosol adalah *Duo SAS Super 360* manakala *Q-Trak IAQ Monitor* digunakan untuk pemantauan suhu dan kelembapan. Pengenalpastian jenis bakteria dan kulat telah dilakukan dengan menggunakan kaedah konvensional dan molekul. Borang soal selidik ISAAC yang diubahsuai telah dijawab oleh 201 kanak-kanak sekolah

bersama-sama dengan ibu bapa mereka bagi menilai gejala pernafasan. Normal flora bakteria adalah jenis bakteria yang paling kerap dijumpai termasuklah *Staphylococcus* spp., *Pseudomonas* spp., dan *Bacillus* spp. *Terribacillus* spp. yang dijumpai dalam kajian ini tidak pernah dilaporkan daripada mana-mana kajian yang dijalankan sebelum ini. Genera kulat yang paling kerap dijumpai ialah *Aspergillus*, *Penicillium, Fusarium, Rhizopus* dan *Zygomycetes*. Kepekatan purata bakteria bagi udara dalaman dan luaran ialah 1025 (±612) CFU/m^3 dan 1473 (±1261) CFU/m^3 dan kepekatan purata kulat bioaerosol bagi udara dalaman dan luaran adalah 292 (±83) CFU/m^3 dan 401 (±235) CFU/m^3. Peratusan sampel bakteria dan kulat di udara yang berada dalam tahap yang disyorkan oleh ACGIH adalah 44% dan 33.8%. Nisbah udara dalaman ke udara luar bagi kepekatan kulat berada di bawah 1.0, mencadangkan sumber generatif dalaman yang minimum untuk kulat bioaerosols. Nisbah udara dalaman ke udara luar yang mendekati 1.0, mencadangkan kehadiran sumber dalaman yang berpotensi menambah kepekatan mikroorganisma dalam bangunan dan pengudaraan yang tidak sempurna. Penghuni bangunan menjadi salah satu faktor yang berpotensi dalam pertambahan mikroorganisma dalam udara dalaman bangunan kerana keputusan kajian menunjukkan kepekatan bakteria tanpa penghuni jauh lebih rendah berbanding dengan penghuni ($p<0.05$). Kanak-kanak sekolah kerap melaporkan batuk yang kronik dengan kahak (29.9%), gatal dan berair mata dan hidung (28.4%) dan berdehit atau bersiul dalam dada (20.9%). Alahan kepada habuk mencatatkan prevalen tertinggi (35.8%) berbanding dengan alahan yang lain. Bakteria yang paling kerap

dijumpai *Staphylococcus* spp. dan *Bacillus* spp. tidak ada kaitan dengan gejala pernafasan kecuali sakit tekak. *Aspergillus* spp. menunjukkan kaitan secara signifikan dengan gejala pernafasan yang dilaporkan di kalangan kanak-kanak sekolah berbanding *Penicillium* dan *Rhizopus* spp. Asma dan alergi menunjukkan kaitan dengan sejarah ibu bapa asma dan alahan tetapi tiada kaitan dengan pendedahan kepada 'bioaerosol'. Pendedahan kepada kepekatan kulat dalam udara di sekolah yang melebihi 500CFU/m^3 merupakan faktor penting yang berkait rapat dengan gejala pernafasan yang dilaporkan di kalangan kanak-kanak sekolah berbanding pendedahan kepada kepekatan bakteria dan jenis 'bioaerosol'.

ACKNOWLEDGEMENT

I would like to express my deepest gratitude to the Almighty Allah. Without the blessing of Him, I would not have been able to complete the thesis entitled 'Exposure of Bacterial and Fungal Bioaerosol in School Environment and Association with Respiratory Symptoms among Primary School Children in Hulu Langat, Selangor'.

My greatest gratitude goes to my dedicated supervisor, Prof. Dr. Zailina Hashim for her sincere guiding, support and outstanding supervising. Her wide knowledge, experience and unflagging encouragement were instrumental. Special thanks to the committee members Prof. Dato Dr. Lye Munn Sann and Assoc. Prof. Dr. Mariana Nor Shamsudin for their kind and sincere assistance and being very supportive throughout this project.

I am indebted beyond the words to the school headmasters and teachers that involved in this research; SK Bandar Baru Bangi, SK Saujana Impian, SK Dusun Tua, SK Sungai Serai and SK Dusun Nanding. Thank you for kind consideration and understanding in approving data collection to be successfully conducted at the schools. My deepest appreciations to all the children and parents for time and participation towards completion of this project.

I would like to thank the academic and non –academic staff of the Department of Medical Microbiology and Parasitology and Department of Environmental Health especially En. Zainan, En. Zainal, En. Nik Khairul Azizi, Cik Haslinda Ayu, Cik Shamsurina, Pn. Norijah and Pn. Norhazlene for their professional help and direct involvement during laboratory work in completing this project. My appreciation to the UPM Research University Grant Scheme (RUGS) 91127 as grant provider that helps a lot in ensuring data collection successfully conducted.

To all my friends, Junaidah Zakaria, Nurul Izzah, Rumaizah, Siti Zalikha, Norazura Ismail, Nurzafirah Mazlan and Khairul Nizam Mat Isa thank you so much for the support and encouragement during the hard time that greatly contributed to the completion of this project.

I wish to express my personal appreciation to my parents and beloved husband Mohd Nazim Mat Nawi for continuous support, prayers, advice and love, who always there in my hours of need. Last but not least, thank you very much for those who are directly or indirectly lends a hand in making of this project success. May Allah bless you.

~~I certify that an Examination Committee has met on September 2011 to conduct the final examination of Nor Husna bt Mat Hussin on her Master of Science thesis entitled "Bakteria dan Kulat „Bioaerosol" dalam Persekitaran Sekolah dan Kaitannya dengan Gejala Pernafasan di Kalangan Kanak- kanak 10-11 tahun di Sekolah dalam Daerah Hulu Langat, Selangor" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the student be~~ awarded the Master of Science.

