Microbiological Assessment of Indoor Air in a University Hospital: Efficiency of Protective and Control Measures

Pawit Chaivisit

A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy in Environmental Management Prince of Songkla University 2016

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I hereby certify that this work has not been accepted in substance for any degree, and is not being currently submitted in candidature for any degree.

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ชื่อวิทยานิพนธ์	การประเมินทางจุลชีววิทยาของอากาศภายในอาคารโรงพยาบาล มหาวิทยาลัย: ประสิทธิภาพของมาตรการป้องกันและควบคุม
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บทคัดย่อ

ข้อมูลคุณภาพอากาศภายในอาคารของโรงพยาบาลมีความสำคัญต่อการประเมินและวางแผนการจัดการคุณภาพอากาศเพื่อป้องกันสุขภาพของบุคลากรและประชาชนผู้มารับบริการที่โรงพยาบาล แต่จากการทบทวนวรรณกรรมพบว่าข้อมูลการศึกษาคุณภาพอากาศภายในโรงพยาบาลของประเทศไทยมีน้อยมาก โดยเฉพาะโรงพยาบาลมหาวิทยาลัยซึ่งมีผู้มารับบริการเป็นจำนวนมาก เนื่องจากโรงพยาบาลประเภทดังกล่าวเป็นสถานที่ที่ให้บริการทางด้านการแพทย์ตั้งแต่ปฐมภูมิถึงตติยภูมิ การศึกษาครั้งนี้จึงมีวัตถุประสงค์เพื่ออธิบายลักษณะการกระจายของขนาดละอองจุลชีพในอากาศภายในอาคารโรงพยาบาลมหาวิทยาลัย และศึกษาความสัมพันธ์ระหว่างปัจจัยทางสิ่งแวดล้อมและความเข้มข้นของละอองจุลชีพ (แบคทีเรียและรา) ในอากาศภายในอาคารเพื่อเป็นข้อมูลพื้นฐานของการพัฒนาระบบการจัดการคุณภาพอากาศภายในโรงพยาบาล นอกจากนี้ยังทำการเทียบเคียงชนิดของจุลินทรีย์กลุ่มเด่นซึ่งแขวนลอยในอากาศและอธิบายความสัมพันธ์กับขนาดของละอองจุลชีพด้วย ตัวอย่างอากาศจากพื้นที่ต่างๆ ทั้งภายในและภายนอกอาคารของโรงพยาบาลมหาวิทยาลัย ถูกเก็บด้วยเครื่อง Six-stage viable impactor ในระหว่างเดือนมิถุนายน พ.ศ. 2554 ถึง เดือนธันวาคม พ.ศ. 2555 ใช้การวิเคราะห์อนุกรมเวลาเพื่อทำนายอิทธิพลของฤดูกาลต่อความเข้มข้นของจุลชีพในอากาศ ใช้การสนทนากลุ่มสำหรับการหามาตรการลดการปนเปื้อนของละอองจุลชีพในพื้นที่ให้บริการของโรงพยาบาลมหาวิทยาลัย

ผลการศึกษาคุณภาพอากาศในพื้นที่อภิบาลผู้ป่วยหนัก หรือ ICUs (Intensive Care Units) พบค่าเฉลี่ยความเข้มข้นของแบคทีเรียและราภายในหออภิบาลผู้ป่วยหนักอายุรกรรม (M-ICU) มีค่าเท่ากับ 214.22±83.27 cfu/m^3 และ 194.25±74.83 cfu/m^3 ส่วนแบคทีเรียและราในหอผู้ป่วยหนักศัลยกรรม (S-ICU) มีค่าเท่ากับ 274.44±140.75 cfu/m^3 และ 234.39±115.60 cfu/m^3 ตามลำดับ

แบคทีเรียและราส่วนใหญ่ในพื้นที่ ICUs มีขนาดอยู่ในช่วง 1.1-3.3 ไมโครเมตร (μm) โดยชนิดของ แบคทีเรียกลุ่มเด่น ได้แก่ *Staphylococcus* spp., *Micrococcus* spp., *Pseudomonas* spp. และ *Bacillus* spp. ส่วนชนิดของรากลุ่มเด่นคือ *Clodosporium* spp. *Penicillium* spp., *Aspergillus* spp. และ *Fusarium* spp. การใช้ระบบฆ่าเชื้อด้วยแสงอัลตราไวโอเลต (UVGI) และความเร็วลมในระบบ ระบายอากาศภายในอาคารเป็นปัจจัยที่สำคัญในการลดการปนเปื้อนของแบคทีเรียภายใน ICU ขณะที่ปริมาณราภายใน ICU ขึ้นอยู่กับการปนเปื้อนจากราภายนอกอาคาร ความเร็วลมภายใน อาคาร ความชื้นสัมพัทธ์ และอุณหภูมิ ในพื้นที่ที่มีการใช้ระบบปรับอากาศ ใช้แผ่นกรองอากาศชนิด HEPA และมีพื้นที่จำกัดเช่น ICU จำเป็นต้องมีการติดตั้งระบบ UVGI และเพิ่มความเร็วลมในระบบ ระบายอากาศเพื่อลดปริมาณจุลชีพและป้องกันผลกระทบต่อสุขภาพของผู้เกี่ยวข้อง

สำหรับปริมาณแบคทีเรียและราในอากาศของพื้นที่ผู้ป่วยนอก (Out-patient departments, OPDs) และหอผู้ป่วยใน (In-patient departments, IPDs) ซึ่งใช้ระบบระบายอากาศแบบใช้อากาศเจือ จาง จากการตรวจวัดและวิเคราะห์ตัวอย่างอากาศในพื้นที่คลินิกผู้ป่วยนอกอายุรกรรม (M-OPD) ผู้ป่วยนอกตา (E-OPD) หอผู้ป่วยในอายุรกรรม (M-IPD) และ หอผู้ป่วยในศัลยกรรม (S-IPD) พบว่า ปริมาณแบคทีเรียและรามีค่าเฉลี่ยต่ำกว่า 1,000 cfu/m^3 แบคทีเรียส่วนใหญ่ในพื้นให้บริการที่มี ประชาชนหนาแน่น มีการกระจายหนาแน่นอยู่ในช่วงขนาดอนุภาคใหญ่กว่า >7 μm และในช่วง ขนาดอนุภาค 2.1-3.3 μm ส่วนราที่แขวนลอยในอากาศพบว่าส่วนใหญ่กระจายอยู่ในช่วงขนาด อนุภาค 2.1-3.3 μm และพบว่าขนาดของจุลชีพที่สามารถเข้าสู่ระบบทางเดินหายใจ (Respirable Fraction, RF) มีสัดส่วนมากกว่า 59% ของปริมาณทั้งหมด สัดส่วนของขนาดแบคทีเรียและราสะสม (d_{50}) คือ 3.17±0.19 μm และ 2.81±0.14 μm ตามลำดับ จุลชีพกลุ่มเด่นที่ตรวจพบในตัวอย่างอากาศ ของพื้นที่บริการทุกแห่งที่ใช้ระบบระบายแบบเจือจางในสองฤดูกาล (ฤดูแล้งและฝน) คือแบคทีเรีย กลุ่ม *Staphylococcus* spp., *Micrococcus* spp., *Corynebacterium* spp., และ *Bacillus* spp. ส่วนเชื้อ รากลุ่มเด่น ได้แก่ *Cladosporium* spp., *Penicillium* spp., *Aspergillus* spp., และ *Fusarium* spp. สำหรับลักษณะการกระจายของขนาดและสัดส่วนของจุลชีพที่สามารถเข้าสู่ระบบทางเดินหายใจ ไม่แตกต่างระหว่างฤดูกาลในเขตสภาพอากาศแบบร้อนชื้น แต่ปริมาณของแบคทีเรียและรามีการ เปลี่ยนแปลงตามสภาพแวดล้อม จากการวิเคราะห์อนุกรมเวลาพบว่าการเปลี่ยนแปลงของเวลามีผล ต่อปริมาณแบคทีเรียและราในทุกพื้นที่ให้บริการ ปริมาณแบคทีเรียใน OPDs เปลี่ยนไปตาม

จำนวนคน ความชื้นภายในอาคาร และปริมาณแบคทีเรียภายนอกอาคาร ขณะที่อุณหภูมิภายใน อาคาร และราภายนอกอาคาร มีผลต่อปริมาณราภายในอาคาร (P<0.05) สำหรับพื้นที่ IPDs พบว่า จำนวนคน และจำนวนแบคทีเรียภายนอกอาคารมีผลต่อปริมาณแบคทีเรียใน IPDs ขณะที่ปริมาณรา ขึ้นอยู่กับความชื้นภายในอาคารและราภายนอกอาคาร จากการสนทนากลุ่มเพื่อระดมสมองในการ หามาตรการลดปริมาณจุลชีพในอากาศของพื้นที่ที่มีการใช้ระบบระบายอากาศแบบใช้อากาศเจือ จาง พบว่ามาตรการที่ต้องดำเนินการ ได้แก่ 1) เพิ่มความเร็วลม 2) ตรวจสอบและป้องกันน้ำรั่ว 3) ทำให้พื้นแห้ง 4) ทำความสะอาดพัดลมเพดานและมุ้งลวด ซึ่งเป็นกิจกรรมที่สามารถปฏิบัติได้ ทันทีเพื่อลดระดับความชื้นและจุลชีพในอากาศ

Thesis Title   Microbiological Assessment of Indoor Air in a University Hospital: Efficiency of Protective and Control Measures

Author   Mr. Pawit Chaivisit

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ABSTRACT

Information on the indoor air quality in a hospital is necessary to determine and plan for indoor air quality management to protect the staff and people in the medical service areas. However, a literature review revealed that little information is available especially for university hospitals. This type of hospital has a high service recipient density because it provides primary through tertiary medical services. This study, therefore, aimed to describe the bioaerosols (bacteria and fungi) in the indoor air of a hospital. The results could be useful for the development of air quality management systems in hospitals. The dominant types of bioaerosols were isolated and their size distributions were described. The air samples were collected from many areas of a university hospital by a six-stage viable impactor instrument during the period of June 2011 to December 2012. A time series analysis predicted the influence of seasonal effects on the bioaerosols and focus group discussions provided recommendations to reduce the bioaerosol concentrations in the service areas of the university hospital.

The results of air quality determination in the areas of intensive care units (ICUs) showed that the average indoor bacteria and fungi concentrations at the Medical Intensive Care Unit (M-ICU) were 214.22 ± 83.27 cfu/m^3 and 194.25 ± 74.93 cfu/m^3, while at the Surgical Intensive Care Unit (S-ICU) site the concentrations were 274.44 ± 140.75 cfu/m^3 and 234.39 ± 115.60 cfu/m^3, respectively. Most of the airborne bacteria and fungi in the ICUs were in the size range of 1.1-3.3 μm. The predominant types of airborne bacteria were *Staphylococcus* spp., *Micrococcus* spp., *Pseudomonas* spp. and *Bacillus* spp. The predominant types of airborne fungi were *Cladosporium* spp., *Penicillium* spp., *Aspergillus* spp. and *Fusarium* spp. The ultraviolet germicidal irradiation (UVGI) system and the room air velocity were significant factors that reduced indoor airborne bacteria contamination while airborne fungi concentrations

depended significantly on the total number of outdoor fungi, air velocity, relative humidity and temperature. In the limited spaces of the ICUs where heating, ventilation and air conditioning systems with high efficiency particulate air filters were used, UVGI systems should be installed and the air velocity should be increased to reduce the levels of bioaerosols and prevent health hazards.

The mean airborne bacteria and fungi concentrations were also determined in the out-patient departments (OPDs) and in-patient departments (IPDs) where dilution ventilation systems were used. In the areas of the Medical Out-patient Department (M-OPD), Eye Out-patient Department (E-OPD), Medical In-patient Department (M-IPD) and Surgical In-patient Department (S-IPD) the airborne bacteria and fungi concentrations were lower than 1000 cfu/m^3. Most of the airborne bacteria in areas of high occupant density were detected in a double peak pattern of size in the range of >7 μm and 2.1-3.3 μm while most of airborne fungi were distributed in the size range of 2.1-3.3 μm. The respiratory factions (RFs) of bioaerosols were more than 59%. The average accumulation size fractions (d$_{50}$) of airborne bacteria and fungi were at 3.17±0.19 μm and 2.81±0.14 μm, respectively. The dominant genera that were identified in the dilution ventilation systems in both dry and wet seasons were *Staphylococcus* spp., *Micrococcus* spp., *Corynebacterium* spp. and *Bacillus* spp. for airborne bacteria while *Cladosporium* spp., *Penicillium* spp., *Aspergillus* spp. and *Fusarium* spp. were found to be the predominant airborne fungi. The characteristics, size distribution and respirable concentrations of the bioaerosols were not affected by the seasons in tropical humid weather, while the airborne bacteria and fungi concentrations were affected by seasonal variations.

The airborne bacteria concentrations were predicted by a time series analysis and a seasonal effect at all service areas. The airborne bacteria concentration in the OPDs depended on the number of people in the areas, indoor relative humidity and outdoor bacteria, while the indoor airborne fungi concentrations depended on the indoor temperature and outdoor fungi concentrations (p<0.05). For the IPDs, the number of people and outdoor airborne bacteria affected the indoor airborne bacteria concentrations, while the indoor airborne fungi concentrations depended on the indoor relative humidity and outdoor airborne fungi concentrations (p<0.05). The recommendations put forward by the focus group to find a strategy to reduce the level

of bioaerosols in the areas that used the dilution ventilation system were 1) increase indoor ventilation, 2) monitor and prevent water leaks, 3) keep floors dry and 4) keep ceiling fans and mosquito screens clean. These actions could be initiated quickly and would be expected to reduce the levels of the indoor relative humidity and bioaerosols.

ACKNOWLEDGEMENTS

TABLE OF CONTENTS

TABLE OF CONTENTS (con.)

TABLE OF CONTENTS (con.)

TABLE OF CONTENTS (con.)

TABLE OF CONTENTS (con.)

TABLE OF CONTENTS (con.)

TABLE OF CONTENTS (con.)

Page

LIST OF TABLES

LIST OF TABLES (con.)

LIST OF FIGURES

Page

LIST OF FIGURES (con.)

LIST OF FIGURES (con.)

Page

CHAPTER 1
General Introduction

1.1 Background

Bioaerosol exposure in an occupational environment can have effects on human health that include acute toxicity effects, allergies, respiratory symptoms, sick building syndrome and nosocomial infection (Douwes et al., 2002; Srikanth et al., 2008; Joshi, 2008; Todorova-Christova et al., 2015). The indoor air environment of health care facilities in the health service areas is occupied by patients who are sick with different diseases such as infectious diseases, chronic diseases, and non-communicable diseases. These areas are also used for other health service activities. A university hospital provides services at the tertiary level to patients who have complex and severe illnesses that can lead easily to a nosocomial infection. The infection rates in university hospitals were the highest among different hospital sizes with rates of 8.6 to 11.9% (Izquierdo-Cubas et al., 2008; Gordts et al., 2010; Dasgupta et al., 2015). The nosocomial infection rates in various departments were compared. It was found that the infection rates in the surgical departments ranged from 4.17% to 24.5% (Shah et al., 2009; Ginawi et al., 2014) and the infection rate was 28.0% in the medical department (Ginawi et al., 2014).

The main cause of nosocomial infection is bacteria (80.8%) (Kallel et al., 2005), especially Gram-negative bacteria, i.e. *Pseudomonas* spp., *Escherichia coli*, *Acinetobacter baumannii* and *Klebsiella* spp. (Liu et al., 2015). Gram-positive bacteria represent 19.2% of the infections (Kallel et al., 2005). These are classified mainly as methicillin-resistant *Staphylococcus aureus*, *Staphylococcus aureus*, coagulase-negative Staphylococci and *Streptococcus* A (Sehulster et al., 2003; Woodford and Livermore, 2009; Jung and Kim, 2015). These are different from fungi which are the main cause of opportunistic infections in patients, i.e. *Aspergillus* spp., (Faure et al., 2002; Chege et al., 2013; Tochigi et al., 2013) Mucorales order, *Acremonium* spp. and *Pseudallescheria boydii.* (Sehulster et al., 2003; Guarner and Brandt, 2011; Qazi et al., 2015).

Airborne microorganisms can be distributed and transmitted though the air that can spread diseases to other persons without meeting face-to-face. Microorganisms can be distributed to a large number of people. The distribution by airborne microorganisms from coughing or sneezing (Garner, 1996; Hospodsky et al., 2012) can contaminate many surfaces such as the equipment and floors (Damaceno et al., 2014). Airborne distribution of bacteria >5 μm can disperse within 3 feet from the original source (Schaal, 1991). However, if the particles are smaller than 5 μm, they can be dispersed farther and stay alive longer in the environment (Garner, 1996). Therefore, information on airborne microorganism contamination is necessary for a program of air contamination reduction in a hospital. Several methods have been applied, such as machine ventilation systems (Balaras et al., 2007), filtration systems (Sehulster et al., 2003), ultraviolet germicidal irradiation (UVGI) (Riley and Nardell, 1989) dilution ventilation and natural ventilation systems (Escombe et al., 2007). These methods prevent the dispersion of airborne microorganisms (Ninomura et al., 2006).

In tropical countries, most hospitals use the dilution ventilation system to reduce air pollution in the building because it produces a high air flow rate and has low energy consumption (Yau et al., 2011) while it reduces the temperature and creates a comfortable feeling. A dilution ventilation system can prevent the accumulation of air pollutants and microorganisms (Wan Hanifah et al., 2000). However, its efficiency depends on the ambient conditions. Sometimes this system performs poorly and causes a risk of infection in hospitals (Yau et al., 2011). Moreover, the outdoor air quality is also able to increase or decrease the contamination and pollution in the building. For example, if the outdoor air is contaminated by airborne fungi, it will increase the indoor air contamination (Zuraimi and Tham, 2008). Furthermore, the seasonal changes related to physical factors such as temperature, humidity, rain fall, and wind speed were also related with the size of particles and microorganism distribution in the air (Walter et al., 1990; Abdel Hameed et al., 2009). For example, the airborne fungi levels increased during the autumn and summer were lower in the winter and spring (Linlin et al., 2013). Each species of fungi can grow in different seasons, for instance, *Bjerkandera* spp. and *Penicillium*

spp. increased during the winter and autumn, whereas *Cladosporium* spp. surged in the spring and summer (Sautour et al., 2009a).

Apart from this, air pollution from carbon dioxide (CO_2), ozone (O_3), sulphur dioxide (SO_2), nitrogen dioxide (NO_2), nitrogen monoxide (NO), carbon monoxide (CO), and formaldehyde (HCHO) can affect the quantity and survival of bacteria and fungi in the air (Lighthart, 1973; Mancinelli and McKay, 1983; Chan et al., 1991; Cao et al., 2009; Wang et al., 2010a) . These pollutants are related to the density of nearby traffic and several other activities (Wang and Xie, 2009). In the case of patient rooms or hospital wards with patients, medical staff and the relatives of patients who came to visit the patients during treatment, it was found that the quantity of bacteria and fungi accumulated in crowded hospital wards due to the peeling of skin (Obbard and Fang, 2003) and diffusion from clothes, especially the diffusion of fungi (Potera, 2001).

Thailand is located in a tropical zone and many hospitals use the dilution ventilation system in both in- and out-patient departments. However, there are few studies that investigated the quantity, size and type of bacteria and fungi, which may change by seasons, including other factors that affect the increase of bacteria and fungi in a building. The results show the inability to control the diffusion of airborne microorganisms in the air effectively. Hence, this research was conducted to investigate the quantity, size and type of bacteria and fungi that are diffused in the intensive care unit, in-patient and out-patient departments throughout the year in a university hospital located in Thailand. In addition, other factors studied were related to the quantity of bacteria and fungi in the air in the university hospital that included physical factors, chemical factors, in-hospital ambience and the management system. The results are expected to be useful for the benefit of planning and managing as well as proposing a method to decrease the contamination of bacteria and fungi in the air of hospitals. It would reduce the risks of infection in hospitals and increase the quality of the health care system.

1.2 Literature review

    1.2.1 Mode of transmission of airborne diseases

    The basic principles of hygiene, prevention and control were considered to be the essence of air quality management in hospitals (Wiseman, 2006). The Centers for Disease Control and Prevention (CDC) and the Hospital Infection Control Practices Advisory Committee (HICPAC) reviewed the guidelines for infection control in hospitals in order to reduce the spread of infection. The transmission-based precautions were three principles: 1) contact precautions can reduce the spread of infection of both direct and indirect contact; 2) droplet precautions were recommended since the droplets that were produced during a cough or sneeze were usually larger than 5 µm and exposed to other persons within 3 feet from the source; 3) airborne precautions were intended to reduce the spread of airborne infection that was produced in small sizes in the range of 1-5 µm (Sehulster et al., 2003). The main transmission of airborne diseases was caused by respiratory droplets from a cough or sneeze (Garner, 1996), the contamination of dust microorganisms (peeling skin) or surface contamination (Sehulster et al., 2003, Luksamijarulkul and Pipitsangjan, 2015). Humans can contract airborne microorganisms into the respiratory system (Pedro-Botet et al., 2002). The small particles (1-5 µm) of microorganisms were associated with respiratory tract infection. It was found that particle sizes ≤ 5 µm could get into the lung and particle sizes 1-2 µm could get into the alveoli (Capstick and Clifton, 2012). Diseases could be spread to other persons without meeting face-to–face. The prevention and control of airborne disease transmission were necessary in hospitals, especially, when small aerosols could survive and be transported over long distances (National Tuberculosis Controllers, Centers for Disease, and Prevention, 2005).

    1.2.2 Situation of airborne bacteria and fungi concentration in hospitals

    1.2.2.1 Situation of airborne bacteria concentration

    The mean indoor airborne bacteria concentration in the hospital ambient air was 404 cfu/m^3 (colony-forming units per cubic meter). It was found that the bacteria aerosol concentrations in the main lobby, ICU, surgical ward and biomedical laboratory were 372 cfu/m^3, 202 cfu/m^3, 293 cfu/m^3 and 218 cfu/m^3,

respectively (Kim at al., 2010) and 586 cfu/m^3 in the postpartum nursing unit (Kim and Kim, 2007). In dental practice, the concentration of bacterial aerosols was around 970 cfu/m^2/h (Rautemaa et al., 2006). The quantity of airborne bacteria was related to the occupancy level and activity of humans in the building (Rajasekar and Balasubramanian, 2011). The predominant groups of airborne bacteria included *Staphylococcu*s spp. (50%), *Micrococcus* spp. (15-20%), *Corynebacterium* spp. (5-20%), and *Bacillus* spp. (5-15%) (Kim and Kim 2007).

The outdoor airborne bacteria level was 1,986 cfu/m^3 (Wu et al., 2012) depending on the locations and time of air sampling (Lighthart, 1973; Lighthart, 2000; Rintala et al., 2008; Bowers et al., 2012; Harper et al., 2013). The predominant genera found were *Staphylococcus* spp., *Micrococcus* spp., *Bacillus* spp. and *Corynebacterium* spp. (Kim and Kim, 2007).

1.2.2.2 Situation of airborne fungi concentration

The mean indoor airborne fungi concentration was 382 cfu/m^3 and it was 371 cfu/m^3 in the postpartum nursing unit (Kim and Kim, 2007), 2.27-4.36 cfu/m^3 in the medical mycology laboratory (Sautour et al., 2009a), 156 cfu/m^3 in the main lobby, 65 cfu/m^3 in the ICU, 96 cfu/m^3 in the surgical ward and 126 cfu/m^3 in the biomedical laboratory. The predominant airborne fungi were *Cladosporium* spp. (30%), *Penicillium* spp. (20–25%), *Aspergillus* spp. (15–20%) and *Alternaria* spp. (10–20%) (Kim and Kim, 2007).

The outdoor airborne fungi quantity varied from 0 cfu/m^3 to 11,884.62 cfu/m^3 (Pei-Chih et al., 2000; Lee and Jo, 2006). Furthermore, the seasonal change had an effect on the concentration of airborne fungi as it was at the lowest in the winter and the highest in the summer (Fang et al., 2013). The highest level of outdoor airborne fungi in hospitals was in the autumn (168 cfu/m^3) followed by summer (138 cfu/m^3), spring (110 cfu/m^3) and the lowest was in the winter (49 cfu/m^3). *Penicillium* spp. was found at the highest level in autumn and *Cladosporium* spp. was high in the spring and summer (Sautour et al., 2009a). However, the *Aspergillus* spp. level was stable throughout the year (Sautour et al., 2009a). This genera included *Aspergillus niger* (39.2%), *Aspergillus flavus* (17.5%), *Aspergillus fumigates* (7.7%) and other

Aspergillus spp. (6%) (*Aspergillus nidulans* and *Aspergillus terreus*) (Panagopoulou et al., 2002).

1.2.3 Microorganisms associated with airborne transmission

The airborne bacteria and fungi were distributed in the air and they were associated with pathologies in humans (Table 1-1).

Table 1-1 Microorganisms associated with airborne transmission.

Frequency	Fungi	Bacteria
Numerous reports in health-care facilities	*Aspergillus* spp. *Mucorales* (*Rhizopus* spp.)	*Mycobacterium tuberculosis*
Atypical occasional reports	*Acremonium* spp. *Fusarium* spp. *Pseudallescheria boydii* *Scedosporium* spp. *Sporothrix cyanescens*	*Acinetobacter* spp. *Bacillus* spp. *Brucella* spp. *Staphylococcus aureus* Group A *Streptococcus*
Airborne in nature; airborne transmission in health care settings not described	*Coccidioides immitis* *Cryptococcus* spp. *Histoplasma capsulatum*	*Coxiella burnetii* (Q fever)
Under investigation	*Pneumocystis carinii*	-

Source: Sehulster et al. (2003)

1.2.3.1 Pathogen characteristics of airborne fungi and disease in humans (Table 1-2)

Table 1-2 Characteristics of fungi and disease induced in humans.

Type of fungi	Characteristic	Disease
Aspergillus spp.	The conidia of *Aspergillus* spp. are 2-6 µm. The colony color is bluish green to gray (Fisher and Cook, 1998).	*Aspergillus* spp. can be found in both indoor and outdoor environments. They are an opportunistic disease in humans and immunocompromised patients (Ascioglu et al., 2002; Dagenais and Keller, 2009) such as surgical patients (Lutz et al., 2003), bone marrow transplant patients, lung transplant recipients (Helmi et al., 2003), cancer and in hematopoietic stem cell transplant patients (Ascioglu et al., 2002). A study by Arnow et al. (1991) showed an increased incidence of aspergillosis in patients who were immunocompromised. Levels of *Aspergillus flavus* and *Aspergillus fumigatu*s higher than 1 cfu/m^3 greatly increased the risk in patients who were immunocompromised. In addition, the *Aspergillus* spore is related to asthma (Taccone et al., 2015) and sick building syndrome (O'Connor et al., 2004).

Table 1-2 Characteristics of fungi and disease in humans. (Cont.)

Type of fungi	Characteristic	Disease
Mucorales (*Rhizopus* spp.)	The base of the sporangiophores of the Mucorales often collapse into umbrella shapes in age while the dry sporangiophores have straight walls (Pitt and Hocking, 2009)	The Mucorales order can cause a variety of diseases such as skin infection (Zaini et al., 2009) and respiratory tract infection (Kontogiorgi et al., 2007).
Acremonium spp.	*Acremonium* spp. colonies are smooth and waxy or velvety that vary in color, such as white, gray and rose (Fisher and Cook, 1998).	*Acremonium* spp. can be found in the environment and can cause infection in immune compromised patients. It can affect the gastrointestinal tract, respiratory tract, and can cause meningitis and endocarditis (Fincher et al., 1991).
Fusarium spp.	The macroconidia of *Fusarium* spp. are fusiform to crescent in shape. Many of them can produce smaller shapes of conidia and microconidia. The color of the colony is cream, salmon pink or orange (Fisher and Cook, 1998).	*Fusarium* spp. infects patients who are immunocompromised (Nucci and Anaissie, 2007) such as cancer patients (Krcmery Jr et al., 1997).

Table 1-2 Characteristics of fungi and disease in humans. (Cont.)

Type of fungi	Characteristic	Disease
Pseudallescheria boydii	The colony color of *P. boydii* is brownish gray to black. The hyphae are 1 to 3 µm wide, hyaline, septate with many branches (Fisher and Cook, 1998).	*Pseudallescheria boydii* can cause infection in immunocompromised patients (Balaras et al., 2007).
Scedosporium spp.	The colony color of *Scedosporium* spp. is light brown. The average diameter of hyphae is 6 µm (2 to 10 µm) (Fisher and Cook, 1998).	*Scedosporium* spp. can infect humans and animals (Swerczek et al., 2001). In humans, it affected patients who were immunocompromised, sick with leukemia, breast cancer or developed immunodeficiency syndrome (Idigoras et al., 2001).
Sporothrix cyanescens	*S. cyanescens* colonies can have lavender, red or blue pigmentation (Sigler et al., 1990).	*Sporothrix cyanescens* is isolated from the blood and skin of patients (Sigler et al., 1990). It can be the cause of pneumonia (Grossi et al., 2000).
Coccidioides immitis	The colony color of *C. immitis* is white with floccose mycelia or gray. The artheroconidia hyphae are 3 to 6 µm wide that are vegetative hyphae. The arthroconidia	*Coccidioides immitis* is found in the desert and immunocompromised patients can increase the incidence of coccidioidomycosis (Kirkland and Fierer, 1996).

Table 1-2 Characteristics of fungi and disease in humans. (Cont.)

Type of fungi	Characteristic	Disease
	are barrel shaped or rectangular (Fisher and Cook, 1998)	
Cryptococcus spp.	*Cryptococcus* spp. are identified as spherical to oval yeast cells and the diameter range is 4-20 μm. They are surrounded by a mucopolysaccharide capsule (Gazzoni et al., 2010).	The *Cryptococcu*s spp. can be isolated from the atmosphere and from bird droppings (Pedroso et al., 2009). It increases the incidence of crytococcosis in patients who have AIDS (Dzoyem et al., 2012).
Histoplasma capsulatum	*H. capsulatum* has various forms, textures, and pigments. The colony color is white or beige to brown and white or yellow to yellow-orange (Fisher and Cook, 1998).	*Histoplasma capsulatum* frequently causes pulmonary disease (Nosanchuk and Gacser, 2008) and chronic meningoencephalitis (Negroni et al., 1995) in immunocompromised patients, such as AIDS patients (Breton et al., 2006) and patients with tumor necrosis (Deepe, 2005).
Pneumocystis carinii	*Pneumocystis* species have two forms. The cyst is a stage in the life cycle that has a diameter	*Pneumocystis carinii can* cause pneumonia in immunocompromised patients (Santamauro et al., 2002)

Table 1-2 Characteristics of fungi and disease in humans (Cont.)

Type of fungi	Characteristic	Disease
	range of 5-12 µm. Free trophozoites are ameboid in shape (5 µm) (Fisher and Cook, 1998).	

1.2.3.2 Pathogen characteristics of airborne bacteria and disease in humans (Table 1-3)

Table 1-3 Pathogen characteristics of airborne bacteria and disease in humans.

Type of bacteria	Characteristic	Disease
Mycobacterium tuberculosis	*M. tuberculosis* is a thin rod-shaped bacterium approximately 0.4 x 3 µm and cannot be identified by Gram staining. Moreover, *M. tuberculosis* can survive in a dry environment for a long time (Jawetz and Brooks, 1991)	*Mycobacterium tuberculosis* is the primary risk of airborne bacteria in a health care center. It can spread to other people by inhaling the droplet nuclei and can infect immune suppressed humans such as the elderly and children (National Tuberculosis Controllers, Centers for Disease, and Prevention, 2005)

Table 1-3 Pathogen characteristics of airborne bacteria and disease in humans.
(Cont.)

Type of bacteria	Characteristic	Disease
Acinetobacter spp.	*Acinetobacter* spp. are aerobic Gram-negative coccobacillus (Bergogne-Bérézin and Towner, 1996) that can be isolated from soil, water, food and human skin	The *Acinetobacter* spp. is the main infectious disease from the environment and medical devices in hospitals (Sehulster et al., 2003). In the intensive care unit of a Sicilian hospital, 66.7% of infections found in the patients were caused by this genera. It caused the failure of the prevention and control program of nosocomial infection in the hospital (Agodi et al., 2006).
Bacillus spp.	*Bacillus* spp. are aerobic Gram-positive rods, 1 x 3-4 μm in size. The spore-forming *bacillus* can survive for a long time in the environment (Jawetz and Brooks, 1991).	*Bacillus anthracis* is the most important bacteria in a medical aspect as it causes anthrax disease. It can be transmitted from animals to humans through eating and inhaling the bacteria spores. A study by Adler et al. (2005) showed that *Bacillus* spp. in the neonatal intensive unit increased the incidence of the nosocomial infection rate in patients.

Table 1-3 Pathogen characteristics of airborne bacteria and disease in humans.
(Cont.)

