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

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By

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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

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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

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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/m³ dan 1473 (±1261) CFU/m³ dan kepekatan purata kulat bioaerosol bagi udara dalaman dan luaran adalah 292 (±83) CFU/m³ dan 401 (±235) CFU/m³. 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 kulut dalam udara di sekolah yang melebihi 500CFU/m³ 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’.

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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)

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Professor and Deputy Dean
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Date:
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Faculty of Medicine and Health Science  
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(Chairman)

**Lye Munn Sann, PhD**  
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Faculty of Medicine and Health Science  
University Putra Malaysia  
(Member)

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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

<table>
<thead>
<tr>
<th>Chapter</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Introduction</td>
</tr>
<tr>
<td>2</td>
<td>Literature Review</td>
</tr>
<tr>
<td></td>
<td>Bioaerosol</td>
</tr>
<tr>
<td></td>
<td>Bacteria bioaerosol</td>
</tr>
<tr>
<td></td>
<td>Fungi bioaerosol</td>
</tr>
<tr>
<td></td>
<td>Exposure to bacteria and fungi bioaerosol</td>
</tr>
<tr>
<td></td>
<td>Bioaerosol associated health effects</td>
</tr>
<tr>
<td></td>
<td>Bacteria bioaerosol and health effects</td>
</tr>
<tr>
<td></td>
<td>Fungi bioaerosol and health effects</td>
</tr>
<tr>
<td></td>
<td>Bioaerosol sampling and measurement</td>
</tr>
<tr>
<td></td>
<td>Bioaerosol identification</td>
</tr>
<tr>
<td>3</td>
<td>Methodology</td>
</tr>
<tr>
<td></td>
<td>Study design</td>
</tr>
<tr>
<td></td>
<td>Study location</td>
</tr>
<tr>
<td></td>
<td>Sampling</td>
</tr>
<tr>
<td></td>
<td>Study population</td>
</tr>
<tr>
<td></td>
<td>Study sample</td>
</tr>
<tr>
<td></td>
<td>Sampling frame</td>
</tr>
<tr>
<td></td>
<td>Sampling method</td>
</tr>
<tr>
<td></td>
<td>Selection criteria</td>
</tr>
<tr>
<td></td>
<td>Sample size</td>
</tr>
<tr>
<td></td>
<td>Data collection</td>
</tr>
<tr>
<td></td>
<td>Environmental sampling</td>
</tr>
<tr>
<td></td>
<td>Bioaerosol sampling</td>
</tr>
<tr>
<td></td>
<td>Calculation of bioaerosol concentration</td>
</tr>
<tr>
<td></td>
<td>Identification of bacteria and fungal bioaerosol</td>
</tr>
<tr>
<td></td>
<td>Measurement of relative humidity and temperature</td>
</tr>
</tbody>
</table>
Questionnaire 44
Quality control 45
Statistical methods 46
Study ethics 47
Study limitation 47

4  PAPER 1  49
Characterization of Bacteria and Fungal Bioaerosol in the Indoor Air of Primary School in Selangor, Malaysia

5  PAPER 2  81
Associations of Bacterial and Fungal Bioaerosol in Selected School Environment with Respiratory Symptoms among Children Aged 10-11 years in Hulu Langat District, Selangor.

6  SUMMARY, GENERAL CONCLUSION AND RECOMMENDATION FOR FUTURE RESEARCH  115
REFERENCES
APPENDICES
BIODATA OF STUDENT
LIST OF TABLES

3.1: Primers nucleotide sequence use for PCR amplification of 16s RNA 43

4.1: Primers nucleotide sequence use for PCR amplification of 16s RNA 59

4.2: Temperature (°C) and relative humidity (%) in the indoor air between November 2008 and June 2009. 44

4.3: Comparisons of temperature and relative humidity in the indoor and outdoor air 63

4.4: Correlation between temperature and relative humidity with bioaerosol concentrations 63

4.5: Comparisons of bioaerosol concentrations in the indoor and outdoor air 64

4.6: Bacteria concentration at sampling stations in selected primary schools between November 2008 and June 2009 67

4.7: Fungi concentration at sampling stations in 68 selected primary schools between November 2008 and June 2009 68

4.8: Colony numbers and percentage of identified bacteria isolated by the study 71

4.9: Colony numbers and percentage of identified fungi isolated by the study 73

5.1: Description of school 92

5.2: Respondents’ background 95

5.3: Prevalence of respiratory and allergy symptoms of respondent 96

5.4: Prevalence of asthma and allergy of respondent 96
5.5: Prevalence of current respiratory symptoms in school the last 3 months

5.6: Bacteria concentration in classrooms at selected primary schools between November 2008 and June 2009

5.7: Fungi concentration at sampling stations in selected primary schools between November 2008 and June 2009

5.8: Difference in bacterial and fungal bioaerosol concentrations in the presence and absence of occupancy

5.9: Association between respiratory symptoms and bioaerosol concentrations in schools

5.10: Association between current respiratory symptoms and bioaerosol concentration in school the last 3 months

5.11: Associations between respiratory symptoms with exposure to the most common type of fungi isolated

5.12: Associations between respiratory symptoms with exposure to most common type of bacteria isolated

5.13: Associations between bioaerosol exposure and respiratory symptoms among school children
LIST OF FIGURES

3.1: Flowchart data collection 35
4.1: Mean indoor and outdoor bacteria concentrations with and without indoor occupants 69
4.2: Mean indoor and outdoor fungi concentrations with and without indoor occupants 69
LIST OF APPENDICES

Appendix A  Questionnaire (Malay version)
Appendix B  Subject information sheet and informed consent (Malay version)
Appendix C  Approval to conduct study
Appendix D  Photo on equipment used for sampling
Appendix E  Photo on bacteria and fungi colonies isolated
Appendix F  Paper accepted for publication
# LIST OF ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACGIH</td>
<td>American Conference of Governmental Industrial Hygienist</td>
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<tr>
<td>CFU/m³</td>
<td>Colony forming unit per meter cubic</td>
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<tr>
<td>DOSH</td>
<td>Department of Occupational Safety and Health, Malaysia</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>IAQ</td>
<td>Indoor Air Quality</td>
</tr>
<tr>
<td>ISAAC</td>
<td>International Study of Asthma and Allergies in Childhood</td>
</tr>
<tr>
<td>MEA</td>
<td>Malt Extract Agar</td>
</tr>
<tr>
<td>NIOSH</td>
<td>National Institute of Occupational Safety and Health</td>
</tr>
<tr>
<td>PCR</td>
<td>Polymerase Chain Reaction</td>
</tr>
<tr>
<td>PEF</td>
<td>Peak Expiratory Flow</td>
</tr>
<tr>
<td>RNA</td>
<td>Ribonucleic acid</td>
</tr>
<tr>
<td>SDA</td>
<td>Sabaroud Dextrose Agar</td>
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<tr>
<td>TSA</td>
<td>Tryptic Soy Agar</td>
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<tr>
<td>WHO</td>
<td>World Health Organization</td>
</tr>
<tr>
<td>NCBI</td>
<td>National Center for Biotechnology Information</td>
</tr>
</tbody>
</table>
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, sluggishness, 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 $\beta(1\rightarrow3)$-glucans that originate from most fungi. Many studies have shown positive association between endotoxins and $\beta(1\rightarrow3)$-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.
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