Members of the Examination Committee were as follows:

Prof. Lekhraj Rampal
Universiti Putra Malaysia
(Chairman)

Dr. Malina Osman
Universiti Putra Malaysia
(Internal Examiner)

Dr. Rukman Awang Hamat
Universiti Putra Malaysia
(Internal Examiner)

Dr. Dan Norback
Department Occupational and Environmental Medicine
Uppsala University
Sweden
(External Examiner)

_____

NORITAH OMAR, PhD
Professor and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master in Science. The members of the Supervisory Committee were as follows:

Zailina bt Hashim, PhD

Professor

Faculty of Medicine and Health Science

University Putra Malaysia

(Chairman)

Lye Munn Sann, PhD

Professor Dato"

Faculty of Medicine and Health Science

University Putra Malaysia

(Member)

Prof. Dr. Mariana Shamsuddin

Professor

Faculty of Medicine and Health Science

University Putra Malaysia

(Member)

_____

BUJANG BIN KIM HUAT, PhD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.

_____

NOR HUSNA BT MAT HUSSIN

Date: 8 September 2011

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

LIST OF ABBREVIATIONS

ACGIH	American Conference of Governmental Industrial Hygienist
CFU/m^3	Colony forming unit per meter cubic
DOSH	Department of Occupational Safety and Health, Malaysia
DNA	Deoxyribonucleic acid
IAQ	Indoor Air Quality
ISAAC	International Study of Asthma and Allergies in Childhood
MEA	Malt Extract Agar
NIOSH	National Institute of Occupational Safety and Health
PCR	Polymerase Chain Reaction
PEF	Peak Expiratory Flow
RNA	Ribonucleic acid
SDA	Sabaroud Dextrose Agar
TSA	Tryptic Soy Agar
WHO	World Health Organization
NCBI	National Center for Biotechnology Information

CHAPTER 1

1.0 INTRODUCTION

1.1 Introduction

Recently, indoor air in school is becoming a major health concern. This is due to the fact that poor indoor air quality (IAQ) can reduce the students' health, productivity and ability to perform specific mental task requiring concentration as they might appear sleepy, coughing, dizzy and experienced other respiratory illnesses (Moglia et al., 2006). Besides, these physically developing and growing children are more vulnerable and likely to suffer from the deteriorated indoor air quality. Children spend a major portion of their time in school during weekdays which exposed them to the indoor air pollutants in school (Meklin et al., 2002).

The quality of indoor air in school environment can be contaminated by various factors including biological and chemical contaminants. Biological contaminants include moulds, bacteria, fungi and allergens (Zhao et al., 2006). These biological agent and their by-products of metabolism released into the air and become airborne particles or bioaerosols (Otten and Burge, 1999). Bioaerosols can consist of pathogenic or non-

pathogenic bacterial cells and cellular fragments, bacterial endotoxins, fungal spores, mycotoxins, peptidoglycans, pollen and plant fibres (Douwes et al., 2003).

Bioaerosol exposures in schools environment have been an area of continuing research interest. Exposure of children to certain airborne microorganisms at elevated concentrations could result in allergenic reactions, irritant responses, toxicosis, rhinitis, asthma, pneumonia and other health effects (Stetzenbach, 2004; Douwes et al., 2003). Among school children, asthma is the leading cause for absenteeism and can influence a child's academic performance and ability to participate in school activities (Bruzzese et al., 2009). Besides, allergic diseases such as nasal allergy and other allergies are the 'number one' chronic childhood illness, accounting for one-third of all chronic conditions occurring annually which affect 20% of school children (Bayer et al., 1999).

Cai et al., (2011) and Quah et al., (1997) reported a significant increase in the number of respiratory symptoms and asthma among school children in Malaysia by 41% and 13% as compared to studies conducted in 1990 to 2001. The rapid increase in the prevalence of respiratory problems in recent decades suggested the potential risk factors on environmental factors rather than genetic factors (Kim et al., 2007).

Bioaerosols are everywhere in the environment. In non-industrial indoor environments like school buildings and offices, the most important source of airborne contaminants is the presence of human or occupant density (Kalogerakis et al., 2005 and Stetzenbach, 2004). Microbial contaminants also can be brought inside by the occupants. Under favourable conditions like inadequate ventilation, poor maintenance and housekeeping, bioaerosols are able to grow and propagate on a variety of building materials and indoor surfaces, causing indoor air pollution (Zhu et al., 2003).

Several field studies have shown that the most common fungal genera in school buildings were *Penicilium* spp., yeasts, *Cladosporium* spp. and *Aspergillus* spp. (Aydogdu et al., 2005 and Meklin et al., 2002). *Penicilium* spp. are common in ambient environmental with high carbon dioxide (CO_2) concentration that frequently shows inadequate ventilation of a building and it affects the students' concentration and teachers' productivity (Clements, 2006). High concentrations of *Cladosporium* spp. and *Aspergillus* spp. had been proven to be associated with few allergic symptoms in respiratory systems (Aydogdu, 2007 and Fischer and Dott, 2003). Moreover, airborne concentrations of *Cladosporidium, Epicoccum, Coprinus* and *Fusarium* spores were associated with peak expiratory flow rates (PEFRs) deficiency indicating decreased in lung functions (Neas et al., 1996).

Presence of fungi with elevated airborne bacteria can cause nasal, optical and physiological changes and sensory symptoms exemplified by irritation, slugginess, sleepiness, headache and reduction of ability to concentrate (Dacarro et al., 2003 and Pan et al. 2000). The dominant airborne bacteria are Gram-positive bacteria included *Staphylococcus* spp., *Micrococcus* spp., *Corynebacterium* spp., and *Bacillus* spp. (Kim and Kim, 2007; Aydogdu, et al., 2005). However, there are also few Gram-negative bacteria found in indoor air like *Pseudomonas* spp., *Aeromonas* spp. and *Escherichia. coli* (Kim and Kim, 2007).

Mechanism of bioaerosol in causing various respiratory symptoms are due to the presence of agents with pro-inflammatory properties like endotoxins (Gram-negative bacteria cell wall components), peptidoglycans that are more prevalent in Gram-positive bacteria and β(1→3)-glucans that originate from most fungi. Many studies have shown positive association between endotoxins and β(1→3)-glucans exposure with health effects in exposed individuals (Douwes et al., 2003 and Rylander, 1999).

