ASSESSMENT OF VENTILATION SYSTEM, COMFORT LEVEL AND MICROBIAL AIR QUALITY OF ACADEMIC AND HOSPITAL BUILDING

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By

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I certify that this research work titled *"Assessment Of Ventilation System, Comfort Level And Microbial Air Quality Of Academic And Hospital Building"* is my own work. The work has not been presented elsewhere for assessment. The material that has been used from other sources as been properly acknowledged/referred.

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ABSTRACT

This study aimed at performing the following objectives (i) investigating and comparing indoor air quality (IAQ) and thermal comfort in classrooms of four departments of NUST having different types of heating ventilation and air conditioning (HVAC) system; (ii) seasonal(winter and spring) assessment of airborne microbial levels in eight hospital sites of a publically managed hospital of Islamabad; (iii) airborne microbial levels assessment in three cafeterias (A, B and C), with different HVAC system. On-site continuous measurements of indoor levels of carbon dioxide (CO_2), temperature (T) and relative humidity (RH) were recorded from fifteen classrooms and two hospital locations at an interval of 1 minute for both weekdays, including occupational and non-occupational hours, as well as weekends. However, microbial samples from six hospital locations (twice a week covering one month for each season) and three cafeterias (each having two indoor locations and one outdoor) were collected during the peak hours. Simultaneous outdoor temperature and relative humidity measurements were also monitored during the study and used in the analysis. Results of mean hourly CO_2values and thermal comfort parameters of selected classroom and hospital site showed significant difference over the weekday and also among different buildings. Exceedance in levels of CO_2 from ASHRAE standards was found to be more in buildings with non-centralized systems as compared to centralized systems. Moreover, thermal comfort parameters were influenced by outdoor climatic conditions and buildings orientation. Bacterial concentration levels of hospital sites didn't exhibit any significant seasonal variation however fungal concentrations were different. Highest bacterial level in hospital and cafeterias were found in OPD and $CafeB_{SC}$, while lowest in OT1 and in $CafeC_{C2}$. Moreover, highest fungal level was found in GMW and $CafeA_{SC}$ while lowest in OT2 and $CafeC_{C2}$. Identified bacterial strains from both monitored locations belonged to genera staphylococcus, micrococcus, kocuria, aerococcus, kytococcus, bacillus and pseudomonas. However, the most dominant fungal genera include cladosporium, aspergillus, penicillium, alterneria, geotrichium, fuserium and ulocladium.

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List of Abbreviations

IAQ	Indoor Air Quality
CO_2	Carbon dioxide
T	Temperature
RH	Relative Humidity
NUST	National University of Science and Technology
PIMS	Pakistan Institute of Medical Sciences
ICU	Intensive Care Unit
PCR	Polymerase Chain Reaction
LCR	Ligase Chain Reaction
PFGE	Pulsed-field Gel Electrophoresis
DNA	Deoxyribonucleic Acid
IESE	Institute of Environmental Sciences and Engineering
NIT	National Institute of Transportation
CASEN	Center of Advanced Studies in Energy
NBS	NUST Business School
NDIR	Non-dispersive Infrared
WD	Weekdays
WE	Weekends
OT1	Emergency Operation Theatre
OT2	General Surgery Operation Theatre

SW	Surgical Ward
GMW	General Medicine Ward
ES	Emergency Services
OPD	Out-Patient Department
OT3	Liver Operation Theatre
ICU	Intensive Care Unit
HVAC	Heating, Ventilation and Air-Conditioning
TSA	Tryptone soy agar
PDA	Potato dextrose agar
WHO	World Health Organization
ACGIH	American Conference of Governmental Industrial Hygienists
PNS	Portuguese National Standards

Chapter 1

INTRODUCTION

1.1. Background

Increasing growth rate of urban population has made air pollution a global issue. Depending upon time and concentration of exposure to pollutants, both indoor as well as outdoor air pollutants may be detrimental to human health (Salthammer et al., 2016). Concentration of indoor air pollutants is found to be higher than outdoor pollutant concentrations in most of the cases (Branco et al., 2015). Also human spend more time in indoor as compared to outdoor which makes a more concerning situation for indoor air quality (IAQ) to ensure health protection of people (Salthammer et al., 2016 ; Branco et al., 2015 ; Madureira et al., 2015) Pollutants from outdoor tend to infiltrate indoor, thus dependence of indoor air quality on outdoor air quality and outdoor climatic conditions (temperature and humidity) can't be denied (Salthammer et al., 2016). In addition to this, present ventilation system and indoor activities of building occupants are also contributors of IAQ (Yoon, Lee, & Park, 2011).

Molds and bacteria in indoor environment are the microbial degrading factors for IAQ. Airborne microbes can enter in indoor environment from outdoor through ventilation system incorporated (natural or mechanical) in that environment (Yassin & Almouqatea, 2010). Indoor and outdoor moisture content and comfort parameters (temperature and relative humidity) are the driving factors for the growth of airborne

micro-organisms (Yassin & Almouqatea, 2010; Kim, Kim, Kim, Nakajima, & Higuchi, 2009; Rajasekar & Balasubramanian, 2011). Moisture problems in buildings can cause increasing growth rate of micro-organisms (Salonen, Lappalainen, Lindroos, Harju, & Reijula, 2007). Detrimental impacts on individuals health has been observed due to their exposure to airborne biological pollutants (bacteria and fungus) (Rajasekar & Balasubramanian, 2011; Teixeira & Oliveira, 2015) which include short-term as well as long-term health issues like respiratory diseases, allergies, infections (Rajasekar & Balasubramanian, 2011), hypersensitivity (Yassin & Almouqatea, 2010) (Gładyszewska-Fiedoruk, 2011), tiredness, rhinitis, headache (Salonen et al., 2007) and asthma (Madureira et al., 2015).

Nature and condition of present ventilation system along with presence of humans, outdoor pollutants, pets, plants etc. contribute as sources of airborne bacteria in indoor environment. Sources for airborne fungi in indoor environments on the other hand can be indoor as well as outdoor where outdoor sources contribute more in case of naturally ventilated building (Teixeira & Oliveira, 2015). People with immune deficiencies (children, elderly people, and patients) are more vulnerable to diseases associated with poor IAQ (Madureira et al., 2015; Yoon et al., 2011; Yassin & Almouqatea, 2010; Teixeira & Oliveira, 2015).

Among the different methods used to assess the quality of indoor air, the globally accepted method by most researchers is taking carbon dioxide (CO_2) levels as a surrogate of quality of indoor air and ventilation, because high CO_2 levels are indicator of poor air exchange of indoor environment from which build-up of concentration of

other pollutants can be inferred (Salthammer et al., 2016; Branco et al., 2015; Gładyszewska-Fiedoruk, 2011). Temperature (T) and relative humidity (RH) on the other hand can be taken as comfort indicators (Branco et al., 2015). Besides this health impacts related to high CO_2 levels include headaches, lack of concentration, tiredness etc. (Krawczyk, Rodero, Gładyszewska-Fiedoruk, & Gajewski, 2016). Increasing indoor CO_2 levels can be due to poor building design and ventilation system (Griffiths & Eftekhari, 2008).

1.2. Study Area

Keeping in view the importance of IAQ in human life, this study investigated the IAQ of three indoor environments (Classrooms and Cafeterias in academic building, and multiple hospitals sites) having different type of indoor pollutant sources. CO_2 concentration levels were taken as ventilation and IAQ surrogate for selected classrooms of National University of Science and Technology (NUST), Islamabad and in selected sites of a publically managed hospital of Islamabad, Pakistan. T and RH on the other hand served as comfort indicators. This study further involved investigations to explore how different ventilation types may influence indoor air quality and the comfort level. Seasonal variation of microbial indoor air concentration of selected sites of a publically managed hospital of Islamabad, Pakistan, has been assessed covering winter and spring. Microbial monitoring also includes three cafeterias of NUST, Islamabad. On-site monitoring of CO_2, T, RH and airborne microorganisms was carried out followed by ventilation performance assessment and comparison of different types of ventilation systems.