Type of bacteria	Characteristic	Disease
Staphylococcus aureus	*S. aureus* is strongly Gram-positive of young cocci and has a diameter of about 1 μm. A colony of *S. aureus* is round, smooth and the color is gray to deep golden yellow (Jawetz and Brooks, 1991).	*Staphylococcus aureus* is a common bacterium in humans that can be found in the skin flora and carried in the nasal cavity. It is a pathogen that can infect the respiratory system and skin in certain patients, such as AIDS, COPD and surgery patients (Kluytmans et al., 1997).
Group A *Streptococcus*	Group A *Streptococcus* is a Gram-positive cocci that can have a mucoid colony (Runoff, 2002)	Group A *Streptococcus* is an important bacteria that causes pharyngitis, rheumatic fever and acute glomerulonephritis (Cunningham, 2000).
Coxiella burnetii (Q fever)	*C. burnetii* is a Gram-negative, coccobacillus with two cell types (large and small cell variants) and has a diameter of 0.2-0.7 μm (Marrie, 1995).	There is laboratory evidence and epidemiology information (Benenson and Tigertt, 1956) that it can infect the respiratory tract and is transmitted from person-to-person (Schack et al., 2014).

1.2.4 Factors affecting the size distribution and quantity of bioaerosols

The size distribution and quantity of microorganisms in the air depend on two main factors: physical characteristics of microorganisms and environmental factors.

1.2.4.1 Physical characteristics of microorganisms

The physical characteristics of microorganisms are the size, density and shape. The particle size can be classified into 3 groups. The aerodynamic diameter of microorganisms was 1-30 µm for airborne fungi spores (Gregory, 1973) and 0.25-8 µm for bacteria (Thompson, 1981) which depended on the species of the bioaerosol (Pasanen et al., 1991; Reponen et al., 1996; Reponen et al., 1998). Furthermore, the age of spores, nutrients for microorganism growth, accumulation and release of bioaerosols also depended on the distribution size and quantity of microorganisms in the air (Harding, 1975; Ellis, 1981).

1.2.4.2 Environmental factors

The environmental factors such as air velocity, humidity and temperature affect the suspension and distribution of microorganisms. They are transported over long distances and 25% of bioaerosols originated from various materials (Jones and Harrison, 2004). However, the quantity of airborne fungi is different between the material sources. The quantity of bioaerosols that originated from wood structures was greater than from concrete structures (Meklin et al., 2002). In addition, it was found that different sites have an effect on the quantity and type of bioaerosols. The fungi spore concentration in a suburban area was higher than the urban area (Pei-Chih et al., 2000; Sakai et al., 2003). For airborne bacteria, it was found that the concentrations in urban areas were higher than in the rural and forest areas (Shaffer and Lighthart, 1997). Moreover, the distribution size of microorganisms was related to the environmental factors of buildings; there was a positive relationship with humidity (Ponce-Caballero et al., 2013). The accumulation of moisture increased the size of the bioaerosols and affected the movement of microorganism particles in the air (Pasanen et al., 1991; Madelin and Johnson, 1992; Reponen et al., 2001). Fungi spore survival was more flexible than that of bacteria and viruses because the spores could dehydrate and rehydrate as well as resist UV

radiation (Cox 1989; Karra and Katsivela, 2007). In buildings that used heating ventilation and air conditioning (HVAC), the particle sizes of airborne bacteria were in the range of 3.3-4.7 μm (Pastuszka et al., 2005). However, in natural ventilation systems the particle size distribution was smaller (1.1-3.3 μm) (Pastuszka et al., 2005). Moreover, air velocity, air movement and human activity showed an effect on the release of microorganism particles in the buildings (Yau et al., 2011; Rajasekar and Balasubramanian, 2011; Prussin et al., 2015a). In addition, the seasonal changes can affect the level and species of microorganisms. The variation of airborne bacteria is at the highest in the autumn and the lowest in the winter (Wang et al., 2010b). Moreover, Gram-positive bacteria cocci can be found in the summer and winter of both indoor and outdoor sites (Tsai et al., 2007).

The airborne fungi quantity is at the highest in the spring and autumn and at the lowest in summer and winter (Awad et al., 2013). However, a study by Grinn-Gofron (2011) revealed that the airborne fungi level was the highest in the summer. The predominant airborne fungi of *Aspergillus* spp. was the highest in the summer, while *Penicillium* spp. were found at the highest in the winter (Pei-Chih et al., 2000). However, *Cladosporium* spp. were the main predominant type of indoor air, while *Aspergillu*s spp. were the main predominant type of outdoor air (Pei-Chih et al., 2000). Thus, the size distribution and quantity of bioaerosols change according to the time and site location (Lighthart, 2000; Raisi et al., 2010). The relationship between airborne bacteria and fungi and environmental factors are shown in Tables 1-4 and 1-5.

Table 1-4 Relationship between the environmental parameters and quantity of airborne bacteria.

Environmental factors	Review
UVGI	The UVGI (ultraviolet germicidal irradiation) could reduce the transmission of airborne bacteria in hospitals (Jensen et al., 2005). It protected personnel and patients from airborne bacteria. NIOSH recommended 2 ways to install the UVGI system: 1) in the upper-room area, at the waiting rooms of the out-patient and emergency departments and 2) at the anti-retroviral treatment facilities (Nardell, 2015).
Time of service	Daily indoor airborne bacteria varied by the occupant densities and human activities (Rajassekar and Balasubramanian, 2011; Park et al, 2013; Prussin and Marr, 2015).
Rainfall	High rainfall increased the humidity level in the air, thus cleaning the air and decreasing the airborne bacteria concentration (Wang et al., 2010a).
Air velocity	Strong air velocity causes an increase of bioaerosols in the air (Sakiyan and Inceoglu, 2003). However, microorganisms in the building can be diluted if the outdoor air has a lower concentration of microorganisms than the indoor air (Yau et al., 2011).

Table 1-4 Relationship between the environmental parameters and quantity of
airborne bacteria. (Cont.)

Environmental factors	Review
Wind direction	Wind direction affected the airborne bacteria quantity. For example, wind directions from the main road which had heavy traffic, a construction site and human activity could lead to an increased quantity of airborne bacteria in the environment (Pei-Chih. 2000).
Humidity	Humidity affects the survival of Gram-negative bacteria. A 50-90% relative humidity can increase the mortality rate of *Serratia marcescens*, *Escherichia coli*, *Salmonella pullorum*, *Salmonella derby*, *Pseudomonas aeruginosa*, and *Proteus vulgaris* (Webb, 1959; Won and Ross, 1966), while Gram-positive bacteria such as *Bacillus* spp. and *Klebsiella pneumoniae* can survive at 60% of relative humidity (Bolister et al., 1992). In addition, a high humidity level in the environment demonstrated that *Staphylococcus albus*, *Streptococcus haemolyticus*, *Bacillus subtilis* and *Streptococcus pneumoniae* had an increased mortality rate (Dunklin and Puck, 1948; Webb, 1959).

Table 1-4 Relationship between the environmental parameters and quantity of
airborne bacteria. (Cont.)

Environmental factors	Review
Temperature	High temperature is an important factor in the inhibition of bacterial growth. However, it was found that in a low temperature environment airborne bacteria could survive for a long period of time (Tang, 2009) and Gram-positive bacteria could survive at higher temperatures (Marthi et al., 1990, Walter et al., 1990). According to a study by Wang et al. (2010a), it was discovered that temperature was positively related to the concentration of the airborne bacteria.
Outdoor wind speed	Outdoor wind speed affected the bioaerosols that can release the microorganisms into indoor and outdoor environments (Abdel Hameed et al., 2009) which could lead to an increased quantity of airborne bacteria in the environment (Fang et al., 2007; Abdel Hameed et al., 2009).
Carbon dioxide (CO_2)	Carbon dioxide is positively related to the concentration of airborne bacteria in buildings (Kim et al., 2009; Wang et al., 2010b; Mahyuddin et al., 2013). Poor ventilation and human activity especially increase the level of carbon dioxide in the work place (Mancinelli and Shulls, 1978).
Carbon monoxide (CO)	Carbon monoxide is a part of urban air pollution. It can affect the growth of some bacteria. For example, *Serratia marcescens* had an increased mortality rate when the concentration of carbon monoxide was 25% in a humid environment. However, if the humidity was up to 90%, it can increase the survival rate (Lighthart, 1973).

Table 1-4 Relationship between the environmental parameters and quantity of
airborne bacteria. (Cont.)

Environmental factors	Review
Methane (CH_4)	A study by Wu et al. (2012) showed that methane was positively related to the level of airborne bacteria.
Formaldehyde (HCHO)	A study by Wang et al. (2010a) demonstrated that formaldehyde was negatively related to the concentration of airborne bacteria.
Nitrogen monoxide (NO)	A study by Mancinelli and McKay (1983) disclosed that a nitrogen monoxide concentration of more than 1 ppm could reduce the number of bacteria because it inhibited the growth of the bacteria.
Ozone (O_3)	A study by Lighthart (1973) found that ozone had a negative relationship with the bacteria concentration as it could destroy the airborne bacteria (Dyas et al., 1983).
Sulfur dioxide (SO_2)	It is found in a study by Wu et al. (2012) that sulfur dioxide was positively related to the bacteria concentration as it affected the growth of bacteria (Lee et al.,1973; Mancinelli and Shulls, 1978).
Patients, medical staff and relatives of patients.	A study by Rajasekar and Balasubramanian (2011) found that airborne bacteria were related to the occupancy level and human activity in the work place.
Outdoor airborne bacteria	Outdoor bioaerosols are an important factor that can be transmitted through windows into the indoor areas. The levels of bioaerosols increased in the work place (Meadow et al., 2014).

Table 1-5 Relationship between the environmental parameters and quantity of
airborne fungi.

Environmental factors	Review
UVGI	UVGI (ultraviolet germicidal irradiation) could reduce the transmission of airborne bioaerosols in a hospital (Jensen et al., 2005; Krishnamoorthy and Tande, 2014) that only slightly affected fungi spores (Riley and Nardell, 1989). It was poorly effective because of high humidity (NIOSH, 2009)
Time of service	Studies by Abdel Hameed et al. (2009) and Chen et al. (2010) found that the concentration of airborne fungi were lower in the afternoon. During the morning, airborne fungi spores were affected by the heat of the sun which could reduce the relative humidity (Joy Royes, 1987), whereas the high temperature and radiation in the afternoon could reduce the levels of airborne fungi (Jones and Harrison, 2004).
Rainfall	Rainfall increased the humidity in the environment (Troutt and Levetin, 2001; Hollins et al., 2004; Peternel et al., 2004) which affected the survival and growth of fungi (Erkara et al., 2008).
Air velocity	Strong air velocity had the effect of increasing the numbers of bioaerosols in the air (Pasanen et al., 1991). However, the microorganisms could be diluted in the building if the outdoor air had a lower concentration of microorganisms than the indoor air (Sautour et al., 2009b).

Table 1-5 Relationship between the environmental parameters and quantity of
airborne fungi. (Cont.)

Environmental factors	Review
Wind direction	Wind direction affected the quantity of airborne fungi quantity. For example, wind directions from a forest can increase the airborne fungi in the environment (Fang et al., 2007).
Humidity	High humidity was related to the concentration of the airborne fungi spores in the environment (Sabariego et al., 2000; Stennett and Beggs, 2004; Rodriguez-Rajo et al., 2005; Erkara et al., 2008; Andersen et al., 2011) because it is an important factor for the growth of fungi. The species of *Aspergillus*, *Penicillium*, *Alternaria*, and *Cladosporium* were predominant fungi that were related with high humidity (Sakiyan and Inceoglu, 2003; Oliveira et al., 2009; Hameed et al., 2012).
Temperature	High temperature positively increased the concentration of fungi spores in the environment (Stennett and Beggs, 2004; Erkara et al., 2008). A large number of fungi spores could be found in the air in an environment with high temperature and humidity (Li and Kuo, 1994; Pei-Chih et al., 2000; Sakai et al., 2003). The species of *Cladosporium*, *Alternaria* and *Aspergillus* were predominant types of fungi that were related with a high temperature in the environment (Troutt and Levetin, 2001; Adhikari et al., 2006; Reyes et al., 2009; Quintero et al., 2010; Hameed et al., 2012).
Outdoor wind speed	A high outdoor wind speed can release bioaerosols into the air and increase the indoor bioaerosol concentrations (Abdel Hameed et al., 2009; Raisi et al., 2010).

Table 1-5 Relationship between the environmental parameters and quantity of airborne fungi. (Cont.)

Environmental factors	Review
Carbon dioxide (CO_2)	A study by Kim et al. (2009) and Wang et al. (2010b) revealed a positive correlation between carbon dioxide and the quantity of airborne fungi at a work place.
Carbon monoxide (CO)	A study by Ho et al. (2005) demonstrated that carbon monoxide was positively related to the total concentration of fungi.
Methane (CH_4)	The concentrations of bioaerosols were positively correlated with methane (Ho et al., 2005).
Formaldehyde $(HCHO)$	A study by Wang et al. (2010b) showed negative correlation indices with formaldehyde.
Nitrogen dioxide (NO_2)	A study by Ho et al. (2005) and Grinn-Gofron et al. (2011) disclosed that nitrogen dioxide was negatively related to the concentration of airborne fungi.
Ozone (O_3)	Ozone can reduce the levels of airborne fungi (Fenn et al., 1989) but it depended on the atmospheric temperature (Adhikari et al., 2006).
Sulfur dioxide (SO_2)	A study by Grinn-Gofron et al. (2011) showed that sulfur dioxide was negatively related to the concentration of airborne fungi.

Table 1-5 Relationship between the environmental parameters and quantity of
airborne fungi. (Cont.)

Environmental factors	Review
Patients, medical staff and relatives of patients.	Airborne fungi can be distributed into the air from clothes and the skin flora of humans (Grice and Segre, 2011; Caggiano et al., 2014).
Outdoor airborne fungi	Outdoor bioaerosols were an important factor that could be transmitted through windows into the indoor areas. The levels of bioaerosols increased in the work place (Sautour et al., 2009a; Ponce-Caballero et al., 2013)

1.2.5. Prevention and control of bioaerosols in hospitals

Poor indoor air quality in health-care facilities affects human health and may cause allergies, sick building syndrome, respiratory diseases and nosocomial infection (El-Sharkawy and Noweir, 2014; Bentayeb et al., 2015). The principle of environmental infection control of airborne contamination such as the use of local exhaust ventilation, general ventilation and air cleaning can prevent hazards to patients and medical staff in hospitals (Mehta et al., 2014). Environmental management can reduce the nosocomial infection rate (Mamishi et al., 2014). Methods of prevention and control of airborne bioaerosols in hospitals include ventilation, filtration, UVGI system and cleaning methods which are used widely in hospitals.

1.2.5.1 Natural ventilation

A natural ventilation system allows the air from the outdoors to flow into a building in order to remove the pollution. It has a high air flow rate and low cost and it is widely used in the tropical zone such as in Malaysia, Singapore and Peru. Therefore, the building design can control the temperature, humidity and flow rate of air ventilation to improve the indoor air quality (Yau et al., 2011). In the tropical zone, the temperature and humidity are between 24-32°C and 50-90%,

respectively, (Mallick, 1996) which is in a suitable range for the growth of microorganisms.

The WHO recommendations for natural ventilation in hospitals are shown in Table 1-6. The natural ventilation system is based on the level of the outdoor pollution flow rate in the building. It can increase or decrease the concentration of the indoor air pollution. The concentration of airborne fungi increases in the building that has low ventilation (Mehta et al., 2014). Meanwhile, although a natural ventilation system is good, in the outdoors there are a number of fungi spores which can have a substantial effect on the indoor air quality (Sautour et al., 2009; Ponce-Caballero et al., 2013).

Buildings that use the natural ventilation system can increase the bioaerosol contamination in the indoor air (Burge, 2002; Wu et al., 2005; Mehta et al., 2014). It increases the contamination of *Aspergillus flavus* and *Aspergillus fumigates* which travel through windows. The contamination can affect immunocompromised patients (Vonberg and Gastmeier, 2006).

Table 1-6 WHO recommendations on natural ventilation in hospitals.

Types of room	Hourly average ventilation rate	Note
General wards and out-patient department	60/l/s/patient	
Precaution room	160/l/s/patient; 80/l/s/patient (Minimum)	This only applies to new health-care facilities and major renovations
Corridors and other transient spaces	2.5 l/s/m^3	The space without a fixed number of patients.

Source: Atkinson et al. (2009)

1.2.5.2 Mechanical ventilation

Mechanical ventilation uses mechanical fans installed directly on the wall or in air ducts to supply air or exhaust air. This ventilation can set both the flow rate and the direction of the ventilation distribution which are the most important in a separate room or an operating room in order to reduce the contamination in the indoor airspace (Atkinson et al., 2009).

1.2.5.3 Dilution ventilation

Hybrid or mixed-mode ventilation is a combination of mechanical ventilation and natural ventilation. The building must be designed for natural ventilation and mechanical ventilation to increase the air flow rate of the natural ventilation system (Heiselberg et al., 2002; Atkinson et al., 2009) to save energy. The indoor air quality depends on the outdoor air quality which is similar to the natural ventilation system.

1.2.5.4 Filtration

A filtration system can remove particles from the air, but it depends on the type of filters. The high efficiency particulate air (HEPA) system is able to remove at least 99.97% of the particles with a diameter ≥ 0.3 μm (Sehulster et al., 2003) but it cannot reduce the level of *Aspergillus* spores. (Oliveira et al., 2005; Abdul Salam et al., 2010; Fournel et al., 2010). Some fungi can pass through a filter into the air conditioning system and be distributed into a hospital room (Perdelli et al., 2006). In addition, the laminar air flow (LAF) system is used widely in an operation room. The LAF system has a higher efficiency than the HEPA filtration system but it cannot remove fungi spores (Araujo et al., 2008). However, an indoor air filtration system contained one-third of the outdoor fungi concentrations. A highly efficient filtration system can reduce the fungi concentration to less than the total concentration of outdoor airborne fungi (Araujo et al., 2008)

1.2.5.5 Ultraviolet Germicidal Irradiation (UVGI)

UVGI is recognized as a method to reduce the transmission of airborne microorganisms in hospitals. It can be installed as duct irradiation and upper-room air irradiation (Xu et al., 2003; Reed, 2010). The wavelength of the UV-C component is 253.7 nm which can damage the DNA of microorganisms. The UV-C component can reduce the spreading of airborne bacteria and viruses while the spores of airborne fungi are only slightly affected (Riley and Nardell, 1989). The efficiency of an upper-room UVGI system is related to the UV-C dose (30-50 $\mu W/cm^2$), humidity (30-60%), ventilation, air mix and temperature (20-24°C) (NIOSH, 2009). The UVGI component can reduce the transmission of tuberculosis and is recommended for use in waiting rooms, out-patient areas, emergency department and anti-retroviral treatment facilities (Escombe et al., 2009).

1.2.5.6 Cleaning

Two reasons indicate the importance of cleaning: 1) for comfort and to strengthen the corporate image of an organization and 2) to reduce the sources of nosocomial infection (Griffith et al., 2000). Cleaning can minimize and control the distribution of microorganisms in the work place (Dancer, 2008). A study by Dancer et al. (2004) proposed standards for clinical surface hygiene to increase cleaning methods and reduce the contamination of microorganisms in hospitals.

1.3 Objectives

1.3.1 To investigate the quantity, size and types of bacteria and fungi distributed in the indoor air of a university hospital.

1.3.2 To investigate the relationships between airborne bacteria and fungi and ambient factors in a university hospital.

1.3.3 To provide recommendations to improve the performance of the management system to further reduce contamination in the air.

1.4 Expected benefits

        1.4.1 To obtain knowledge on the quantity, type and size distribution of the ambient airborne bacteria and fungi in the university hospital.

        1.4.2 To know the relationships between the environmental factors which have a significant impact on the distribution of ambient airborne bacteria and fungi in the university hospital.

        1.4.3 To know the appropriate method to reduce the contamination of airborne bacteria and fungi in university hospitals.

1.5 Dissertation organization

        This study investigates the quantity, size, and type distribution of airborne bacteria and fungi in a general ventilation system of a university hospital and the relationships between the environment factors and bioaerosols. The results provide recommendations to improve the performance of management systems to reduce bioaerosol contamination in the air. The steps of the study process are presented as follows.

        1.5.1 Determination of the time for sampling

        The bioaerosols were collected by a six-stage viable cascade impactor (Model 10-800, Andersen Inc, USA). The indoor locations of air sampling were: 1) the Surgical Intensive Care Unit (S-ICU) and 2) Medical Intensive Care Unit (M-ICU) which used mechanical ventilation systems; 3) Eye Out-patient Department (E-OPD) which used the dilution ventilation system. Nutrient agar was used for airborne bacteria cultures and incubated at 37°C for 1-2 days. Potato dextrose ager was used for airborne fungi and incubated at 25°C for a period of 3-5 days. The sample collection times were 3, 5, 10, and 15 minutes. The preliminary results found that 5-minute air sampling was the optimal time for specimen collection for this research because from all sampling sites the bioaerosol colonies on the agar were easier to count.

1.5.2 Study site

The university hospital has 853 beds and over 2,000 staff employees and provides primary to tertiary care treatment. The dilution ventilation system was used as the main mechanism for the reduction of indoor air pollution. There was no temperature difference between the interior and exterior areas studied. The service areas that used the dilution ventilation system and had a high population density included the Medical Out-patient Department (M-OPD), the Surgical Out-patient Department (S-OPD), the Eye Out-patient Department (E-OPD), the Medical In-patient Department (M-IPD), and the Surgical In-patient Department (S-IPD). All of these sites were selected for air sampling except S-OPD. Since all OPDs were located on the first floor of the main building in same open space next to the corridor (Figure 1-1), only two air sampling points (E-OPD and M-OPD) were set with an appropriate distance between the sampling points. Ventilation fans were installed on the walls of these sites. The IPDs were located on the 5th and 9th floors of the surgical and medical building (13th floor). Ceiling fans were installed on the ceiling between the patient beds and the nurse's station. The windows were open all of the time and mosquito screens were installed. All indoor sampling sites collected the bioaerosols in the middle of the rooms because the rooms were large sites (Yassin and Almougata, 2010; Kalwasińska et al, 2012).

The M-OPD was built over 20 years ago and is located on the first floor of the main building. The total area is approximately 430 m^2. Ultraviolet germicidal irradiation (UVGI) systems were installed which had an average power density of 986 μW/cm^2 (9 light bulbs) and a cleaning solution was used twice a day to clean the floor. Air samples were collected in the middle of the room, sitting area and next to the nurse's station.

The E-OPD was an area of about 430 m^2. This included a waiting area for the Ear/Nose/Throat Department, the Eye Department and the treatment rooms. The average power density of the UVGI was 518 μW/cm^2 (12 light bulbs). The sampling methods and locations were similar to the M-OPD including the waiting area of the Eye Clinic.

The location of the M-IPD was on the 9th floor (23.4 m) with an approximate area of 700 m^2 of the surgical and medical building that is over 20 years old and is the

tallest building in this hospital. Dilution ventilation is used to reduce indoor air pollutants via flow through the windows and doors. It has 40 beds and cleaning the floor was performed 4 times per day. The samples were collected near the nurse's station. The capacity of the S-IPD was 34 beds and the floor was also cleaned 4 times per day. It was located on the 5th floor (13 m) of the same building as the M-IPD. The sampling sites were similar to the M-IPD. Outdoor air samples were also taken for comparison with indoor air quality.

The outdoor air sampling sites were the outside of the main building (OPD), outside the 5th floor and outside the 9th floor of the surgical and medical building on the connecting bridges between this building and other buildings.
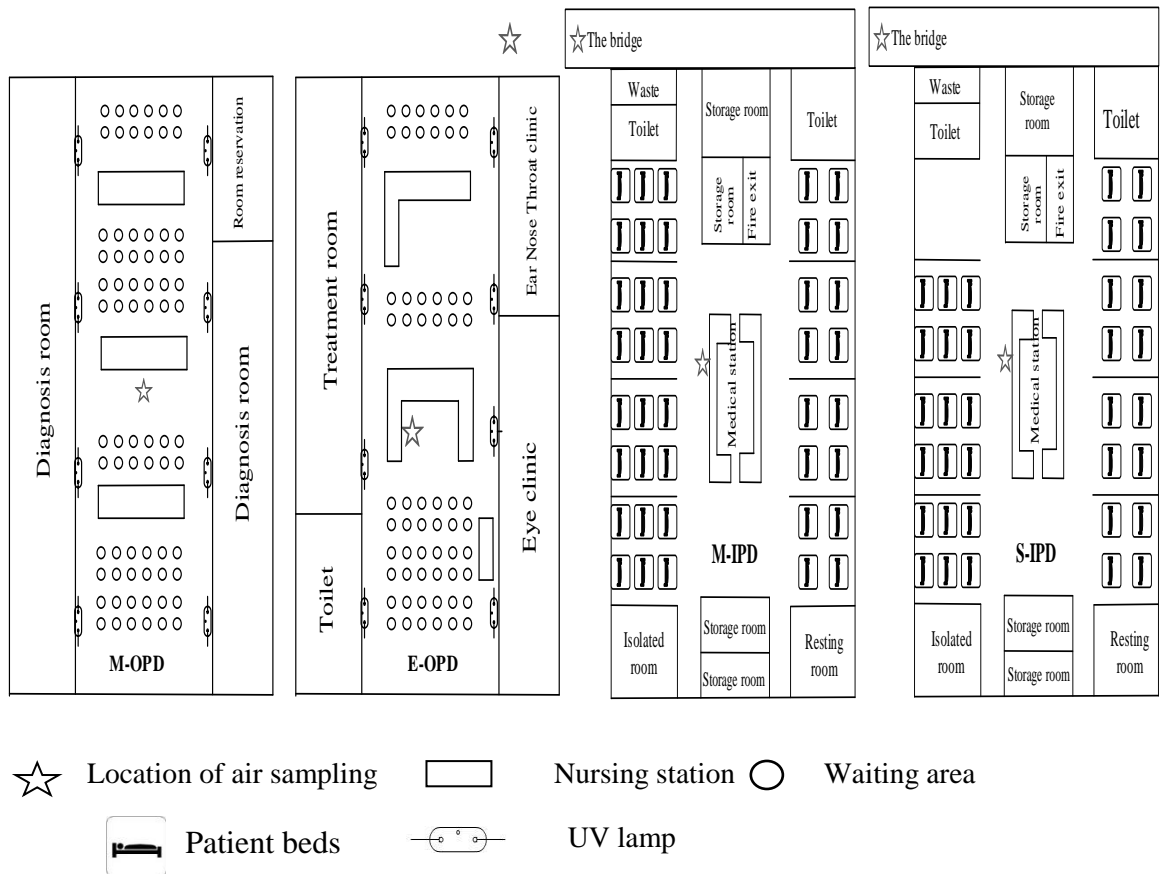
Figure 1-1 Layouts of M-OPD, E-OPD, M-IPD and S-IPD

1.5.3 Sample collection

Investigation of the bacteria and fungi contamination was performed in the air of a university hospital. The effectiveness the protective measures of this study can be classified into 3 aspects.

1.5.3.1 Quantity and size distribution of bioaerosols

The quantity and size distribution of bacteria and fungi in the air were collected from January to December 2012 in the M-OPD, E-OPD, M-IPD and S-IPD. Samples were collected 4 times a day in the morning (09:00-12:00) and afternoon (13:00-16:30) twice a month through 1 year or until 768 samples (4,608 plates) (bacteria, 384 samples; fungi, 384 samples) were collected. The outdoor environment samples were collected outdoors of the Out-patient Department (O-OPD), Medical In-patient Department (OM-OPD) and Surgery In-patient Department (OS-IPD). A total of 576 (3,456) samples (bacteria, 288 samples; fungi, 288 samples) were collected at these 3 areas each time samples were collected inside the building.

1.5.3.2 Bioaerosol collections and measurements

A six-stage viable cascade impactor (Model 10-800, Andersen Inc, USA) was used to classify the particles of bioaerosols (airborne bacteria and fungi) according to different aerodynamic diameter ranges: stage 1 (>7.0μm), stage 2 (4.7-7.0μm), stage 3 (3.3-4.7μm), stage 4 (2.1-3.3μm), stage 5 (1.1-2.1μm) and stage 6 (0.65-1.1μm) (Anderson, 1658). The air sampling time was 5 minutes and the flow rate was adjusted to 28.3 L/min. Trypticase soy agar (TSA) was used to culture the airborne bacteria and incubated at 37°C for 1-2 days. Malt extract agar (MEA) was used to culture the fungi and incubated at 25°C for 5 days. The airborne bacteria and fungi concentrations were reported in colony forming units per cubic meter (cfu/m^3) according to these equations (Kim and Kim, 2007).

Total bioaerosol concentration

$$\text{cfu/m}^3 = \frac{\text{Colony counted on agar stage 1-6}}{\text{Air volume (m}^3)} \qquad (1)$$

$$\text{Air volume (m}^3) = \frac{28.3 \text{ L/min x sampling time (min)}}{10^3} \qquad (2)$$

The respirable fractions of the bioaerosols were defined as follows:

$$B_i = C_i/C_b \text{ x } 100\% \tag{3}$$

Where B_i = the airborne bacteria fraction (%); C_i is the i stage of airborne bacteria concentration, which is 1 for the first stage (>7 µm), 2 for the second stage (4.7-7 µm), 3 for the third stage (3.3-4.7 µm), 4 for the fourth stage (2.1-3.3 µm), 5 for the fifth stage (1.1-2.1 µm), 6 for the sixth stage (0.65-1.1 µm) and C_b = is the total airborne bacteria concentration.

$$F_i = C_i/C_f \text{ x } 100\% \tag{4}$$

Where F_i = the airborne fungi fraction (%); C_i is the i stage of airborne fungi concentration, and C_f = is the total airborne fungi concentration.

$$R_b = (C3 + C4 + C5 + C6)/ C_b \text{ x } 100 \% \tag{5}$$

Where R_b = Respirable fraction of airborne bacteria (Kim and Kim, 2007).

$$R_f = (C3 + C4 + C5 + C6)/ C_f \text{ x } 100 \% \tag{6}$$

Where R_f = Respirable fraction of airborne fungi

1.5.3.3 Study on the type of bacteria and fungi by size

Types of bacteria and fungi in the dilution ventilation system of the university hospital were collected from April 2012 (dry season) and from November 2012 (wet season). The location sites were the M-OPD, E-OPD, M-IPD and S-IPD for indoor bioaerosols and the O-OPD, OM-IPD and OS-IPD for outdoor bioaerosols.

The type distributions of airborne bacteria and fungi in the morning period of both seasons were identified by size. Since this period has a high occupant density and human activity, it is a critical time of service especially in the ICUs, OPDs and IPDs. Therefore, the results of the airborne bacteria and fungi characteristics can be used for environmental planning to reduce the health hazard of medical staff and patients. The pure colonies of the bioaerosols were classified by Gram stain technique and biochemical tests for airborne bacteria while the airborne fungi were classified by form, spore, shape and color of colony on a slide by optical

microscope. After that, the pure colonies of bacteria and fungi were identified by PCR technique.

                1.5.4 Relationship between environmental factors and quantity of bioaerosols

        The factors included UVGI, time of service, rainfall, outdoor wind speed, wind direction, relative humidity, air velocity and temperature, carbon dioxide (CO_2), carbon monoxide (CO), sulfur dioxide (SO_2), ozone (O_3), formaldehyde (HCHO), methane (CH_4), Nitrogen monoxide (NO), nitrogen dioxide (NO_2), occupancy level, outdoor bacteria and fungi. They were collected and processed at the same time as the airborne bacteria and fungi (Table 1-7). The level of CO_2, CH_4, CO, HCHO, NO_2, NO, O_3 and SO_2 in the indoor air were monitored continuously by a Gasmet^{TM} DX-4030 FTIR Gas Analyzer. The indoor and outdoor physical parameters (velocity, humidity and temperature) were recorded by a VelociCalc® Air Velocity Meter (Model 9555). In addition, the rainfall, outdoor wind speed and wind direction were secondary data that was available from the Metrological Department. A UV meter was used for the UVGI parameter. The occupancy level was recorded during bioaerosol sampling. A spearman correlation and time series analysis were used to evaluate and predict the relationship between the airborne bacteria and fungi and environmental factors.

Table 1-7 Relationship between the bioaerosols and environmental factors

Environmental factors	Relationship		Methods and sources of data
	Bacteria	**Fungi**	
UVGI	√	√	UV meter
Time of service	√	√	-
Rainfall	√	√	Meteorological Department
Outdoor wind speed	√	√	
Outdoor wind direction	√	√	
Indoor and outdoor air velocity	√	√	VELOCICALC Air Velocity Meter
Indoor and outdoor humidity	√	√	Model 9555/Analyzed during sampling
Indoor and outdoor temperature	√	√	of bioaerosols
Carbon dioxide (CO_2)	√	√	Gasmet^{TM} DX-4030 FTIR Gas
Methane (CH_4)	√	√	Analyzer/Analyzed during bioaerosol
Carbon monoxide (CO)	√	√	sampling
Formaldehyde (HCHO)	√	√	
Nitrogen dioxide (NO_2)	√	√	
Nitrogen monoxide (NO)	√	-	
Ozone (O_3)	√	√	
Sulfur dioxide (SO_2)	√	√	
The number of people	√	√	Counted during bioaerosol sampling
Outdoor bacteria and fungi	√	√	Six-stage viable cascade impactor

√ Factors related to quantity of airborne bacteria and fungi

- No relationship to airborne fungi

1.5.5 Focus group discussions to reduce bioaerosol concentrations

The results of a time series analysis were the main points to review and document concerning environmental management to reduce the airborne bacteria and fungi in the work place. The aim of the focus group discussions was to find methods to decrease the contamination in a building that used the dilution ventilation system. The interviews included eight stakeholders who worked in the M-OPD, E-OPD, M-IPD, and S-IPD and five professionals from the Occupational Safety Unit, Division of Health Promotion, Infection Control Unit, and the Faculty of Environmental Management who had substantial work experience. The experimental framework is shown in Figure 1-2.