1.2 Problem Statement

Fungi and bacteria are ubiquitous microorganisms found in the indoor environments and they are not all pathogenic. Some of them may exist as normal flora and do not cause disease but there are few types of microbes that can take a role as human airborne pathogens (Stetzenbach, 2004 and Dacarro et al., 2003). According to Zeldin et al., (2006) microbes may play a role as immunoregulators or pathogens. Exposure to microbes at early age demonstrated a reduction in allergen sensitization in children of farmers and in children raised in day-care centres due to microbial stimulation of the immune system. However, in some studies documented exposure to bacterial endotoxins can inflame airways, elicit asthmatic attack and bronchial hyperreactivity as well as deficiency in peak expiratory flow rates (PEFRs) in children (Ross et al., 2000).

Exposure to these indoor air contaminants particularly among school children needs tremendous attention as they are more susceptible to the infection and respiratory problems and they spend large portion of their weekday time in school. Schools have been considered as the most important indoor environments for children beside homes. Many research findings have reported possible health effects of school environment and microbial exposures in school as potential risk factors for respiratory

symptoms, asthma and allergy among children (Kim et al., 2007; Meklin et al., 2005 and Meyer et al., 2004).

However, the information available on the types of airborne microbes and exposures in school buildings are currently limited (Dacarro et al., 2003). Most of the studies on school environment were published from western countries (Mendell and Heath, 2005 and Daisey et al., 2003) whereas there are relatively few from the Asian countries. Investigation of the air quality in classrooms helped to characterize pollutant levels and implement corrective measures.

High airborne microorganism concentrations were resulted from inadequate maintenance, poor housekeeping and inadequate ventilation. Besides, the elevated airborne microorganisms also can be brought into the building by the occupants through the normal shedding of skin cells or through aerosolization from mucosal surfaces by talking, coughing or sneezing (Bartlett et al., 2004)

Furthermore, respiratory tract infections in childhood may have long-term effect, including loss of lung functions after severe episodes of lower respiratory tract infection, the development of asthma or bronchitis and an increased risk of developing chronic obstructive pulmonary disease in adulthood (Heikkinen et al., 2005). However, while clinicians are trying to educate schools administration regarding environmental

control measures for children with asthma, little information about the conditions and the preventive measures are available in schools (Stanley, 2009).

1.3 Study Justification

Presence of some airborne bacteria and fungi may deteriorate the IAQ in school and potentially play a role as human airborne pathogens. Increased in bioaerosol level could reduce children's concentration or caused headaches and might affect comprehension capacity and motivation as well as increased in asthma attacks and bronchial hyperreactivity (Aydogdu et al., 2005 and Ross et al., 2000). Occurrence of airborne contaminants indicated the presence of moisture damage, inadequate ventilation rate, increase relative humidity and low temperature in school building which may affect the performance of the building occupant as a whole. Presence of moisture damage and inadequate ventilation in school buildings posed significant risk factors for respiratory symptoms in school children (Meklin et al., 2002).

Due to the rapid development of urbanization, urban traffic and climate change, increase in chronic disease like asthma is believed to be due to environmental changes rather than due to genetic factors. In Malaysia, few studies conducted on asthma and other respiratory problems have shown the relationship between exposures to air pollutants such as

particulate matter (PM_{10}), nitrogen dioxide (NO_2), asbestos, formaldehyde etc. (Jamal et al., 1998 and Zailina et al., 1997). However, no study has been conducted to study the correlation between airborne microorganisms and human health especially among school children. There is also a lack of scientific and quantitative information on causal relationship between health symptoms, exposure and dose-response relationship for indoor bioaerosol. Besides, there is different cut off points and no universally standards threshold limit value (TLV) for safe microorganism exposure and the mitigative measures should be taken by authorities and community to minimize the level of indoor microbial contaminants (ACGIH, 2004 and Shelton et al., 2002).

Therefore, this study is proposed to characterize the bacteria and fungi in school environment and the potential health risk associated with exposure among the school children. This study will also propose microbial indices as one of a tool and parameters in evaluating IAQ in schools. Besides, the relationship between the airborne bacteria and fungi and selected indoor parameters such as temperature and relative humidity would be studied to determine any correlations. This is to ensure that the students' health, well-being, performance and school environment can be improved.

1.4 Objectives

1.4.1 General Objectives

To characterize the indoor bioaerosol (bacteria and fungi) in school environment and its association with respiratory problems among school children in Selangor.

1.4.2 Specific Objectives

1. To isolate and identify the types of bacteria and fungal bioaerosols that exists in the selected school environments.

2. To determine the indoor and outdoor bioaerosol concentrations in selected primary schools.

3. To compare the levels of bacterial and fungal bioaerosol encountered in the indoor and outdoor environment of primary schools.

4. To correlate between presence of building occupants and level of indoor bacterial and fungal bioaerosol concentrations.

5. To determine the correlations between relative humidity and temperature with the viable bacterial and fungi counts in the indoor air of schools.

6. To determine the association between students' respiratory problems and exposures to bacterial and fungal bioaerosol in the indoor air of schools.

1.5 Study Hypotheses

1. There is a significant difference between bacterial and fungal bioaerosol concentrations in the indoor and outdoor environment of primary schools.

2. There is a significant correlation between presence of building occupants and level of indoor microbial contaminants.

3. There is a significant correlation between relative humidity and temperature with viable counts of bacterial and fungi in the indoor air.

4. There is a significant association between student's respiratory problem and exposure to bacterial and fungal bioaerosol in the indoor air.

1.6 Definition of variables

1.6.1 Conceptual definition

Bioaerosol exposure

Bioaerosols are collections of airborne biological material that can consist of bacterial cells and cellular fragments, fungal spores and by-products of microbial metabolism (Stetzenbach et al., 2004).

Primary school children

Male and female school children 7 to 12 years old (MOE, 2010).

Wheezing

Whistling sound in the chest mainly during exhalation. It is due to the constriction or obstruction of throat, pharynx, trachea or bronchi (Elizabeth, 1994).

Allergy

A disorder in which the body become hypersensitive to particular antigen/ allergens, which provoke characteristic symptoms (Elizabeth, 1994).

Respiratory symptoms

Respiratory symptoms comprise a vast range of inflammation and discomfort throughout respiratory track. Symptoms that affect the nose, throat and lings are typically considered respiratory symptoms (Mekin et al., 2005).

Asthma

Asthma is a disease characterized by recurrent attacks of breathlessness and wheezing, which vary in severity and frequency from person to person (WHO, 2005)

1.6.2 Operational definition

Bioaerosol exposure

Exposure of airborne bacteria and fungi in indoor and outdoor air of school environment that have been sampled and cultured on TSA and SDA media. Reported as concentration of colony forming unit (CFU) per meter cubic volume of air sampled.