1.3. Objectives

The objectives of this study include,

- Performance evaluation and comparison of ventilation systems of class rooms of selected buildings of NUST by monitoring CO_2, temperature and relative humidity
- Seasonal assessment of airborne microbial levels in selected hospital sites and evaluation of ventilation system performance of operation theatre and intensive care unit (ICU)
- Assessment of microbial levels in selected sites of food courts in NUST

Chapter 2

LITERATURE REVIEW

2.1. Indoor Air Quality (IAQ)

Indoor air quality (IAQ) refers to air quality within the building that has an influence on human health and comfort (Gładyszewska-Fiedoruk, 2011). Scientists are more concerned about IAQ due to the fact that human beings spend most of their life time indoor thus making them more exposed to indoor air pollutants as compared that in outdoor (Lee, 2008; Branco et al., 2015; Ai, Mak, Cui, & Xue, 2016; Kinnane, Sinnott, & Turner, 2016; Roda et al., 2011; Madureira et al., 2015). The decisive factor for IAQ is the presence of diversity of pollutants which depends upon building characteristics, climatic conditions, present ventilation system, cultural behavior and indoor activities (Yoon et al., 2011; Roda et al., 2011). Along with all these mentioned factors, thermal comfort also contributes in affecting the IAQ (Kumar et al., 2016; Lee, 2008). Good IAQ has been linked to increase in productivity and well-being of a person (Theodosiou & Ordoumpozanis, 2008; Pei, Lin, Liu, & Zhu, 2015; Kalimeri et al., 2016).

IAQ is strongly controlled by indoor as well as outdoor sources of pollutants which tend to infiltrate indoor. Thus dependence of IAQ on outdoor air quality and outdoor climatic conditions like temperature and humidity can’t be denied which require proper control measures (Salthammer et al., 2016; Kaunelienė et al., 2016; Kumar et

al., 2016; Jung, Wu, Tseng, & Su, 2015; Lee, 2008; Ferdyn-Grygierek, 2016). Concentration of indoor air pollutants is found to be higher than outdoor pollutant concentrations in most of the cases (Branco et al., 2015).

IAQ is becoming a public concern due to the evidence of existence of chemical and biological contaminant in indoor environment which may cause respiratory issues (Roda et al., 2011). Indoor air pollution has been reported to cause detrimental impacts on human health (Kumar et al., 2016) depending upon time and concentration of their exposure (Salthammer et al., 2016). Short-term as well as long-term health issues can be the results of poor IAQ (Madureira et al., 2015). Poor IAQ affects a person with immune deficiencies more than normal healthier persons (Yoon et al., 2011). Most common diseases related to poor IAQ are asthma and allergy (Madureira et al., 2015).

2.2. Indoor Air Quality Monitoring

Monitoring of quality of air in which human beings breathe is important to manage IAQ and overcome the health consequences of related to poor IAQ. Among the methods used to monitor IAQ, two are discussed in detail below.

2.2.1. Ventilation Quality Monitoring

Ventilation is the process of replacement of indoor air with fresh outdoor air. Ventilation is indispensable for maintaining good IAQ as more than 50% of the time of humans, spend in indoor environment (Ai et al., 2016). Concentration of outdoor pollutants affecting IAQ is largely dependent on the type and present condition of ventilation system of buildings. An efficient building ventilation system is essential to

maintain IAQ within acceptable limits (Kumar et al., 2016; Lee, 2008; Gładyszewska-Fiedoruk, 2011; St-Jean et al., 2012). Tightening of buildings, with a focus on achieving energy efficiency, has reduced the IAQ by limiting the use of efficient ventilation system, resulting in harmful effects on human health (Kaunelienė et al., 2016; Kinnane et al., 2016; Ng, Persily, & Emmerich, 2015; Krawczyk et al., 2016). Previous research on ventilation system concluded that an efficient ventilation system helps in diluting levels of indoor air pollutants (Jung et al., 2015; Kinnane et al., 2016).

Globally to assess IAQ, CO_2 levels are taken as a surrogate for ventilation quality assessment as CO_2 levels above a certain limit indicate poor ventilation which shows possibility of build-up of higher levels of other pollutants having negative impact on human health (Salthammer et al., 2016; Branco et al., 2015; Ai et al., 2016; Gładyszewska-Fiedoruk, 2011; St-Jean et al., 2012; Kalimeri et al., 2016). On the other hand, indoor T and RH levels are taken as comfort indicators (Branco et al., 2015). Adverse health impacts related to high CO_2 levels include headaches, lack of concentration, tiredness etc. (Krawczyk et al., 2016). Poor building design and ventilation system is the main reason of increasing indoor CO_2 levels which can affect learning ability of persons (Griffiths & Eftekhari, 2008). Indoor air exchange rate is largely dependent on occupation density. High occupation density demand high air exchange rate (Salthammer et al., 2016) which can be improved by opening windows and doors, utilizing ventilation dampers in case of window type air conditioners and also by using exhaust fans (Ai et al., 2016).

Ventilation systems can be broadly classified into two types i.e. natural ventilation and mechanical ventilation (Jung et al., 2015). A brief description of both is given below.

2.2.1.1. Natural Ventilation

Natural ventilation is a phenomenon of replacement of indoor air with fresh outdoor air without utilization of any mechanically operated device. Replacement of air in the process of natural ventilation occurs by the use of natural forces like wind and buoyancy (Kleiven & Art, 2003; Jiang & Chen, 2001). IAQ can be improved by natural ventilation by opening of windows and doors. In many public buildings, like educational institutes, have natural ventilation system (Gładyszewska-Fiedoruk, 2011). A properly designed naturally ventilated system can help in maintain good IAQ without a compromising indoor thermal comfort with less energy consumption (Jiang & Chen, 2001).

Although a natural ventilation system helps in exchanging indoor air with outdoor, but at the same time it is also responsible for issues like compromising noise (Ai et al., 2016). It also doesn't incorporate changes in levels of comfort parameters (T and RH) due to changes in outdoor climatic conditions if it is not properly designed. Thus heat recovery in those systems is not easy to manage (Kleiven & Art, 2003). It can also result in increasing levels of airborne microbes in indoor environment (Gładyszewska-Fiedoruk, 2011).

2.2.1.2. Mechanical Ventilation

Mechanical ventilation is the process of utilization of mechanically driven forces to replace indoor air with outdoor (Kleiven & Art, 2003). Mechanical ventilation can be centralized as well as non-centralized (Jung et al., 2015). In hot and humid regions of the world, mechanical cooling systems are used to maintain thermally comfortable environment. These cooling units can be of window type or split type. In an air conditioning room, ventilation rates are generally found quite low than the required limit, thus often resulting in buildup of CO_2 levels above the standard limit defined by ASHRAE standards (1000 ppm). Window-type air conditioners have a provision of ventilation dampers, which can help in increasing IAQ by lowering CO_2 concentration levels. Studies showed that mechanical ventilation through exhaust fans can cause rapid decrease in CO_2 concentration levels i.e. from 3000 ppm to 1000 ppm depending upon size of fans as well as spaces under study (Ai et al., 2016).

The advantages of this system include stable airflow, heat recovery and good IAQ. Moreover, these systems are complex having less life and requiring large space, energy consumption and high running cost (Kleiven & Art, 2003).

One possible solution to address all the issues linked with both types of ventilation systems (natural and mechanical) includes use of hybrid ventilation system. This type of ventilation system utilized all the good features of both systems by minimizing their disadvantages (Kleiven & Art, 2003). Another solution, proposed in a study of United Kingdom on IAQ of naturally ventilated classrooms, is the use of purge ventilation with opening the windows. Study showed that this type of ventilation can be able to

decrease indoor CO_2 levels without compromising indoor thermal comfort (Griffiths & Eftekhari, 2008).

2.2.2. Monitoring of Indoor Bacteria, Dampness and Fungi

Molds and bacteria in indoor environment are the common microbial damaging factors of IAQ. Airborne micro-organisms, which are matter of great concern for environment and public health, show variation in concentration with time, indoor as well as outdoor conditions and geographic locations (Rajasekar & Balasubramanian, 2011). Various natural and anthropogenic sources are the contributors of high concentration of micro-organisms in indoor air (Wang et al., 2012; Mashat, 2015) like biological, physical and chemical factors (Lee, 2008), outdoor sources, number of occupiers (Mashat, 2015; Lee, 2008; Forthomme et al., 2014; Rajasekar & Balasubramanian, 2011). Uncontrolled internal environment and particularly indoor air of buildings due to absence of adequate control system, pose health risk as well as comfort problems, thus requiring scheduled monitoring followed by comparison with standards (Ferdyn-Grygierek, 2016).

There is growing frequency of infections caused by airborne micro-organisms due to the recent concept of air-tight buildings (Ferdyn-Grygierek, 2016). Some of the health issues linked with the exposure of airborne micro-organisms are infectious diseases, toxic reactions (Forthomme et al., 2014), pneumonia, hypersensitivity, bronchitis (Yassin & Almouqatea, 2010), tiredness, headache (Yassin & Almouqatea, 2010), asthma, allergies (Madureira et al., 2015), alveolitis (Mashat, 2015), rhinitis (Lee & Jo, 2006) and hay fever etc. where intensity being function of pathogenicity of micro-

organisms, immune system of persons and environmental conditions (Breza-Boruta, 2016). Nature and condition of present ventilation system along with humans, outdoor pollutants, pets, plants etc. contribute as sources of airborne bacteria in indoor environment. Sources for airborne fungi in indoor environments on the other hand can be indoor or outdoor where outdoor sources contribute more in case of naturally ventilated building (Mashat, 2015).