1.6 Experimental framework

Survey site of study to set up sampling site			
OPDs	IPDs	ICUs	
Sampling sites and time optimization			

Airborne bacteria and fungi sampling, numeration, identification		
Size distribution	Dominant type	Quantities

Environmental factor studies

UVGI, time of service, rainfall, air velocity, relative humidity, wind speed, wind direction, temperature, CO_2, CO, HCHO, NO_2, NO, O_3, SO_2, number of people and outdoor bioaerosols

Relationships of ambient airborne bacteria and fungi in the hospital and environmental factors

Data analysis

Focus group to find a strategy to enhance the indoor air management system of a university hospital

Figure 1-2 Experimental framework

CHAPTER 2

Bioaerosol Assessment in the Intensive Care Units of a Tertiary Care Hospital

Abstract

This cross-sectional study describes the characteristics and size distributions of bioaerosols in the medical intensive care unit (MICU) and surgical intensive care unit (SICU) of a university hospital. The relationship of environmental factors on indoor bioaerosol concentrations was clarified. A six-stage viable cascade impactor was used to assess the concentrations and size distributions of bioaerosols in the ICUs from June 2011 to February 2012. The predominant bioaerosols were further analyzed by the PCR technique. The meteorology factors were simultaneously measured with the viable microbes.

The total indoor bacteria and fungi concentrations at the MICU were 214.22 ± 93.27 and 194.25 ± 74.83 cfu/m^3, while at the SICU during on-ultraviolet germicidal irradiation (UVGI) the concentrations were 274.44 ± 140.75 and 234.39 ± 115.60 cfu/m^3 and off-UVGI they were 515.12 ± 247.75 and 531.41 ± 337.65 cfu/m^3, respectively. Since air passed though the MICU at a velocity of less than 1 m/s from a nearby construction site, accumulation of outdoor bacteria and fungi such as *A. fumigatus* and *A. flavus* were sampled at the site. The predominant bacteria and fungi in the ICUs were *Staphylococcus* spp., *Micrococcus* spp*., Bacillus* spp., *Pseudomonas* spp. and *Cladosporium* spp., *Penicillium* spp., *Aspergillus* spp. and *Fusarium* spp., respectively. The functioning of the UVGI and the room air velocity depended significantly on the indoor bacteria concentration in the SICU while the indoor fungi concentration depended significantly on the outdoor fungi concentration, room air velocity, indoor relative humidity and indoor temperature. To decrease the indoor bioaerosol concentrations, the room air velocity should be increased and the UVGI system should be installed in the limited space of the ICUs.

Keywords: Airborne bacteria; airborne fungi; construction work; environmental factor; size distribution

2.1 Introduction

Microbiological contamination in terms of poor indoor air quality can lead to major respiratory system infections in immunocompromised patients (Haddad et al., 2004, Kim et al., 2010) and sick building syndrome of hospital occupants (Leung and Chan, 2006). Nosocomial infections in hospitals were determined to be a major contribution to the morbidity and mortality rates in patients (Kallel et al., 2005; Lyytikainen et al., 2008) especially patients admitted into the intensive care units (ICUs) which had the highest infection rates (Danchaivijitr et al., 2007). Hence, several methods to reduce the levels of airborne microorganisms were implemented in the ICUs such as isolated and closed rooms and controlling the admittance of relatives of patients (Vonberg and Gastmeier, 2006). However, bioaerosols can be distributed from the peeling of skin, the gastrointestinal tract and the clothing of personnel (Obbard and Fang, 2003). The airborne bacteria which can affect humans are *Mycobacterium tuberculosis*, *Acinetobacter* spp., *Bacillus* spp., *Staphylococcus aureus*, and the airborne fungi are *Aspergillus* spp., *Rhizopus* spp., *Acremonium* spp., *Fusarium* spp. and *Pseudallescheria boydii* (Sehulster et al., 2003). Bioaerosols can be generated from several sources that include the inside and outside of the ICUs. The outdoor air may have an effect on the indoor air quality in ICUs via the moving in and out of people and the conductive air. In addition, many studies reported the effects of nearby activity on the air quality in a hospital, for example heavy truck traffic can increase the amount of particulate matter in the environment of hospital (Fournel et al., 2010). Therefore, the air quality management outside an ICU is also important and should be a concern.

The ICUs in tertiary care hospitals should be influenced less by the outdoor environmental factors because ultraviolet germicidal irradiation (UVGI) systems and heat, ventilation and air conditioning (HVAC) systems were installed in the ICUs to reduce viable *Mycobacterium tuberculosis* and other microorganisms (Reed, 2010; Yau et al., 2011). Appropriate intensity UVGI systems and good efficiency of heat, ventilation and air-conditioning (HVAC) systems may reduce the presence of microorganisms (Leung and Chan, 2006; Reed, 2010; Yau et al., 2011). However, if a good maintenance program of these systems is not in place, their performance will decrease over time. There are limited data on indoor air quality management and

environmental factors related to indoor bioaerosol concentrations in the ICUs of Thailand. Therefore, bioaerosol samples were taken in the ICUs of a tertiary care hospital to determine the efficiency of the maintenance program.

This study aimed to investigate the airborne microbiological characteristics in the ICUs of a tertiary care hospital. The concentration, size distribution, and types of airborne bacteria and fungi are presented. The relationships of outdoor air quality and some environmental factors both from inside and outside of the ICUs included occupant density, frequency of room cleaning, UVGI operation, temperature, relative humidity, air flow velocity and wind direction, and the distances from construction sites were also determined. These results will be useful in the planning and management of indoor air quality especially in the ICUs or other special wards in hospitals to prevent nosocomial infections of patients and also prevent sick building syndrome of health care workers in the tropical zone.

2.2 Materials and methods

2.2.1 Study area

This is a cross-sectional study that was performed in the intensive care units (ICUs) of Songklanagarind Hospital, Prince of Songkla University, Thailand from August to September 2011. The bioaerosol samplings were taken during the rainy season (August to September 2011) and there was construction work near the study area. An aerial view of the bioaerosol sampling sites is shown in Figure 2-1. The Internal Medical Intensive Care Unit (MICU) (No. 2) and Surgical Intensive Care Unit (SICU) (No. 5) represent the indoor environments of the ICUs. The outdoor environment of the ICUs is represented by the open-air areas which were located away from the construction work site at 43 (No.1, O_c), 81 (No. 3, O_1) and 143 meters (No.4, O_2). Outdoor samples were collected simultaneously on the same day as the indoor bioaerosol samples.
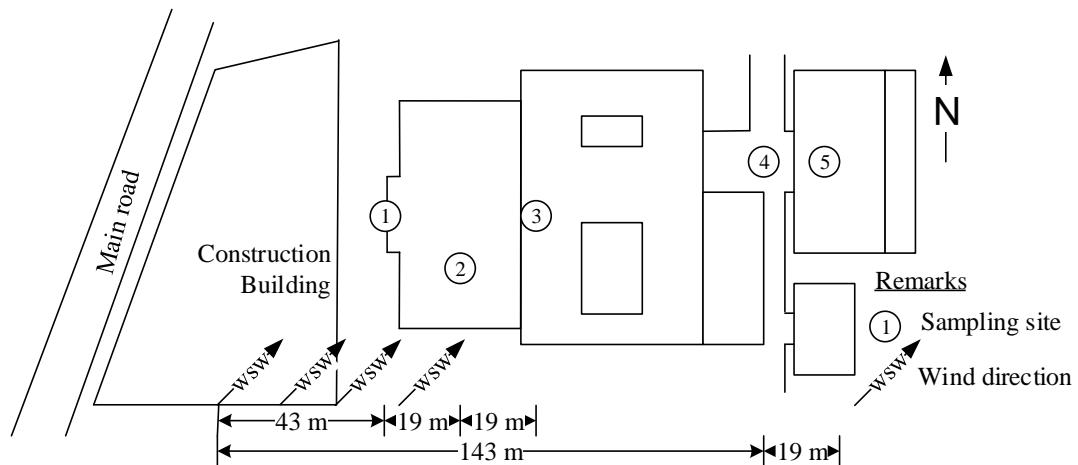
No. 1 = nearby the construction work site (O_c); No. 2 = MICU;

No. 3 = outdoor reference for MICU (O_1); No. 4 = outdoor reference for SICU (O_2); No. 5 = SICU;

WSW=west-southwest

Figure 2-1 Map of the construction site and sampling locations.

2.2.2 Bioaerosol sampling and meteorology data

The six-stage viable Andersen cascade impactor (The Thermo Scientific®, USA) used in this study was designed and developed to measure the viable microbial load. The aerodynamic diameter ranges for each stage in the cascade impactor were: >7.0 μm (stage 1), 4.7–7.0 μm (stage 2), 3.3–4.7 μm (stage 3), 2.1–3.3 μm (stage 4), 1.1–2.1 μm (stage 5) and 0.65–1.1 μm (stage 6). To determine the bioaerosol size distribution and concentration with Andersen cascade impactor, the air flow rate at 28.3 L/min (NIOSH Method 0801) and the sampling time was 5 minutes in order to prevent overloading the plates (pilot runtimes of bioaerosol sampling were taken at 5 and 10 minutes). All samples were taken at a height of 1.5 meters from the floor to represent the human breathing zone environment. Moreover, the indoor bacteria and fungi quantities in the S-ICU were investigated between the off and on UVGI operations for 1 hour to evaluate the effectiveness of the UVGI system.

At each location site, the sampling was between 09:00 and 12:00 to represent the morning period, and between 13:00 and 16:00 for the afternoon period. Therefore, each bacteria and fungi sample at the MICU was collected 3 days X 4 times (twice in the morning and twice in the afternoon) X 2 duplicate samples, whereas at the SICU

during the UVGI off and on samples were collected 3 days X 2 times (once in the morning and once in the afternoon) X 2 duplicate samples. The meteorology factors such as temperature ($^{\circ}$C), relative humidity (%RH) and wind velocity (meters per second, m/s) were measured simultaneously with bioaerosol sampling by direct-reading instruments (VelociCalc, TSI, Germany). The outdoor wind velocity and wind direction data were from the Khohong agrometeorological station (Songkhla). The highest frequencies of wind direction at each sampling location are represented in percentage (Table 2-1).

2.2.3 Bioaerosol and Polymerase Chain Reaction (PCR) analysis

A 70% ethanol solution was used to disinfect the instruments prior to use for air collection. Nutrient agar was used for bacterial cultures and potato dextrose agar was used for fungal cultures. Normally, the bacterial cultures were incubated at 37 $^{\circ}$C for 2 days and the fungi cultures at 25°C for 5 days. The concentrations of microorganisms were expressed in colony forming units per cubic meter, cfu/m^3 following these converting equations.

The concentrations of microorganisms of each stage (cfu/m^3)
= colony forming count at the stage (colony forming units, cfu) x 10^{-3}
  sampling flow rate (liter per minute, Lpm) x sampling times (minute)

The concentrations of microorganisms of each stage (cfu/m^3)
= the summation of colony forming count 1 to 6 (colony forming units, cfu) x 10^{-3}
  sampling flow rate (liter per minute, Lpm) x sampling times (minute)

Only airborne bacteria and fungi samples of the morning phase were uniformly dispersed in all of the six stages. They were purely cultured and classified according to Bergey's manual (Buchanan and Gibbons, 1974) for bacteria. The fungi were classified by form, shape, spore color and color according to the St-Germain and Summerbell (2011) method. After that, the pure colony samples were sent to the Faculty of Science, Mahidol University for identification according to the following methods.

The DNA extractions of bacteria and fungi used the modified boiled-cell method by Keegan et al. (2005). The pellet was dissolved in 100-500 µl of TE Buffer

(10 mM Tris-HCL pH 8.0 and 1 mM DTA), vortexed and held at 100°C for 10-15 min. The suspension was centrifuged at 10,000-12,000 rpm for 5-10 minutes and 50-200 µl were kept in a freezer at 0-5°C.

The molecular method was used to identify the airborne bacteria and fungi. The complete 16S rDNA gene (bacteria) was amplified by PCR using the primers UFUL-f: 5'-gCC TAA CAC ATg CAA gTC gA-3' and 802-r: 5'-TAC Cag ggT ATC TAA TCC-3'. While 26S rDNA gene (fungi) was amplified using the F63-f: 5'-GCA TAT CAA TAA GCG GAG GAA AAG-3' and LR-r: 5'-GGT CCG TGT TTC AAG ACG G -3'.

The reaction mixture consisted of 2-5 µl DNA template, 0.4 µM dNTP, 0.4 µM each primer, 1xPCR buffer (10 mMKCL, 10 mM $(NH_4)_2SO_4$, 20 mM Tris-HCL, 2 mM $MgSO_2$, 0.1% Triton X-100), 2 mM $MgCL_2$, 1 U Taq DNA Polymerase (BioLab) and deionized water was added to a volume of 20 µl. DNA amplification was performed by initial denaturation at 94°C for 5 min, 30 cycles at 94°C for 30 second (60 sec for fungi), annealing at 55°C for 30 sec (52°C for 60 second for fungi), extension 72°C for 30 sec (120 second for fungi) and final extension at 72°C for 5 min.

The PCR products were purified and checked by electrophoresis on 1.0% agarose gel electrophoresis. The DNA sequences of bacteria used the same primers for bacteria (UFUL-f and 802-r) and for fungi (F63-f). The reaction mixture was 8.0 µl BigDye v3.1, 3-10 ng DNA Template, 3.2 pmol primer and deionized water was added to a volume of 20 µl. The 16rRNA and 26 rRNA sequences were as follows: an initial denaturation at 95°C for 5 min, 30 cycles at 95°C for 30 sec (60 second for fungi), annealing 50°C for 10 sec (30 second for fungi), extension 60°C for 4 min and final extension at 60°C for 4 min.

The 16 rRNA and 26 rRNA products were sequenced by an automated sequence analyzer (3100-Avant genetic analyzer). Sequence associations were determined using the nucleotide-nucleotide BLAST, which has known bacteria and fungi listed in the official databases of the National Centre for Biotechnology Information (http://ncbi.nlm.nih.gov).

2.2.4 Statistical analysis

The descriptive statistics, that included percentage, mean, median, and standard deviation, were used to explain the bioaerosol concentrations and environmental parameters and the data of the other general characteristics. To compare the differences of the bioaerosol concentrations during the morning and afternoon periods, the t-test and Wilcoxon rank test were used to compare the results of normal and skewed distributions, respectively. The general linear model was performed to investigate the association between all variables and indoor bioaerosol concentrations by the R program. A p-value of <0.05 was considered significant.

2.3 Results

2.3.1 General characteristics of ICUs

The main structure of the ICUs was a concrete block building. The MICU was a large area of approximately 360 m^2 with a capacity of 10 beds for medical treatment of severe illnesses. It was located on the fourth floor and was 62 meters from a nearby construction work site and a main traffic road. The MICU area was separated into 2 sections: an infection control area which employed a UVGI system and a non-infection control area. The SICU area consisted of only an infection control area. The SICU was smaller than the MICU. The area of the SICU was about 80 m^2 and it had 10 beds for the care of critically ill surgical patients. The location of the SICU was on the third floor and was 162 meters from the construction site (Figure 2-1). The UVGI system in the SICU operated only when infectious-disease patients were admitted into the ward. The HVAC systems of both MICU and SICU were the same type and the double doors were established to protect the areas from contamination from outdoor bioaerosols. The general characteristics of the two ICUs and the meteorology data of each sampling site are shown in Table 2-1.

All of the bacteria and fungi concentrations, even when the UVGI system was on, were higher in the SICU than in the MICU. The occupant density of the SICU was higher than that of the MICU. When the UVGI system was on, the relative humidity was lower than when it was off. The highest concentrations of outdoor bacteria and fungi were observed at O_c and were lower at O_1 and at O_2.

Table 2-1 Indoor and outdoor general characteristics of ICUs and meteorology data at all sampling sites.

Sampling site	Occupant density (people/m^2)	Cleaning (times/day)	UVGI system	Temperature (^{0}C)	Relative humidity (%)	Air flow velocity (m/s)	Wind direction (%)	Total fungi	Total bacteria
MICU	0.1±0.03	4	no	26.0±1.07	67.0±5.61	0.14±0.03	-	194.25±75.83 (47.56%)	214.22±93.27 (35.82%)
Outdoor 1 (O$_1$)	-	-	no	29.4±1.02	70.5±3.54	0.80±0.73	WSW (50.0)	688.60±80.40 (64.18%)	384.28±170.82 (35.82%)
SICU	0.39±0.05	3	UVGI Off	25.6±0.71	66.7±3.60	0.14±0.06	-	531.41±337.65 (50.78%)	515.12±246.75 (49.22%)
	0.34±0.09		UVGI On	26.2±1.26	57.0±2.27	0.11±0.03	-	234.39±115.60 (46.06%)	274.44±140.75 (53.94%)
Outdoor 2 (O$_2$)	-	-	no	30.5±1.39	64.0±6.43	1.35±0.65	ESE (33.2)	585.39±532.77 (85.84%)	96.58±38.49 (14.16)
Nearby construction site (O$_c$)	-	-	no	29.9±0.07	70.5±0.31	1.24±0.43	WSW (50.0)	2,065.37±234.20 (81.92%)	455.83±134.65 (18.08)

Remark:

Mean and standard deviation (SD) of number determinations are presented

WSW = West-southwest, ESE = East-southeast, % of wind direction represented the most direction on wind in that sampling day.

The occupant density = the number of people in each area/the room area (m^3).

The UVGI concentration = 168.13 µW/cm^2

2.3.2 Size distribution of airborne bacteria and fungi in the ICUs

The total concentrations of airborne fungi and bacteria at the MICU were 194.25±74.83 and 214.22±93.27 cfu/m^3, respectively. The total concentrations of fungi and bacteria at the SICU when the UVGI was on were 234.39±115.60 and 274.44±140.75 cfu/m^3, respectively, and 531.41±337.65 and 515.12±246.75 cfu/m^3, respectively, when the UVGI was off (Table 1-1). UVGI operation for 1 hour and at an average radiation of 168.13 μW/cm^3 decreased the concentration of airborne fungi and bacteria by 55.83% and 46.78%, respectively. There was no significant difference of the bioaerosol concentrations between the morning and afternoon periods except at O$_1$ and the functioning of UVGI at the SICU (Tables 2-2 and 2-3). The size distribution of the fungi (Table 2-2) in the MICU peaked at 1.1-2.1 μm while the size distribution of the fungi in the SICU peaked at 2.1-3.3 μm (UVGI system on and off).

The indoor/outdoor ratio (I/O) that can predict ambient air penetration into the indoor environment in this study showed that most fungi in the ICUs were lower than the outdoor counterparts (I/O <1). The size distribution of bacteria (Table 2-3) in the MICU peaked at 2.1-3.3 μm. However, the size distributions of bacteria in the SICU during the off and on conditions of the UVGI system peaked at 1.1-2.1 μm. In the SICU, the I/O ratios of bacteria were higher (I/O >1) in both situations of UVGI system on or off. However, the I/O ratio of bacteria in the MICU was lower than 1.

The indoor concentrations of the bioaerosols in the ICUs were in the range of 46.06-47.56% for airborne fungi and 52.44-53.94% for airborne bacteria while the outdoor airborne bacteria and fungi concentrations were 14.16-35.82% and 64.18-81.92%, respectively.

2.3.3 Factors related to indoor bioaerosol concentration

The outdoor bioaerosol concentration (cfu/m^3), period of day (morning =1/ afternoon=2), functioning of UVGI system (No=0/yes=1), indoor air velocity (m/s), indoor RH (%), indoor temperature (°C), outdoor air velocity (m/s), outdoor RH (%), outdoor temperature (°C), and number of people in each period of the day were the factors used to determine the effects on the indoor bioaerosol concentrations (cfu/m^3). Finally two equations were used to predict the indoor bioaerosol concentrations in this

study. The prediction calculation for the total bacteria colony count in the SICU was done based on significant parameters by the following equation:

(1) Y = 1114.93 - 304.40 (UVGI, on, off) - 4556.22 (indoor velocity, m/s)

Remark: where Y = total bacteria (cfu/m^3).

However, there were no significant parameters to predict the total colony count of bacteria in the MICU.

The prediction calculation for the total fungi colonies in SICU employed the following equation:

(2) Y = - 6501.84 + 0.43 (total outdoor fungi, cfu/m^3) − 5236.58 (indoor velocity, m/s) + 75.61 (indoor relative humidity, %) + 110.71 (indoor temperature, ^{o}C)

Remark: where Y = (total fungi, cfu/m^3).

Likewise, there were no significant parameters to predict the total number of fungi colonies in MICU.

The total concentration of indoor bacteria depended on UVGI usage (on or off). The bioaerosol concentrations during UVGI-off decreased when the room air velocity increased, whereas the outdoor fungi concentrations, indoor air flow velocity, relative humidity and temperature significantly influenced the indoor fungi concentrations in the SICU.

Table 2-2 Fungi concentrations (cfu/m^3) in different size ranges at all sampling points.

Stage No. (size range, μm)	MICU (n=12; 12)		O1 (n=4;4)		SICU					
					UVGI Off (n=6; 6)		UVGI On (n=6; 6)		O2 (n= 4; 4)	
	Morning	Afternoon	Morning	Afternoon	Morning	Afternoon	Morning	Afternoon	Morning	Afternoon
1 (>7)	11.19±5.20	10.69±5.48	40.64±7.50	25.61±1.25	21.20±14.13	24.74±15.40	31.80±22.07	15.31±10.20	37.68±27.45	42.40±19.67
2 (4.7-7)	12.37±5.81	17.08±11.12	36.10±22.49	21.79±14.16	42.40±15.46*	32.78±10.03	23.56±22.72	15.31±10.20	30.98±26.76	54.18±38.92
3 (3.3-4.7)	31.80±20.48	20.51±10.81	113.07±14.99	113.37±35.40	74.20±54.39*	87.75±25.75	43.58±42.16	41.22±17.43	74.20±56.09	209.66±215.09
4 (2.1-3.3)	66.54±49.29	62.23±40.78	337.46±52.47	326.86±112.44	213.19±202.69*	153.12±56.35	67.14±42.99	81.27±15.40	148.41±159.17	259.13±276.44
5 (1.1-2.1)	64.78±29.87	76.95±34.04	167.84±22.49	182.27±37.06	212.01±257.27**	176.48±59.78	73.03±50.01	58.89±7.36	109.54±93.69	179.34±169.48
6 (0.65-1.1)	6.48±6.86**	7.85±8.28*	3.53±4.99	7.66±5.83	12.69±8.16*	11.79±7.46	8.24±4.08	9.42±2.04	9.42±2.04	14.13±10.60
Total	193.17±89.24	195±66.0	699.65±64.96	677.56±121.18	576.97±517.53*	487.85±105.94	247.35±174.87	221.47±48.23	412.45±337.68	758.54±711.09
P-value	>0.05+		>0.05+		>0.05+		>0.05++		>0.05+	

Remark: Mean and its standard deviation (SD) of number determinations are presented

* means I/O > 1 which the indoor air was contaminated with the microorganisms.

** means I/O > 1.5 which the indoor air was contaminated with the microorganisms.

+ Wilcoxon rank sum test   ++t-test

Table 2-3 Bacteria concentrations (cfu/m^3) in different size ranges at all sampling points

Stage No. (size range, μm)	MICU (n=12; 12)		O1 (n=4;4)		SICU					
					UVGI Off (n=6; 6)		UVGI On (n=6; 6)		O2 (n= 4; 4)	
	Morning	Afternoon	Morning	Afternoon	Morning	Afternoon	Morning	Afternoon	Morning	Afternoon
1 (>7)	47.11±20.44	29.64±18.84*	50.65±44.18	22.37±1.67	68.32±30.05**	45.15±15.99**	31.80±16.19**	38.86±9.35**	17.67±9.35	14.13±9.35
2 (4.7-7)	34.16±21.97	22.48±6.37	42.40±410.75	25.91±3.33	88.34±76.69**	51.63±35.53**	38.87±12.74**	25.91±7.36**	10.60±6.12	10.60±9.35
3 (3.3-4.7)	50.06±18.75	27.36±7.14*	97.76±92.44	24.74±9.99	103.65±57.12**	73.81±37.18**	71.85±5.40**	29.45±5.40*	21.20±10.60	21.20±22.07
4 (2.1-3.3)	55.36±22.86	42.70±29.70	86.98±74.48	58.01±27.90	128.39±56.13**	123.09±64.00**	94.23±73.30**	36.5±20.1**	24.74±10.60	20.02±15.93
5 (1.1-2.1)	55.36±37.90*	38.48±21.74	45.94±42.84	96.88±82.87	173.14±99.51**	122.30±47.23**	88.34±49.47**	56.5±55.2**	17.67±9.35	21.20±7.07
6 (0.65-1.1)	14.13±8.94*	11.58±10.03	12.96±12.41	37.10±52.47	30.62±13.38**	21.79±4.45**	21.20±10.60**	15.31±7.36**	9.42±5.40	4.71±2.04
Total	256.18±99.41	172.26±70.92	503.53±7.50	265.02±174.90	592.46±313.12**	438.78±190.33**	346.29±166.53**	202.59±79.43**	101.30±10.20	91.87±59.44
P-value	>0.05$^+$		<0.05$^+$		>0.05^{++}		<0.05^{++}		>0.05^{++}	

Remark: Mean and its standard deviation (SD) of number determinations are presented

* means I/O > 1 which the indoor air was contaminated with the microorganisms.

** means I/O > 1.5 which the indoor air was contaminated with the microorganisms.

$^+$ Wilcoxon rank sum test $^{++}$t-test

2.3.4 The predominant and size distributions isolated from the construction site and prevalent bioaerosols in the ICUs

The prevalences of bacteria and fungi were analyzed using PCR. The indoor sites at both ICUs found a similar predominance of airborne fungi. The most predominant fungi genera in the ICUs were *Cladosporium* spp. (64-85 cfu/m^3), *Penicillium* spp. (42-78 cfu/m^3), *Aspergillus* spp. (14-42 cfu/m^3) and *Fusarium* spp. (14-21 cfu/m^3). Moreover, the percentages of yeast species of indoor and outdoor air were found in the range of 1.09-3.20% of total fungi. In addition, the predominant indoor airborne bacteria in both ICUs were *Staphylococcus* spp. (106-205 cfu/m^3), *Micrococcus* spp. (57-78 cfu/m^3), *Pseudomonas* spp. (21-42 cfu/m^3) and *Bacillus* spp. (14-35 cfu/m^3) (Table 2-4). When the UVGI was turned on, the predominant airborne bacteria and fungi in the S-ICU decreased in concentrations.

Table 2-4 Isolated indoor airborne fungi and bacteria in ICUs (cfu/m^3)

Predominant of Bioaerosol	MICU	SICU	
		UVGI off	UVGI on
Fungi (cfu/m^3)			
Cladosporium spp.	78	85	64
Penicillium spp.	64	78	42
Aspergillus spp.	14	42	21
Fusaruim spp.	14	21	14
Bacteria (cfu/m^3)			
Staphylococcus spp.	106	205	120
Micrococcus spp.	64	78	57
Pseudomonas spp.	21	42	35
Bacillus spp.	14	35	28

The size distributions of each prevalent bacteria and fungi are shown in Figure 2-2. The 4 predominant bacteria, *Staphylococcus* spp. and *Micrococcus* spp. were found to be the highest at stage 4 (2.1-3.3μm), while *Pseudomonas* spp. peaked at stage 5 (1.1-2.1μm) and *Bacillus* spp. peaked at stage 1 (>7 μm).

The predominant fungi were *Penicillium* spp., *Cladosporium* spp., *Aspergillus* spp., and *Fusarium* spp. The *Cladosporium* spp., *Aspergillus* spp., and *Penicillium* spp., also showed peaks at the same stage 4 (2.1-3.3 μm) while *Fusarium* spp. showed a peak at stage 1 (>7 μm). *Aspergillus fumigatus* and *Aspergillus flavus* were found at O_c and at O_1, while at O_2 only *Aspergillus fumigatus* was found. However, those species were not found at the indoors of either the MICU or SICU.
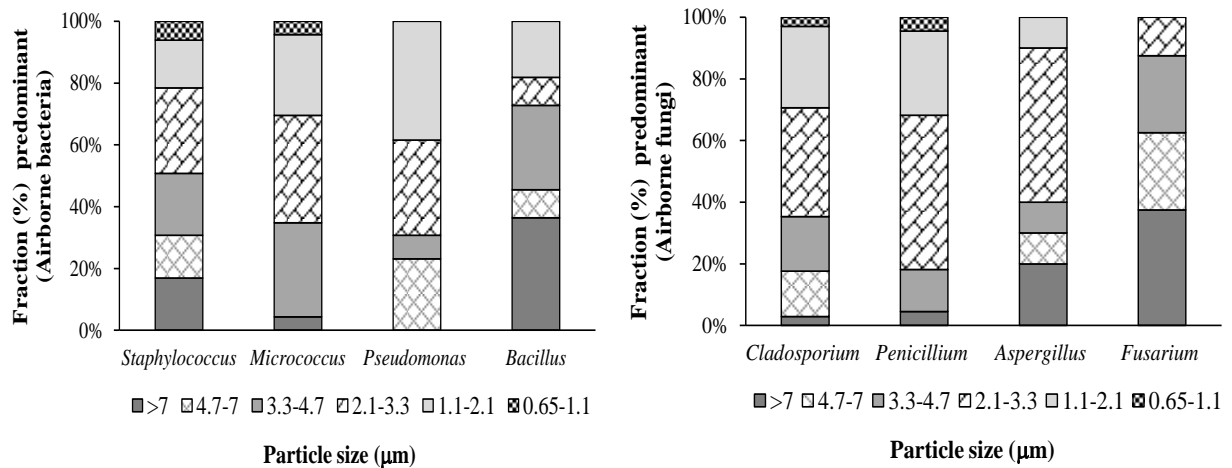
Figure 2-2 Size distribution of predominant bioaerosols at the ICUs.

2.4 Discussion

2.4.1 Indoor bioaerosol characteristics and size distributions

The difference of bioaerosol outdoor concentrations may be due to a better air flow (1.35±0.65 m/s with an ESE direction) at O_2. At O_1 and at O_c, a lower air flow (0.80±0.73-1.24±0.43 m/s) with a WSW direction passed though the construction site. Therefore the outdoor bioaerosol concentrations with lower air flow velocity at O_c and O_1 were higher than O_1 and O_2. The higher bioaerosol concentrations in the SICU probably occurred from the limited space (80 m^2) which could not separate the SICU area into infection and non-infection areas as in the MICU (320 m^2). The activities of surgical treatment, such as wound care dressing and wound debridement, have the potential to spread bioaerosols into the environment (Pegues et al., 2002). The bioaerosols in the areas of high occupant density and human activity showed slightly different proportions between the airborne bacteria and fungi. This conforms to the

Rajasker and Balaubramanian (2011) study which found the indoor bacteria and fungi at 50.5% and 49.50%, respectively.

Normally, the aerodynamic diameters of indoor bacteria in the clean environment ranged from 1 to 3 μm while indoor fungal spores ranged from 2 to 4 μm (Baron and Willeke, 2005). All of the bioaerosol size diameters were related to particles that are inhalable and susceptible to deposit in the respiratory tract. These bioaerosols might be generated from droplet nuclei and infectious aerosols which were smaller than 2 and 5 μm, respectively (Pegues et al., 2002; Haddad et al., 2004; Kim and Kim, 2007; Kim et al., 2010; Mandal and Brandl 2011). The size distributions of fungi were similar to the results found in ambient fungi in the Wang et al. (2010) study in China and in the study by Lin and Li (2000). In contrast, the results differed from what the Kim et al. (2010) study (i.e., the size distribution of fungi peaked in a range >7 μm). However, different peaks of each microorganism especially in the SICU possibly was influenced by the UVGI system which controlled not only *Mycobacterium tuberculosis* (Reed, 2010; Yau et al., 2011) but also other microorganisms (Table 2-2 and 2-3)

There was little impact of the ambient air, especially from the construction site, that penetrated the MICU (I/O <1) and these ratios were also lower than the I/O ratio (2.1 times) reported by Kim et al. (2010). The size distributions of bacteria were consistent with that of the Kim et al. (2010) and Wang et al. (2010) studies. From the wind direction in Figure 2-1, there was no influence of the construction site on the reference outdoor sampling for the SICU (O_2) and there was a very low bacteria concentration (96.60±38.50 cfu/m^3). Therefore, the ratios of bacteria in the SICU, while the UVGI system was either turned on or off, were higher than the outdoor ratios (I/O>1) which was higher than the Kim et al. (2010) study by about 2 and 4 times, respectively. However, the I/O ratio of bacteria in the MICU was lower than 1. Perhaps there was some contamination in the indoor environment that was due to the limitation of space in the SICU, occupant density and the low efficiency of the HVAC system (Kim and Kim 2010; Mandal and Brandl, 2011). However, Pastuszka et al. (2005) found that a room with an HVAC had a high level of bacteria size distribution in the range of 3.3-4.7, respectively.