Level of bacteria concentrations

Bacteria concentration has been classified into three levels that are low (<500 CFU/m^3), moderate/ medium (500-1000 CFU/m^3) and high (>1000 CFU/m^3) based on WHO and ACGIH recommendation.

Level of fungi concentrations

Fungi concentration has been classified into three levels that are low (<200 CFU/m^3), moderate/ medium (200-500 CFU/m^3) and high (>500 CFU/m^3) based on research finding by Robertson, 1997.

Wheezing

Symptoms of wheezing or whistling sound in breathing and breathlessness on most days and night, obtain from ISAAC questionnaire.

Asthma

Ever had asthma, asthma attacks in 12 months and doctor's diagnosed asthma, obtain from ISAAC questionnaire.

REFERENCES

ACGIH (1989). Guideline for the Assessment of Bioaerosols in the Indoor Environment. Cincinnati: ACGIH

ACGIH (2004). TLVs and BEIs, Based on the Documentations of the Threshold Limit Values for Chemical Substances and Physical Agents and Biological Exposure Indices. American Conference of Governmental Industrial Hygienists, 1330 Kemper Meadow Dr., Cincinnati, OH 45240-1643.

Alsmo T, and Holmberg, S. (2007). Sick buildings or not: Indoor air quality and health problems in schools. *Indoor and Built Environment*, 16(6), 548.

Ammor, S., Rachman, C., Chaillou, S. p., PrÃ©vost, H., Dousset, X., Zagorec, M., et al. (2005). Phenotypic and genotypic identification of lactic acid bacteria isolated from a small-scale facility producing traditional dry sausages. *Food Microbiology, 22*(5), 373-382.

Aydogdu, H., Asan, A., Otkun, M.T., Ture, M. (2005). Monitoring of bacteria and fungi in the indoor air of primaty school in Edirne City, Turkey. *Indoor And Built Environment*, 14 (5), 411-425.

Bartlett, K. H., Kennedy, S. M., Brauer, M., van Netten, C., & Dill, B. (2004). Evaluation and determinants of airborne bacterial concentrations in school classrooms. *Journal of Occupational and Environmental Hygiene*, 1(10), 639-647.

Bayer, C.W., Crow, S., Fischer, J. (1999). *Causes of indoor air quality problems in schools*. Energy Division, Oak Ridge National Laboratory. In: Summary of Scientific Research, US Department of Energy: Oak Ridge, TN. 24–27.

Bjornsson, E., Janson, C., Norback, D., & Boman, G. (1998). Symptoms related to the sick building syndrome in a general population sample: associations with atopy, bronchial hyper-responsiveness and anxiety. *The International Journal of Tuberculosis and Lung Disease, 2*(12), 1023-1028

Bruzzese, J.-M., Evans, D., & Kattan, M. (2009). School-based asthma programs. *Journal of Allergy and Clinical Immunology,* 124(2), 195-200.

Burch, M., & Levetin, E. (2002). Effect of meteorological conditions on spore plumes. *International Journal of Biometeorology*, 46, 107–117.

Buttner, M.P., Willeke, K., Grinshpun, S. (2002). Sampling and analysis of airborne microorganisms. In Manual of Environmental Microbiology, edn 2. Edited by Hurst CJ, Crawford RL, Knudsen G, McInerney M, Stetzenbach LD. Washington DC: ASM Press; 814-826.

Cai, G.H., Jamal, H.H., Zailina, H., Faridah, A., Bloom, E., Larsson, L., Lampa, E., Norback, D., (2011). Fungal DNA, allergens, mycotoxins and associations with asthmatic symptoms among pupils in schools from Johor Bahru, Malaysia. *Pediatric Allergy and Immunology*. 22: 290-297.

Clements-Croome, D., Awbi, H., Bako-Biro, Z., Kochhar, N., & Williams, M. (2008). Ventilation rates in schools. *Building and Environment*, 43(3), 362-367.

Cooley, J.D., Wong, W.C., Jumper, C.A. and Straus, D.C. (1998). Correlation between the prevalence of certain fungi and sick building syndrome, *Occupational Environmental Medicine*, 55, 579–584.

Corden, J.M., Millington, W.M. (2001). The long-term trends and seasonal variation of the aeroallergen Alternaria in Derby, UK. *Aerobiologia* 17:127-136.

Cox, C. S., & Wathes, C. M. (1995). Bioaerosols handbook. NY: Lewis Publishers.

Croome, C.D.J., Awbi, H.B., Bako'-Biro', Z., Kochhar, N. and William, M. (2008). Ventilation rates in schools. *Building and Environment*, 48,362-367.

Dacarro, C., Picco, A.M., Grisoli, P and Rodolfi, M. (2003). Determination of aerial microbiological contamination in scholastic sports environment. *Journal of Applied Microbiology*, 95, 904- 912.

Dales, R.E., Miller, D and McMullen, E. (1997). Indoor air quality and health: validity and determinants of reported home "dampness and mould". *International Journal of Epidemiology*,26, 120-125.

Dales, R.E., Zwanenburg, H., Burnett, R., Franklin, C.A., 1991. Respiratory health e!ects of home dampness and molds among children. *American Journal of Epidemiology*, 134, 196-203.

Daisey, J. M., Angell, W. J., & Apte, M. G. (2003). Indoor air quality, ventilation and health symptoms in schools: an analysis of existing information. *Indoor air*, 13(1), 53-64.

Daneault, S., Beausoleil, M., Messing, K., (1992). Air quality during winter in Quebec day care centers. *American Journal of Public Health*, 82, 432–434.

De-Wei, L. and Kendrick, B. (1995) Indoor aeromycota in relation to residential characteristics and allergic symptoms. *Mycopathologia,* 131, 149–157.

Department of Occupational Safety and Health (DOSH) Malaysia (2010): Code of Practise on Indoor Air Quality. JKKP DP (S) 127/379/4-39.

di Giorgio, C., Krempff, A., Guiraud, H., Binder, P., Tiret, C., & Dumenil, G. (1996). Atmospheric pollution by airborne microorganisms in the city of Marseilles. *Atmospheric Environment*, *30*(1), 155-160.