Effect of environmental factors such as carbon dioxide (CO_2), T and RH on the indoor concentration of airborne micro-organisms is considered important for their growth (Kim et al., 2009; Sudharsanam et al., 2012). In normal conditions, species of fungi are not supposed to cause any infection, but they are found to spread diseases in immunosuppressed patients of hospitals (Cabo Verde et al., 2015; Sautour et al., 2009; Sudharsanam et al., 2012). Although World health organization (WHO) showed concern towards indoor biological agents and building moisture (Teixeira & Oliveira, 2015), majority of countries don't have clear regulations or proposed guidelines for acceptable concentrations of micro-organisms in hospital environment particularly (Salonen et al., 2007; M. Gao et al., 2015).

The most suitable way for indoor and outdoor airborne microbial examination is air sampling. Two air sampling approaches are used for microbial quality assessment i.e. active sampling and passive sampling (Lee, 2008).

2.2.2.1. Active Sampling

In active sampling technique, a known volume of air is drawn to a certain collection medium. The collection medium is usually a nutrient medium which is then incubated for a specified period of time according to the type of organisms (bacteria: 37°C for 24-48 hours, fungus: 28.5ºC for 72 hours). The colonies recovered are then expressed in the form of CFU/m^3. Airborne microbes can be analyzed qualitatively as well as quantitatively in this method of sampling. There are certain disadvantages of this type of sampling which include the cost and noise of the instruments used. Moreover, samplers need to calibrate again and again after a specific period of time (Pasquarella, Pitzurra, & Savino, 2000).

2.2.2.2. Passive Sampling

In passive air sampling, also known as settle plate sampling, petri dish having desired nutrient medium is made to expose to air for some specified time period. The settled particles on the medium are then incubated and counted. The recovered colonies in this type of sampling are expressed as CFU/plate or CFU. It is a non-quantitative method of microbial study and is used for only qualitative microbial analysis (Pasquarella et al., 2000).

2.3. Identification of Micro-organisms

2.3.1. Phenotypic Identification

Phenotypic identification of bacteria for medical purposes includes biochemical and serological reactions, susceptibility to anti-microbial agents, bacteriocins and phages

and cell protein profiles (Castro-Escarpulli et al., 2015). However, for research purposes, most reported studies used biochemical reactions like gram staining, oxidase test, catalase test (Cabo Verde et al., 2015), motility test, methyl red test (Rajasekar & Balasubramanian, 2011) etc.to identify bacteria phenotypically. However, fungal phenotypic identification was done on the basis of spore color, shape and microscopic appearance of the colonies (Kim et al., 2009).

2.3.2. Genotypic Identification

Genotypic identification for microbes for medical purposes include polymerase chain reaction (PCR), ligase chain reaction (LCR), hybridization, restriction enzyme digestion, ribotyping, multilocus sequence typing, plasmid profile, analysis of plasmids polymorphism and reaction and separation by pulsed-field gel electrophoresis (PFGE). However, previous studies reported PCR for identification of species up-to genus level. Polymerase chain reaction (PCR) is an amplification technique in which extracted fragments of deoxyribonucleic acid (DNA) of micro-organisms are amplified by the use of paired primers sets. It is a quicker, versatile and accurate method having more sensitivity as compared to other methods for genotypic identification (Castro-Escarpulli et al., 2015).

2.4. Indoor Air Quality of Educational Institute

Educational institutes are the places where students and teachers spend more time as compared to any other indoor environment after homes making them the most important indoor environment to be studied (Yoon et al., 2011; Cavaleiro Rufo et al., 2016). It is expected that academic buildings maintain good thermal comfort and IAQ

that contributes towards increase in students' educational performance and minimize health risks (Sarbu & Pacurar, 2015). Also learning ability of students is found to be associated with fresh air circulation in the classrooms (Turanjanin, Vučićević, Jovanović, Mirkov, & Lazović, 2014). In most of the educational institutes, natural ventilation is the only way of fresh air circulation inside the building (more than 90%), thus controlling and maintaining good IAQ is difficult in these buildings (Gładyszewska-Fiedoruk, 2011; Turunen et al., 2014). Additionally, in classrooms due to high occupation density, ventilation demand increases which makes IAQ and comfort a more important concern (Theodosiou & Ordoumpozanis, 2008). Ventilation quality in these facilities is found to be insufficient causing number of health related issues (Bakó-Biró et al., 2012; Wargocki & Wyon, 2013; J. Gao, Wargocki, & Wang, 2014).

In educational institutes, for assessment of IAQ, CO_2 levels are taken as surrogate while T and RH, as a comfort parameters, are considered important for assessing airborne microbial growth inside the building (Gładyszewska-Fiedoruk, 2011). Performance and learning abilities of students have been observed to be affected due to high indoor CO_2 levels (Krawczyk et al., 2016; Bakó-Biró et al., 2012; Griffiths & Eftekhari, 2008; Mijakowski & Sowa, 2017) as short-term and long term health problems associated with poor IAQ result in decrease in productivity of students and staff (Lee, 2008). These high levels of CO_2 can be associated with poor building design and absence/ insufficient use of ventilation provisions or occupation density higher than that considered while ventilation system design phase (Griffiths & Eftekhari,

2008). In Representatives of European Heating and Ventilating Associations (REHVA) Guidebook 13 limiting value for CO_2 level is 1500 ppm (REHVA, 2010) while according to American Society of Heating, Refrigeration and Air Conditioning Engineers (ASHRAE) standards 62-1989 limiting value during occupational period is 1000 ppm (ASHRAE, 1989).

2.5. Indoor Air Quality of Hospitals

Incorporation of advanced technologies in medical practices added complexity to hospital environment making it a source of risk from health perspective which needed special attention for prevention (Jung et al., 2015; Pasquarella et al., 2000). In hospital environment, airborne microbial population is present in diverse range (Bali, Sharma, Nagrath, & Gupta, 2014), where concentrations depend primarily on type of patients and transfer medium from one individual to another is air through coughing and sneezing (Qudiesat, Elkarmi, Hamad, & Abussaud, 2009). In addition to this, medical activity, cleaning frequency and cleaning procedures of hospitals (Jung et al., 2015), weather and ventilation rate (Cabo Verde et al., 2015), building design (Sudharsanam et al., 2012) are also the decisive factors for their concentration levels which all together make challenging situation to maintain satisfactory IAQ (Jung et al., 2015).

High ventilation rates can help in decreasing the concentration levels of airborne micro-organisms. Thus, ventilation can help in the control of concentration levels of airborne micro-organisms in hospitals (Jung et al., 2015). People with immune deficiencies (children, elderly people, and patients) are more vulnerable to diseases

associated with poor indoor air quality (Teixeira & Oliveira, 2015; Yassin & Almouqatea, 2010).

Sensitivity and complexity of hospitals vary from one place to another. OTs are supposed to be the most sensitive places and required strict maintenance of levels of airborne micro-organisms. Similarly, different wards required control measures according to the sensitivity of patients present there.

2.6. Indoor Air Quality of Cafeterias

Cafeterias are the places with large occupant density. The major activities of these facilities that can impact quality of indoor air include food handling activities (Lee & Jo, 2006), cooking and cleaning activities along with the number of occupants. Airborne bacterial concentration levels are highly dependent upon the type of food materials, cleaning frequency, ventilation systems, outdoor climatic conditions and cleaning of cooking utensils. Microbial concentration levels in a closed space can be found higher as compared to open cafeterias (Rajasekar & Balasubramanian, 2011). Moreover, indoor fungal levels can be related to moisture problems in building (Kalliokoski, Lignell, Meklin, Koivisto, & Nevalainen, 2002) and outdoor sources more than the indoor sources (Rajasekar & Balasubramanian, 2011).

Chapter 3

MATERIALS AND METHODS

The overview of the methodology followed is shown in Figure 3.1.