2.4.2 Factors related to indoor bioaerosol concentration

The total concentration of indoor bacteria colonies depended on UVGI usage (turn on and off) and room air flow velocity. The UVGI system was established in hospital areas to control infectious agents such as *Mycobacterium tuberculosis* (Reed, 2010; Yau et al., 2011). However, the efficiency of the UVGI system depends on the relative humidity and room air circulation (Lin and Li, 2000). A relative humidity between 30-60% can increase the UVGI efficiency. Moreover, indoor air movement increased the contact between the bioaerosols and the upper room UVGI system, and decreased the indoor bioaerosol concentration (NIOSH, 2009; Memarzadeh et al., 2010). There should be a focus on monthly or quarterly cleaning of the UVGI systems in the problem areas to remove the accumulated dust. Monitoring the performance of the UVGI systems is also important to reduce the levels of bioaerosols because the performance of UVGI lamps decline at 20% per 9000 hours (NIOSH, 2009).

The appropriate effectiveness of a UVGI system with 42 ± 19 μW/cm^2 upper-zone irradiance were at the conditions of 50% RH, room size around 87 m^2 and 6 air changes per hour (CDC, 2009). The outdoor fungi concentration, indoor air velocity, relative humidity and temperature significantly influenced the indoor fungi concentrations in the SICU. An increase in the indoor air velocity resulted in a decrease of indoor fungi concentrations. Even if the HVAC had a high-efficiency particulate absorption (HEPA) filter installed in the ICU, extended use of the HEPA filter, the location of the ward doors (Yau et al., 2011), and anthropogenic sources might have an influence on the levels of indoor ICU fungi. The SICU had 2 conditions of UVGI on and off while the MICU had only one condition. While the UVGI was functioning the indoor velocity and relative humidity were low and the indoor bioaerosols in the SICU had decreased by 2 times (Tables 2-1, 2-2 and 2-3).

2.4.3 Predominant bioaerosols

Staphylococcus spp., *Micrococcus* spp., *Bacillus* spp., and *Pseudomonas* spp. were the common bacteria found in this study and in other hospitals (Kim et al., 2010; Memarzadeh et al., 2010). *Staphylococcus* spp. and *Micrococcus* spp. are common skin and respiratory flora. Therefore, they are found easily in indoor environments

especially in areas of high occupant density and human activity (Górny et al., 1999; Pastuszka et al., 2005; Kim et al., 2010). Among these predominant types of bacteria, *Pseudomonas* spp. is a gram negative bacterium and an endotoxin agent .The sources of these bacteria in the hospital environment can be found from water leakage, sink drains, tap water and secretion of patients (Boyer et al., 2011). *Pseudomonas* spp. are hardy survivors in a wide variety of antiseptics (Wunderink and Mendoza, 2005).and for this reason they can be found in the indoor environment of ICUs.

      Bacillus spp. presented as the fourth most common indoor airborne bacteria which was similar to the studies of Kim and Kim (2007) and Kim et al. (2010) which were done in public buildings. However, these results were different from Gotofit-Szymczak and Górny (2010) who found that *Bacillus* spp. were the second most common of the predominant genera in office buildings. *Bacillus* spp. form resistant endospores which survived in chemical disinfectant conditions and in the harsh condition of ultraviolet-C radiation (Setlow, 2006; Gotofit-Szymczak and Górny, 2010).

      The sources of these bacteria in a hospital environment can be from tap water leakage and sink drains. Occupant density and preparation of patient beds should be of great concern in order to avoid infections (Rajasekar and Balasubramanian, 2011; Gribert et al., 2010). The predominance as percentages of airborne bacteria was different between the indoor and outdoor sites which was similar to those of Kim and Kim (2010). *Staphylococcus* spp. had the highest concentrations in the indoor air of a public building (Pastuszha et al., 2005; Kim and Kim, 2007; Yagoub and Agbash, 2010), while *Micrococcus* spp. had the highest concentration in the outdoor air (Kim and Kim, 2007; Harper et al., 2013).

      These results were different from the studies of Kim and Kim (2007) and Kim et al. (2010) which found that *Staphylococcus* spp. and *Micrococcus* spp. were identified most often at stage 4 (2.1-3.3 μm), *Pseudomonas* spp. was found at stage 5 (1.1-2.1 μm) and *Bacillus* spp. were found at stage 1 (>7 μm). The sources of these bacteria in the hospital were from human activities and environmental conditions such as talking, coughing, breathing, making beds, tap water, and building materials which should be more of a concern in order to lower their concentrations and avoid infections (Gilbert et al., 2010; Balasubramanian et al., 2011).

The predominant fungi were *Penicillium* spp., *Cladosporium* spp., *Aspergillus* spp. and *Fusarium* spp. These findings were similar to other studies (Lee and Jo, 2006; Kim et al., 2010; Abdel Hameed et al., 2012; Sepahvand et al., 2013). Moreover, the percentages of yeast genera in this study were similar to the Sepanand et al. (2012) study that found 0.7-1.6% yeast in the indoor and outdoor environments of an ICU. *Aspergillus* spp. and *Fusarium* spp. can infect immunocompromised patients who have undergone surgery or bone marrow transplants and cancer patients **(**Krcmery et al., 1997; Lutz et al., 2003; Grow et al., 2002; Nucci and Anaissie, 2007**)**. These fungi showed a peak at stage 4 (2.1-3.3 µm) while *Fusarium* spp. peaked at stage 1 (>7 µm). However, *Cladosporium* spp. and *Penicillium* spp. also showed a peak at stage 4 (2.1-3.3 µm).

These results were in contrast to the Kim et al. (2010) studies that found *Penicillium* spp. and *Cladosporium* spp. were at both stage 1 (>7 µm) and at stage 3 (3.3-4.7 µm). These fungi genera are commonly found on the surfaces of armrests, beds, sinks, tables and medical devices. Outdoor airborne fungi and their spores can spread easily into indoor environments (Jones and Harrison, 2004; Kumar et al., 2011; Garcia-Cruz et al., 2012). Allergies, inflammation and infections from these fungi genera are of considerable concern, especially for low-immunity patients with respiratory allergic symptoms and allergen sensitization (Cooley et al., 1998; Bornehag et al., 2001; Burge 2002; Lugauskas and Krikštaponis, 2004). In addition, there were no threshold limit values or cut-off levels for interpreting environmental measurements of bioaerosols for health and safety levels (ACGIH, 2014), but the results of Vonberg and Gastmeier (2006), who reviewed nosocomial aspergillosis, found that *Aspergillus* spp. below a concentration of 1 cfu/m^3 can be enough to infect immunocompromised patients. Implementation of a program to control airborne infection should be based on the findings of this study that showed the predominant bioaerosols were of respirable size with peaks of 2.1-3.3 µm. Only an HVAC system with an HEPA filter might not be enough to filter particulate matter smaller than 3 µm. In particular, wet areas that serve as anthropogenic sources, air changes per hour, and indoor air velocity should be observed annually and continuously monitored.

2.5 Conclusions

The sizes of the bioaerosols in both ICUs were of respirable size. The MICU used a split HVAC system to control room temperature and the occupant density was lower; therefore, the airborne bacteria and fungi concentrations were lower than in the SICU. In the SICU, the central HVAC system with a HEPA filter and UVGI system were not enough to control the bioaerosols. The prevalent indoor airborne bacteria in both ICUs were *Staphylococcus* spp., *Micrococcus* spp., *Pseudomonas* spp. and *Bacillus* spp., and the prevalent indoor airborne fungi were *Cladosporium* spp., *Penicillium* spp., *Aspergillus* spp. and *Fusarium* spp. The concentrations and size distributions of the bioaerosols in the ICUs were influenced by the bioaerosol species, location of sampling, outdoor environment such as building construction sites, indoor environment, HVAC with HEPA, UVGI, anthropogenic sources, and the outdoor environment such as buildings that were under construction and meteorological factors such as air flow velocity, relative humidity and temperature. To decrease the indoor bioaerosol concentrations, room air velocity should be increased and UVGI systems should be installed in the limited spaces of the ICUs. In particular, wet areas, air changes per hour and indoor air velocity should be continuously observed and monitored.

CHAPTER 3

Bacterial and fungi aerosol characteristics of the environment in the service areas of a university hospital

Abstract

This study investigates the characteristics and respirable fractions of indoor bioaerosols and the influence of environmental factors on bioaerosol concentrations in the outpatient and inpatient department (OPD and IPD) service areas of a university hospital. A total of 336 bioaerosol samples (2,016 plates) were collected by a six-stage viable cascade impactor in the OPDs and IPDs during the dry and wet seasons. The bioaerosol types were identified by standard methods. The environmental parameters were recorded during the bioaerosol sampling periods. The average concentrations of bacteria at the OPDs in the dry and wet seasons were 853.06 ± 407.35 cfu/m^3 and 855.86 ± 423.12 cfu/m^3 and 750.15 ± 454.27 cfu/m^3 and 747.20 ± 309.60 cfu/m^3 for airborne fungi, respectively. The concentrations at the IPDs in the dry and wet seasons were 334.22 ± 194.90 cfu/m^3 and 160.63 ± 78.45 cfu/m^3 for airborne bacteria and 770.61 ± 659.32 cfu/m^3 and 886.04 ± 671.18 cfu/m^3 for airborne fungi, respectively. The indoor air of the OPDs was contaminated by higher bioaerosol concentrations than in the IPDs. The dominant airborne bacteria identified in the wet and dry seasons were *Staphylococcus* spp., *Micrococcus* spp., *Corynebacterium* spp., and *Bacillus* spp., while *Cladosporium* spp., *Penicillium* spp., *Aspergillus* spp., and *Fusarium* spp. were the predominant airborne fungi. Most of them were detected in stage 4 (2.1-3.3 µm). The respiratory fractions (RFs) of bioaerosols were more than 59% in both seasons for the OPDs and IPDs. The time of service, CO_2, indoor relative humidity, indoor temperature, outdoor bacteria and rainfall were significant factors on indoor bacteria levels whereas indoor fungi levels were significantly influenced by outdoor fungi, indoor relative humidity, and wind direction. The seasons in the humid tropical environment affected the indoor airborne bacteria concentrations in the IPDs but not the OPDs. However, airborne fungi concentrations in both seasons were not different while the RFs were similar in all areas studied.

Keywords: Airborne bacteria; airborne fungi; season; service area; hospital

3.1 Introduction

Cross-contamination of airborne microbes suspended in the indoor air of hospitals among humans affects the patients, relatives of patients, and the medical staff. It is a cause of respiratory tract disease and increases health risks such as nosocomial infection, asthma, allergies, and sick building syndrome (Tang et al., 2006; Knutsen et al., 2012). Therefore, an indoor air quality management program has to be established and strictly monitored to reduce the risks to public health.

The majority of hospitals in Thailand use dilution ventilation systems to reduce air pollutants and the accumulation of bioaerosols in the buildings. However, the effectiveness is affected by several factors, such as temperature, relative humidity, CO_2, air velocity, rainfall, and outdoor air quality and seasonal variations (Chao et al., 2002; Kim et al., 2009; Qudiesat et al., 2009; Wang et al., 2010a; Heo et al., 2014). In addition, the environmental conditions and types of bioaerosols were also related to the aerodynamic diameters of airborne bacteria and fungi (Kim and Kim, 2007). Small particles are able to travel into the pulmonary system and affect human health. Therefore, it is important to establish indoor air quality management programs especially in large hospitals that provide primary to tertiary care (Leung and Chan, 2006). Previous studies indicated that the nosocomial infection rates in university hospitals in Thailand showed higher rates of infection (7.3-7.6%) than in provincial and regional hospitals (4.9-6.0%) (Danchaivijitr et al., 2005; Danchaivijitr et al., 2007).

The aim of this study is to investigate the seasonal variations and bioaerosol characteristics during two seasons in tropical humid weather. The relationship between environmental factors and the bioaerosol levels was also determined. The results of this study can provide fundamental information for indoor air quality improvement in university hospitals that are located in the humid tropical zone of Thailand and use dilution ventilation systems.

3.2 Materials and methods

3.2.1 Site description

The study site was a university hospital located in southern Thailand. The hospital has 853 beds and over 2,000 staff employees and provides primary to tertiary care treatment. The location of the hospital is close to a main road in front of a hill surrounded by greenery. The hospital has a humid tropical climate. The annual average temperature was 27.6°C with a range of $25.9\text{-}30.0^{\circ}$C. The seasons in this area were classified by the amount of rainfall to be dry and wet seasons. During the dry season (February to mid-May), the average rainfall is 100.1 mm with a range of 2.6-287.19 mm. The average amount of rainfall in the wet season (mid-May to January) is 248.8 mm with a range of 29.4-924.0 mm.

3.2.2 Air sampling site

Air sampling was done in service areas that used dilution ventilation systems. These service areas, that included the Medical Outpatient Department (M-OPD), Eye Outpatient Department (E-OPD), Medical Inpatient Department (M-IPD) and Surgical Inpatient Department (S-IPD), had high occupant densities. All OPDs were located on the first floor of the main building with an open space next to the corridor where the floor areas were approximately 430 m^2/site (Figure 3-1). Ultraviolet germicidal irradiation systems were used to reduce tuberculosis in the E-OPD and M-OPD areas with average power densities of 986 $\mu W/cm^2$ and 518 $\mu W/cm^2$, respectively. The IPDs were located on the 5th and 9th floors of a surgical and medical building that were about 700 m^2/site. Ceiling fans were installed between the patient beds and the nurse's station. The windows were open all the time and protected by mosquito screens. Air samples were collected in the middle of the working areas near the nurse's station. Three outdoor bioaerosol samples were collected: outside of the Outpatient Department, Medical Inpatient Department, and Surgical Inpatient Department. The samplings at the outdoor sites were performed on the same days as the indoor air sampling.
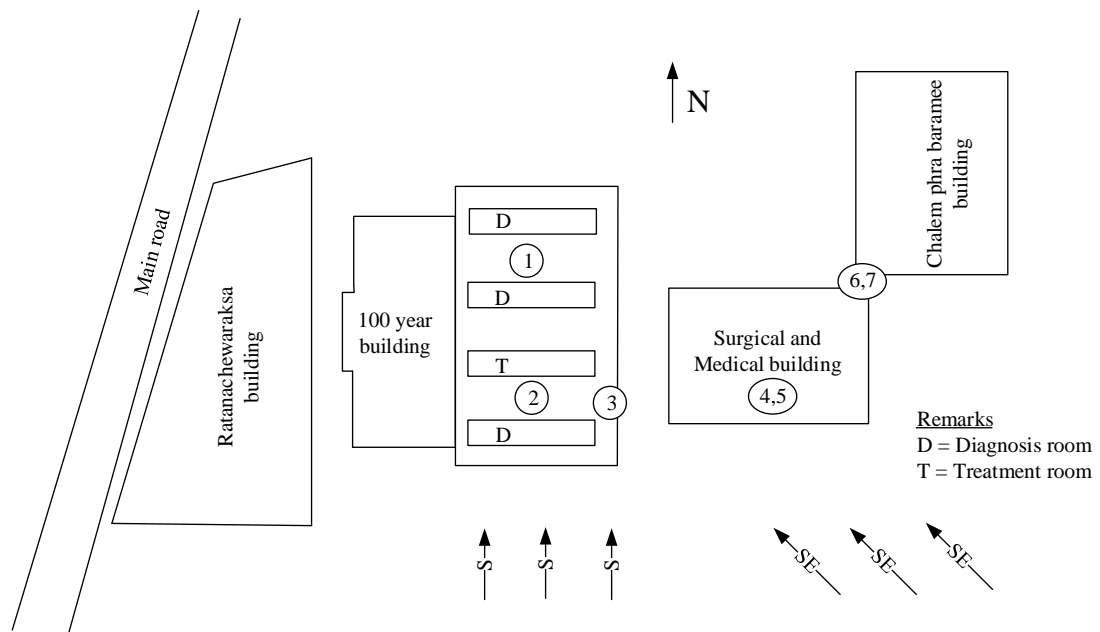
No. 1 = M-OPD; No. 2 = E-OPD; No. 3 = Outdoor reference for OPD; No. 4 = M-IPD; No. 5 = S-IPD; No. 6 = Outdoor reference for M-IPD; No. 7 = Outdoor reference for S-IPD; S = Wind direction from South at OPDs (27%); SE = Wind direction from Southwest at IPDs (41%).

Figure 3-1 Sampling locations at the M-OPD, E-OPD, M-IPD and S-IPD.

3.2.3 Sampling times

The air samplings were carried out from February to April (dry season) and from October to December (wet season). These periods were selected because they were the peak period of low rainfall, relative humidity, and highest temperature in the dry season and the peak period of high rainfall, relative humidity, and the lowest temperature in the wet season.

The air sampling at each investigation site included four points at the four indoor sites and three points at the outdoor sites. The samplings were performed 8 times once every two weeks for six months. Two sets of samples, in the morning (09:00-12:00) and in the afternoon (13:00-16:00), were obtained on each visiting day. Each set of samples at each sampling site contained 12 plates (6 plates for bacteria analysis and 6 plates for fungi analysis) and the samplings were done in duplicate. All sites were visited on the same day. The total number of samples was 336 (2,016

plates) which included 192 samples (1,152 plates) from indoor sites and 144 samples (864 plates) from outdoor sites.

   3.2.4 Airborne bacteria and fungi measurement

   The distribution of the particle sizes was determined using a six-stage viable cascade impactor (Model 10-800, Andersen Inc, USA) which had six ranges of aerodynamic diameters: stage 1 (>7 µm), stage 2 (4.7-7.0 µm), stage 3 (3.3-4.7µm), stage 4 (2.1-3.3µm), stage 5 (1.1-2.1µm) and stage 6 (0.65-1.1µm). Before conducting the sampling, the cascade impactor was sterilized with 70% alcohol to prevent external contamination. The air samples were collected at 1.5 m above the floor (respirable zone) with an adjusted flow rate of 28.3 L/min for 5 min. The flow rate was calibrated at intervals and kept constant during each sampling. The time of sampling was obtained from optimization. A trypticase soy agar medium was used for bacterial cultures and incubated at 37°C for 1-2 days. A malt extract agar medium was used for fungal cultures and incubated at 25°C for 3-5 days. The culture temperature was set at the preferable temperature of most mesophilic airborne microbes. The concentrations of airborne bacteria and fungi were expressed as colony forming units per cubic meter (cfu/m^3) by the following formula:

$$\text{cfu/m}^3 = \frac{\text{colony forming count on agar plate (stages 1-6) x } 10^{-3}}{\text{sampling flow rate (L/min) x sampling times (min)}}$$

   Respirable fraction concentration of bioaerosols:
   = Sum of airborne bacteria or fungi concentration at stages 3, 4, 5, and 6

   3.2.5 Identification of airborne bacteria and fungi

   The pure cultures of airborne bacteria were classified by Bergey's manual (Buchanan et al., 1974), Gram stain technique, and biochemical test, whereas pure cultures of fungi were classified by form, spore, shape and color of the colony on a slide by optical microscope (St-Germain and Dummerbell, 2011).

3.2.6 Environmental factor measurements

The environmental parameters that included temperature, relative humidity, air velocity, and CO_2 were collected during airborne microorganism samplings at the same sampling height. The temperature, relative humidity, and air velocity were measured using a VelociCalc® Air Velocity Meter (TSI Alnor, Model 9555, USA). The level of CO_2 was monitored by a Gasmet™ FTIR gas analyzer (Gasmet Technologies, Model DX4000, Finland). The number of people in the sampling area, including patients, medical staff employees, and relatives of patients were counted during the sampling time. In addition, the wind speed, wind direction, and rainfall data were from the Khohong agrometeorological station in Songkhla Province.

3.2.7 Data analysis

The Wilcoxon sign-ranked test was used to test the statistical differences of airborne bacteria and fungi concentrations, and environmental factors between dry and wet seasons. The Mann Whitney U-test was used to compare the concentration of bioaerosols between indoor and outdoor sites and the concentrations of bioaerosols in the OPDs and IPDs. However, the results of the bacteria and fungi levels were transformed using base 10 logarithms and natural logs to approximate normality in multiple regression analysis. Multiple regressions were used to test the relationships within the indoor areas between the quantities of bacteria and fungi and the environmental factors in the OPDs and IPDs.

3.3 Results

3.3.1 Seasonal concentration distribution of airborne bacteria and fungi

Table 3-1 presents the concentrations of bioaerosol and environmental parameters detected at the sampling sites. The indoor and outdoor airborne bacteria concentrations in the dry season at the IPD sites were significantly higher than those during the wet season ($p<0.05$). The airborne fungi concentrations in the dry season were similar to the wet season ($p>0.05$). However, the airborne bacteria and fungi showed a difference between the indoor and outdoor concentrations ($p<0.05$). Accordingly, the indoor/outdoor ratio (I/O) can predict the ambient air penetration into the indoor environment to investigate the contamination of indoor air (I/O >1). In

this study, the I/O ratio values at all sites investigated exceeded 1.0 but the I/O ratios of bacteria were obviously higher than those for fungi. Moreover, the respirable fractions (RFs) of airborne bacteria and fungi also showed similar values in the OPDs and IPDs (p>0.05) and were not significantly different between the seasons at all sites (p>0.05). The RFs of bacteria and fungi were 59.98–64.26% and 84.14–86.28%, respectively. The proportion of indoor airborne bacteria and fungi in both seasons in OPDs was 53.21-53.39% and 46.16-46.79% while in IPDs were 15.32-30.25% and 69.75-84.68%, respectively.

Table 3-1 Levels of bioaerosol concentrations and environment parameters in the OPDs and IPDs of a university hospital

Data	OPDs		P-value	IPDs		P-value
	Dry (N=48)	Wet (N=48)		Dry (N=48)	Wet (N=48)	
Indoor bacteria						
Total (cfu/m^3)	853.06±407.35	855.86±423.12	>0.05	334.22±194.90	160.63±78.45	<0.05*
I/O[a]	2.13	2.59		2.21	2.92	
Res.[b] (cfu/m^3)	514.72±250.23	523.70±280.60	>0.05	216.43±138.83	160.63±78.45	<0.05*
RF[c] (%)	60.62±8.08	59.98±10.59	>0.05	64.26±10.14	61.94±12.17	>0.05
Indoor fungi						
Total (cfu/m^3)	750.15±454.27	747.20±309.60	>0.05	770.61±659.32	886.04±671.18	>0.05
I/O[a]	1.18	1.22		1.30	1.18	
Res.[b] (cfu/m^3)	639.87±424.14	629.71±249.87	>0.05	666.67±563.58	760.01±594.16	>0.05
RF[c] (%)	84.14±5.98	84.76±5.20	>0.05	86.28±5.46	85.32±6.33	>0.05
Indoor factors						
Occupancy level (Persons/m^3)	0.34±0.29	0.44±0.35	<0.05*	0.09±0.02	0.10±0.02	<0.05*
CO$_2$ (ppm)	495.99±31.31	520.92±56.70	<0.05*	470.96±26.13	451.06±18.76	<0.05*
Temperature (°C)	29.55±0.85	28.81±0.89	<0.05*	29.65±1.09	29.19±1.30	>0.05
Relative humidity (%)	69.04±4.33	73.77±3.96	<0.05*	67.73±6.56	72.61±7.44	<0.05*
Air velocity (m/s)	0.28±0.06	0.30±0.08	>0.05	0.18±0.07	0.17±0.07	>0.05
Outdoor factors	Dry (N=24)	Wet (N=24)				
Bacteria (cfu/m^3)	399.59±146.15	330.09±202.94	>0.05	150.62±84.61	55.06±34.96	<0.05*
Fungi (cfu/m^3)	603.95±290.11	608.36±233.29	>0.05	589.96±351.00	749.26±527.59	>0.05
Temperature (°C)	30.35±1.08	29.25±1.01	<0.05*	30.84±2.00	29.22±1.92	<0.05*
Relative humidity (%)	67.31±5.32	72.65±4.85	<0.05*	63.52±7.79	72.15±9.91	<0.05*
Air velocity (m/s)	0.29±0.08	0.26±0.08	>0.05	1.41±0.57	1.42±0.55	>0.05
Wind speed	5.72±3.86	5.96±3.41	>0.05	4.91±3.48	3.61±2.68	<0.05*
Rainfall (mm)	0.23±0.53	3.52±1.37	<0.05*	11.87±26.07	3.80±5.14	>0.05
Proportion (%) (bacteria: fungi)	53.21:46.79	53.39:46.61	-	30.25:69.75	15.32:84.68	-

[a] I/O ratio = indoor airborne concentration/outdoor airborne concentration.

[b] Resp. = respirable concentration or sum of bioaerosols quantity measured at stages 3-6.

[c] RF = respirable fraction, percentage of respirable concentration/total concentration

Mean and its standard derivation (SD) of number determinations are presented.

Significant at p<0.05* (Wilcoxon rank sum test)

3.3.2 Daily variation of airborne bacteria and fungi concentration

A graph of daily airborne bacteria and fungi concentrations in the OPDs and IPDs is presented in Figure 3-2. The indoor airborne bacteria concentrations at the OPDs were significantly higher in the morning than in the afternoon ($p < 0.05$) while the IPDs did not have a variation between morning and afternoon service periods ($p > 0.05$). The OPDs showed a higher occupant density in the morning than in the afternoon ($p < 0.05$). On the other hand, the average concentrations of airborne fungi in the OPDs and IPDs were not significantly different between the morning and afternoon periods ($p > 0.05$).
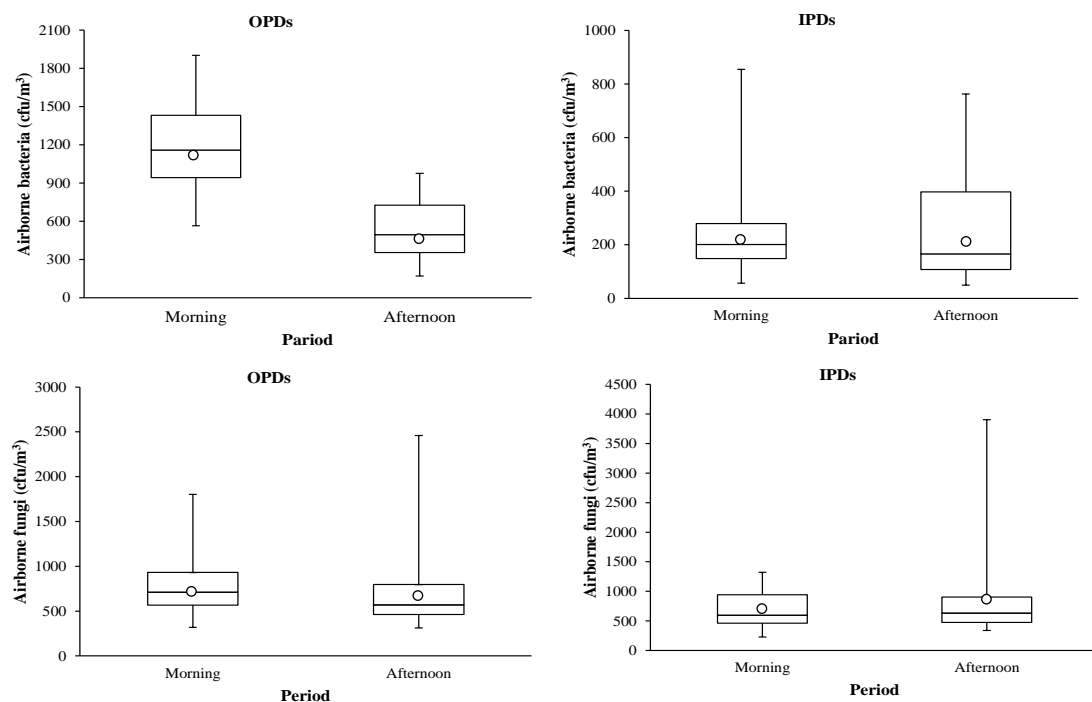
Box and whisker plots of airborne bacteria and fungi concentration between the morning and afternoon periods; the box frames represent the upper and lower quartiles, the lines represent the median, whiskers represent the upper and lower of the range and the O = Represent mean.

Figure 3-2   Daily variation of airborne bacteria and fungi concentrations.

3.3.3 Relationship between bioaerosols and environmental factors

Table 3-2 presents the relationship between each environmental factor and the geometric indoor bioaerosol levels in the OPDs and IPDs. The results showed that the time of service period, CO_2, indoor temperature and indoor relative humidity had an

impact on the indoor bacteria levels (R^2=0.679), while the concentrations of outdoor airborne fungi were the only factors significantly related to the indoor fungi concentrations in the OPDs (R^2=0.512). In addition, the CO_2, indoor temperature, indoor relative humidity, rainfall and outdoor bacteria were important environmental factors related to indoor airborne bacteria (R^2=0.555) whereas the indoor relative humidity, wind direction, and outdoor fungi impacted the indoor fungi concentrations in the IPDs (R^2=0.733).

Table 3-2 Multiple regression models of geometric indoor airborne bacteria and fungi in OPDs and IPDs of a university hospital

	β coefficient	SE	P-value	
OPDs				R^2=0.679
Ln airborne bacteria				
Constant	-0.810	2.146	0.707	
Time of service	-0.640	0.085	0.000	
CO_2 (ppm)	0.003	0.001	0.002	
Indoor temperature (°C)	0.175	0.051	0.001	
Indoor relative humidity (%)	0.025	0.011	0.021	
Ln airborne fungi				
Constant	5.816	0.077		R^2=0.512
Outdoor airborne fungi (cfu/m^3)	0.001	0.00	0.000	
IPDs				
Log10 airborne bacteria				R^2=0.555
Constant	-1.780	1.069	0.099	
CO_2 (ppm)	0.003	0.001	0.002	
Indoor temperature (°C)	0.066	0.028	0.023	
Indoor relative humidity (%)	0.009	0.005	0.064	
Rainfall (mm)	0.005	0.001	0.000	
Outdoor bacteria (cfu/m^3)	0.002	0.000	0.000	
Log10 airborne fungi				R^2=0.733
Constant	1.978	0.123	0.000	
Indoor relative humidity (%)	0.008	0.002	0.000	
Outdoor fungi (cfu/m^3)	0.000	0.000	0.000	
Wind direction	0.020	0.008	0.016	

3.3.4 Seasonal distribution of airborne bacteria and fungi types

The results of the identification of indoor airborne bacteria in the university hospital during the dry and wet seasons are shown in Table 3-3. The predominant genera of indoor and outdoor airborne bacteria identified in both seasons were *Staphylococcus* spp. (55.10-60.31%), followed by *Micrococcus* spp. (23.19-26.06%), *Bacillus* spp. (6.47-10.91%), and *Corynebacterium* spp. (7.53-9.57%) for indoor

airborne bacteria. For outdoor airborne bacteria, the main types were *Micrococcus* spp. (39.34-46.70%), *Staphylococcus* spp. (31.98-40.08%), *Bacillus* spp. (9.92-17.77%) and *Corynebacterium* spp. (3.55-12.44%). In addition, the same predominant airborne fungi were found in the samples of both seasons from indoor and outdoor air sampling (i.e., *Cladosporium* spp. [41.47-67.30%], followed by *Penicillium* spp. [17.56-38.25%], *Aspergillus* spp. [8.66-13.02%] and *Fusarium* spp. [7.26-13.64%]). The percentages of yeast species of indoor and outdoor air were found between 1.09-3.20% of total airborne fungi. Moreover, the most common fungi isolated from the indoor and outdoor airborne fungi were septate hypha (99.07% to 97.69%) and non-septate hypha (0.93% to 2.31%).

Table 3-3 Airborne bacteria isolated (cfu/m^3) from various sites of a university hospital in the dry and wet seasons

		OPDs		O-OPD		IPDs		O-IPDs	
		Dry	**Wet**	**Dry**	**Wet**	**Dry**	**Wet**	**Dry**	**Wet**
Bacteria									
Staphylococcus spp.	cfu/m^3	926	1,258	198	141	389	268	170	63
	%	59.47	60.31	40.08	35.79	55.10	57.63	34.91	31.98
Micrococcus spp.	cfu/m^3	361	495	205	155	184	113	212	92
	%	23.19	23.73	41.50	39.34	26.06	24.30	43.53	46.70
Corynebacterium spp.	cfu/m^3	149	198	42	49	56	35	42	7
	%	9.57	9.49	8.50	12.44	7.93	7.53	8.62	3.55
Bacillus spp.	cfu/m^3	121	135	49	49	77	49	63	35
	%	7.77	6.47	9.92	12.44	10.91	10.54	12.94	17.77
Total	cfu/m^3	1,557	2,086	494	394	706	465	487	197
	%	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Fungi									
Cladosporium spp.	cfu/m^3	601	551	311	170	1,484	360	693	594
	%	54.10	50.64	59.35	46.32	67.30	41.47	57.99	56.04
Penicillium spp.	cfu/m^3	261	311	92	127	417	332	212	233
	%	23.49	28.58	17.56	34.60	18.91	38.25	17.74	21.98
Aspergillus spp.	cfu/m^3	128	135	57	35	191	113	127	120
	%	11.52	12.40	10.88	9.54	8.66	13.02	10.63	11.32
Fusarium spp.	cfu/m^3	121	91	64	35	113	63	163	113
	%	10.89	8.36	12.21	9.54	5.12	7.26	13.64	10.66
Total	cfu/m^3	1,111	1,088	524	367	2,205	868	1,195	1,060
	%	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00

3.3.5 Seasonal size distribution of viable bacteria and fungi

The average size distribution of airborne bacteria and fungi in the indoor air showed similar patterns in both seasons (Figure 3-3). The results showed that the size distribution of airborne bacteria during occupancy was the highest in stage 4 (2.1-3.3

µm) and stage 1 (>7 µm) while airborne fungi were at their highest at stage 4 (2.1-3.3µm). Figures 3-4 present the predominant genera of indoor airborne bacteria and fungi in both seasons at the sampling sites and revealed a similar pattern.
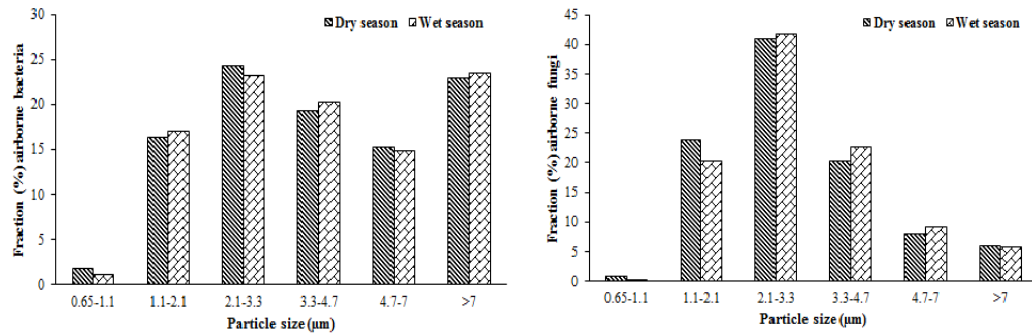
Figure 3-3 Size distributions of airborne bacteria and fungi during the two seasons in a university hospital.