Douwes, J. (2005). β(1→3)-glucans and respiratory health: a review of the scientific evidence. *Indoor Air*, *15*(3), 160-169.

Douwes J., Thorne P., Pearce, N., Heederik, D. (2003). Bioaerosol health effects and exposure assessment: progress and prospects. *Annals of Occupational Hygiene.* 47:187-200.

Drancourt M, Bollet C, Carlioz A, Martelin R, Gayral J, Raoult D (2000). 16S ribosomal DNA sequence analysis of a large collection of environmental and clinical unidentifiable bacterial isolates. *Journal of Clinical Microbiology.* 38:3623-3630.

Dungby, C.I., Kozak, P., Gallup, J. and Gallant, S. (1986). Aeroallergen exposure in the elementary school setting. *Annals of Allergy, Asthma and Immunology*, 56, 218–221.

El-Morsy, E. S. M. (2006). Preliminary survey of indoor and outdoor airborne microfungi at coastal buildings in Egypt. *Aerobiologia,* 22, 197–210.

Elizabeth, M. A. (Ed.) .(1994). Concise Medical Dictionary (4 ed.). Oxford University Press.

Fang, Z., Ouyang, Z. Y., Hu, L. F., Wang, X. K., & Lin, X. O. (2005). Community structure and ecological distribution of airborne microbes in summer in Beijing. *Acta Ecologica Sinica*, 25, 83–88.

Fischer, G., Dott, W. (2003). Relevance of airborne fungi and their secondary metabolites for environmental, occupational and indoor hygiene. Archives of Microbiology, 179(2), 75-82.

Foarde, K., & Berry, M. (2004). Comparison of biocontaminant levels associated with hard vs. carpet floors in nonproblem schools: results of

a year long study. *Journal of Exposure Science and Environmental Epidemiology,* 14, S41-S48.

Fogelmark, B., Goto, H. and Yuasa, K. (1992). Acute Pulmonary Toxicity of Inhaled *B* -1,3-Glucan and Endotoxin. *Agents Action* ,35,50–56.

Franke, D. L., Cole, E. C., Leese, K. E., Foarde, K. K., & Berry, M. A. (1997). Cleaning for improved indoor air quality: an initial assessment of effectiveness. *Indoor air*, 7(1), 41-54.

Giardino, N.J. (2004). Summary of Currently Available Guidelines for Fungal Levels in Indoor Spaces. *Indoor Environment Review*,1, 32.

Gilliland, F.D., Li, Y.F., Dubeau, L., Berhane, K., Avol, E., McConnell, R., (2002). Effects of glutathione S-transferase M1, maternal smoking during pregnancy, and environmental tobacco smoke on asthma and wheezing in children. American Journal of Respiratory and Critical Care Medicine, 166 (4),457-463.

Goh, J.C.C., Juliana, J., Malina, O., Ngah, Z.U. and Norhafizalena, O. (2007). Prevalence of *Penicillium* specific Ig E level and allergy symptoms among office workers in a selected company in Bangi, Malaysia. *Journal of Tropical Biomedicine*, 24 (1), 37-46.

Gorny, R., Dutkiewicz, J. (2002). Bacterial and fungal aerosols in indoor environment in Central and Eastern European countries. *Annals of Agricultural and Environmental Medicine*, 9 (1), 17-23.

Gutarowska, B., Sulyok, M., Krska, R. (2010). A study of the toxicity of moulds isolated from dwelling. *Indoor and Built Environment*, 19(6), 668–675.

Gyntelberg, F., Suadicani, P., Nielsen, J.W., Skov, P., Valbiørn, O., Nielsen, P.A., Schneider, T., Jørgensen, O.(1994). Dust and the sick building syndrome, *Indoor Air,* 4, 223–238.

Halonen, M., Stern, D. A., Wright, A. L., Taussig, L. M., & Martinez, F. D. (1997). Alternaria as a major allergen for asthma in children raised in a desert environment. *American Journal of Respiratory and Critical Care Medicine, 155*(4), 1356.

Handal, G., Leiner, M., Cabrera, M., & Straus, D. (2004). Children symptoms before and after knowing about an indoor fungal contamination. *Indoor Air,* 14(2), 87-91.

Hargreaves, M., Parappukkaran, S., Morawska, L., Hitchins, J., He, C., & Gilbert, D. (2003). A pilot investigation into associations between indoor airborne fungal and non-biological particle concentrations in

residential houses in Brisbane, Australia. *The Science of the Total Environment, 312*(1-3), 89-101.

Herr, C., Zur Nieden, A., Jankofsky, M., Stilianakis, N., Boedeker, R., & Eikmann, T. (2003). Effects of bioaerosol polluted outdoor air on airways of residents: a cross sectional study. *Occupational and Environmental Medicine*, 60(5), 336.

Holt, P.G., Sly, P.D., Bjo¨rksten, B. (1997). Atopic versus infectious diseases in childhood: a question of balance. *Paediatric Allergy Immunology*, 8, 53–58.

Hossain, M. A., Ahmed, M. S., & Ghannoum, M. A. (2004). Attributes of Stachybotrys chartarum and its association with human disease. *Journal of Allergy and Clinical Immunology, 113*(2), 200-208.

Husman, T. (1996). Health effects of indoor-air microorganisms. *Scandinavian Journal of Work, Environment and Health, 22*(1), 5.

ISAAC (The International Study of Asthma and Allergy in Childhood) (1998). Steering Committee: Worldwide variation in the prevalence of symptoms of asthma, allergic, rhinoconjunctivitis and atopic asthma. ISAAC. *Lancet*, 351, 1225-1232.

Jamal, H.H., Zailina, H., Juliana, J., Syarif, H.L., and Ridzwan, H. (1998). Respiratory functions of elementary school children exposed to Kuala Lumpur haze. Abstract. *Epidemiology,* 9 (4) July Supplement: 103

Jazrawi, S.F., Al-Shahwani, M.F. (1983). Concentration of viable microorganisms in indoor settled dust and its relation with meteorological parameters in Baghdad city. *Journal of Environmental Science Health*, 18A, 145-154.

Jensen, P. A., and Schafer, M. P. (1998). Sampling and characterization of bioaerosols. *NIOSH manual of analytical methods. Cincinnati, OH: US Department of Health and Human Services, National Institute for Occupational Safety and Health*, 82-112.