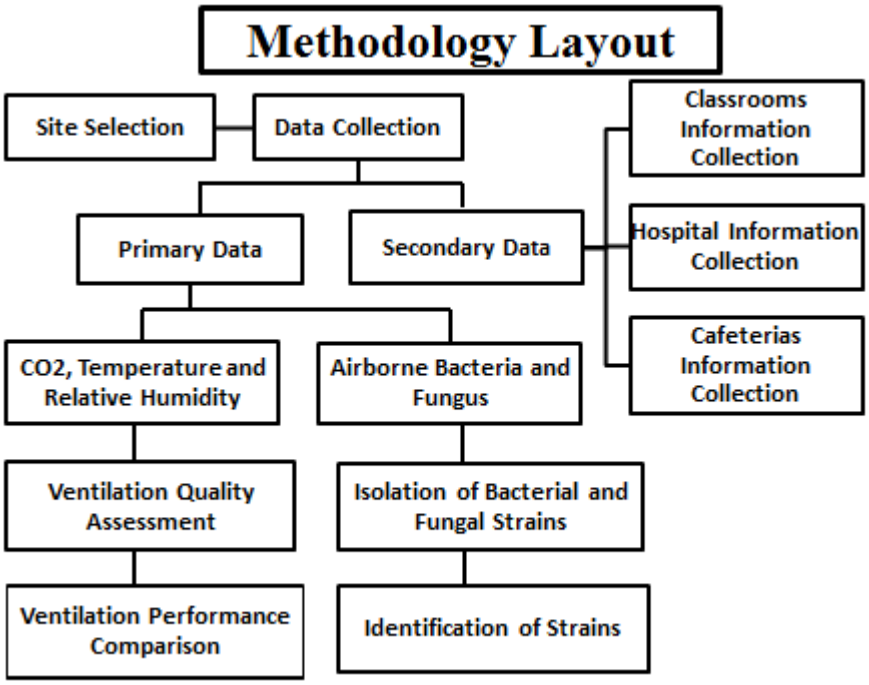


Figure 3.1: Layout of Methodology

3.1. Site Selection

Three sites were selected for the purpose of study brief description of which is given below.

3.1.1. Academic Site Description

Assessment of ventilation system and IAQ of academic buildings was performed in the main campus of National University of Sciences and Technology (NUST), Islamabad, Pakistan (33.73° N, 73.09° E). It is a semi-government university of Pakistan founded in 1991. Keeping the variation in HVAC systems incorporated in view, classrooms in four departments was selected for monitoring purpose that includes; Institute of Environmental Sciences and Engineering (IESE), NUST Institute of Civil Engineering (NICE), Center of Advanced Studies in Energy (CASEN) and NUST Business School (NBS). The buildings are represented as Building A, Building B, Building C and Building D respectively. Onsite measurements were recorded during the months of March to June when fans and air-conditioners were supposed to be switched on. Construction year of selected buildings ranged from 2005 to 2017. A prior survey was conducted to obtain the information about the activities and factors which may impact the parameters being monitored. Table 3.1 shows the detailed description of the monitored sites.

Buildings A and B were built in 2005 and 2008 respectively, each having two levels. Four classrooms (CR1A, CR2A, CR3A, CR4A) from building A and five from building B (CR1B, CR2B, CR3B, CR4B and CR5B) were selected for monitoring. Among them CR1A, CR2A, CR2B, CR3B, CR4B AND CR5B were on ground floor while remaining t were on 1^{st} floor. Lecture sessions of undergraduate programs were scheduled in the morning (09:00 AM to 01:00 PM) while post-graduate lecture sessions were scheduled from 05:00 PM to 08:30 PM with a half hour break from 06:30 PM to 07:00 PM. Both buildings had natural ventilation system and during

monitoring period of building A, air-conditioners were switched off while in case of building B air-conditioners (split units) were switched on.

(a) (b)

(c) (d)

Figure 3.2: Monitored Academic Buildings (a) Building A, (b) Building B, (c) Building C, (d) Building D

Building C was newly built having four levels in which academic activities started few months before monitoring period (in January 2017). Building D was built in 2010 and had 2 levels. Both buildings, C and D had centralized HVAC system which was functional during the monitoring period. Observed classrooms of building C were located on 1st floor while that of building D were on ground floor. Three classrooms from each building (C1C, C2C, C3C and C1D, C2D, C3D) were selected for monitoring purpose having lecture sessions scheduled from 09:00 AM to 05:00 PM.

Table 3.1: Description of studied classrooms

Buildings	Room	Floor	Windows	Doors	Area (m^2)	Construction Year	Occupation[a]	Occupation Period	Sampling Days	Occupation Density (People/m^2)[a]
A	CR1A	Ground Floor	2	2	76	2005	37+20	09:00 AM-08:30 PM	3+2	0.5 + 0.3
	CR2A	Ground Floor	2	2	76	2005	35+25	09:00 AM-08:30 PM	3+1	0.5 + 0.3
	CR3A	1st Floor	2	2	76	2005	29+21	09:00 AM-08:30 PM	4+2	0.4 + 0.3
	CR4A	1st Floor	2	2	76	2005	30+24	09:00 AM-08:30 PM	2+2	0.4 + 0.3
B	CR1B	Ground Floor	2	2	77	2008	43+0	09:00 AM-08:30 PM	4+1	0.6 + 0

Buildings	Room	Floor	Windows	Doors	Area (m^2)	Construction Year	Occupation[a]	Occupation Period	Sampling Days	Occupation Density (People/m^2)[a]
B	CR2B	Ground Floor	2	2	86	2008	45+23	09:00 AM-08:30 PM	5+1	0.5 + 0.3
	CR3B	1st Floor	2	2	77	2008	41+33	09:00 AM-08:30 PM	3+1	0.5 + 0.4
	CR4B	1st Floor	2	2	86	2008	42+8	09:00 AM-08:30 PM	4+1	0.5 + 0.09
	CR5B	Ground Floor	1	1	93	2010	40+17	09:00 AM-08:30 PM	3+2	0.4 + 0.2
C	CR1C	1st Floor	2	2	59	2017	34+0	09:00 AM-05:00 PM	4+1	0.6 + 0

Buildings	Room	Floor	Windows	Doors	Area (m^2)	Construction Year	Occupation[a]	Occupation Period	Sampling Days	Occupation Density (People/m^2)[a]
C	CR2C	1st Floor	2	2	66	2017	30+0	09:00 AM-05:00 PM	4+2	0.5 + 0
	CR3C	1st Floor	4	2	61	2017	33+0	09:00 AM-05:00 PM	4+2	0.5 + 0
D	CR1D	Ground Floor	3	2	107	2010	40+0	09:00 AM-05:00 PM	4+1	0.4 + 0
	CR2D	Ground floor	3	2	107	2010	47+0	09:00 AM-05:00 PM	3+1	0.5 + 0
	CR3D	Ground floor	3	2	107	2010	42+0	09:00 AM-05:00 PM	4+1	0.4 + 0

[a] (Morning + Evening)

3.1.2. Hospital Site Description

Assessment of airborne bacterial and fungal levels and ventilation system of hospitals was performed ina publically managed hospital of Islamabad, Pakistan.. It was a 950-bed publically managed hospital of Pakistan, founded in 1985 and covered an area of 5.1 hectares. Six sites were selected for the purpose of airborne microbial investigation including two operation theatres (OT1=emergency operation theatre, OT2= general surgery operation theatre), two wards (SW=surgical ward, GMW=general medicine ward), emergency services (ES) and out-patient department (OPD). Moreover, two sites were selected for the purpose of ventilation quality assessment, including one operation theatre (OT3) and one intensive care unit (ICU).

OT1 remain operational 24 hour a day having 15-20 patients operated per day. In OT2 and OT3, on the other hand, patients were operated from 8:00 hours to 14:00 hours, having average 8 patients operated per day. Selected wards for study purpose had a capacity for 8 patients each with 2-3 attendants. ES remain operational 24 hours while working timing hours for OPD was from 8:00 hours to 14:00 hours. Both of them were heavily crowded places. Moreover, intensive care unit (ICU) has 8 beds with 8-9 hospital staff. Table 3.2 shows the complete description of monitored sites.

Table 3.2: Hospital Sampling Site Description

Locations	Symbol	Floor	Building Type	Area (m^2)	Maximum Capacity	Occupational Period
Emergency Operation Theatre	OT1	1st Floor	Closed	31.11	11*	24 hours
Surgical Operation Theatre	OT2	1st Floor	Closed	37.47	11*	8:00 am- 2:00 pm
Liver Operation Theatre	OT3	1st Floor	Closed	34.03	11*	8:00 am- 2:00 pm
Intensive Care Unit	ICU	1st Floor	Closed	84.31	16-18**	24 hours
Out-patient Department	OPD	Ground Floor	Semi-closed	701.88	>1200	8:00 am- 2:00 pm
Emergency services	ES	Ground Floor	Closed	177.05	>200	24 hours
Surgical Ward	SW	1st Floor	Closed	56.21	20-30***	24 hours
General medicine Ward	GMW	Ground Floor	Closed	56.21	20-30***	24 hours

* 1 patient and 10 hospital staff
** 8 patients with 8-9 hospital staff
*** 8 patients with 2-3 attendants

3.1.3. Cafeteria Site Description

Assessment of airborne bacteria and fungi was performed in three cafeterias (A, B and C) of National University of Sciences and Technology (NUST), Islamabad. Microbial

investigation was carried out between April 2017 and May 2017. Two indoor locations and one outdoor location from each sampling site were selected. Samples in duplicate were collected from each indoor measurement location of three cafeterias during the peak hours for three consecutive days. A brief overview of the details of each sampling location is given in Table 3.3.