However, the size distributions of *Staphylococcus* spp. and *Bacillus* spp. in both seasons were detected mainly at >7µm. *Micrococcus* spp. and *Corynebacterium* spp. were at 1.1-3.3 µm and 2.1-4.7 µm, respectively. The airborne fungi showed that *Cladosporium* spp. and *Penicillium* spp. had the highest size distributions at 2.1-3.3 µm, while *Fusarium* spp. had the highest size distribution at >7 µm, and *Aspergillus* spp. had the highest distribution at 2.1-4.7 µm (Figures 3-4).
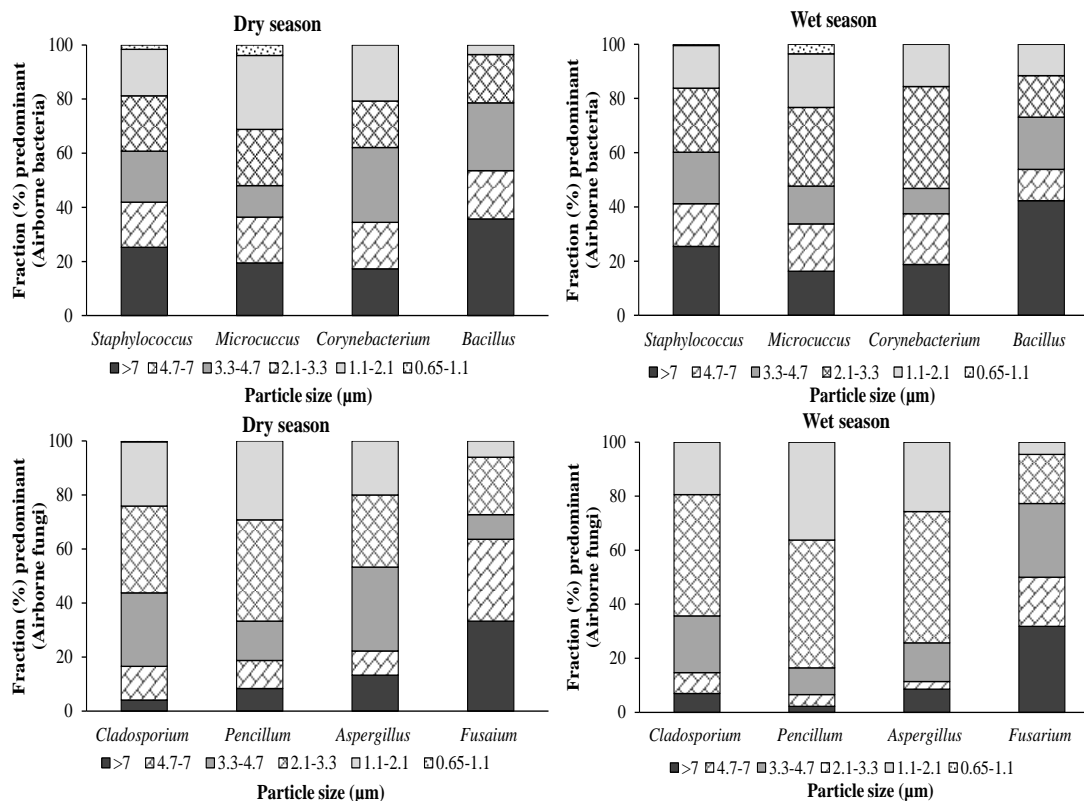
Figure 3-4 Size distributions of predominant airborne bacteria and fungi during the two seasons in a university hospital.

3.4 Discussion

3.4.1 Seasonal concentrations of airborne bacteria and fungi

The IPD areas were located on the 5th and 9th floors of the building and had fewer visitors. Furthermore, the outdoor airborne bacteria concentrations in the wet season were lower because the wet season in the study area had high rainfall and after the rainfall the relative humidity level increased in the air, thus cleaning the air and decreased the airborne bacteria levels (Wang et al., 2010a). The low concentration of outdoor airborne bacteria diluted the indoor bacteria and decreased the airborne bacteria concentrations especially in the wet season at the IPDs. However, the OPDs which were in the center of the hospital were located on the first floor and had a high density of people throughout the year. In addition, the OPDs were close to areas of human activity and near the main road. These were the causes of the outdoor airborne bacterial contamination. Thus, the seasons do not have an effect on the concentrations at this site because the indoor air had a higher level of human activity and a greater

number of occupants which were the main sources of airborne bacteria. Although meteorological factors such as relative humidity and temperature showed a statistical difference between the seasons, they varied in a narrow range because in a tropical humid environment, especially in southern Thailand, rainfall and high temperatures persist throughout the year. Therefore they had no effect on the fungi concentrations.

These results were different compared with reports by Sautour et al. (2009a) and Ponce-Caballero et al. (2010) who found that the airborne fungi concentrations varied with seasonal changes; however, those studies were conducted in meteorological conditions with more fluctuations.

The high I/O ratios of indoor airborne bacteria depended on the levels of human activity and numbers of occupants which were the main sources of airborne bacteria (Hospodsky et al., 2012). The I/O ratios of airborne bacteria in this study were similar to a report by Kim et al. (2010), while the I/O ratios of airborne fungi were different compared with this study. Hence, a study on the relationships of the I/O ratio variations and indoor air quality levels may be useful for the development of an indoor air quality index in a public health work place environment.

The RFs in this study were similar to a study by Wang et al. (2010b) who found the %RFs of indoor bacteria and fungi were 62.8% and 81.4%, respectively. The particle sizes of bioaerosols below 5 μm (respirable fraction) have the potential for health risks in vulnerable patients, such as immunocompromised individuals (Kim et al., 2010; Sturm et al., 2012). The high RF means high risk of infection in these people. However, the RFs of bacteria and fungi in this study were not different between the dry and wet seasons. This was due to the humid tropical environment that had only slightly different weather conditions in the area studied. The indoor bioaerosol proportions of the OPDs were similar to the Rajasker and Balaubramanian (2011) study in high occupant density that found the indoor bacteria and fungi at 50.5% and 49.50%, respectively, except in the IPDs. The proportion of indoor airborne bacteria and fungi varied due to the occupant density and human activity. For this reason the overall concentration of the indoor airborne bacteria was lower than the airborne fungi in the IPDs.

3.4.2 Relationship between bioaerosols and environmental factors

The CO_2 was a significant factor that impacted the bioaerosol concentrations in the OPDs and IPDs. Patient treatment, human activity, and occupant density can increase the CO_2 concentration in the indoor air that was significantly related to airborne bacteria in this study. These results were similar to those reported in previous studies (Fox et al., 2003; Mahyuddin et al., 2014). The indoor airborne bacteria concentrations during the morning service hours at the OPDs were higher than in the afternoon service hours. These work sites had higher levels of human activities and occupant densities due to the numbers of patients, relatives of patients, and medical personnel. These factors were the main sources of airborne bacteria in the building. The airborne bacteria concentrations in the IPDs were lower where the occupant densities and human activities were constant all day. Moreover the indoor temperature and relative humidity were significant factors related to the bacteria concentrations in the OPDs and IPDs. These results were similar to the studies of Tang (2009) and Wang et al. (2010b).

For the IPDs, a positive correlation was found between the periods of rainfall and the indoor airborne bacteria concentrations. These results conformed to a study by Heo et al. (2014) who found that the outdoor airborne bacteria level increased during rainy conditions and then the high bacteria concentration could affect the indoor airborne bacteria levels in any area where dilution ventilation was used. It was also found that the outdoor fungi levels contributed to the indoor fungi concentrations and a similar phenomenon was found in the OPDs and IPDs. The outdoor fungi can flow through the open windows and doors into the inside workplaces which increased the indoor fungi concentrations (Sautour et al., 2009b; Ponce-Caballero et al., 2013). In addition, the IPDs were located on the 9th and 5th floors of the surgical and medical building where the wind flows into the building without obstruction from the green environment. Therefore, the levels of airborne fungi in the indoor environment increased (Pei-Chih et al., 2000). Moreover, the high relative humidity of indoor air from human activity and building materials in the IPDs can be potential factors to increase the indoor airborne fungi concentrations (Andersen et al., 2011).

3.4.3 Seasonal distribution of airborne bacteria and fungi types

Generally, the type of airborne bacteria depended on the location of the sampling (Harper et al., 2013), but the main genera found in this study were mostly similar to those in other studies that investigated the indoor air of hospitals (Pastuszka et al., 2005; Kim and Kim, 2007). The predominant genera of airborne bacteria were also the most frequently isolated genera in hospitals (Pastuszka et al., 2005; Kim et al., 2010). As described in the literature, the source of *Staphylococcus* spp. is mainly from humans. Therefore, in high occupant density and human activity conditions *Staphylococcus* spp. can be found in higher concentrations than other genera (Pastuszka et al., 2005; Kim and Kim, 2007; Kim et al., 2010). Normally, *Staphylococcus* spp. are found in hospitals and the indoor air concentrations are higher than in the outdoor air. However, the levels of *Micrococcus* spp. in the outdoor areas were higher than the indoor environments in all work sites. Similar results were reported by Fang et al. (2007). The predominant genera were consistent with other studies that showed *Cladosporium* spp., *Penicillium* spp., *and Aspergillus* spp. were the main flora in buildings (Kim et al., 2010; Ponce-Caballero et al., 2013).

Normally, all predominant genera can be isolated from the environment when they are confirmed from an outdoor source (Sautour et al., 2009a). However, the indoor levels were higher than the outdoor levels which demonstrated that it was due to the accumulation of bioaerosols with insufficient ventilation, greater indoor human activity, and the building materials (Soutour et al., 2009a; Andersen et al., 2011). However *Cladosporium* spp., *Penicillium* spp., and *Aspergillus* spp. are increasingly considered as health risks for hematologic, cancer, and transplantation patients. The frequency of airborne fungi exposure is a cause of adverse human health effects such as allergy, asthma, and sick building syndrome (Tang et al., 2006; Knutsen et al., 2012). Moreover, yeast genera of fungi can be found in the indoor and outdoor hospital environments. The Sepahvand et al. (2012) study found yeast genera at 0.7-1.6% of total fungi in the outdoor and indoor environments of a hospital. The percentages of septate and non-septate hypha were slightly different than the Luksamijiarulkul et al. (2012) study that found them at 99.40% and 0.60%, respectively, in the air of a child home-care center in Thailand.

3.4.4 Seasonal size distribution of viable bacteria and fungi

The percentages of size fractions of the predominant bacteria isolated were not different between the two seasons. This may be because most bacteria came from human sources such as human activity, speaking, coughing, and breathing that can account for all particle sizes from the smallest to the largest (<0.8 to 125μm) (Morawska et al., 2009; Xie et al., 2009). This is why the same genera were found in all size fractions and were not different between the two seasons. *Staphylococcus* spp. and *Bacillus* spp. were found in large size fractions which were in accordance with the results of Kim and Kim (2007) and Kim et al. (2010) who also reported that *Staphylococcus* spp. and *Bacillus* spp. were found in stage 1 (>7 μm). Normally, the size of *Staphylococcus* spp. is in the range of 0.5-1.0 μm in diameter (Faster, 1996), but in this study the particle size distribution of airborne *Staphylococcus* spp. was in the fraction of large particles. The explanation may be that the bacteria were attached to large particles such as dead human skin (10-20 μm) and floor dust (Wilson, 2006; Hospodsky et al., 2012). The bacteria found in this study were also found in cluster forms. *Bacillus* spp., *Micrococcus* spp. and *Corynebacterium* spp. have a typical single cell size of 0.5-4.0 μm but they were found in the larger size fraction (Bennerman et al., 2007; Brooks et al., 2013). It could be said that free cell airborne bacteria are normally found in the range of 1 to 2.0 μm while bacteria clusters are found in a size range from 3.0 to 7.0 μm (Tham and Zuraimi et al., 2005).

The size distribution of airborne fungi was found mainly at 2.1-3.3 μm. When considering the %size fraction of the predominant fungi isolated in this study, the airborne fungi were in conidia and spore forms. The %size fractions of predominant fungi were mostly in the particle size range of 2.1-3.3 μm that fit with the spores of *Penicillium* spp. (2.1-3.4 μm) (Reponen et al., 2001) and *Cladosporium* spp. spores (2.0-4.0 μm) (Samson and van Reenen-Hoekstra, 1988). Moreover, the pathogenic *Aspergillus* spp., which has a spore size of about 2.5-3.0 μm in diameter (Morris et al., 2000; Rivera et al., 2006), can penetrate into the lungs and be highly hazardous for human health. In addition the *Fusarium* spp. also has a large size (>7 μm). This is caused by the *Fusarium* morphology that can appear in the form of macroconidia with large diameters (Khokhar et al., 2015).

3.5 Conclusions

In addition to bacteria concentration, the %respirable size fractions of airborne bacteria and fungi are important factors that can be utilized for indoor air quality control. Higher concentrations of bacteria were found in the OPDs because of higher occupant densities than in the IPDs, whereas the airborne fungi levels in the indoor air were found at similar levels across all working sites of the hospital. Differences in the airborne fungi contamination levels in the hospital indoor environment between the wet and dry seasons were not found in this study. The bacteria size distribution study showed that the bacteria were mostly in cluster forms attached to larger particles, whereas the indoor airborne fungi were conidia and spore forms distributed from indoor and outdoor sources. However, the %RFs of airborne bacteria and fungi in all working sites were higher than 59%. In addition, it was found that the airborne bacteria and fungi contaminations in the indoor air of the hospital were highly influenced by outdoor microorganisms. Therefore, the air quality management system should not only attend to the indoor environment but also be concerned with the surrounding environments.

CHAPTER 4

**Relationships between Environmental Factors and Airborne Bacteria and Fungi
in the Service Areas and Recommendations for Environmental Management**

Abstract

This study was undertaken to investigate and understand the behavior of indoor airborne bacteria and fungi concentrations in the out-patient and in-patient departments (OPDs and IPDs) where dilution ventilation systems operated from January to December 2012. The focus group discussions investigated possible recommendations to reduce the bioaerosol contamination. The yearly average airborne bacteria and fungi concentrations in the service areas were lower than 1000 cfu/m^3. The respiratory factions (RFs) of airborne bacteria and fungi were more than 59%. The airborne bacteria in areas of high occupant densities showed double peaks at >7 μm (stage 1) and 2.1-3.3 μm (stage 4), and a peak at 2.1-3.3 μm (stage 4) for airborne fungi. The average accumulation size fractions (d_{50}) of airborne bacteria and fungi were 3.17±0.19 μm and 2.81±0.14 μm, respectively. Daily variations affected the particle size of airborne fungi at 1.1-2.1 μm (stage 5) and 3.3-4.7 μm (stage 3). Time series regression showed a seasonal effect on the indoor airborne bacteria concentrations in the OPDs which varied by the number of people, indoor relative humidity and outdoor airborne bacteria concentrations ($p < 0.05$), while the number of people and outdoor airborne bacteria concentrations were significantly related to the indoor airborne bacteria concentrations in the IPDs ($p < 0.05$). The concentrations of the outdoor airborne fungi and indoor temperature were significantly related to the concentrations of the indoor airborne fungi in the OPDs ($p < 0.05$) and indoor relative humidity and outdoor airborne fungi were significantly related to the indoor fungi concentrations in IPDs ($p < 0.05$). The focus group discussions recommended reducing the bioaerosols at the work sites by increasing the ventilation in the work sites, monitoring plumbing leaks, keeping dry and clean floors, and cleaning the ceiling fans and mosquito screens which can reduce the levels of relative humidity and airborne fungi. This study provides the fundamental data for improvement of the indoor air management in a university hospital that is located in a tropical humid zone and uses a dilution ventilation system.

Keywords: Relationships; airborne; dilution ventilation; environment management; dynamic

4.1 Introduction

      Airborne microorganisms are a potential health risk to the respiratory system of people. Airborne microorganisms are associated with the occurrence of nosocomial infection, sick building syndrome, asthma, allergies, rhinitis, chronic obstructive pulmonary disease, respiratory infection, and other pneumonia and respiratory diseases (Toews, 2005; Fisk et al., 2007; Ege et al., 2011; Sahlberg et al., 2013; Dadbakhsh et al., 2015). In a hospital, patients, medical staff and the relatives of patients can be exposed to bioaerosols in the workplace. Of special concern are immunocompromised patients who are highly susceptible to a nosocomial infection.

      In tropical areas, most hospitals use a dilution ventilation system to reduce and prevent accumulated pollutants in the workplaces. A dilution ventilation system provides high flow rates, low energy consumption (Atkinson et al., 2009) and reduces cross-infection risk of airborne diseases (Qian et al., 2010). However, it is difficult to control the indoor air quality which is affected by the surrounding environmental conditions because the levels of bioaerosols depend on meteorological and environmental factors (Troutt and Levetin, 2001; Grinn-Gofroń and Strzelczak, 2008; Tang, 2009; Wang et al., 2010b; Grinn-Gofroń and Bosiacka, 2015).

      Indoor air used in dilution ventilation systems was affected by outdoor and indoor natural and anthropogenic sources including plants, agriculture (Abdel Hameed and Khodr, 2001), human activities (Wang et al., 2010a) and occupant density. Moreover, the humidity and temperature are important factors that were positively related to airborne levels of bioaerosols (Webb, 1959; Sakai et al., 2003; Tang, 2009; Wang et al., 2010). Outdoor wind speed and wind direction are potential factors that increased indoor bioaerosol levels (Zhu et al., 2003; Abdel Hameed et al., 2009; Fang et al., 2010b), while the indoor air velocity diluted the air pollution in a building (Yau et al., 2011). In addition, the outdoor urban areas were the source of pollutants such as carbon dioxide (CO_2), carbon monoxide (CO), nitrogen dioxide (NO_2), nitrogen monoxide (NO), ozone (O_3), formaldehyde (HCHO), methane (CH_4), sulfur dioxide (SO_2) and bioaerosols produced by industrial factories, department

stores, automobile traffic and forests (Gómez-Perales et al., 2004; Singer et al., 2004; Han and Naeher, 2006; Cao et al., 2009; Dadbakhsh et al., 2015). They affected the survival and quantity of bioaerosols (Lighthart, 1973; Chan et al., 1991; Ho et al., 2005; Cao et al., 2009; Kim et al., 2009; Wang et al., 2010b). Moreover, air pollutants and bioaerosols increased the health risk of headache, weakness, bronchitis, allergy, sick building syndrome and respiratory diseases (Douwes et al., 2003; Chang et al., 2015; Dadbakhsh et al., 2015; Shakerkhatibi et al., 2015).

Moreover, the levels of bioaerosols varied with environmental factors, time and locations. Thus, time series analysis is a good method to perform an analysis of the time series and dynamics of airborne pollution (Rodriguez-Rajo et al., 2006; Aznarte et al., 2007; Puc 2012). For instance, it was used for the environment conditions to predict the airborne Alnus pollen concentrations in Spain (Rodriguez-Rajo et al., 2006).

Hence, the aim of this study was to investigate the behavior of bioaerosols and environmental factors to predict the airborne bacteria and fungi concentrations in a dilution ventilation system of a university hospital by using time series analysis. Further, this study aims to protect personnel and control the airborne bacteria and fungi in the workplace. The results of this study provide the dynamics and background of the bioaerosols for protective and control measures in a hospital where the climate is predominantly humid and subtropical.

4.2 Materials and methods

4.2.1 Sampling

In this study, indoor ambient air was collected at the M-OPD, E-OPD, M-IPD and S-IPD of a university hospital (Figure 4-1). The airborne bacteria and fungi samples were taken over a period of one year (January to December 2012). The samplings investigated were taken during the morning (09:00-12:00) and afternoon (13:00-16:00) for a total of 16 times/month. The environmental parameters included the levels of CO_2, CO, CH_4, HCHO, NO, NO_2, SO_2, O_3, air velocity, relative humidity and temperature. The indoor bioaerosol and environment sampling included a total of 768 samples: M-OPD (192 samples), E-OPD (192 samples), S-IPD (192 samples) and

S-IPD (192 samples). The outdoor bioaerosol and environmental parameters included 576 samples: O-OPD (192 samples), M-IPD (192 samples) and S-IPD (192 samples).
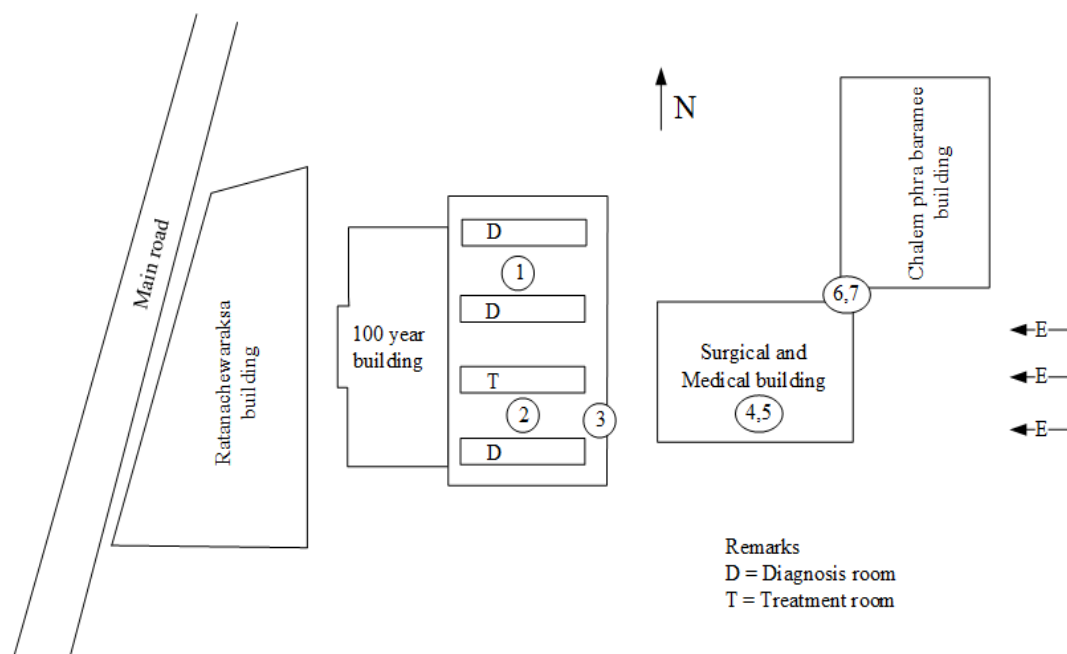
Where: No. 1=M-OPD; No. 2 = E-OPD; No. 3 = Outdoor reference for OPD; No. 4 = M-OPD; No. 5 = S-IPD; No. 6 = Outdoor reference for M-IPD; No. 7 = Outdoor reference for S-IPD; E = wind direction from East at OPDs (29.17%) and IPDs (28.13%).

Figure 4-1 Sampling site locations of the M-OPD, E-OPD, M-IPD and S-IPD

4.2.2 Measurement and analysis

The bioaerosols were collected by a six-stage viable cascade impactor (Model 10-800, Andersen Inc, USA). The aerodynamic diameters were stage 1 (>7 µm), stage 2 (4.7-7.0 µm), stage 3 (3.3-4.7 µm), stage 4 (2.1-3.3 µm), stage 5 (1.1-2.1 µm) and stage 6 (0.65-1.1 µm). Air sampling was done for a period of 5 min at a flow rate of 28.3 L/min and set at 1.5 m above the floor to sample airborne bacteria and fungi. Before the sampling procedure, 70% alcohol was used to disinfect and prevent contamination. The total amount of bacteria was cultured using Trypticase soy agar medium and incubated at 37ºC for 2 days. Malt extract agar medium was used for all fungi and incubated at 25ºC for 5 days. The concentration of airborne bacteria and fungi were presented as cfu/m^3.

4.2.3 Environmental parameters

The measurements of indoor air pollution included carbon dioxide (CO_2), carbon monoxide (CO), methane (CH_4), formaldehyde (HCHO), nitrogen dioxide (NO_2), nitrogen monoxide (NO), ozone (O_3) and sulfur dioxide (SO_2) which were monitored by a Gasmet^{TM} (Model DX4000, Finland). The relative humidity, temperature and velocity were collected by a VelociCalc air velocity meter (Model 9555, USA). The numbers of occupants were counted during the sampling of the bioaerosols. The wind direction, wind speed and rainfall data were collected from the Khohong agrometeorological station. The average of all duplicate samples was used in all samples in this study.

4.2.4 Statistical analysis

The environmental factors and bioaerosols were explained by descriptive statistics such as percentage, mean and standard deviation. The Kruskal-Wallis test examined the differences of the airborne bacteria and fungi concentrations and environment factors at the four sampling sites. A comparison of the indoor and outdoor bioaerosol and environment factors in the work sites used the Mann Whitney U-test. The Spearman correlation test was used to investigate the relationship between the environmental factors and bioaerosols. ARMA models were used to predict the airborne bacteria and fungi concentrations. The ARMA model used two parameters to explain changes in time that included autoregressive (p) modeling which is the number of autoregressive parameters of the model, and (q) which is the order of the running mean of the process. Sine and cosine were used to build a smooth variation of the seasonal model.

4.2.5 Focus group discussion

After an analysis of the relationships between the environmental factors and bioaerosols, the results of the significant factors were reviewed for recommendations to reduce the airborne bacteria and fungi in the indoor ambient sites that used the dilution ventilation system. The focus group included 15 people. Seven professionals had substantial work experience: Occupational Safety Health (1), Division of Health Promotion (2), Infection Control Unit (3), and the Faculty of Environmental

Management (1). In addition, other stakeholders consisted of four head nurses and four nurses who worked at the M-OPD, E-OPD, M-IPD and S-IPD (8). The aim of the focus group in this study was to investigate methods to reduce the airborne bacteria and fungi at the workplaces. The focus group meetings were held at the research unit for Holistic and Safety Management in the Community Medicine Department at the Faculty of Medicine. The total duration of the focus group meetings was 120 minutes and all proceedings were audiotaped. Participants were introduced to the concepts and topic decisions concerning the results of this study that included the situations of airborne bacteria and fungi and the relationships between the environmental factors and the quantities of airborne bacteria and fungi. The main topic for the focus group was how to reduce the concentrations of airborne bacteria and fungi in the workplaces that used the dilution ventilation system.

4.3 Results

4.3.1 Concentrations of airborne bacteria and fungi in the service areas

The yearly average concentrations of airborne bacteria and fungi in all four indoor environments of a university hospital are shown in Table 4-1. The concentration of indoor bacteria was the highest at the M-OPD followed by the E-OPD, M-IPD and E-IPD. However, the average concentration of airborne fungi was the highest at the S-IPD followed by the M-IPD, E-OPD and M-OPD. The indoor airborne bacteria concentrations in the OPDs were significantly higher than in the IPDs ($p<0.05$). However, there were no significant differences in the indoor airborne bacteria between the two OPDs (M-OPD and E-OPD) or the IPDs (M-IPD and S-IPD) ($p>0.05$). In addition, the indoor and outdoor airborne fungi levels were no different between the OPDs and IPDs ($p>0.05$). However, the indoor airborne bacteria and fungi concentrations at all work sites were higher than the outdoor air ($p<0.05$). The proportions of indoor airborne bacteria and fungi of the OPDs were 45.38-50.15% and 49.85-54.64% while at the IPDs the proportions were 22.09-22.89% and 77.21-77.91%, respectively.

Variations of the indoor airborne bacteria levels were found in the M-IPD and S-IPD that were located at the 9th and 5th floors, respectively, of the surgical and medical building. The concentrations were found to be the highest in April and lower

from August to December (Figure 4-2), while the indoor airborne bacteria concentrations in the OPDs were high in April to June. Statistically, the airborne fungi levels were not diffident at all work sites ($p > 0.05$). The airborne fungi concentrations had high peaks in January, April to July, August to October and December with the same pattern as the outdoor airborne fungi (Figure 4-3).

The densities of people in the M-OPD and E-OPD were higher than in the IPDs ($p < 0.05$) (Table 4-1). In addition, a comparison between the M-OPD and E-OPD found that the occupant density in the M-OPD was higher than in the E-OPD ($p < 0.05$) but the airborne bacteria concentrations were not different ($p > 0.05$). These results had a similar pattern in the IPDs where the occupant density at the M-IPD was higher than at the S-IPD ($p < 0.05$) while the airborne bacteria concentrations were not different ($p < 0.05$). Moreover, the outdoor airborne bacteria levels at the OPDs were higher than at the outdoor IPDs ($p < 0.05$).

The airborne bacteria I/Os at the four service areas were more than 2.00 and 1.00 for airborne fungi. In this study, all sites of investigation showed that the I/O ratios exceeded 1.0. However, the I/O ratios of bacteria were obviously higher when compared with fungi ($p > 0.05$).

In addition, respiratory fractions (RFs) were calculated from the total number of airborne microorganisms counted in stages 3-6 divided by the summation of all stages. The RF ranges of airborne bacteria and fungi in the four service areas were 59.05%-61.75% and 84.56%-85.68 %, respectively. The RFs of airborne bacteria and fungi also showed similar values at all work sites ($p > 0.05$).

Table 4-1 Yearly average airborne bacteria and fungi concentrations and environmental factors in four service areas of a university hospital

Data	Service areas			
	M-OPD	E-OPD	M-IPD	S-IPD
Airborne bacteria				
Total (cfu/m^3)	859.10±441.66	758.24±347.01	285.59±178.12	279.27±186.06
	(50.15%)*	(45.38%)*	(22.89%)*	(22.09%)*
I/O[a]	2.36	2.08	2.00	2.21
Respirable[b]	516.42±273.42	452.37±222.69	176.16±115.70	175.94±141.23
RF[c] (%)	59.34±9.52	59.05±7.30	61.75±11.22	61.29±12.16
Airborne fungi				
Total (cfu/m^3)	853.80±561.84	913.21±766.70	961.87±739.76	985.20±853.71
	(49.85%)*	(54.64%)*	(77.21%)	(77.91%)*
I/O[a]	1.32	1.41	1.27	1.27
Respirable[b]	724.75±479.10	774.51±647.04	824.06±660.02	846.51±736.86
RF[c] (%)	84.56±5.32	84.96±5.66	84.95±6.21	85.68±6.17
Occupancy level (Person/m^2)	0.52±0.36	0.22±0.13	0.11±0.13	0.08±0.01

[a]I/O ratio - indoor airborne concentration/outdoor airborne concentration

[b]Respirable – respirable concentration or sum of bioaerosol quantities measured at stages 3-6.

RF – respirable fraction, percentage of respirable concentration/total concentration

* Proportions of indoor airborne bacteria and fungi
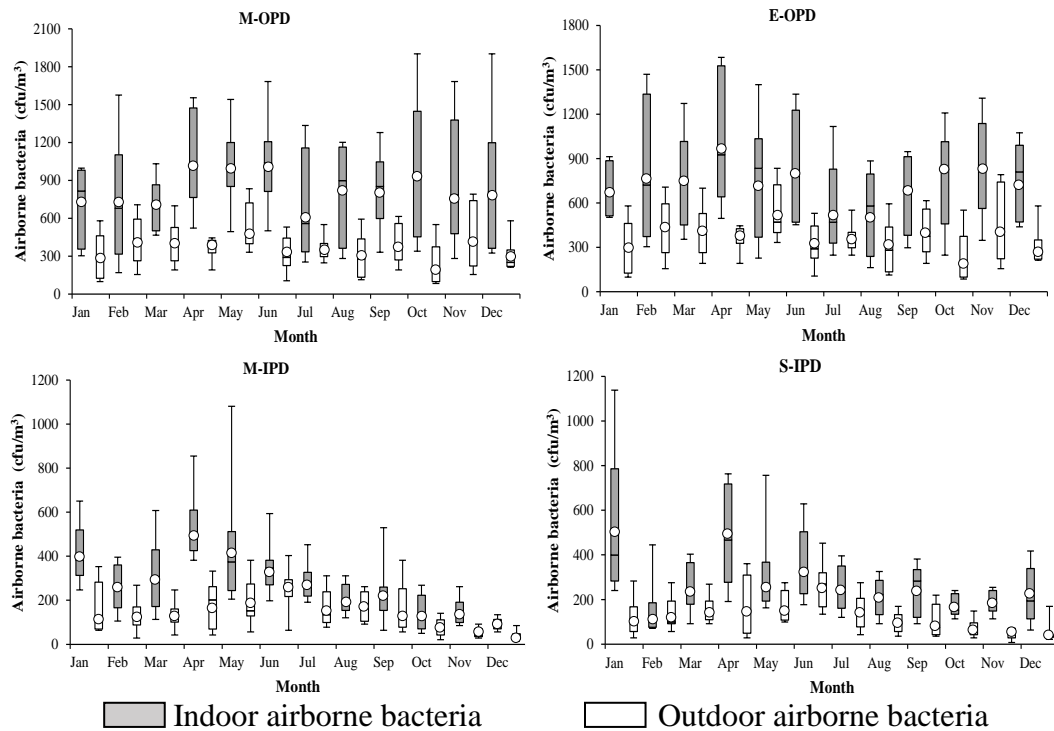
Box and whisker plots of airborne bacteria and fungi concentration between the indoor and outdoor of service areas; the box frames represent the upper and lower quartiles, the lines represent the median, whiskers represent the upper and lower of the range and the O = Represent mean.