Jo, W. K., & Seo, Y. J. (2005). Indoor and outdoor bioaerosol levels at recreation facilities, elementary schools, and homes. *Chemosphere, 61*(11), 1570-1579.

Johansson, P., Samuelson, I., Ekstrand-Tobin, A., MjÃ¶rnell, K., Sandberg, P., & Sikander, E. (2005). Microbiological growth on building materials critical moisture levels. *State of art, SP Swedish National Testing and Research Institute, BorÃ¥s*.

Kalogerakis, N., Paschali, D., Lekaditis, V., Pantidou, A., Eleftheriadis, K., & Lazaridis, M. (2005). Indoor air quality--bioaerosol measurements in domestic and office premises. *Journal of Aerosol Science,* 36(5-6), 751-761.

Karen, H.B., Susan, M.K., Michael, B., Chris, vN. and Barbara, D. (2004). Evaluation and Determinants of Airborne Bacterial Concentration in School Classrooms. *Journal of Occupational and Environmental Hygiene*, 1, 639-647.

Kaur, B., Anderson, H. R., Austin, J., Burr, M., Harkins, L. S., Strachan, D. P., et al. (1998). Prevalence of asthma symptoms, diagnosis, and treatment in 12â€"14 year old children across Great Britain (international study of asthma and allergies in childhood, ISAAC UK). *BMJ (Clinical Research Ed.), 316*(7125), 118-124.

Kim, J.L., Elfman, L., Mi, Y., Wieslander, Smedje, G., Norback, D. (2007). Indoor molds, bacteria, microbial volatile organic compounds and plasticizers in school- associations with asthma and respiratory symptoms in pupils. *Indoor Air*, 17,153-163.

Kim, K. Y., & Kim, C. N. (2007). Airborne microbiological characteristics in public buildings of Korea. *Building and Environment,* 42(5), 2188-2196.

Kim, K. Y., Park, J. B., Jang, G. Y., Kim, C. N., & Lee, K. J. (2007). Assessment of bioaerosols in the public buildings of Korea. *Indoor and Built Environment,* 16(5), 465.

Kline, J. N., Cowden, J. D., Hunninghake, G. W., Schutte, B. C., Watt, J. L., Wohlford-Lenane, C. L., et al. (1999). Variable airway responsiveness to inhaled lipopolysaccharide. *American Journal of Respiratory and Critical Care Medicine,* 160(1), 297.

Koskinen, O. M., Husmana, T. M., Hyvarinen, A. M., Reponen, T. A., & Nevalainen, A. I. (1997). Two moldy day-care centers: A follow-up study of respiratory symptoms and infections. *Indoor Air*, 7, 262–268.

Larone, D.H. (1976). Medically Important Fungi. A Guide to Identification. New York: Harper & Row.

Levetin, E., Shaughnessy, R., Fisher, E., Ligman, B., Harrison, J. and Brennan, T. (1995). Indoor air quality in schools: exposure to fungal allergens. *Aerobiologia* ,11, 27–34.

Li, C. S. and Kuo, Y. (1993). Microbiological indoor air quality in subtropical areas. *Environment International*, 19(3),233-239.

Li, C.S., Kuo, Y.M. (1994). Characteristics of airborne microfungi in subtropical homes. *The Science of Total Environment*, 155, 267–271.

Macher, J. (1999). Bioaerosols: assessment and control: ACGIH.

Macher, J., Huang, F. and Flores, M. (1991). A two-year study of microbiological indoor air quality in a new apartment. *Achieve Environmental Health*, 46, 25-29.

Mahon, C.R., Lehman, D.C., Manuselis, G. (2007). *Textbook of Diagnostic Microbiology*. In Textbook of Diagnostic Microbiology. 3rd edition. India: Elsevier.

Maidack, B. L., G. J. Olsen, N. Larsen, R. Overbeek, M. J. McCaughey, and C. R. Woese. (1996). *The ribosomal data base project (RDP)*. Nucleic Acids Res, 24,82–85.

Martinez, F.D., Holt, P.G. (1999). The role of microbial burden in the aetiology of allergy and asthma. *Lancet,* 11,12–15.

Meklin, T., Husman, T., Vahteristo, M., Koivisto, J., Nevalainen, A. (2002). Indoor air microbes and respiratory symptoms of children in moisture damaged and reference schools. *Indoor Air*, 12, 175-183.

Meklin, T., Potus, T., Pekkanen, J., Hyva¨ rinen, A., Hirvonen, M.R., Nevalainen, A. (2005). Effects of moisture damage repairs on microbial exposure and symptoms in schoolchildren. *Indoor Air*, 15,40–47.

Menetrez, M. Y., & Foarde, K. K. (2004). Emission exposure model for the transport of toxic mold. *Indoor Built Environment*, 13, 75–82.

Mendell, M. J., & Heath, G. A. (2005). Do indoor pollutants and thermal conditions in schools influence student performance? A critical review of the literature. *Indoor Air,* 15(1), 27-52.

Meyer, H.W., Wu¨ rtz, H., Suadicani, P., Valbjørn, O., Sigsgaard, T. and Gyntelberg, F. (2004) Molds in floor dust and building-related symptoms in adolescent school children. *Indoor Air*, 14, 65–72.

Mims, C., Dockrell, H.M., Goering, R.V., Roitt, I., Wakelin, D., Zuckerman, M. (2004). Medical Microbiology. 3rd edition. London: Elsevier.

Ministry of Education MOE (2010). Portal Rasmi Kementerian Pelajaran Malaysia. (Online) downloaded from http://www.moe.gov.my on 3 January 2010.

Moglia, D., Smith, A., MacIntosh, D.L. (2006). Prevalence and implementation of IAQ programs in U.S. schools. *Environmental Health Perspective*, 114,141–146.

Moon, H.J., and Yoon, Y.R. (2010). Investigation of physical characteristics of houses and occupants' behavioural factors for mould infestation in residential buildings. *Indoor and Built Environment*, 19(1), 57-64.

Mui, K., Chan, W., Wong, L., & Hui, P. (2007). Fungi in an indoor air quality assessment parameter for air-conditioned offices. *Building Services Engineering Research and Technology*, 28(3), 265-274.