Table 3.3: Details of sampling locations of cafeterias

Cafeterias	**Locations**	**Area (m^2)**	**Type of location**	**Ventilation**	**Maximum Occupancy**
A	$CafeA_C$	123.9	Closed	Natural Ventilation	75
	$CafeA_{SC}$	400	Semi-closed	Natural Ventilation	385
B	$CafeB_C$	119.7	Closed	Natural Ventilation	50
	$CafeB_{SC}$	395	Semi-closed	Natural Ventilation	132
C	$CafeC_{C1}$	175.6	Closed	Centralized HVAC	80
	$CafeC_{C2}$	229.7	Closed	Centralized HVAC	100

Sampling locations of cafeteria A and B (also known as Concordia 1 and Concordia 2 respectively) were almost similar having naturally ventilated closed and semi-closed space. Both locations were open for students and faculty and had an occupation period from 09:00 am to 09:30 pm with peak hours during the lunch breaks from 12:00 pm to 02:00 pm. Cafeteria A has more occupational density as compared to B. All the cooking activities were carried out within each cafeteria. In the semi-closed area there

was a juice corner in the middle. Total area of closed and semi-closed space of cafeteria A was 123.9 and 400 m^2 while occupation density was 75 and 385 respectively. Similarly, area of closed and semi-closed space of cafeteria B was 119.7 and 395 m^2 while occupation density was 50 and 132 respectively. Both sampling locations were surrounded by plants.

(a) (b)

(c)

Figure 3.3: Monitored Cafeterias (a) Cafeteria A, (b) Cafeteria B, (c) Cafeteria C

Indoor sampling locations of cafeteria C (also known as faculty cafeteria), on the other hand were both closed spaces having a controlled environment due to presence of centralized heating, ventilation and air-conditioning system (HVAC). It was for faculty only, having an occupation period only during the lunch break (12.00 to 2.00 PM). Both the locations are well maintained and no cooking activity was performed within

the sampling locations. Total area of sampling locations was 175.6 and 229.7 m^2 while occupation density was 80 and 100 respectively.

3.2. Data Collection

3.2.1. CO_2, Temperature and Relative Humidity

3.2.1.1. NUST Classrooms

Monitoring for indoor levels of carbon dioxide (CO_2), temperature (T) and relative humidity (RH) in classrooms of NUST, was done from March to June 2017 when maximum, minimum and average outdoor temperatures were 43, 11 and 28.9°C and ambient humidity values were 77, 4.425 and 33.31% respectively which were recorded from the nearest weather station (33.62°N, 73.10°E). Occupancy and occupational period varied among studied classrooms and is given in Table 3.1 accordingly.

HT-2000 equipped with non-dispersive infrared (NDIR) CO_2 sensor was used for monitoring of the selected parameters (CO_2, RH and T) having features given in Table 3.4. Measurements were recorded continuously at an interval of 1 minute from four to six days including weekdays (WD) and weekends (WE) as given in Table 3.1.

Table 3.4 Characteristics of sensors used

Sensors	Range	Accuracy
Carbon dioxide	0-9999 ppm	±5% reading
Temperature	-10 - 70ºC	±1.2ºC
Humidity	0.1-99.9%	±3%

3.2.1.2. Hospital Sites

Monitoring of indoor CO_2, temperature and relative humidity in hospital include two sites i.e. operation theatre (OT3) and intensive care unit (ICU). Monitoring period

include month of January 2017, when average outdoor temperatures and relative humidity recorded from nearest weather station were 11°C and 74%. Occupancy and occupational periods are given in Table 3.2. Instrument used for monitoring was the same as described before and details of which are given in Table 3.4.

3.2.2. Airborne Bacteria and Fungus

3.2.2.1. Hospital Sites

Seasonal (winter, spring) assessment of airborne bacteria and fungus had been performed covering one month for each season. Winter sampling covered month of January 2017 when average outdoor temperature and humidity, recorded from nearest weather station (33.62°N, 73.10°E), were 11°C and 74% respectively while for spring sampling, month of April 2017 had been selected when average outdoor temperatures and humidity were 26ºC and 46.5% respectively.

Airborne microbial samples were collected twice a week during the peak hours of each sampling location using Gilian 5000 operated at flow rate of 5l/min for 10 min. To represent breathing zone, sampling height was kept at 1.5m above ground. Cellulose nitrate filter paper with a pore size of 0.45µm was used as a collecting medium for microbes. Tryptone soy agar (TSA) for bacterial colonies and potato dextrose agar (PDA) for fungal colonies, autoclaved at 121°C for 15-20 min, were used as a culture medium for airborne microbes. After sampling plates were sealed and transferred to laboratory where bacterial colonies were incubated at 37°C for 24-48 hours while fungal colonies were given incubation of 28.5°C for 72 hours. Colonies recovered were then counted and expressed as CFU/m^3. Environmental factors such as indoor

and outdoor temperature and humidity were also measured simultaneously to observe their influence on the concentration of airborne micro-organisms.

3.2.2.1. Cafeterias

Samples for airborne bacteria and fungus were collected at a flow rate of 4 l/min for 13 minutes. Representation of breathing zone of occupants was done by collecting samples at a height of 1.5 m from ground. Collected bacterial samples on Tryptone Soy Agar (TSA) plates and fungal samples on Potato Dextrose Agar (PDA) plates, were then sealed and transferred to laboratory where bacterial samples were incubated for 48 hours at 37ºC while fungal samples for 72 hours at 25ºC. Colonies recovered were then counted and expressed in the form of CFU/m^3. Measurements for outdoor temperature and relative humidity were noted from nearest weather station of Islamabad (33.62°N, 73.10°E).

3.3. Data Analysis

3.3.1. Ventilation Analysis

For descriptive statistical analysis of ventilation data, MS Excel (Microsoft Corporation, USA) was used while all other statistical analysis was performed using SPSS 14 (IBM Corp., USA). Using significance level (α) of 0.05, non-parametric Kruskal-Wallis Test was used to analyze the difference of CO_2, RH and T values obtained on two or more sampling days for each classroom. On the basis of test results, mean hourly values of the three monitored parameters for each studied classroom were calculated for WD and WE. Difference of the three parameters along the day and

between any two buildings was analyzed using non-parametric Wilcoxon Signed Rank Test.

3.3.2. Microbial Analysis

3.3.2.1. Isolation of Bacteria and Fungus

Bacterial and fungal colonies recovered on their respective nutrient medium were counted and expressed in the form of CFU/m^3 by dividing number of colonies recovered by the total volume of air sample drawn. Frequently observed bacterial colonies were then isolated by sub-culturing on sterile TSA and observed with naked eye for their shape, color, texture, elevation, margins and pigmentation and stored in glycerol broth at -20ºC. Moreover, fungal colonies were isolated on sterile PDA plates and observed with naked eye for their front and back color, shape and texture.

3.3.2.2. Biochemical Characterization of Bacteria

Biochemical characterization of bacterial colonies includes observation of microscopic appearance of strains under an optical microscope at 100x magnification after gram-straining, on the basis of which colonies were characterized into gram-positive and gram-negative groups. Further biochemical characterization of bacterial strains was then performed by modified oxidase and catalase test.

3.3.2.3. Identification of Bacterial Strains

i. Genomic DNA Extraction

Genomic DNA of frequently observed bacterial isolates were extracted by following the below mentioned steps.

- Selected bacterial isolates were inoculated in 10 ml nutrient broth and incubated in a shaking water bath at 37ºC for over-night.
- Over-night grown bacterial culture was transfered in 2ml Eppendorf tube and centrifuged in a micro-centrifuge for 5 minutes at 13000 rpm.
- The resultant supernatant was disposed of while the settled pallet was suspended in 570µl TE buffer and 30µl 10% SDS. After a thorough mix in vortex, the mixture was incubated for 1 hour at 37ºC.
- The mixture was again incubated for 10 minutes at 65ºC in heat block after the addition of 100µl of 5M NaCl and 80µl of CTAB/NaCl which was mixed thoroughly in vortex.
- 700µl of mixture of chloroform/isoamyl alcohol (24:1) was added in the mixture and centrifuged for 5 minutes in micro-centrifuge at 13000 rpm.
- Two layers of mixture were formed. Top layer was picked in a fresh 2ml Eppendorf and 700µl mixture of phenol/chloroform/isoamyl alcohol (25:24:1) was added and again centrifuged at 13000 rpm for 5 minutes.
- Two layers were again formed and top layer was picked in fresh 1.5ml Eppendorf. DNA was precipitated by the addition of 600µl chilled isopropanol and incubated for 1 hour at -20ºC.
- Precipitated DNA was then allowed to settle in the form of compact pellet by centrifuging in a micro-centrifuge at 13000 rpm for 5 min.
- Supernatant was discarded and the settled pellet was washed with 200µl of 70% ethanol and centrifuged for 2 minutes at 11000 rpm.