Figure 4-2 Indoor and outdoor airborne bacteria concentrations in the M-OPD, E-OPD, M-IPD and S-IPD
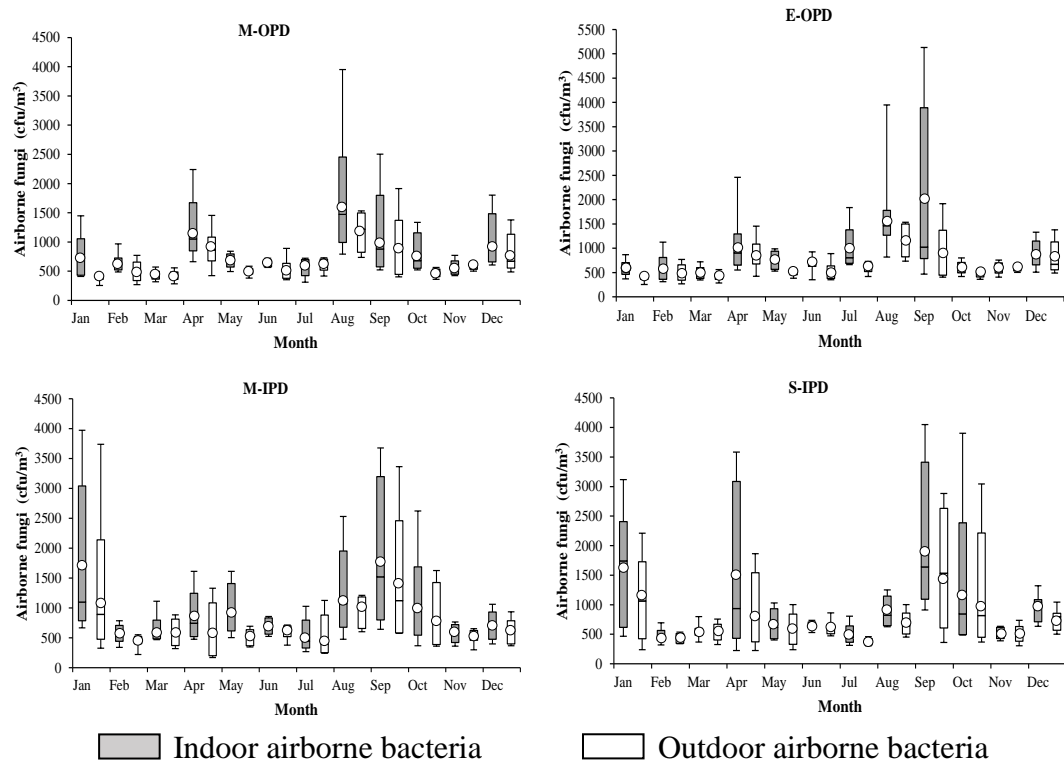
Figure 4-3 Indoor and outdoor airborne fungi concentrations in the M-OPD, E-OPD, M-IPD and S-IPD

4.3.2 Daily average of bioaerosols and environmental factor concentrations

The airborne bacteria and fungi concentrations in the OPDs and IPDs are shown in Table 4-2. The indoor airborne bacteria concentrations at the M-OPD and E-OPD were significantly higher in the morning than in the afternoon (p<0.05). However, the M-IPD and S-IPD did not have an increase in the airborne bacteria concentrations (p>0.05). On the other hand, the average concentrations of indoor airborne fungi in the M-IPD were significantly lower in the afternoons (p<0.05). However, at the E-OPD and S-IPD the average concentrations of indoor airborne fungi were significantly higher in the afternoon (p<0.05).

The average temperature and relative humidity in the morning were higher than in the afternoon in all service areas (p<0.05). In addition, the occupant densities in the morning at the M-OPD and E-OPD were higher than in the afternoon period (p<0.05), while in the M-IPD and S-IPD it was similar in both periods (p>0.05).

Table 4-2 Daily average of bioaerosol and environmental factors

Environmental factors	M-OPD		E-OPD		M-IPD		S-IPD	
	Morning	Afternoon	Morning	Afternoon	Morning	Afternoon	Morning	Afternoon
Indoor								
Airborne bacteria (cfu/m^3)	1131.77±332.41*	586.43±283.13*	1011.34±270.11*	505.15±198.57*	286.81±148.31	284.31±205.82	281.95±159.19	276.59±211.25
Airborne fungi (cfu/m^3)	847.47±349.81	860.13±718.04	818.76±314.36*	1007.66±1041.27*	1033.13±748.30*	890.61±731.99*	771.20±512.38*	1199.20±1056.92*
Temperature (ºC)	29.07±0.79*	29.75±1.33*	29.21±0.87*	29.70±1.18*	28.60±1.18*	30.15±1.80*	29.59±1.68*	30.01±1.88*
Relative humidity (%)	71.33±4.93*	66.71±7.12*	70.76±5.14*	67.02±7.59*	73.75±7.09*	65.78±9.07*	68.70±8.44*	66.63±9.74*
Occupant density (person/m^2)	0.86±0.13*	0.18±0.08*	0.31±0.100*	0.12±0.06*	0.11±0.01	0.11±0.01	0.08±0.01	0.08±0.01
outdoor								
Airborne bacteria (cfu/m^3)	423.73±194.43*	305.65±127.05*	423.73±194.43*	305.65±127.05*	158.72±94.31*	127.06±98.82*	121.02±89.82	132.80±93.46
Airborne fungi (cfu/m^3)	638.99±314.36	656.51±346.57	638.99±314.36	656.51±346.57	785.34±606.67	724.09±585.33	614.84±392.94*	935.07±705.83*

*Significant difference between the morning and afternoon periods (p<0.05) (Wilcoxon rank sum test)

4.3.3 Size distribution of airborne bacteria and fungi in the service areas

The fractions of the indoor airborne bacteria size distributions in high occupant density areas of the M-OPD, E-OPD and M-IPD were greatest at the first stage (>7 μm) followed by the third stage (2.1-3.3 μm), while the S-IPD had the highest fraction at the third stage followed by the first stage (Figure 4-4). However, the double peak pattern of airborne bacteria at all work sites was slightly different. On the contrary, airborne fungi were found mainly in stage 4 (range, 2.1-3.3μm) followed by stage 3 (range, 3.3-4.7) and stage 5 (range, 1.1-2.1μm) (Figure 4-5).
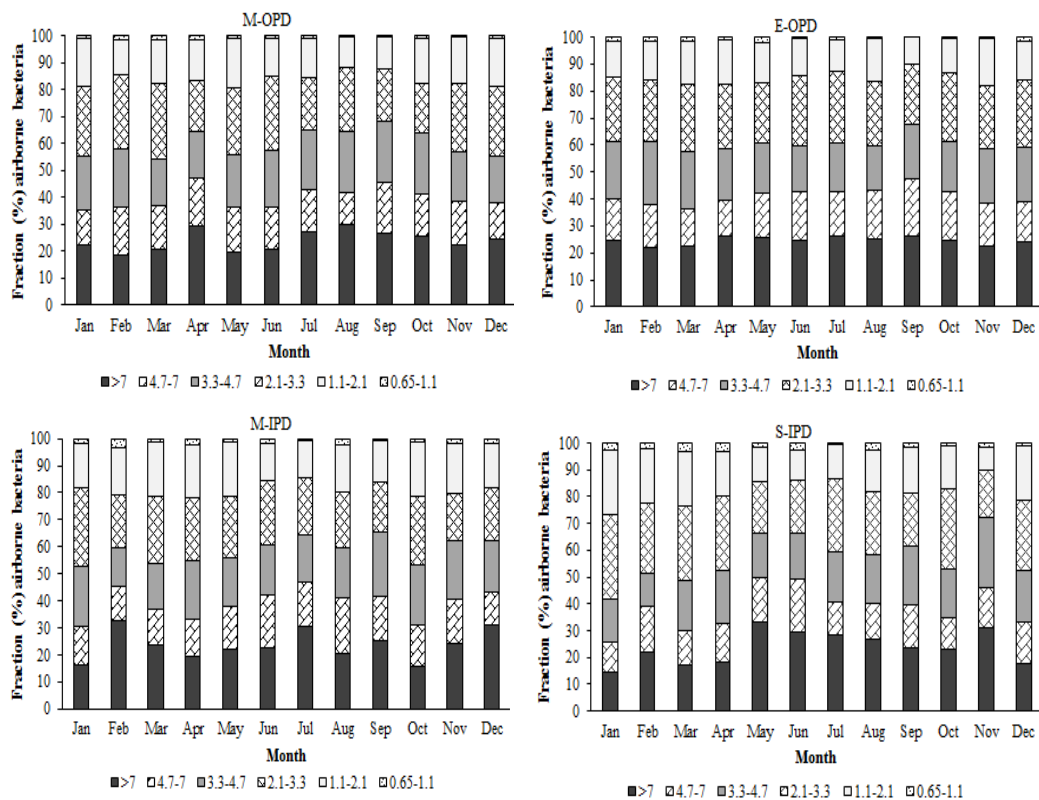
Figure 4-4 Size distributions of airborne bacteria in the service areas of a university hospital.
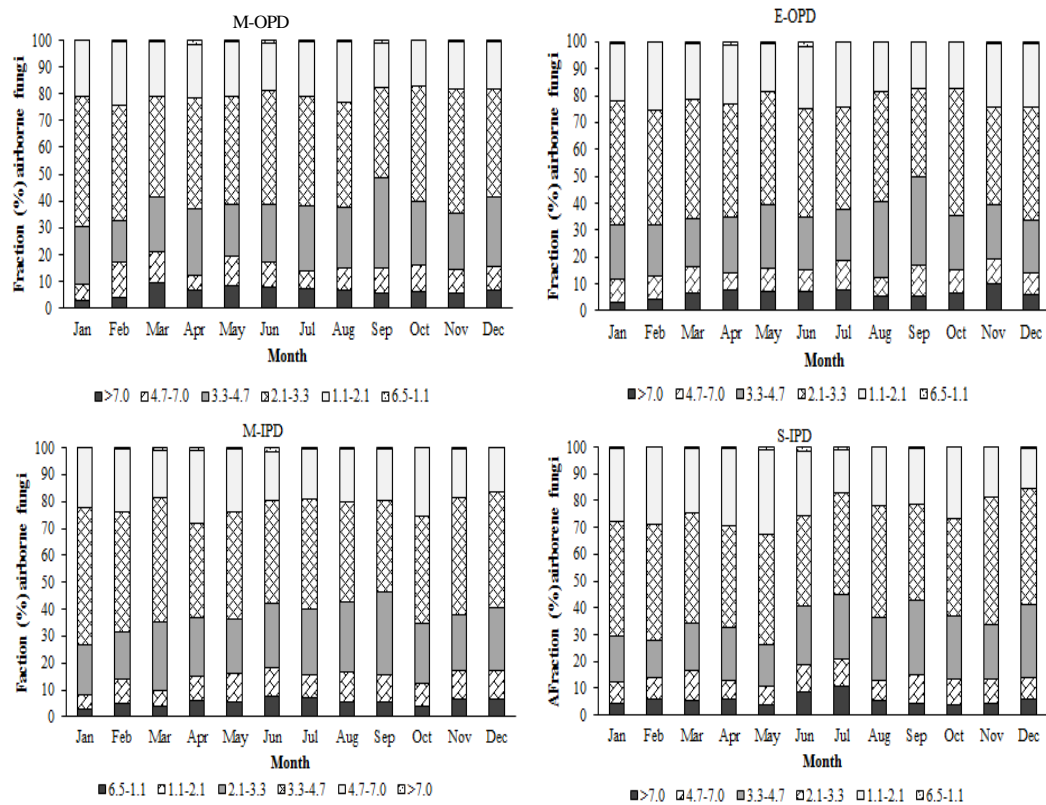
Figure 4-5 Size distributions of airborne fungi in the service areas
of a university hospital.

4.3.4 Accumulation of oversized airborne bacteria in the four service areas

Figures 4-6 and 4-7 show the accumulation size fractions (d_{50}) of airborne bacteria and fungi. The d_{50} results of airborne bacteria from January to December in the M-OPD, E-OPD, M-IPD and S-IPD were 3.22±0.16 μm, 3.22±0.13 μm, 3.13±0.16 μm and 3.10±0.27 μm ($\bar{X}$=3.17±0.19 μm), respectively, while the d_{50} results of the airborne fungi were 2.86±0.11 μm, 2.83±0.14 μm, 2.80±0.14 μm and 2.76±0.17 μm, respectively ($\bar{X}$=2.81±0.14 μm). The d_{50} results of the airborne bacteria and fungi were not statistically different at the four sampling sites (p>0.05).
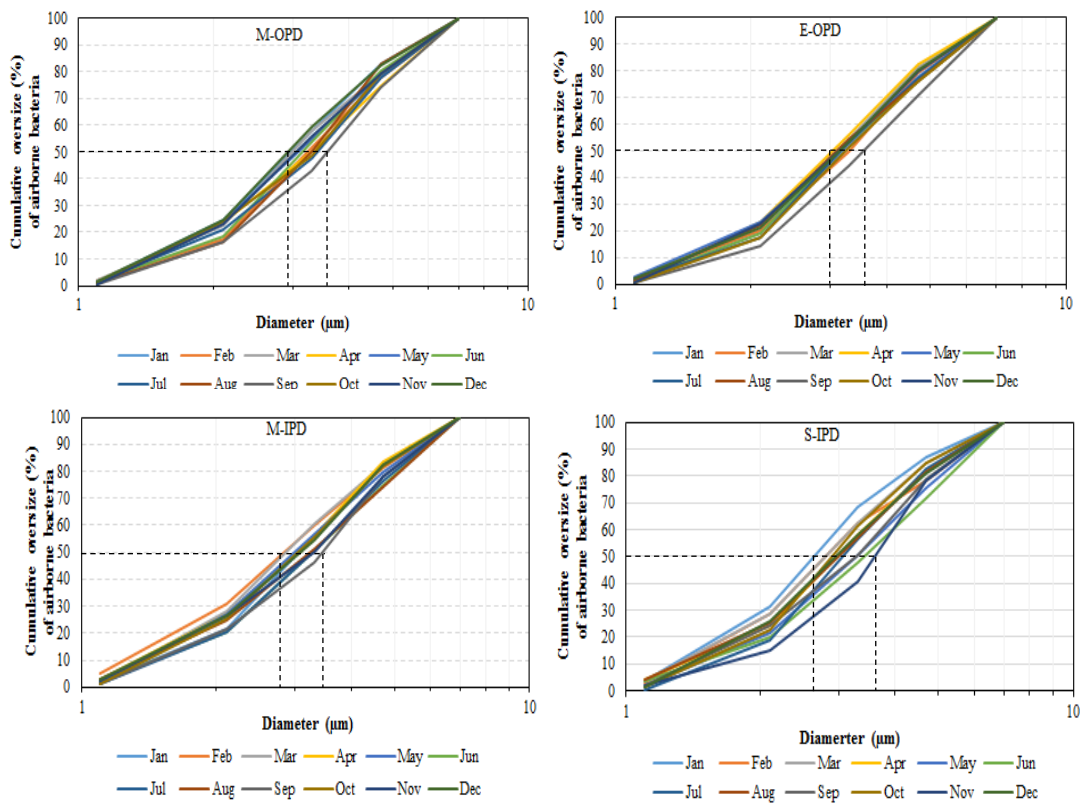
Figure 4-6 Cumulative size distributions of airborne bacteria in the service areas of a university hospital
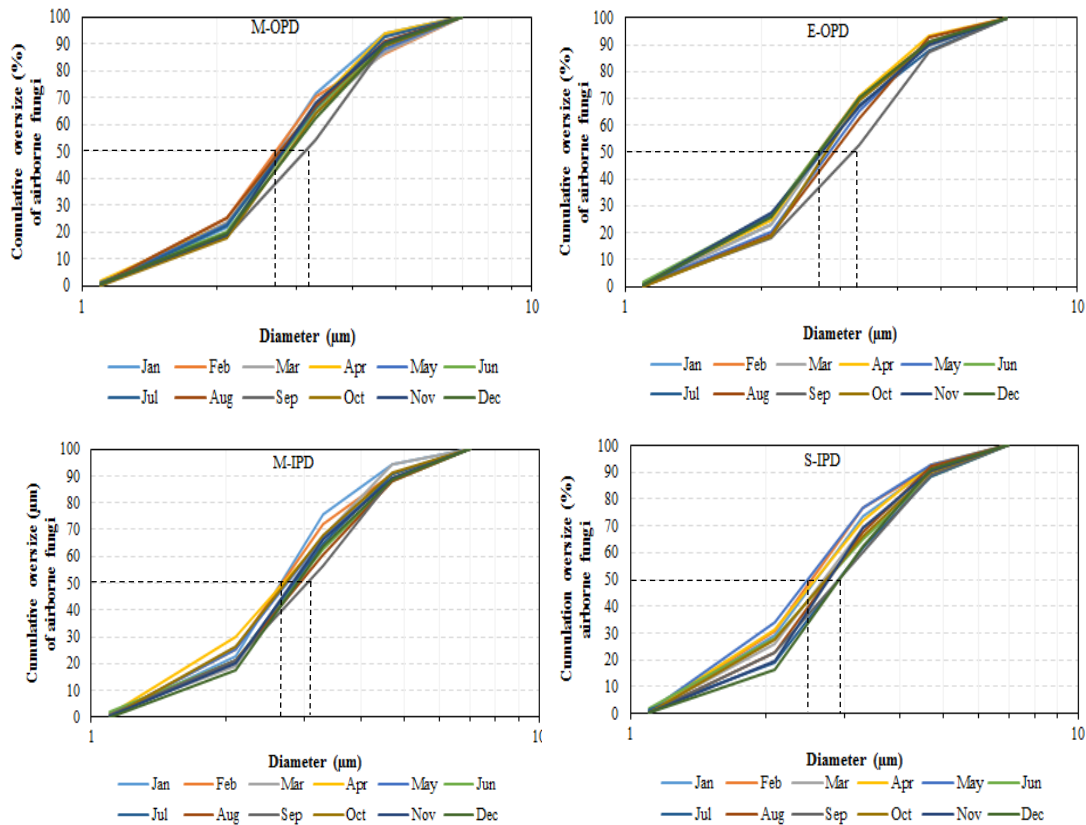
Figure 4-7 Cumulative size distributions of airborne fungi in the service areas
of a university hospital

4.3.5 Size fractions of airborne bacteria and fungi particles in the morning and afternoon of the service areas

Figure 4-8 shows the percentages of the airborne bacteria size distributions in the morning and afternoon. Most of the airborne bacteria of all sizes in the areas of high occupant densities did not vary according to the time of the day. They presented similar size fractions of all particle size distributions in the morning and in the afternoon periods except in the S-IPD. In addition, the percentages of airborne fungi size distributions in the morning and afternoon periods are illustrated in Figure 4-9. The results indicated that most airborne fungi increased in stage 3 (3.3-4.7) in the morning but increased in the afternoon in stage 5 (1.1-2.1) in the work areas.

Percentages of airborne bacteria (%)
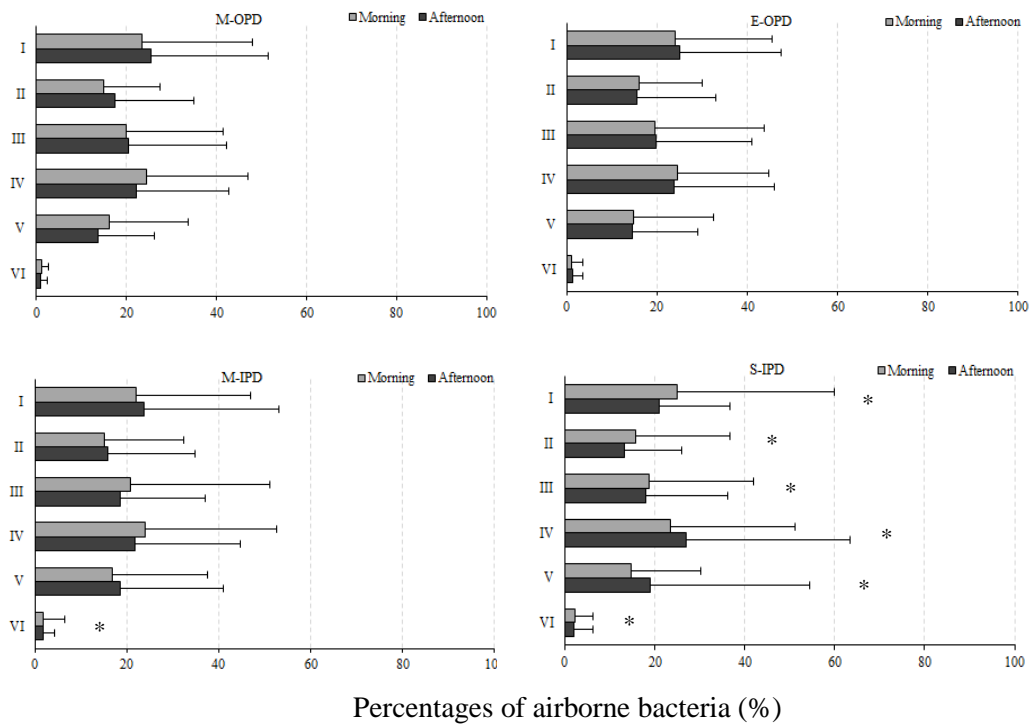
* Wilcoxon rank sum test (p>0.05)

Figure 4-8 Percentages of airborne bacteria in the morning and afternoon periods
at the sixth stage (Stage I: >7 µm; stage II: 4.7-7.0 µm; stage III: 3.3-4.7 µm;
stage IV: 2.1-3.3 µm; stage V: 1.1-2.1 µm and stage VI: 0.65-1.1 µm)
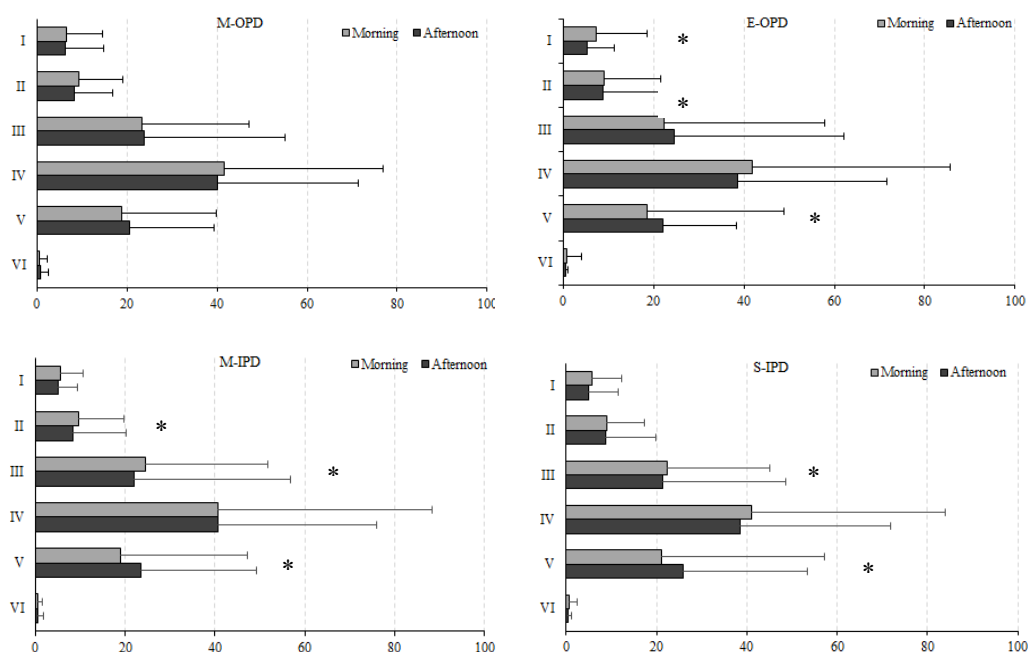
Percentages of airborne fungi (%)

* Wilcoxon rank sum test (p>0.05)

Figure 4-9 Percentages of airborne fungi in the morning and afternoon periods
at the sixth stage (Stage I: >7 µm; stage II: 4.7-7.0 µm; stage III: 3.3-4.7 µm;
stage IV: 2.1-3.3 µm; stage V: 1.1-2.1 µm and stage VI: 0.65-1.1 µm)

4.3.6 Average concentration and variation of environmental factors in the
service areas

The environmental factors collected were the concentrations of CO_2, CO, CH_4,
relative humidity, temperature, rainfall, number of people, UV-C light, wind speed
and wind direction at the four location sites of a university hospital that used the
dilution ventilation system to control the air pollution (NO_2, NO, SO_2, HCHO and O_3
not detected).

The mean concentrations of CO_2 in the M-OPD and E-OPD were
500.54±54.85 ppm and 488.17±32.84 ppm, respectively, which were higher than in
the M-IPD and S-IPD at 451.83±25.02 ppm and 458.36±21.28 ppm, respectively
(p>0.05) (Figure 4-10). However, the concentration of indoor CO_2 did not exceed the

American Society of Heating, Refrigeration and Air Condition Engineers (ASHRAE) standard of 700 ppm (ASHRAE, 2007).

The CH_4 mean concentrations in the indoor M-OPD and E-OPD were 1.73±0.51 ppm and 1.84±0.45 ppm, respectively, while the indoor M-IPD and S-IPD concentrations were 1.85±0.48 ppm and 183.0.45 ppm, respectively (Figure 4-11). The CH_4 concentrations in three service areas were statistically significantly higher than in the M-OPD ($p<0.05$). Moreover, there were many sources such as the toilets, medical waste and general waste that could increase the CH_4 concentration. CH_4 is emitted from natural sources and organic waste that is decomposed by bacteria. Therefore, a lower temperature could also decrease the CH_4 concentration in the indoor air of all sampling sites because in November and December there was high precipitation in the rainy season.

In addition, the CO concentrations in the M-OPD, E-IPD, M-IPD and S-IPD were 0.12±0.23 ppm, 0.18±0.31 ppm, 0.12±0.48 ppm and 0.23±0.38 ppm, respectively (Figure 4-12). However, the CO concentrations in all service areas were not significantly different ($p>0.05$). From the middle of May to October the CO concentration was high because this location was influenced by the wind direction of the southwest monsoon. Even though automobile traffic was not far from all work sites of the university hospital, the indoor CO concentration did not exceed the USEPA standard of 9 ppm in an 8-hour period (USEPA, 2008).

The average concentrations of the indoor relative humidity at the M-OPD, E-OPD, M-IPD and S-IPD were 69.02±6.52%, 68.89±6.72%, 69.77±9.04% and 67.66±9.13%, while the average outdoor relative humidity concentrations were 67.28±7.82%, 67.28±7.82%, 65.96±10.34% and 65.84±12.03%, respectively. The relative humidity was not significantly different between all service areas and indoor and outdoor locations ($p>0.05$). Moreover, the variation of indoor and outdoor relative humidity had a similar pattern that showed higher concentrations in April and September for the OPDs and in January and September for the IPDs (Figure 4-13).

The average temperature levels at the inside work sites were 29.41±1.14^{o}C, 29.46±1.06^{o}C, 29.38±1.70^{o}C and 29.80±1.79^{o}C for the M-OPD, E-OPD, M-IPD and S-IPD, while the average outside temperature levels were 30.01±1.61^{o}C,

30.01±1.61ºC, 30.37±2.39ºC and 30.33±2.76ºC, respectively. The indoor and outdoor temperatures were similar at all sampling areas for the indoor and outdoor areas ($p > 0.05$) except in the M-IPD that was different between the indoor and outdoor areas ($p < 0.05$). However, the variations in the indoor and outdoor temperatures in the OPDs and IPDs presented the same patterns (Figure 4-14).

The mean indoor air velocities in the M-OPD, E-OPD, M-IPD and S-IPD were 0.31±0.07 m/s, 0.26±0.06 m/s, 0.18±0.06 m/s and 0.20±0.08 m/s, and the outdoor air velocities were 0.35±0.13 m/s, 0.35±0.13, 1.45±0.78 m/s and 1.56±0.79 m/s, respectively. The indoor air velocity levels in the OPDs were higher than the IPDs ($p < 0.05$), while a comparison of the outdoor air velocities found higher velocities at the IPDs than the OPDs ($p < 0.05$). The variation of indoor and outdoor air velocities showed a similar pattern at the OPDs and IPDs (Figure 4-15).

The cumulative means of rainfall levels during bioaerosol sampling in the M-OPD, E-OPD, M-IPD and S-IPD were 1.75±4.35 mm, 1.85±4.38 mm, 4.39±14.14 and 4.61±14.16, respectively. The comparisons found that the cumulative rainfall concentrations at the IPDs were higher than the OPDs ($p < 0.05$) and showed a variable pattern that was similar at the OPDs and IPDs (Figure 4-16A). The total rainfall levels at these location sites were higher in January and December (Figure 4-16B).

The UVGI systems installed at the OPDs showed that the average UV intensities were 944.65±54.85 $\mu W/cm^2$ (M-OPD) and 468.50±55.00 $\mu W/cm^2$ (E-OPD) and there was a downward trend over time in both work sites (Figure 4-17). Moreover, the mean numbers of people were 155±155 persons (M-OPD), 93±55 persons (E-OPD), 77±8 persons (M-IPD), and 58±8 persons (S-IPD) and the trends varied differently at all service areas (Figure 4-18).

In addition, the mean outdoor wind speeds in the M-OPD, E-OPD, M-IPD and S-IPD were 5.62±3.46 m/s, 4.79±3.36 m/s, 5.41±3.64 m/s and 3.97±3.10 m/s, respectively, which were different at all sites ($p < 0.05$). Moreover, the variation in the wind speeds in the OPDs and IPDs had a similar pattern (Figure 4-19). The major wind direction at the OPDs and IPDs was from the East and Southeast (Figure 4-20).