Mussalo-Rauhamaa, H., Nikulin, M., Koukila-Kähkölä, P., Hintikka, E.L., Malmberg, M., & Haahtela, T. (2010). Health Effects of Residents Exposed to *Stachybotrys* in Water-damaged Houses in Finland. *Indoor and Built Environment*, 19(4), 476-495.

Murray, P. R., Baron, E. J., Jorgensen, J. H., Pfaller, M. A., & Yolken, R. H. (2003). Manual of clinical microbiology (8th edn.). Washington, D.C.: ASM Pres.

Neas, L.M., Dockery, D.W., Burge, H., Koutrakis, P. and Speizer, F. E. (1996) Fungus spores, air pollutants and other determinants of peak expiratory flow rate in children*. American Journal of Epidemiology*, 143, 797–807.

Nevalainen, A., Pasanen, A. L., Niininen, M., Reponen, T., Kalliokoski, P., & Jantunen, M. (1991). The indoor air quality in Finnish homes with mold problems. *Environment International,* 17(4), 299-302.

Norback, D., Zhao, Z. H., Wang, Z. H., Wieslander, G., Mi, Y. H., & Zhang, Z. (2007). Asthma, eczema, and reports on pollen and cat allergy among pupils in Shanxi province, China. *International Archives of Occupational and Environmental Health*, 80(3), 207-216.

Nouwen, J.L., Alewijn, O., Marjolein, F.Q., Vanderbergh, K., Boelens, H.A.M., Hofman, A., Alex van Belkum and Verbrugh, H.A. (2004). Predicting the *Staphylococcus aureus* Nasal Carrier State: Derivation and Validation of a 'Culture Rule". *Clinical Infectious Diseases.* 39, 806-11.

Otten, J.A and Burge, H.A. (1999). Bacteria. In: Macher JM, Ammann HM, Burge HA, Milton DK, Morey PM, editors. Bioaerosals: assessment and control, Cincinnati, OH. American conference of governmental industrial hygienists, 18-1–18-10.

Owen, M. K., Ensor, D. S. & Sparks, L. E. (1992). Airborne particle sizes and sources found in indoor air. *Atmospheric Environment,* 26, 2149-2162.

Pan, Z., Mølhave, L. and Kjærgaard, S.K. (2000) Effects on eyes and nose in humans after experimental exposure to airborne office dust. *Indoor Air* 10, 237–245.

Pasanen, A. L., Juutinen, T., Jantunen, M., & Kalliokoski, P. (1992). Occurrence and moisture requirements of microbial growth in building materials. *International biodeterioration & biodegradation,* 30(4), 273-283

Pastuszka, J. S., Kyaw Tha Paw, U., Lis, D. O., Wlazo, A., & Ulfig, K. (2000). Bacterial and fungal aerosol in indoor environment in Upper Silesia, Poland. *Atmospheric Environment,* 34(22), 3833-3842.

Patel, J. (2001). 16S rRNA gene sequencing for bacterial pathogen identification in the clinical laboratory. *Molecular Diagnosis*, 6,313-321.

Patino, C.M. and Martinez, F.D. (2001). Interaction between genes and environment in the development of asthma. *Allergy.* 56 (4), 279-286.

Pei-Chih, W., Huey-Jen, S., & Chia-Yin, L. (2000). Characteristics of indoor and outdoor airborne fungi at suburban and urban homes in two seasons. *The Science of the total Environment,* 253(1-3), 111-118.

Peter, S.T., Kulhankova, K., Yin, M., Cohn, R., Samuel, J.A. and Zeldin, D.C. (2005). Endotoxin Exposure Is A Risk Factor for Asthma, The National Survey of Endotoxin in United States Housing. *American Journal of Respiratory and Critical Care Medicine*, Vol 172.

Peterman, T. K., Jalongo, M. R., & Lin, Q. (2002). The Effects of Molds and Fungi on Young Children's Health: Families' and Educators' Roles in Maintaining Indoor Air Quality. *Early Childhood Education Journal,* 30(1), 21-26

Picco, A.M. and Rodolfi, M. (2000) Airborne fungi as biocontaminants at two Milan underground stations. *International Biodegradation and Biodeterioration*, 45, 43–47.

Pitt, J. I. (2000). A laboratory guide to common *Penicillium* species (p. 197, 3rd ed.). Australia: Food Science.

Portnoy, J.M., Barnes, C.S., and Kennedy, K. (2005). Sampling for indoor fungi, current reviews of allergy and clinical immunology. *Journal of Allergy Clinical Immunology,* 113, 189-198.

Quah, B.S., Razak, A.R. and Hassan, M.H. (1997). Prevalence of asthma, rhinitis and eczema among schoolchildren in Kelantan, Malaysia. *Acta Paediatric Japonica*, 39, 329-335.

Rao, C.Y., Burge, H.A. and Chang, J. (1996). Review of quantitative standards and guidelines for fungi in indoor air. *Journal of the Air & Waste Management Association,* 46(9), 899.

Relman, D. A., Schmidt, T. M., MacDermott, R. P., & Falkow, S. (1992). Identification of the uncultured bacillus of Whipple's disease. *New England Journal of Medicine, 327*(5), 293-301.

Ren, P., Jankun, T., Leaderer, B. (1999). Comparisons of seasonal fungal prevalence in indoor and outdoor air and in house dusts of dwellings in one Northeast American county. *Journal of Exposure Science and Environmental Epidemiology*, 9,560-568.

Renz, H and Herz, U (2002). The bidirectional capacity of bacterial antigens to modulate allergy and asthma. *European Respiratory Journal*, 19 (1), 158-171.

Robertson, L.D. (1997). Monitoring viable fungal and bacterial bioaerosol concentrations to identify acceptable levels for common indoor environments. *Indoor and Built Environment*, 6,295-300.

Rolka, H., Krajewska-Kulak E., Łukaszuk, C., Oksiejczuk, E., Jakoniuk, P., Leszczyńska, K., Niczyporuk, W., Penar-Zadarko B. (2005). Indoor air studies of fungi contamination of social welfare home in Czerewki in north-east part of Poland. *Annales Academiae Medicae Bialostocensis*, 50.

Ross, M.A., Curtis, L., Scheff, P.A., Hryhorczuk, D.O., Ramakrisnan, V., Wadden, R.A, Persky, V.W. (2000). Association of asthma symptoms and severity with indoor bioaerosols. *Allergy*, 55,705-711.