- Pellet was stored in 20µl TE buffer at -20ºC after air-drying at room temperature.

ii. Agarose Gel Electrophoresis

Extracted DNA was analyzed by using agarose gel electrophoresis. 0.5 grams of agarose was mixed in 50 ml of 1X TAE buffer (25 mM Tris, 5mM Glacial Acetic acid, 1mM EDTA, pH 8.0) to make 1% agarose gel. 0.5µl of 0.01% ethidium bromide was added after obtaining a transparent clear solution by heating for 2 minutes in a microwave oven. DNA samples, mixed with loading buffer (0.4% bromophenol blue, 0.4% xylene cyanol and 25% Ficol), and DNA markers [10 kilobase pair (kb)] were loaded in the wells of solidified agarose submerged in 1X TAE buffer. Electrophoresis was performed at 80 volts for 45 minutes and visualized with the help of Dolphin-Doc plus Image System.

iii. Genomic DNA Amplification

Extracted fragments of DNA were amplified by polymerase chain reaction (PCR) using primers by Eurofins Scientific, details of which is given in Table 3.5.

Table 3.5: Primers used for DNA amplification

Primers	**Sequence (5'→3')**	**Target genes**	**Bp**
FD1 f	AGAGTTTGATCCTGGCTCAG	16S rDNA	1500
rp2 r	AAGTCGTAACAAGGTAGCCT	16S rDNA	1500

50µl PCR reaction mixture was prepared having composition provided in Table 3.6. Processing of PCR reaction mixture involved 5 minutes at 94ºC for template denaturation followed by 30 cycles of amplification, each consist of three steps i.e. 94ºC for 30 seconds for DNA denaturation into single strand, 58ºC for 30 seconds for hybridization of primer at annealing temperature, 72ºC for 2 minutes complementary DNA strand extension from each primer followed by 72ºC for 5 minutes for Taq DNA polymerase to synthesize any unextended strand left. The resultant PCR product was by using agarose gel electrophoresis and visualized with the help of Dolphin-Doc plus Image System.

Table 3.6: Composition of PCR reaction mix

Reagents	Concentration
PCR Water	33.5µl
Taq Buffer	6µl
$MgCl_2$	2.5µl
dNTPs	2µl
Forward Primer	1µl
Reverse Primer	1µl
Template	3µl
Taq Polymerase	1µl

3.3.2.4. Identification of Fungal Strains

Identification of fungal strains includes preparation of wet-mount slides of respective colonies using lacto-phenol blue. Slides were then observed under optical microscope of magnification 40 x. Fungal colonies were then identified up-to their genus level by the color and shape of their hyphae.

3.3.2.5. Statistical Analysis

The data was analyzed in MS Excel (Microsoft Corporation, USA) and SPSS 14 (IBM Corp., USA). Normality of data was checked by Kolmogrov-Smirnov test and Shapiro-Wilk test. One-Way ANOVA was used to analyze the statistical difference among different sampling sites and t-test was used to analyze the statistical difference between observations of two seasons for same site. Correlation of bacterial and fungal colonies with outdoor climatic conditions i.e. temperature and relative humidity was found using Spearman's correlation test.

Chapter 4

RESULTS AND DISCUSSION

4.1. Academic Institute

Descriptive statistical parameters (mean, median, minimum, maximum and standard deviation) for the hourly mean indoor CO_2 levels have been summarized in Table 4.1 while Table 4.2 summarizes the parameters for hourly mean ambient and indoor RH and T data of studied classrooms.

4.1.2. Variation of CO_2 and Comfort Parameters

4.1.2.1. CO_2 Variation

Monitoring of all four selected buildings during WD showed significant variation ($p<0.05$) in CO_2 levels along the day while during WE no significant variation ($p>0.05$) along the day was observed. Moreover, the indoor concentration remained almost equal to 400 ppm in most of the cases on WE. 24-hour mean hourly CO_2 concentration profiles have been shown in Fig. 4.1 for all classrooms. A 24-hour mean WD and WE profile for each classroom was obtained by averaging all WD 24-hour profiles available for that classroom.

Fig. 4.1(a) shows the observed 24-hour mean hourly CO_2 profiles during WD and WE of four classrooms of building A. All classrooms of building A were naturally ventilated and air-conditioners were not in use during the sampling period. During WD

two peaks of CO_2 levels were observed; one during the morning session whereas other during the evening session.

Table 4.1: Descriptive statistics of hourly CO_2 concentration in classrooms

Building	Rooms	CO_2 (Weekdays/ Weekends)					
		Mean	Median	Mode	Max	Min	StDev
A	CR1A	620/ 462	484/ 447	461/ 439	2443/ 523	437/ 432	357/ 27
	CR2A	883.7/ 463.1	726/ 443	535/ 420	2029/67 2	515/41 4	373/ 58.8
	CR3A	575.4/ 430.0	484/ 417	410/ 410	2019/ 484	404/ 405	254.2/ 24.8
	CR4A	674.6/ 416.2	476/ 405	406/ 393	1885/ 487	400/ 387	344.8/ 26.3
B	CR1B	1331.2/ 421.7	855/ 420	719/ 430	6294/ 457	392/ 376	1137.8/ 13.7
	CR2B	1302.2/ 409	1180/ 410	607/ 397	4383/ 429	489/ 391	719.5/ 11.7
	CR3B	1545.9/ 552.9	1510/ 554	527/ 557	5040/ 566	529/44 7	713.9/ 6.8

Building	Rooms	CO_2 (Weekdays/ Weekends)					
		Mean	Median	Mode	Max	Min	StDev
B	CR4B	985.9/ 572.1	841/ 576	730/ 571	3236/ 606	525/ 444	423.3/ 20.7
	CR5B	1510.9/ 509.6	1096/ 500	485/ 488	4647/ 594	481/ 479	1053.9/ 28.3
C	CR1C	582.1/ 489.5	545/ 491	521/ 491	1831/ 514	453/ 439	128.1/ 13.6
	CR2C	535.1/ 399.6	494/ 401	408/ 401	1005/ 423	364/ 361	133.2/ 10.6
	CR3C	517.7/ 412.3	494/ 413	429/ 412	1034/ 433	375/ 363	124.5/ 12.9
D	CR1D	613.3/ 390.8	531/ 390	400/ 390	2486/ 401	386/ 378	307.6/ 4.6
	CR2D	754.1/ 497.6	691/ 498	497/ 497	2533/ 511	496/ 489	267.1/ 3.3
	CR3D	531.2/ 395.1	455/ 395	432/ 404	2009/ 407	399/ 379	167.4/ 7.5

Table 4.2: Descriptive statistics of hourly values of temperature and relative humidity in classrooms

Building	Room		Temperature (Weekdays/ Weekends)					Relative Humidity (Weekdays/ Weekends)				
			Mean	Median	Max	Min	StDev	Mean	Median	Max	Min	StDev
A	CR1A	A	26.8/25.8	25.7/25.2	33.7/33.5	19.7/20	4.8/4.4	42.1/35.9	41.2/36.7	65.5/65.5	16.7/7.2	13.8/15.4
		In	27.7/26.7	26.9/26.6	32.9/27.5	25.6/26.2	2.3/0.3	43.4/39.6	44.1/39.8	52.5/ 48	34.3/34.3	3.8/3
	CR2A	A	26.4/25.9	25.3/ 25	33.7/ 33	20.3/ 21	4.7/3.6	44.3/39.5	43.7/38	64.7/53	18/21	13.3/8.9
		In	26.1/26.4	26.1/26.3	27.4/26.7	25.2/26.2	0.5/0.1	50.9/46.2	50.9/45.9	58.6/50.8	45.8/41.9	2.8/2.2
	CR3A	A	26.7/25.8	25.7/25.2	33.7/33.5	19.7/20	4.8/4.4	42.1/39.7	41.2/37.7	65.5/65.5	16.7/17.5	13.8/12.1
		In	28.8/29.6	28.9/29.8	30.7/30.3	25.8/27.6	0.9/0.6	38.1/33	39.8/33.6	48.4/41.2	20.9/26.1	6.5/3.1