90
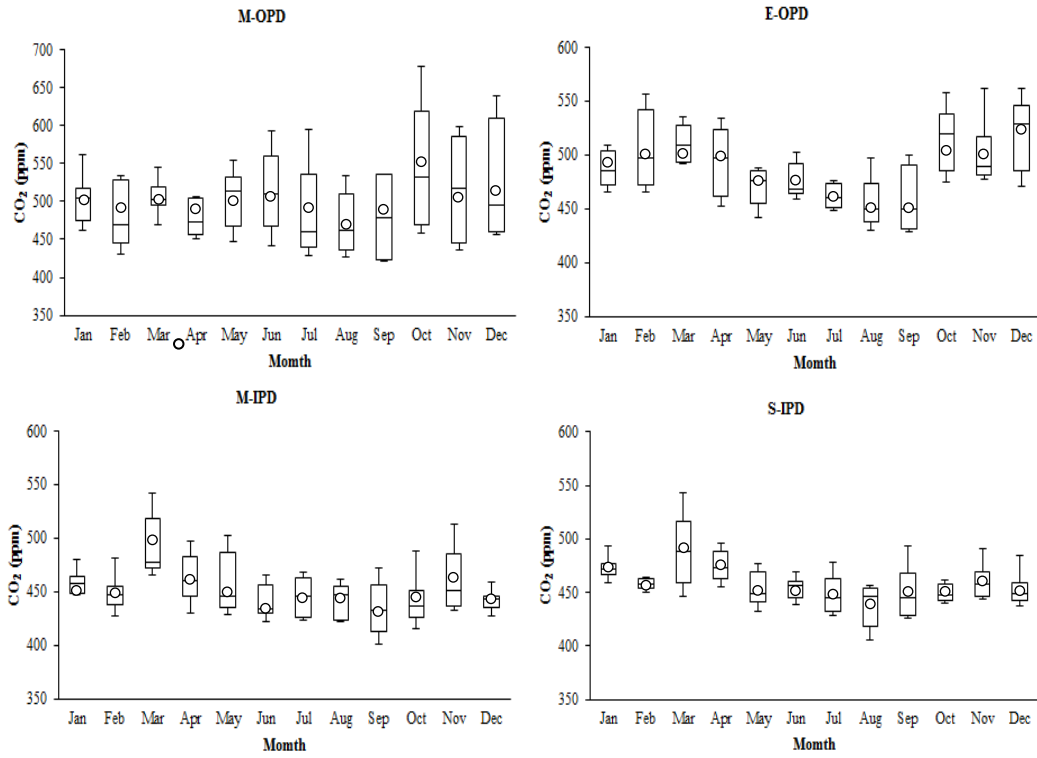
Figure 4-10 Variations of CO$_2$ concentration (ppm) in the service areas
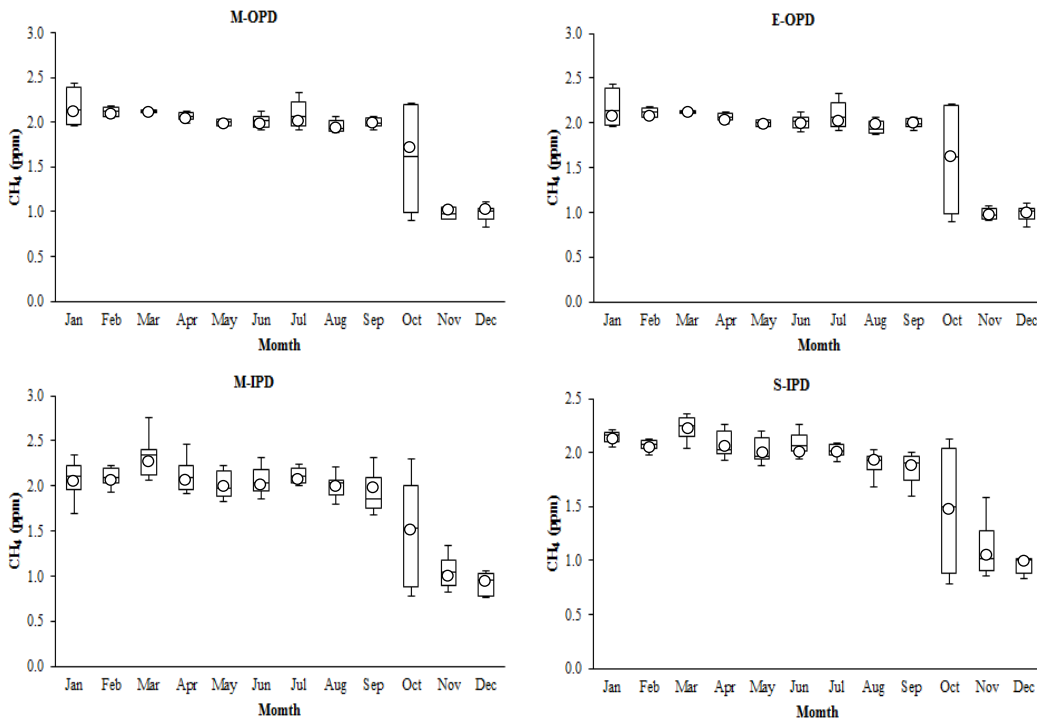
Figure 4-11 Concentrations of CH$_4$ (ppm) in the service areas of a university hospital
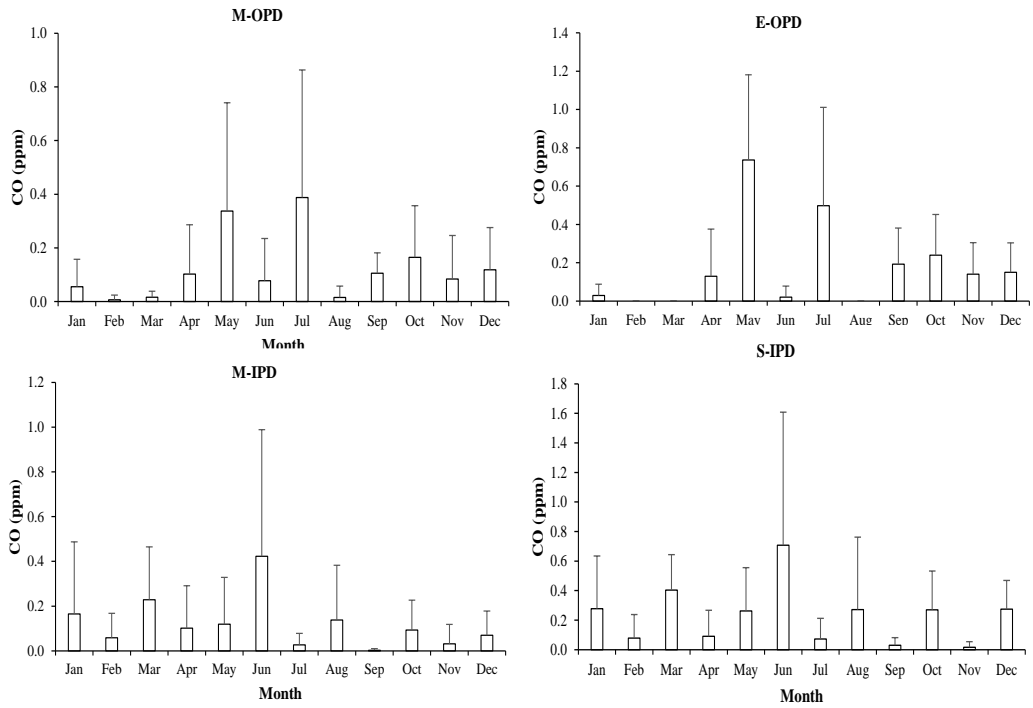
Figure 4-12 Concentrations of CO (ppm) in the service areas of a university hospital
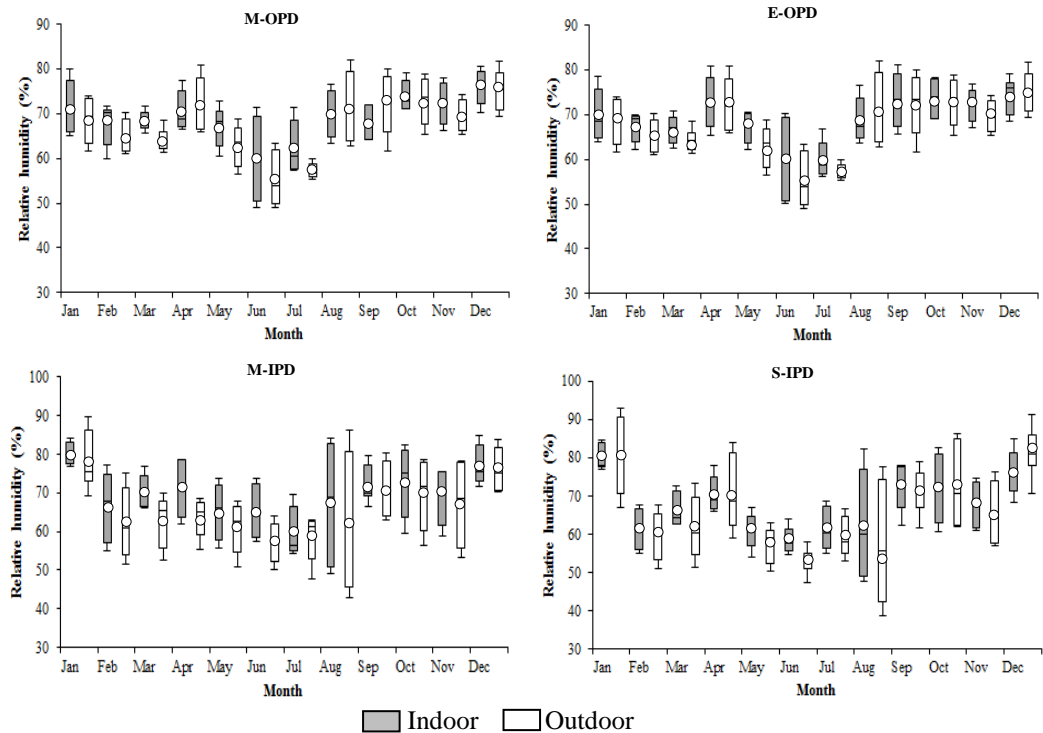
Figure 4-13 Variations of indoor and outdoor relative humidity at the service areas
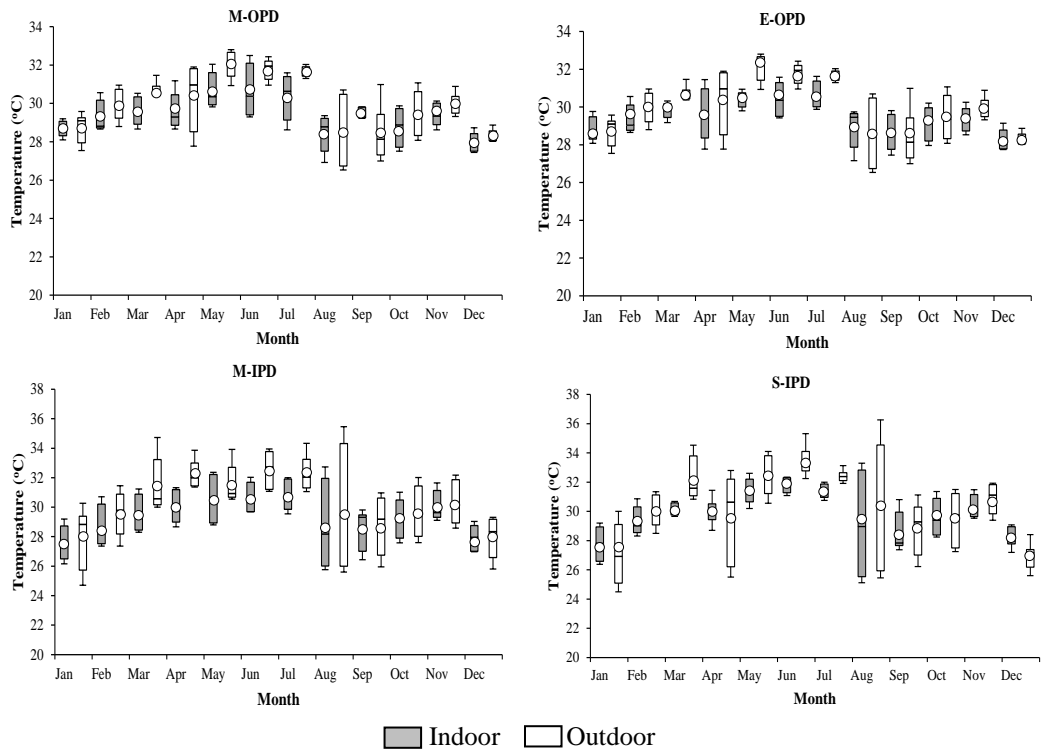
Figure 4-14 Variations of indoor and outdoor temperature (^{o}C) at the service areas
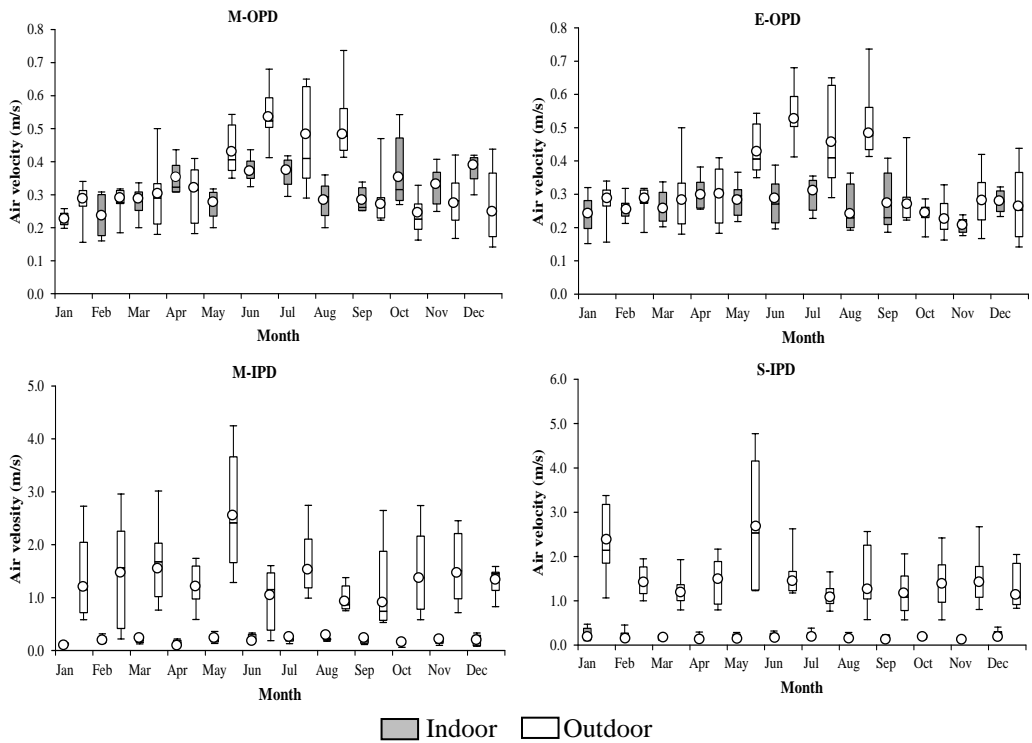
Figure 4-15 Variations of indoor and outdoor air velocity (m/s) at the service areas
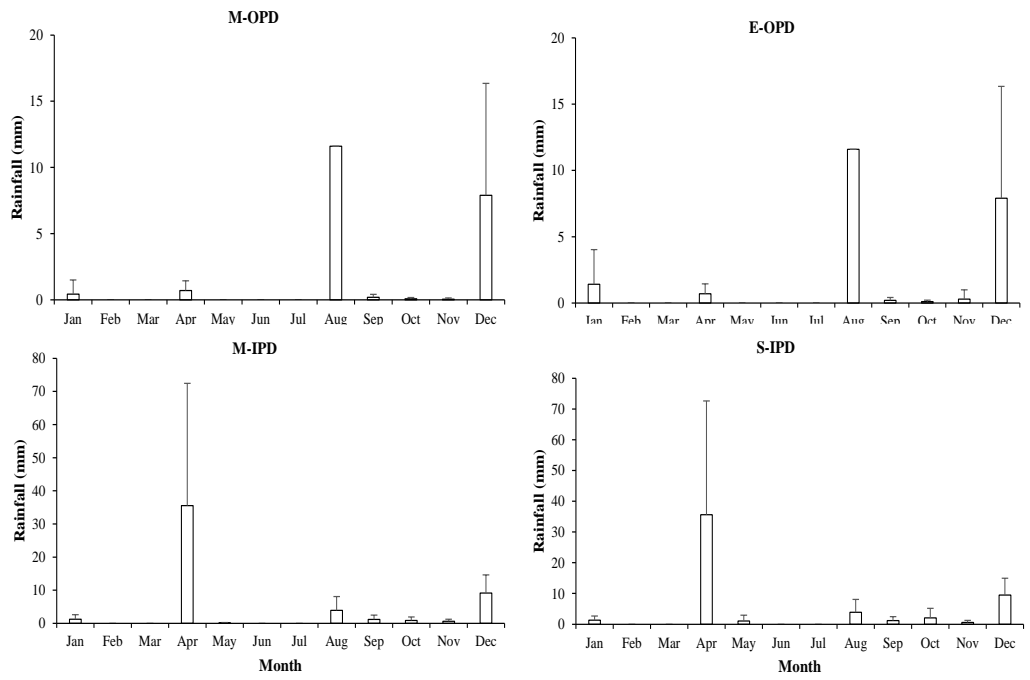
(A)
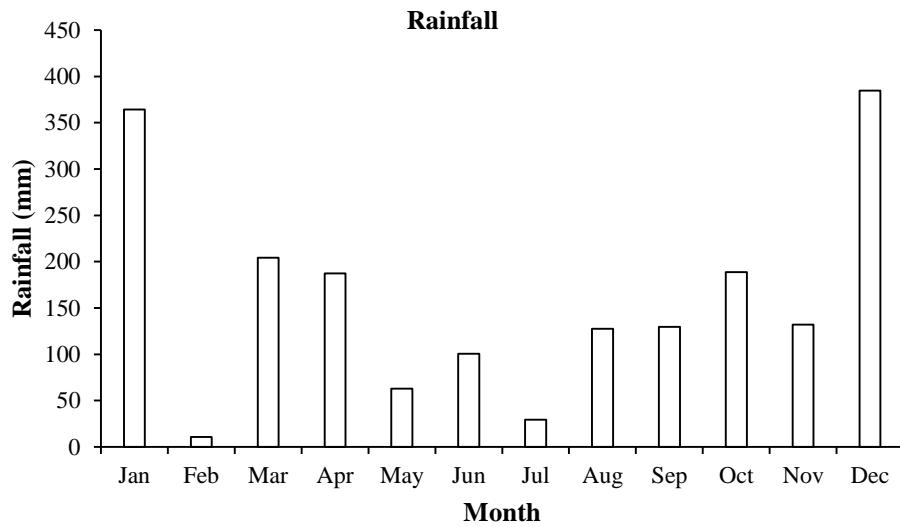
(B)

Figure 4-16 Variations of cumulative rainfall at the service areas (A)

and total rainfall (B) (Kohong agrometeorological station)
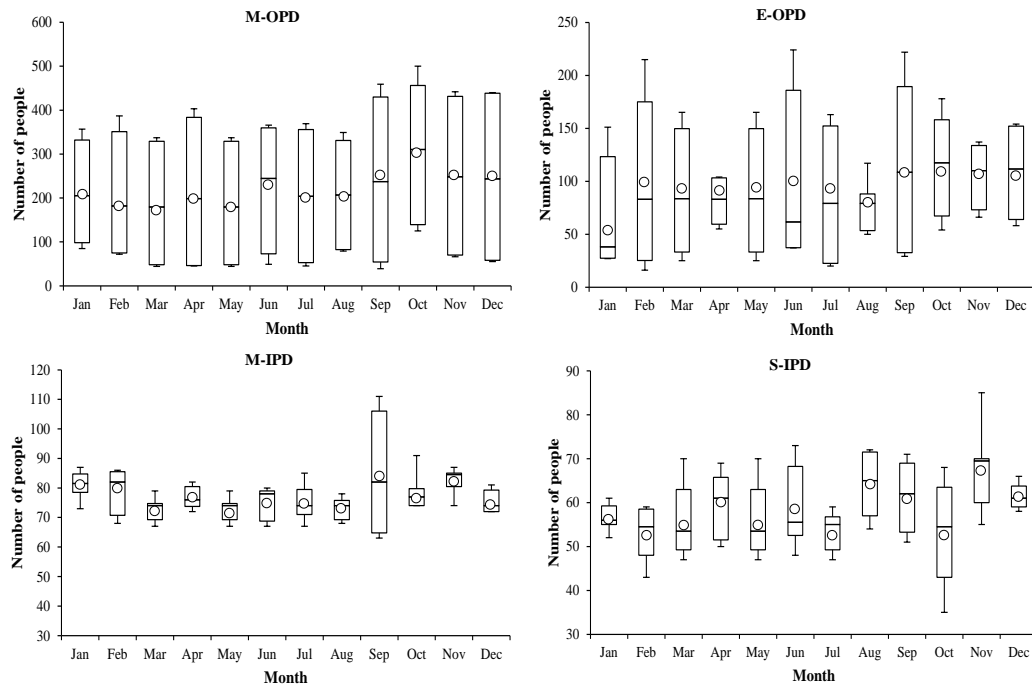
Figure 4-17 Variations in the number of people at the service areas
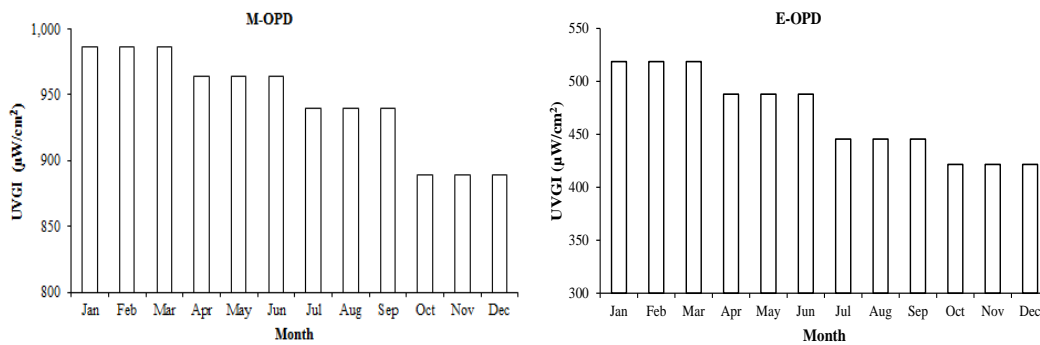
Figure 4-18 Variations of UVGI, UV intensity (μW/cm^2) at the OPDs
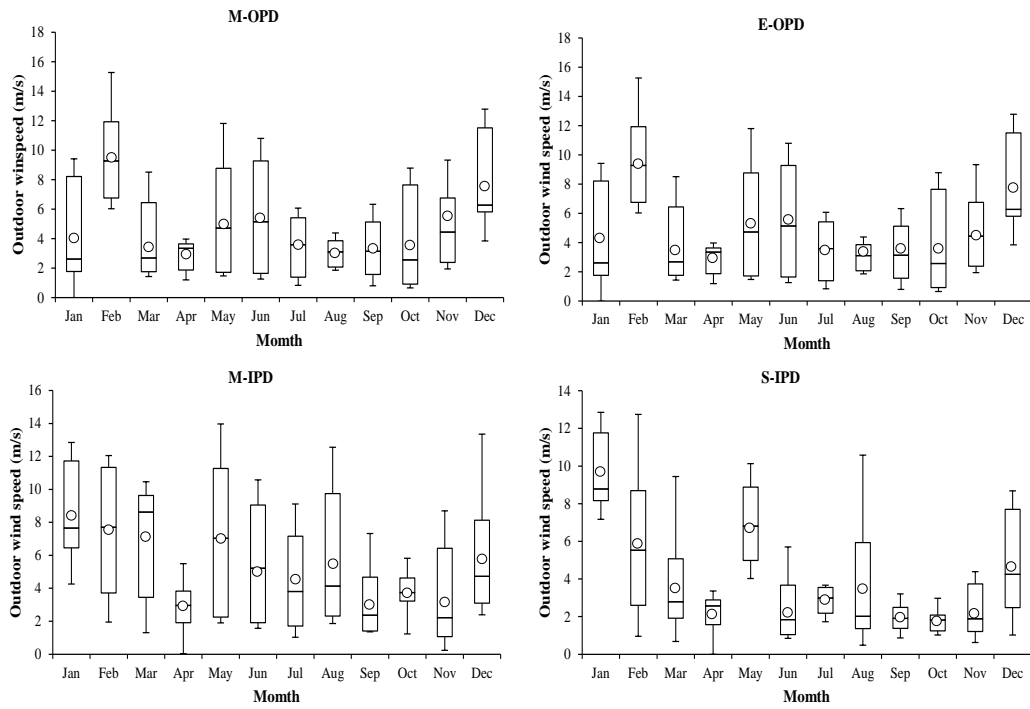
Figure 4-19 Variations of outdoor wind speeds at the service areas

(Kohong agrometeorological station)
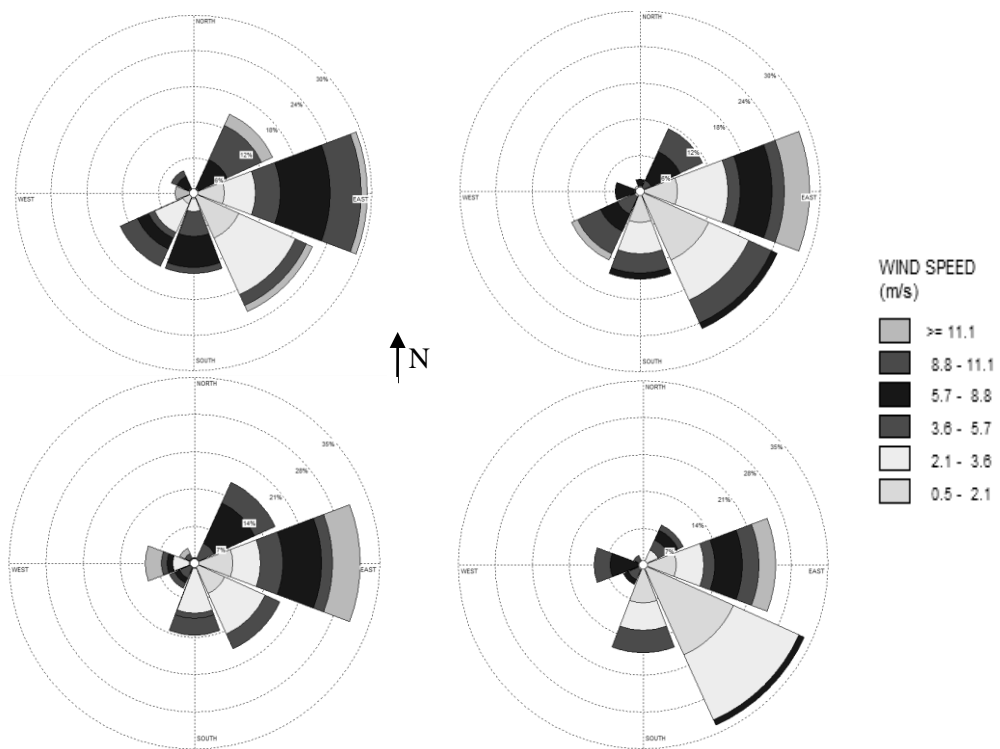
Figure 4-20 Wind directions at the service areas

(Kohong agro meteorological station)

4.3.7 Correlation of environment factors in the OPD and IPD service areas.

A relationship did exist among the environmental factors in the service areas of the OPDs (Table 4-3) that presented a positive correlation between the number of people and the levels of CO_2 (r = 0.663), indoor and outdoor temperatures (r = 0.778), indoor relative humidity and outdoor humidity (r = 0.733), rainfall and outdoor relative humidity (r = 0.598), while the number of people and period of service (r = -0.784), CO_2 and period of service (r = -0.657), indoor temperature and indoor relative humidity (r = -0.770), indoor temperature and outdoor relative humidity (r = -0.655) and outdoor temperature and outdoor humidity (r = -0.828) presented negative relationships.

In addition, the correlation among the environmental factors in the IPDs (Table 4-4) showed positive relationships between the indoor and outdoor temperatures (r = 0.864), indoor relative humidity and outdoor relative humidity (r = 0.868), indoor relative humidity and rainfall (r = 0.567) and outdoor relative humidity and rainfall (r = 0.550), whereas indoor temperature and indoor relative humidity (r = -0.888), indoor temperature and outdoor relative humidity (r = -0.826), indoor relative humidity and outdoor temperature (r = -0.781) and outdoor temperature and outdoor relative humidity (r = -0.907) had negative relationships.

Table 4-3 Spearman correlation coefficient of environmental factors in the OPDs

Environment factors	Period(I)	UV-C(I)	People(I)	CO_2(I)	CO(I)	CH_4(I)	Velocity(I)	Temperature (I)	Humidity (I)	Wind speed(O)	Wind direction (O)	Rainfall(O)	Velocity (O)	Temperature (O)	Humidity (O)
Period of service(I)	1.000														
UV-C(I)	0.00	1.000													
People(I)	-0.784**	0.246**	1.000												
CO_2(I)	-0.657**	0.052	0.663**	1.000											
CO(I)	0.421**	-0.156*	-0.345**	-0.246**	1.000										
CH_4(I)	-0.036	0.126	-0.185*	0.013	-0.105	1.000									
Velocity(I)	0.130	0.200**	-0.004	-0.177*	0.085	-0.294**	1.000								
Temperature(I)	0.228**	0.055	-0.261**	0.185*	0.242**	0.140	0.022	1.000							
Relative humidity(I)	-0.262**	-0.126	0.289**	0.310**	-0.095	-0.174*	-0.012	-0.770**	1.000						
Wind speed(O)	-0.426**	0.149*	0.448**	0.388**	-0.277**	-0.181*	0.012	-0.157*	0.150*	1.000					
Wind direction(O)	-0.126	-0.050	0.042	-0.114	-0.003	0.100	0.207**	0.176*	-0.072	-0.135	1.000				
Rainfall(O)	0.084	0.163*	-0.007	-0.104	-0.030	-0.113	-0.099	-0.465**	0.394**	0.186**	0.021	1.000			
Velocity(O)	-0.070	0.045	-0.061	-0.218	-0.095	0.000	0.188**	0.328**	-0.363**	-0.127	0.467**	-0.179*	1.000		
Temperature(O)	-0.108	0.119	-0.011	0.031	0.74	0.120	0.127	0.778**	-0.579**	0.038	0.226**	-0.559**	0.436**	1.000	
Relative humidity(O)	0.036	-0.173*	0.072	0.091	0.006	-0.169	-0.039	-0.655**	0.733**	-0.087	0.103	0.598**	-0.448**	-0.828**	1.000

** Correlation is significant at the 0.01

* Correlation is significant at the 0.05

I = Indoor

O = Outdoor

Table 4-4 Spearman correlation coefficient of environmental factors in the IPD$_s$

Environment factors	Period$^{(I)}$	People$^{(I)}$	$CO_2^{(I)}$	CO$^{(I)}$	$CH_4^{(I)}$	Velocity$^{(I)}$	Temperature (I)	Humidity (I)	Wind speed$^{(O)}$	Wind direction (O)	Rainfall$^{(O)}$	Velocity (O)	Temperature (O)	Humidity (O)
Period of service	1.000													
People$^{(I)}$	-0.069	1.000												
$CO_2^{(I)}$	-0.134	0.071	1.000											
CO$^{(I)}$	-0.629**	-0.258*	0.095	1.000										
$CH_4^{(I)}$	-0.027	-0.095	0.493**	0.130	1.000									
Velocity$^{(I)}$	0.171	-0.116	-0.185	0.068	0.117	1.000								
Temperature$^{(I)}$	0.465**	-0.074	0.075	0.459**	0.153	0.002	1.000							
Relative humidity$^{(I)}$	-0.425**	0.019	-0.039	-0.389**	-0.145	-0.418**	-0.888**	1.000						
Wind speed$^{(O)}$	-0.617**	0.017	0.114	-0.342**	0.120	-0.100	-0.514**	0.391**	1.000					
Wind direction$^{(O)}$	0.092	-0.224*	-0.165	-0.055	-.030	0.130	0.174	-0.131	-0.017	1.000				
Rainfall$^{(O)}$	0.035	-0.136	-0.147	-0.102	-.335**	-0.250*	0.471**	0.567**	-0.089	0.130	1.000			
Velocity$^{(O)}$	0.415**	-0.117	-0.021	0.252*	0.097	0.170	0.337**	0.271**	-0.101	0.095	-0.045	1.000		
Temperature$^{(O)}$	0.218*	-0.113	0.130	0.388**	0.262**	0.206*	0.864**	-0.781**	-0.371**	0.119	-0.446**	0.166	1.000	
Relative humidity$^{(O)}$	-0287**	0.101	0.010	-0.379**	-0.225*	-0.367**	-0.826**	0.868**	0.311**	-0.080	0.550**	-0.230*	-0.907**	1.000

** Correlation is significant at the 0.01

* Correlation is significant at the 0.05

I = Indoor

O = Outdoor

4.3.8 Correlation of environmental factors and bioaerosol quantities

The Spearman correlation coefficients between the airborne bacteria and fungi concentrations and the environmental factors are shown in Table 4-5. In the OPDs, the number of people, CO_2, indoor relative humidity, outdoor wind speed, outdoor temperature and outdoor bacteria had positive correlations to the indoor airborne bacteria concentration ($p < 0.05$); however, time of service and CO had negative relationships ($p < 0.05$). In addition, the indoor relative humidity, wind direction, rainfall, outdoor relative humidity and outdoor fungi had a positive influence on the indoor fungi concentration ($p < 0.05$); however, time of service, UVGI, indoor temperature and outdoor temperature had negative relationships on airborne fungi concentration ($p < 0.05$).

On the anther hand, the indoor airborne bacteria concentrations in the IPDs were related positively to CO_2, CH_4, outdoor temperature and outdoor airborne bacteria ($p < 0.05$). The indoor airborne fungi concentration had strong positive correlations with the indoor relative humidity, outdoor wind speed, wind direction, rainfall, outdoor relative humidity and outdoor airborne fungi, while the period of service, CO_2, CO, indoor temperature and outdoor temperature had negative correlations ($p < 0.05$).

Table 4-5 Spearman correlation coefficients between environmental factors and
indoor airborne bacteria and fungi in the OPDs and IPDs.

Environmental factors	OPDs		IPDs	
	Indoor bacteria	Indoor fungi	Indoor bacteria	Indoor fungi
Time of service	-0.716**	-0.175*	-0.090	-0.203*
UVGI	0.097	-0.148*	-	-
The number of people	0.662**	0.101	0.119	0.021
CO_2	0.655**	-0.097	0.350**	-0.204*
CO	-0.277**	-0.044	-0.003	-0.332**
CH_4	-0.129	-0.061	0.507**	-0.074
Indoor velocity	-0.039	0.101	0.050	-0.093
Indoor temperature	-0.036	-0.291**	0.129	-0.491**
Indoor relative humidity	0.209*	0.304**	-0.048	0.563**
Outdoor wind speed	0.361**	-0.029	0.097	0.261*
Wind direction	0.015	0.219**	0.070	0.245*
Rainfall	-0.125	0.424**	-0.129	0.351**
Outdoor air velocity	0.011	0.138	0.065	0.072
Outdoor temperature	0.168*	-0.258**	0.251*	-0.550**
Outdoor relative humidity	0.021	0.329**	-0.147	0.578**
Outdoor bacteria	0.363**	-	0.623**	-
Outdoor fungi	-	0.577**	-	0.784**

** Correlation is significant at 0.01
* Correlation is significant at 0.05

4.3.9 Time series analysis and forecasting of airborne bacteria and fungi with environmental factors

High correlation coefficient factors were used to predict the airborne bacteria and fungi concentrations in the OPDs and IPDs. The CO_2 presented a high correlation coefficient factor with the airborne bacteria at both sites. However, this study used the number of people instead CO_2 to predict the airborne bacteria because the source of CO_2 is from human activities and the results of the statistical analysis can be used easily for application in other hospitals. Figures 4-21 and 4-22 show the harmonic seasonal time series between the observed and predicted of indoor bacteria and fungi concentrations in the OPDs. The number of people, indoor relative humidity and outdoor bacteria were highly significantly related to the indoor airborne bacteria concentrations ($p<0.05$) that showed a pseudo R^2 at 56.75 (AIC = 1363.50), while the airborne fungi presented a pseudo R^2 at 48.97% (AIC 1430.76) that found indoor temperature and outdoor fungi were related to the indoor fungi concentrations ($p<0.05$).