Ruotsalainen, R., Jaakkola, N., Jaakkola, J.J.K., (1993). Ventilation and indoor air quality in Finnish daycare centers. *Environment International*, 19, 109–119.

Ryan, K., Ray, C., Sherris (2004). Medical microbiology: an introduction to infectious diseases. McGraw-Hill Medical.

Rylander, R. (1996) Airway responsiveness and chest symptoms after inhalation of endotoxin or (1→3)-β-D-glucan. *Indoor Built Environment*, 5,106–111.

Sarica, S., Asan, A., Otkun, M. T., & Ture, M. (2002). Monitoring indoor airborne fungi and bacteria in the different parts of Trakya University Hospital (Edirne-Turkey). *Indoor and Built Environment*, 11, 285–292.

Savilahti, R., Uitti, J., Roto, P., Laippala, P and Husman, T. (2001). Increased prevalence of atopy among children exposed to mold in a school building. *Allergy,* 56, 175-179.

Shariat, C., & Collard, H. R. (2007). Acute lung injury after exposure to *Stachybotrys chartarum*. *Respiratory Medicine Extra*, 3(2), 74-75.

Shelton, B.G., Kirkland, K.H., Flanders, W.D., Morris GK (2002) Profiles of airborne fungi in buildings and outdoor environments in the United States. *Applied Environmental Microbiology*, 68, 1743– 1753.

Sibbald, B.M.E. and Horn, E.A., Brain. (1980). Genetic factors in childhood asthma. *Thorax.* 36 (9), 671-674.

Singh, J. and Yu, C.W.F. (2010). Building Pathology, Investigation of Sick Buildings—Toxic Moulds. *Indoor and Built Environment*, 19(1), 40-47.

Smedge, G., Norback, D. and Edling, C. (1997). Asthma among secondary school children in relation to the school environment. *Clinical and Experimental Allergy,* 27, 1270-1278.

Stanley J, S. (2009). Managing asthma and allergies in schools: An opportunity to coordinate health care. *Journal of Allergy and Clinical Immunology,* 124(2), 201-204.

Stetzenbach, L.D. (1998). Microorganisms and indoor air quality. *Clinical Microbioliogy Newsletter,* 20,157– 161.

Stetzenbach, L.D., Buttner, M.P., Cruz, P. (2004). Detection and enumeration of airborne biocontaminants. *Current Opinion in Biotechnology*. 15, 170-174.

Stone, B.A. and Clark, A.E. (1992) Chemistry and Biology of (1→3)-β-D-glucan, Victoria Australia, La Trobe University Press.

Su, H. J. J., Wu, P. C., & Lin, C. Y. (2001). Fungal exposure of children at homes and schools: a health perspective. *Archives of Environmental Health,* 56, 144–149.

Tsai, F.C., Macher, J.M., Hung, Y.Y. (2002). Concentrations of Airborne Bacteria in 100 U.S. Office Building. *Indoor Air*.

Tsai, F. and Macher, J. (2005). Concentrations of airborne culturable bacteria in 100 large US office buildings from the BASE study. *Indoor air*, 15, 71-81.

USEPA, (2002). Child specific exposure factors handbook (Interim Report). EPS- 600-P-0-002B. Washington, DC: U.S. Environmental Protection Agency, Office of Research and Development, National Center of Environmental Assessment.

Wallace, L.A., Nelson, C.J., Highsmith, R. & Dunteman, G. (1993). Association of personal and workplace characteristics with health, comfort and odor: A survey of 3948 office workers in three buildings. *Indoor Air,* 3, 193-205.

Weisburg, W. G., S. M. Barns, D. A. Pelletier, and D. J. Lane. (1991). 16S ribosomal DNA amplification for phylogenetic study. *Journal Bacteriology,* 173**:**697–703.

Wu, P.C., Li, Y.Y., Chiang, C.M. (2005). Changing microbial concentrations are associated with ventilation performance in Taiwan's air conditioned office buildings. *Indoor Air,* 15, 19-25.

World Health Organization (2003). Manual of Basic techniques for a Health Laboratory, 2nd edition, Geneva

World Health Organization (1990). Indoor Air Quality: Biological Contaminants. WHO Regional Publications European Series 31; Geneva, WHO

World Health Organisation, (2002). Guidelines for concentration and exposure–response measurements of fine and ultra fine particulate matter for use in epidemiological studies. Geneva:World Health Organisation

WHO. (2005). Air quality guidelines for particulate matter, ozone, nitrogen dioxide and sulfur dioxide Global update 2005 Summary of risk assessment. World Health Organization 2006.

WHO. (2007). Development of WHO guidelines for indoor air quality: dampness and mould, report on a working group meeting Bonn, Germany, 17–18 October

Zailina, H., Juliana, J., Norzila, M.Z., Azizi, H.O. and Jamal, H.H. (1997). The relationship between Kuala Lumpur haze and asthmatic attacks in children. *Malaysian Journal of Children Health*, 9 (2), 151-159

Zeldin, D.C., P. Eggleston, M. Chapman, (2006). How exposures to biologics influence the induction and incidence of asthma. *Environmental Health Perspective*. 114 (4), 620-626.

Zhao, Z.H., Elfman, L., Wang, Z.H., Zhang, Z. and Norba ck, D. (2006). A comparative study of asthma, pollen, cat and dog allergy among pupils and allergen levels in schools in Taiyuan city, China and Uppsala, Sweden. *Indoor Air*, 16, 404–413.

Zhu, H., Phelan, P., Duan, T., Raupp, G., & Fernando, H. (2003). Characterizations and relationships between outdoor and indoor bioaerosols in an office building. *China Particuology, 1*(3), 119-123.

Zock, J.P., D Jarvis, C Luczynska. (2002). Housing characteristics, reported mold exposure, and asthma in the European Community Respiratory Health Survey. *Journal Allergy Clinical Immunology,* 110 (2), 285-292

Zorman, T and Jersˇek, B. (2008). Assessment of bioaerosol concentrations in different indoor environments. *Indoor Built Environment,* 17, 155-163.

Zuraimi, M.S., Tham, K.W. (2008). Indoor Air Quality and its determinants in tropical child care centers. *Atmospheric Environment.* 42 (9), 2225-2239.