Building	Room		Temperature (Weekdays/ Weekends)					Relative Humidity (Weekdays/ Weekends)				
			Mean	Median	Max	Min	StDev	Mean	Median	Max	Min	StDev
A	CR4A	A	22.3/18.8	21.8/19	29.6/25.5	14.8/11	4.9/5.2	41.5/45.1	40.5/43.2	72.2/77	15.3/13	15.7/20.8
		In	24.6/23.1	24.6/23.1	26.5/23.9	21.8/22.2	0.9/0.5	39.1/36.9	37.4/36.3	50.5/44.1	27.9/30	6.5/3.8
B	CR1B	A	30.9/28.7	29.5/29	39/37	24.2/20	5.1/5.7	36.5/32	33.9/28	58.5/56	16.2/14	12.9/11.4
		In	35.9/35.4	36.4/35.8	38.5/36.1	31.4/33.4	1.9/0.7	27.4/26.1	27/25.8	45.5/29.7	21.2/24.5	3.4/1.4
	CR2B	A	22.3/29.9	21.8/30.5	29.6/39	14.8/20	4.9/6.3	40.9/34.5	40.1/33	72.2/68	13.9/7	16.2/16.2
		In	25.7/35.7	25.6/36.2	27.8/36.6	24.5/33.2	0.8/0.9	46.2/27.5	46/27.4	56.9/30.2	29.7/26.2	4.9/0.9
	CR3B	A	24.2/20.3	23.5/21	32.7/26	15.5/12	5.8/5.2	32.4/37.4	30.9/35.5	66.5/67	4.4/14	18.1/17.2
		In	32/30.7	32.4/30.9	34.7/31.2	24.1/29.4	1.8/0.5	31.1/38.9	30.8/38.5	49.1/41.6	19.5/37.4	4.7/0.9

Building	Room		Temperature (Weekdays/ Weekends)					Relative Humidity (Weekdays/ Weekends)				
			Mean	Median	Max	Min	StDev	Mean	Median	Max	Min	StDev
B	CR4B	A	30.3/28.7	29.5/29	38.5/37	22/20	5.8/5.7	35.6/32	30.5/28	62.5/56	14/14	15.5/11.4
		In	27.7/30.9	27.5/31.2	31.9/31.6	24.8/29.2	1.4/0.7	46.1/40.1	48/39.9	52.1/43.4	32/37.7	4.4/0.9
	CR5B	A	29/29.1	28.7/29	34.7/37.5	23.7/24	3.4/3.7	44.5/39.4	43.1/40.5	66/57	20.7/19.5	11.5/9.4
		In	29.5/29.8	29.6/29.8	30.2/29.9	28.6/29.6	0.3/0.1	40.6/39.4	40.8/39	47.7/41.7	31.7/38.1	2.9/1.1
C	CR1C	A	31.1/31.5	31.4/31	37.4/38.5	24.7/25.5	4.2/4.4	41/36.3	39.4/36.5	62/55.5	16/17	12.2/11.1
		In	33.2/34.8	34/34.9	36.2/35.4	25.2/33.7	2.2/0.5	31.9/28.1	31.6/28.5	40/31.4	25.8/26.4	2.8/1.6
	CR2C	A	31.1/31.5	31.4/31	37.4/38.5	24.7/25.5	4.2/4.4	41/36.3	39.4/36.5	62/55.5	16/17	12.2/11.1
		In	33.8/34.3	34.5/34.4	35.8/35.1	27.1/32.2	1.6/0.6	32.4/33.7	32.5/34.1	40.7/35.3	24.8/30.7	2.3/1.2

Building	Room		Temperature (Weekdays/ Weekends)					Relative Humidity (Weekdays/ Weekends)				
			Mean	Median	Max	Min	StDev	Mean	Median	Max	Min	StDev
C	CR1C	A	31.1/31.5	31.4/31	37.4/38.5	24.7/25.5	4.2/4.4	41/36.3	39.4/36.5	62/55.5	16/17	12.2/11.1
		In	33.2/34.8	34/34.9	36.2/35.4	25.2/33.7	2.2/0.5	31.9/28.1	31.6/28.5	40/31.4	25.8/26.4	2.8/1.6
	CR2C	A	31.1/31.5	31.4/31	37.4/38.5	24.7/25.5	4.2/4.4	41/36.3	39.4/36.5	62/55.5	16/17	12.2/11.1
		In	33.8/34.3	34.5/34.4	35.8/35.1	27.1/32.2	1.6/0.6	32.4/33.7	32.5/34.1	40.7/35.3	24.8/30.7	2.3/1.2
	CR3C	A	31.1/31.5	31.4/31	37.4/38.5	24.7/25.5	4.2/4.4	41/36.3	39.4/36.5	62/55.5	16/17	12.2/11.1
		In	34.9/35.2	34.8/35.4	37.8/37.5	27.9/31.8	2/1.5	32.4/34.2	32.5/34	41.1/40.6	23.8/ 30.7	3.3/ 2.3
D	CR1D	A	31.9/33.2	31.8/35	38.5/38	24.8/24	4.6/4.7	40.9/34.5	38.6/29.5	62/57	18.4/19	12.2/10.7
		In	27.7/28.5	27.7/28.6	30.5/28.9	23.8/28.1	1.2/0.2	47.7/47.4	48.5/47.9	61.2/48.9	35.9/45	4.9/1.2

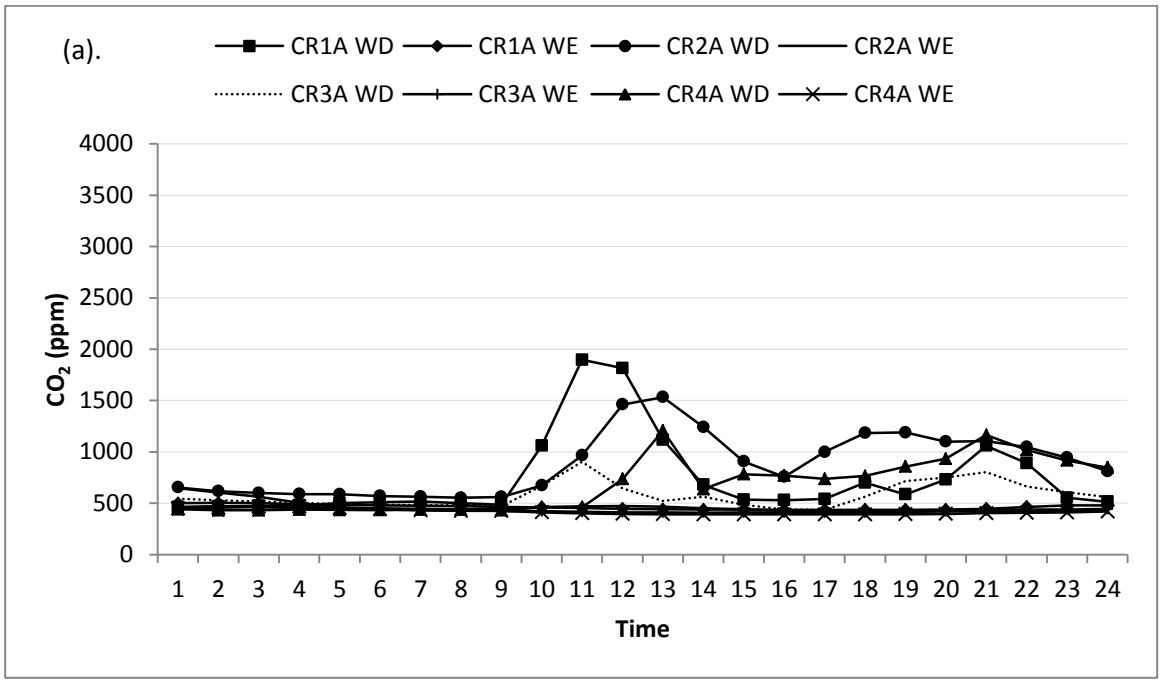
Building	Room		Temperature (Weekdays/ Weekends)					Relative Humidity (Weekdays/ Weekends)				
			Mean	Median	Max	Min	StDev	Mean	Median	Max	Min	StDev
D	CR2D	A	30.1/30.7	29.7/31	37.7/39	23.5/23	4.7/5.2	42.6/34.9	40.5/36	66/53	16.2/16	13.8/9.5
		In	29.6/30.7	29.7/30.7	32.2/31	24.7/30.3	1.8/0.2	42.9/45.8	44.8/45.9	55.1/46.8	32.9/44.4	3.6/0.6
	CR3D	A	29.9/37.2	29.7/39	36.5/43	23.2/26	4.6/5.5	43.9/23.8	40.6/19	69.2/54	20.6/9	14/12.9
		In	27.1/27.7	27.8/27.8	29.5/28.2	21.3/27.1	1.8/0.3	49.9/47.5	49.7/47.1	66.7/49	37/45.7	4.9/0.8