Figures 4-23 and 4-24 show the harmonic seasonal time series between the observed and the predicted of indoor bacteria and fungi in the IPDs. The outdoor bacteria and the number of people were significantly correlated with the indoor airborne bacteria (Pseudo R^2 48.90%; AIC 1224.54) and indoor relative humidity and the outdoor fungi concentrations were correlated with the indoor airborne fungi concentrations (R^2=88.15; AIC=1357.34) (p<0.05).
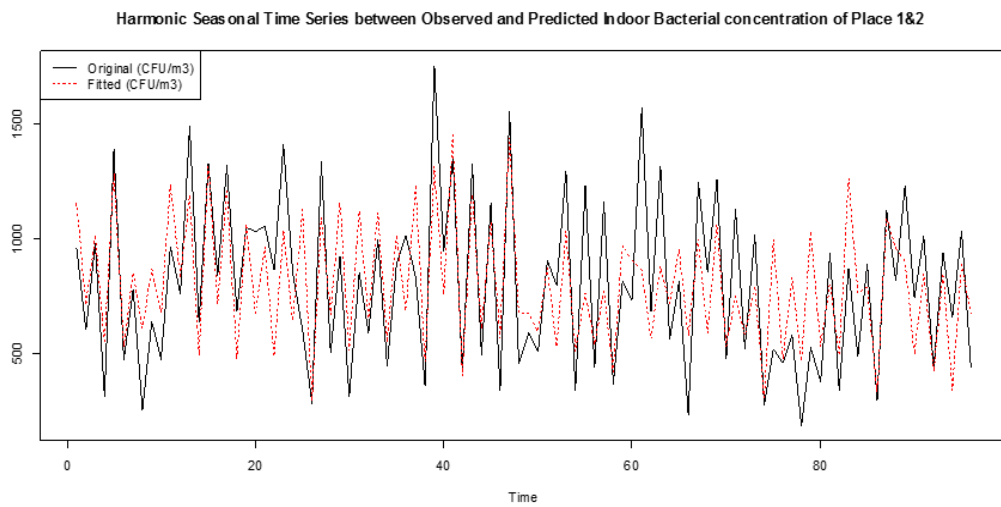
Figure 4-21 Seasonal time series between observed and prediction of indoor airborne bacteria in the OPDs
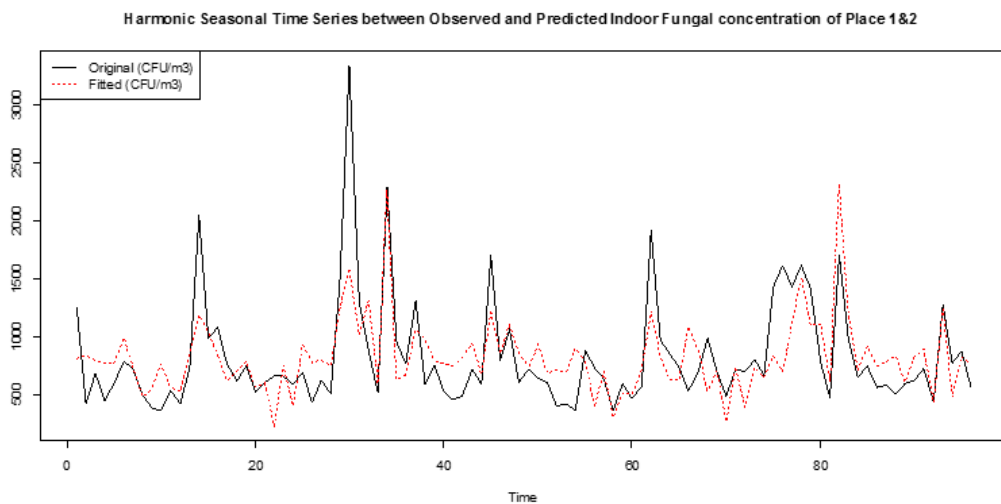
Figure 4-22 Seasonal time series between observed and prediction of indoor airborne fungi in the OPDs
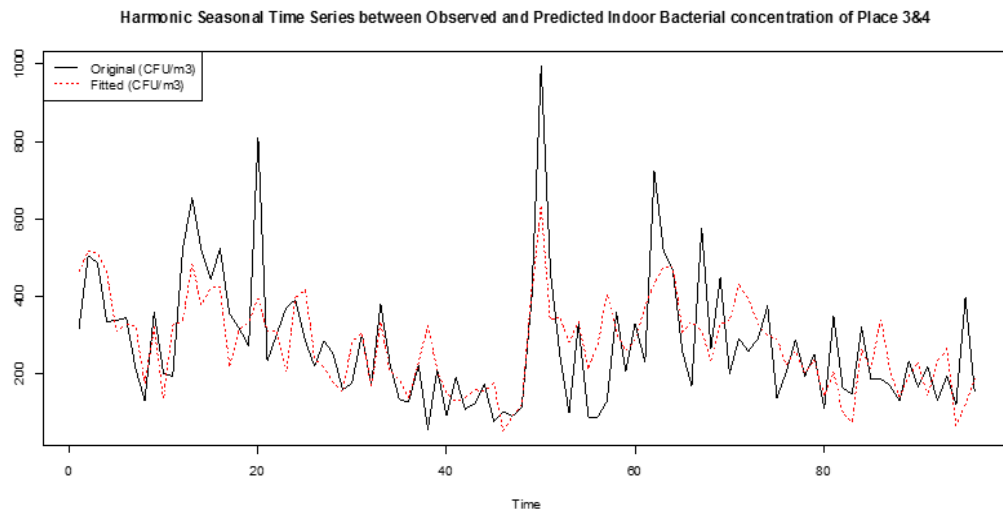
Figure 4-23 Seasonal time series between observed and prediction of indoor airborne

bacteria in the IPDs
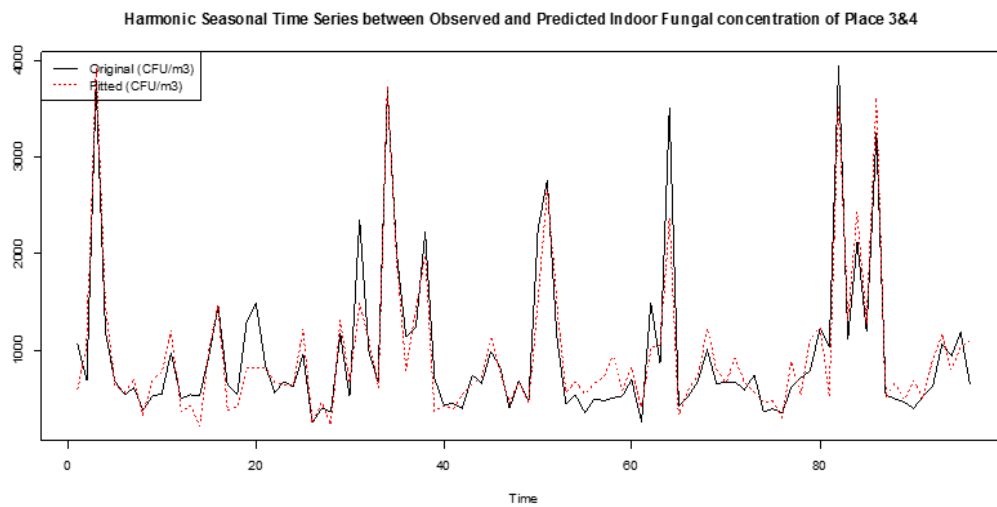
Figure 4-24 Seasonal time series between observed and prediction of indoor airborne

fungi in the IPDs

4.3.10 Protective and control measures of bioaerosols in dilution ventilation

A study of how to decrease the levels of bacteria and fungi in a building that utilizes the dilution ventilation system was carried out through focus group discussions. The study found that the participants were concerned more about how to reduce fungi than bacteria since the analyses found that the airborne bacteria that were discovered do not cause diseases or illnesses. Further, bacteria that spread sicknesses are usually through contact with contaminated medical personnel. However, *Mycobacterium tuberculosis* is known to spread through the air and nowadays is an issue in public health as well. Therefore, the focus group discussions concentrated on the drastic reduction of fungi in the IPDs because many patients come for services while there are many inpatients who are at risk for infection on the premises. Especially, *Aspergillus* spp. and *Fusarium* spp. are the major types of airborne fungi that induce nosocomial infections. The focus group discussions considered these recommendations to prevent airborne fungi in the IPDs.

1) Increasing the air circulation would help reduce the fungi concentrations in the building, especially in the M-IPD which is located on the 9th floor of the surgical and medical building which is the least wind-blocked by the surrounding buildings. Open doors and windows would assist in better air circulation and would result in less fungi reproduction. The air exchange rate should increase to over 6 air changes and this would apply to the inside of the S-IPD as well. This would not only reduce the amount of fungi reproduction but also control or reduce the humidity. If mechanical ventilation were employed to increase the air exchange rate, a high cost for electricity would be expected.

2) UVGI is one of the methods used by several hospitals in Thailand. It decreases the spread of TB and is installed in areas where the airborne diseases are being monitored, for example isolation rooms, ICUs and OPD areas that have a high number of visitors every day. However, a UVGI system needs constant maintenance and the UV lamps need to be cleaned very often. The humidity is also related to the effectiveness of UVGI, since a humidity level of over 60% together with dust in the area reduces the ability to kill germs. In hindsight, UVGI is probably not the best for a

hospital in the southern region of Thailand because of the high humidity and rain during much of the year.

3) The analyses from the statistics research found that the relative humidity inside the building with the IPDs was the main reason for the accumulation of fungi. In the IPD$_S$ there are many activities from humans such as medical treatment, shower/bath, breathing, cleaning and washing that can increase the relative humidity and affect the growth of indoor fungi. Moreover, some structural materials of a building, such as wood and gypsum board, can accumulate dampness and promote the growth of fungi in humid tropical environments. Moreover, the building with the IPDs in this study is older than 20 years and has water on the ceilings throughout the building. Leakage into the gypsum promotes the humidity that finally starts the growth of fungi. The participants of this group discussion had previously suggested that the gypsum ceilings be removed but the idea was rejected because removing the ceiling would affect the building design cosmetically. Besides that, the water fittings in the IPDs are also the reason for the humidity in the building. Hand washing and use of the washrooms frequently by the medical staff and visitors creates a huge amount of water use activities. Although, the ventilation fans are used widely, they still cannot cope with the heavy use of the toilets. It has been suggested that the premises be kept dry by mopping the floors. The pipelines and water system need to be monitored regularly to prevent the system from becoming worn out resulting in water leakage. If an incident of leakage occurs, the maintenance department shall be informed and repair performed within 3 days. However, the participants also gave examples of overseas hospitals that make repairs of the same type of incident within 12 hours. They also have the same concerns of wasting water and the increased humidity inside the building. Another way to create air circulation inside the building is to use ceiling fans located only in the patient wards. However, these ceiling fans would need regular cleaning to prevent the diffusion of dust particles and bioaerosols in the area.

4) The IPDs are cleaned and mopped four times per day with detergent to stop floor contamination which can reduce the bioaerosols from diffusing into the air. Mosquito nets are thoroughly cleaned fortnightly resulting in fewer dust particles and

bioaerosols from the outside. This method has been carried out very well by the hospital.

4.4 Discussion

4.4.1 The concentration of airborne bacteria and fungi in four service areas

The results of the bioaerosol concentrations indicated that people density was significantly related to the bacteria levels in line with other previous studies (Rajasekar and Balasubramanian, 2011; Park et al., 2013; Prussin and Marr, 2015). The sloughing of skin, human activity, speaking, breathing and hair particles of humans are significant human-associated reservoirs (Morawska et al., 2005; Xie et al., 2009; Hospodsky et al., 2012). Even though the indoor E-OPD and S-IPD had lower occupant densities, they had similar airborne bacteria concentrations as the indoor M-OPD and M-IPD. The phenomenon can be explained by the poor ventilation in both work sites.

In addition, variations in the indoor airborne bacteria levels were found in the M-IPD and S-IPD that were located on the 9th and 5th floors, respectively, of the surgical and medical building that showed lower concentrations in the wet season. Since the wet season has a high rainfall and the rainfall increased the relative humidity level in the air, it can clean the air and decrease the outdoor airborne bacteria level and decrease the indoor bacteria concentration at both work sites. These results were similar to other studies (Bhowmick and Rashid, 2004; Wang et al., 2010a; Hospodsky et al., 2012; Rangaswamy et al., 2012). However, this was not the same in the M-OPD and E-OPD that were located on the first floor and had a high density of people throughout the year. The occupant density affected the indoor airborne bacteria concentrations in the OPDs which resulted in a similar pattern throughout the year. Moreover, the indoor airborne bacteria and fungi proportions varied according to the occupant density.

On the other hand, the indoor fungi concentrations depended on the outdoor fungi levels and showed a similar pattern for the indoor and outdoor airborne fungi. These results were similar to several other studies (Sautour et al., 2009; Ponce-Caballero et al., 2013). The main sources of outdoor fungi were from the environment

surrounding the hospital (Shelton et al., 2002; Lee et al., 2006). The outdoor fungi passed through the windows and doors into the inside of the work sites which could increase the levels of the indoor fungi (Sautour et al., 2009; Nasir and colbeck, 2010; Ponce-Caballero et al., 2013). However, indoor organic materials in a relatively humid condition and the relative humidity from human activity could also lead to fungi growth in a building environment, and can increase the indoor airborne fungi concentration (WHO, 2009; Dedesko and Siegel, 2015).

The indoor to outdoor ratio (I/O) of airborne microorganisms can be applied to check spaces that were contaminated with airborne bacteria and fungi (Li and Kuo, 1994; Kim and Kim, 2007). In this study, all sites of investigation showed that the I/O ratios exceeded 1.0. However, the I/O ratios of bacteria were obviously higher compared with fungi. These results were similar to the Li et al. (2015)study. The indoor air had greater human activity and a greater number of occupants that were the main factors affecting the levels of airborne bacteria. The I/O ratio of airborne bacteria reported by Kim et al. (2010) was similar. They reported the I/O of airborne bacteria at 2.4 in a surgical ward while the I/O ratio of airborne fungi had the same pattern as the Li et al. (2015) study in a clinic and dormitory which found that the I/O ratio was between 1.1-1.4. However, the Kim and Kim (2007) study reported different ranges of I/O ratios of the airborne bacteria (0.28-0.71) and fungi (0.33-0.72) in a public building. The high concentrations of indoor airborne fungi were from anthropogenic sources. However, the I/O ratio varied according to the environmental conditions, ventilations and indoor sources.

The RFs of airborne bacteria and fungi also showed similar values at all work sites (p>0.05). Compared to a previous study by Kim et al. (2010), these results were different but the results were similar to a study by Wang et al. (2010b) who found the %RF of indoor bacteria and fungi at 62.8% and 81.4%, respectively. Other studies of %RF showed various results in other indoor environments (Nasir and Colbeck, 2010; Wang et al., 2010b; Li et al., 2015). However, the study by Li et al. (2015) explained that the %RF of airborne bacteria and fungi did not depend on the type of building or geographical and meteorological factors. It may depend more on the type of airborne bacteria and fungi. The RFs of bioaerosols below 5 μm have the potential to deposit in

the lower respiratory system and have potential health risks for medical personnel, relatives of patients and patients who are immunocompromised (McCluskey et al., 1996; Kim et al., 2010; Sturm, 2012).

4.4.2 Size distribution of airborne bacteria and fungi in four service areas

The size distributions of airborne bacteria in this study were similar to the Li et al. (2015) study. They found a peak pattern of airborne bacteria at the first stage (>7 μm) and third stage (2.1-3.3 μm). However, this result was different from the studies by Rajasekar and Ralasubramanian (2011) and Wang et al. (2010) who reported that the particle sizes of airborne bacteria were the highest at the fifth stage (1.1-2.1 μm). The large particles of airborne bacteria were the main fraction of the indoor air especially in areas of human activity and high temperature conditions. These results conformed to the Tham and Zuraimi (2005) study that found large particles in areas of high human activity and high temperature which can cause the formation of larger particles of airborne bacteria than at a lower temperature. Single cells of bacteria were in the size range of 1.0-2.0 μm (Tham and Zuraimi, 2005). Therefore, bacteria may agglomerate and combine with other particles to form larger particles of airborne bacteria than when they exist as single cells. These results also conform to the study of Wilson (2006) and Hospodsky et al. (2012). Moreover, the studies of Chao et al. (2009) and Xie et al. (2009) found that human indoor activities can also produce particles from the smallest to the much larger sizes (<0.8-125 μm). Therefore, indoor air can find airborne bacteria in all stages of sampling.

The size distribution of airborne fungi was similar to a study by Lin and Li (1996) and Zuraimi et al. (2009) that found a high peak of airborne fungi at 2.1-3.3 μm in a subtropical condition and in a building damaged by moisture, respectively. However, the results were different in other weather conditions that found the size distributions of airborne fungi at 1.1-2.1 μm and >7 μm (Kim et al. 2010; Wang et al. 2010b). The size distributions of airborne bacteria and fungi showed different particle sizes. They varied due to the environmental conditions, genus, types, nutrients and sources of bioaerosols (Meklin et al., 2003; Tham and Zuraimi, 2005; Yamamoto et al., 2012; Li et al., 2015). However, this study cannot explain the phenomenon of size

distribution of bioaerosols due to a lack of information to confirm the variance of particle size.

The accumulation size fractions of airborne bacteria and fungi were similar to Li et al. (2015) who found the particle sizes of the airborne bacteria were bigger than the airborne fungi. However, the particle sizes of airborne bacteria and fungi were different (2.77-2.99 µm and 2.51-2.83 µm). It may be due to different types of bioaerosols, sources, indoor activities and spore agglomerations (Li et al., 2015).

The average accumulative size fraction presented a similar pattern in the morning and in the afternoon periods. These results confirmed a study by Cox (1989) that found the size distribution of bacteria changed only slightly with the environmental conditions, while fungi spores can dehydrate and rehydrate faster than bacteria. Therefore, the particle size of fungi spores can increase to a larger particle and combine with other spores and other particles in a higher relative humidity condition. However, in the afternoon higher temperatures can decrease the relative humidity in the environment that can cause the dehydration of fungi spores and decrease the particle size. These results conformed to the studies of Cox et al. (1989) and Reponen et al. (1996).

4.4.3 Environmental factors and seasons affected the concentrations of airborne bacteria and fungi

The Spearman correlation coefficients showed differences between the airborne bacteria and fungi concentrations and environmental factors. The period of service showed a difference in the concentrations of airborne bacteria in the OPDs. In the mornings, a greater amount of human activity and higher occupant densities at the OPDs can produce airborne bacteria and increase the CO_2 concentrations in the indoor air to levels greater than in the afternoon period. Moreover, the increase of indoor CO_2 concentration was significantly related to the airborne bacteria accumulation in the building. These results were similar to the reports in some studies (Fox et al., 2003; Mahyuddin et al., 2013). However, the CO_2 can be predicted from the building ventilation and human emissions. It can affect the quantity of airborne bacteria. Relative humidity is one factor that is very important for the survival and growth of

bacteria in the environment and was shown to have a positive relationship with airborne bacteria. The results were similar to other studies (Obbard and Fang, 2003; Park et al., 2013). In addition, the indoor CO showed a negative correlation with airborne bacteria because it can reduce some bacteria in a high humidity environment (Lighthart, 1973). Outdoor temperature was positively related with indoor airborne bacteria. A high temperature condition may increase the level of dust particles in the outdoor environment which has an impact on the indoor service areas. Choi et al. (2005) found a positive correlation between total dust and airborne bacteria. In addition, an increase of the outdoor wind speed can augment the concentration of bacteria because outdoor microorganisms can increase the indoor numbers via the conductive air of a dilution ventilation system (Meadow et al., 2014; Gandolfi et al., 2015).

The semi-closed areas of the IPDs showed a positive relationship of CO_2, outdoor temperature and outdoor airborne bacteria with indoor airborne bacteria concentrations. The reasons for these factors were similar to the OPD sites. Moreover, the service areas of the OPDs showed that the CH_4 levels had a positive correlation with the indoor airborne bacteria. The sources of CH_4 in the indoor air were from decomposition of organic matter in the bins, toilets and human activities. CH_4 can accumulate in the building and it showed a positive relationship with airborne bacteria which was similar to the results found in another study (Wu et al., 2012).

The fungi concentration levels in the outdoor air contributed to the indoor fungi concentration levels at all of the OPD and IPD sites. A high wind speed around a building and wind direction from a green environment can lead especially to outdoor airborne fungi entering the indoor air (Abdel Hameed et al., 2009) except for the outdoor wind speed at the OPD sites because they were located on the first floor of the building where the wind is blocked by the surrounding buildings. The fungi in the wind passes through the windows and doors into the inside work sites that can increase the indoor fungi levels (Sautour et al., 2009; Ponce-Caballero et al., 2013). Rainfall increases the humidity level in the indoor and outdoor environments which affected the survival and growth of fungi (Erkara et al., 2008). Especially, the high relative humidity of the indoor air from human activity and building materials can

potentially increase the indoor fungi concentration as well (Andersen et al., 2011). The time of service is one important factor that affects the concentration of indoor airborne fungi in the work sites especially in the afternoon period that showed higher concentrations than in the morning. The indoor airborne fungi concentration in the afternoon possibly was affected by the outdoor fungi that were present in higher concentrations in the afternoon.

However, the indoor temperature was negatively related to the airborne fungi concentration. A high temperature can decrease the relative humidity of an indoor environment which affects fungal growth. Moreover, some types of fungi, such as *Penicillium* spp., *Coprinus* spp., *Didymella* spp., *Leptosphaeria* spp. and *Pleospora* spp. had low concentrations in a high temperature environment (Oliveira et al., 2008; Quintero et al., 2009). These results were different compared with other studies which found that spore fungi had a positive relationship with temperature (Stennett and Beggs, 2004; Erkara et al., 2008). Another study found that temperature had no significant relationship with the fungi concentration (Hoseini et al., 2012). A UVGI system in the OPDs can reduce the airborne bioaerosol concentration in the work sites which is recommended in high occupant density environments (Escombe et al., 2009). However, a UVGI system had a low efficiency when the relative humidity increased to more than 60% (NIOSH, 2009), especially in a humid tropical environment.

In addition, the CO_2 and CO in the IPDs were shown to have a negative relationship with indoor airborne fungi. The indoor airborne fungi in the IPDs had a high correlation with outdoor fungi. A dilution ventilation system increases the indoor fungi and decreases the accumulation of CO_2 and CO indoor air which confirmed the negative correlation between these factors and airborne fungi. This result was similar to another study on CO_2 (Chao et al., 2002), while the studies of Ho et al. (2005), Kim et al. (2009), and Wang et al. (2010b) found a positive correlation between CO_2, CO and airborne fungi at a work place.

A time series analysis found a seasonal effect on the airborne bacteria and fungi concentrations. The seasonal pattern of airborne bacteria in the OPDs depended on the number of people, indoor relative humidity and outdoor airborne bacteria, while the number of people and outdoor airborne bacteria affected the indoor airborne

bacteria in the IPDs. In addition, the indoor fungi concentrations in the OPDs varied with the indoor temperature and the outdoor airborne fungi concentrations. Meanwhile, the indoor airborne fungi concentrations depended on the indoor relative humidity and outdoor airborne fungi concentrations in IPDs.

4.3.4 Protective and control measures

The focus group discussion summarized the strategies to reduce the level of airborne fungi in the hospital. 1) Improve the air circulation inside the building by opening the windows and doors and not blocking the air passages. This would provide better air ventilation and lower the humidity that causes fungi to grow inside the buildings. 2) Regular monitoring and maintenance of the water system especially in areas of high levels of activity such as hand basins and washrooms. 3) Adjust the mopping schedule to meet the requirements, for example mopping the washroom floor in public areas after every use to keep the area clean and dry which can reduce the indoor relative humidity. 4) Always have clean floors, ceiling fans and mosquito nets to stop diffusion of bioaerosols and for better air circulation. This practice has been implemented and satisfies the standard. These actions can be implemented quickly at a low cost and are expected to reduce the indoor relative humidity and bioaerosols in the service areas of the hospital.

4.5 Conclusions

The airborne bacteria concentration varied in different indoor environments of the service areas which used the dilution ventilation system. The total mean concentration of airborne bacteria was the highest in the M-OPD followed by the E-OPD, M-IPD and the S-IPD which had the lowest concentration. The airborne fungi had the highest concentrations in the S-IPD followed by the M-IPD, E-OPD and it was the lowest in the M-OPD. This can be explained by differences in the occupant densities, human activities and environmental factors. The I/O ratios of airborne bacteria and fungi concentrations were more than 1 at all of the service areas. The RF of the airborne bacteria was between 59% and 62% while the airborne fungi were in a range of 84% to 86%. The size distribution of airborne bacteria did not vary by the

environment which showed particle sizes at >7 µm and 2.1-3.3 µm for airborne bacteria and 2.1-3.3 µm for airborne fungi in the service areas. Time series regression analysis in the OPDs showed seasonal variations of indoor airborne bacteria concentrations which had a highly significant relationship with the number of people, indoor relative humidity and outdoor bacteria concentration while the indoor airborne fungi had a highly significant relationship with the outdoor airborne fungi concentration and indoor temperature. For the IPDs, the number of people and outdoor bacteria were significantly correlated with the indoor airborne bacteria, and the indoor relative humidity and outdoor fungi were significantly correlated with indoor airborne fungi. The recommendations to reduce the contamination of bioaerosols in the dilution ventilation system were to increase the ventilation at the work sites, prevent leaks from the plumbing system in the building, keep the floors dry, clean the ceiling fans and mop up the water after patients visited the bathrooms and toilets. These activities can be implemented quickly and can reduce indoor humidity and indoor bioaerosols.

CHAPTER 5
General Conclusion

This study investigated the size, type and quantity of airborne bacteria and fungi and the relationship between the environmental factors and airborne bacteria concentrations in a university hospital. Moreover, this study provides recommendations to improve the performance of the management system and reduce the bioaerosol contamination. The study sites were the S-ICU and M-ICU where heat, ventilation and air-conditioning (HVAC) systems were used and the M-OPD, E-OPD, M-IPD and S-IPD that used the dilution ventilation system. The types and size distributions of the airborne bacteria and fungi in the MICU and SICU were investigated. The average concentrations of airborne bacteria in the MICU and SICU were 214.22 ± 93.27 cfu/m^3 and 194.25 ± 74.83 cfu/m^3 and 274.44 ± 140.75 cfu/m^3 and 234.39 ± 115.60 cfu/m^3 for airborne fungi, respectively. Most airborne bacteria and fungi in the ICUs showed a size distribution at 1.1-3.3 μm. The four predominant types of bacteria were *Staphylococcus* spp., *Micrococcus* spp., *Bacillus* spp. and *Pseudomonas* spp., while the fungi specimens were predominantly *Cladosporium* spp., *Penicillium* spp., *Aspergillus* spp. and *Fusarium* spp. The size distribution of fungi showed peaks of *Cladosporium* spp., *Penicillium* spp. and *Aspergillus* spp. at high concentrations in the range of 2.1-3.3 μm. The results found a similar size distribution of airborne *Staphylococcus* spp., *Micrococcus* spp. and *Pseudomonas* spp. The bioaerosols from the construction work site and at 15 meters from the building found two important pathogens (*A. fumigatus and A. flavus*) but they were not found in the indoor air at either of the ICUs. The total number of indoor bacteria colonies at the opening of the UVGI system and the room air velocity were significant factors, while the indoor fungi colony count depended significantly on the total number of outdoor fungi, room air velocity, relative humidity and temperature. However, the use of HEPA filters, anthropogenic sources and the existence of under-construction buildings around the hospital remain points of concern in terms of controlling the concentrations and size distributions of the bioaerosols in the ICUs of the university

hospital. Especially, the UVGI system and air velocity were very important factors to decrease the bioaerosol concentrations in the limited spaces of the ICUs.

On the other hand, data on the types and size distributions of airborne bacteria and fungi in the M-OPD, E-OPD, M-IPD and S-IPD were collected from January to December 2012 that used the dilution ventilation system. The average concentrations of indoor airborne bacteria in the service areas of the M-OPD, E-OPD, M-IPD and S-IPD were 859.10 ± 411.66 cfu/m^3, 758.24 ± 347.01 cfu/m^3, 285.59 ± 178.12 and 279.27 ± 186.06 cfu/m^3, while the concentration of airborne fungi was 853.80 ± 561.84 cfu/m^3, 913.21 ± 766.70 cfu/m^3, 961.87 ± 739.76 cfu/m^3 and 985.20 ± 853.71 cfu/m^3, respectively. The airborne bacteria concentrations in the OPDs were higher than in the IPDs. The main sources of indoor airborne bacteria were from human activity and occupant density. However, the indoor and outdoor airborne bacteria in the IPDs from August to December showed lower concentrations. The high rainfall can increase the humidity level and decrease the airborne bacteria while the airborne fungi levels depended on the outdoor fungi levels and were similar at all service areas. The I/O ratios ranged between 2.00 and 2.36 for airborne bacteria and between 1.27 and 1.41 for airborne fungi. In all service areas, the RFs of airborne bacteria were in the range of 59.05%-61.75% while the airborne fungi were in the range of 84.56%-85.68%. The size distribution of airborne bacteria showed mainly a double-peak pattern in stage 1 (>7.0 µm) and stage 4 (range, 2.1-3.3 µm). Otherwise the airborne fungi were mainly found in stage 4 (range, 2.1-3.3 µm) followed by stage 5 (range, 1.1-2.1 µm) and stage 3 (range, 3.3-4.7 µm). Moreover, the average d_{50} results of the indoor airborne bacteria and fungi in the service areas were 3.17 ± 0.19 µm and 2.81 ± 0.14 µm, respectively. These results were similar to the high peaks of the dry (February to April) and wet seasons (October to December).

The respiratory fractions and particle sizes of airborne bacteria and fungi had similar patterns in the dry and wet seasons. The main genera found were also not different in the two seasons. *Staphylococcus* spp., *Micrococcus* spp., *Bacillus* spp. and *Corynebacterium* spp. were the predominant genera of airborne bacteria while *Cladosporium* spp., *Penicillium* spp., *Aspergillus* spp. and *Fusarium* spp. were the predominant airborne fungi. They are isolated mainly from the environment and

human activity. The bioaerosol genera were often found in the indoor air of the hospital and the types were not different between the dry and wet seasons. The size distribution of the dominant genera also revealed most of the airborne bacteria in the hospital indoor air. The airborne bacteria were in free and combined forms with other particles, whereas the indoor airborne fungi were the conidia and spore forms distributed in the indoors and outdoors.

The number of people, CO_2, indoor relative humidity, outdoor wind speed, outdoor temperature and outdoor bacteria were positively correlated to indoor airborne bacteria concentrations in the OPDs. However, the period of service and CO had a negative relation while indoor relative humidity, wind direction, rainfall, outdoor relative humidity and outdoor fungi were a positive influence on the indoor airborne fungi concentrations. However, the period of service, UVGI and indoor temperature had a negative relationship. On the another hand, indoor airborne bacteria concentrations in the IPDs had high positive correlations with CO_2, CH_4, outdoor temperature and outdoor bacteria, whereas the indoor airborne fungi concentrations had a strong positive correlation with indoor relative humidity, outdoor wind speed, wind direction rainfall, outdoor relative humidity and outdoor fungi but were negatively related to the period of service, CO_2, CO, indoor temperature and outdoor temperature. The time series regression analysis found a seasonal effect on the airborne bacteria that varied by the number of people, indoor relative humidity and outdoor bacteria in the OPDs and the number of people and outdoor bacteria in the IPDs. The indoor airborne fungi in the OPDs were affected by the indoor temperature and outdoor fungi while indoor airborne fungi in the IPDs depended on the indoor relative humidity and outdoor fungi. The focus groups discussed how to reduce the airborne bacteria and fungi in the workplaces that used the dilution ventilation system. The participants recommended improving the performance management system to decrease the levels of bioaerosols in the hospital. The recommendations included: 1) Greater air turnover would have a great influence on the reduction of the bioaerosols in the building; 2) Proper operation and maintenance of the water supply to prevent plumbing leaks in the building; 3) The bathrooms and toilets need to have dry floors that require mopping up the water after patient use and 4) Environmental cleaning can

reduce the levels of airborne fungi in the workplaces by cleaning the floors and ceiling fans inside the building which can reduce the relative humidity and the spread of bioaerosols in the air.

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