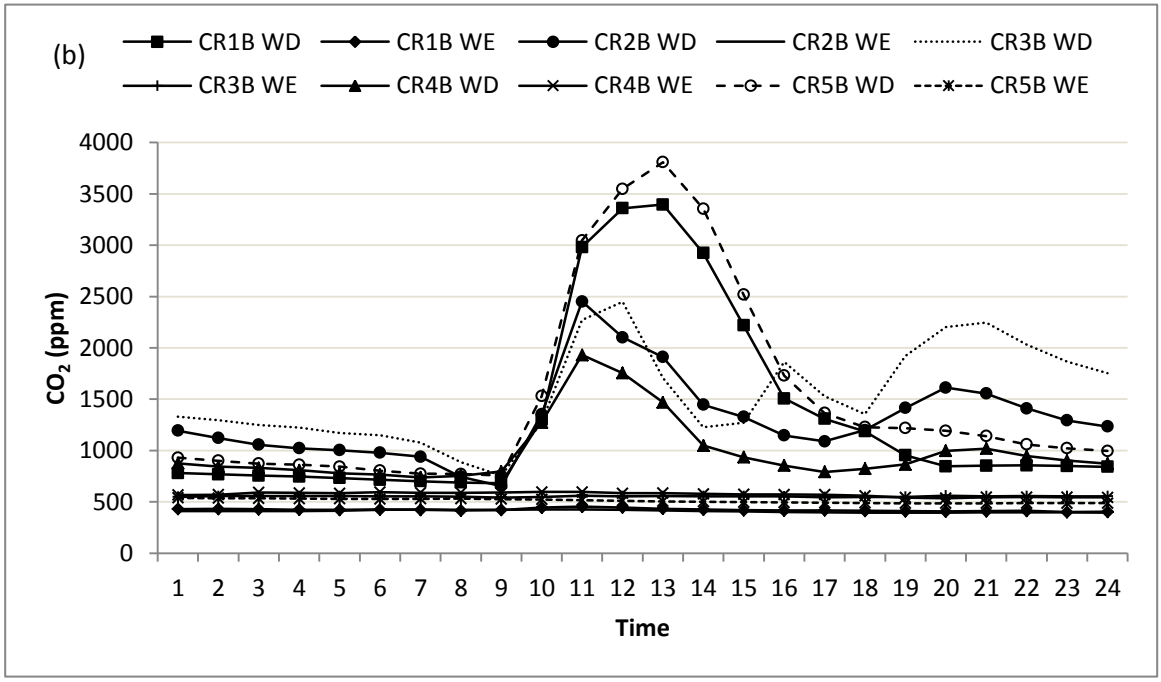
Morning sessions start at 09:00 AM before which CO_2 concentrations were observed to be stable. However, 09:00 AM onwards occupational period of the classrooms starts which results in increase of CO_2 levels until 01:00 PM (end of morning session), when the CO_2 levels start to subside. CO_2 concentrations again start increasing at 05:00 PM (start of evening sessions) until the session ends at 08:30 PM. Number of students was greater in morning sessions resulting in higher peaks as compared to evening sessions. Besides, longer and consistent occupational period was also a contributing factor for higher CO_2 peaks during morning session. Maximum observed values of CR1A, CR2A, CR3A and CR4A were 2444, 2029, 2019 and 1885ppm while minimum values were 437, 515, 404 and 400 ppm respectively. During WE on the other hand no significant difference ($p>0.05$) in values were observed over the day.

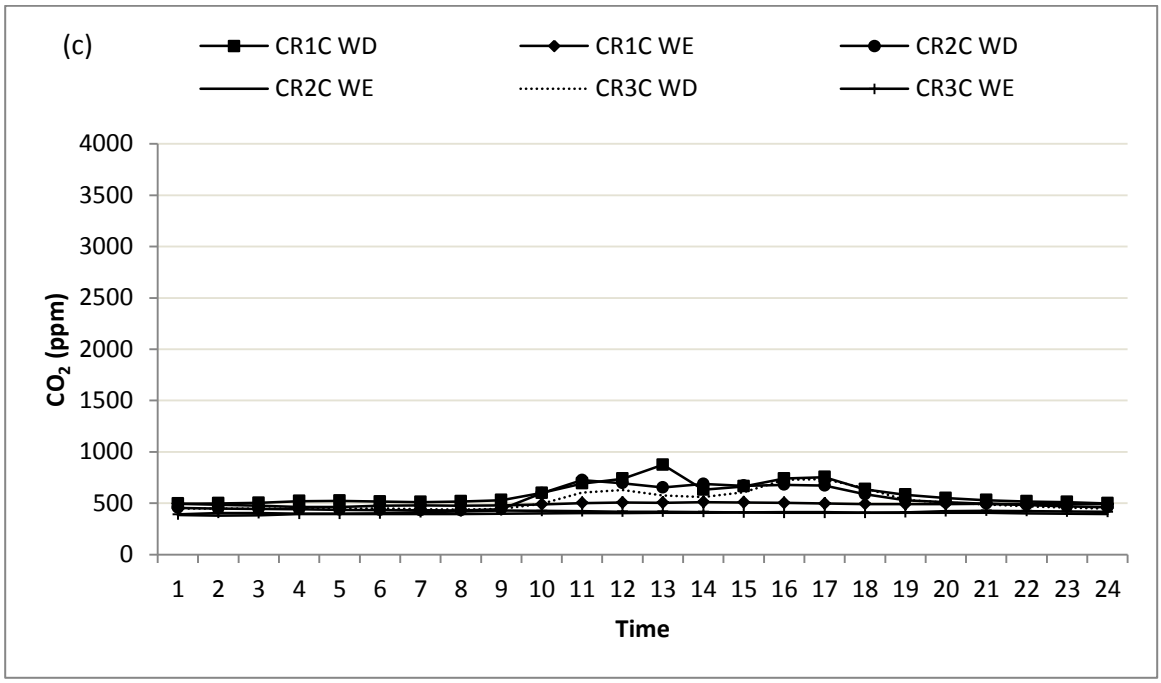
Mean hourly CO_2 levels of five observed classrooms of building B followed almost the same trend as that of classrooms of building A as shown in Fig, 4.1(b) as it was also naturally ventilated. However, during sampling period of these classrooms air-conditioners were in use (hence windows closed) resulting in major increase in CO_2 concentration. Maximum values were observed between 12:00 PM to 01:00 PM (end of morning sessions). CR2B, CR3B and CR4B showed 2 peaks similar to that of classrooms of building A, each, during morning and evening session. CR1B and CR5B on the other hand showed only one peak of CO_2 levels which may be explained by the fact that in CR1B there was no evening session held, where as in CR5B, the number of students in evening session were too low as compared to morning session. Maximum observed CO_2 values of CR1B, CR2B, CR3B, CR4B and CR5B were 6294, 4383,

5040, 3236 and 4647 ppm while minimum values were 392, 489, 529, 525 and 481 ppm respectively. No significant variation ($p>0.05$) in values was observed during WE.

Building C and D had centralized HVAC system. Mean hourly CO_2 levels of both buildings are shown in Fig. 4.1(c) and Fig. 4.1(d) respectively. Like other two buildings, CO_2 levels during WE remained without significant variation ($p>0.05$) over the day. Occupational period of these buildings was from 09:00 AM to 05:00 PM with an hour break in between (01:00 to 02:00 PM). Consequently, CO_2 levels increase from 09:00 AM to 01:00 PM and again from 02:00 to 05:00 PM. Building D had higher occupation density as compared to building C, thus showing greater peaks. Maximum observed CO_2 levels in CR1C, CR2C, CR3C, CR1D, CR2D and CR3D were 1831, 1005, 1034, 2486, 2533 and 2009 ppm while minimum levels were 453, 364, 375, 386, 496 and 399 ppm respectively.


(a).
CR1A WD
CR1A WE
CR2A WD
CR2A WE
CR3A WD
CR3A WE
CR4A WD
CR4A WE
4000
3500
3000
2500
2000
1500
1000
500
0
CO2 (ppm)
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24
Time


(b)
CR1B WD
CR1B WE
CR2B WD
CR2B WE
CR3B WD
CR3B WE
CR4B WD
CR4B WE
CR5B WD
CR5B WE
4000
3500
3000
2500
2000
1500
1000
500
0
CO2 (ppm)
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24
Time



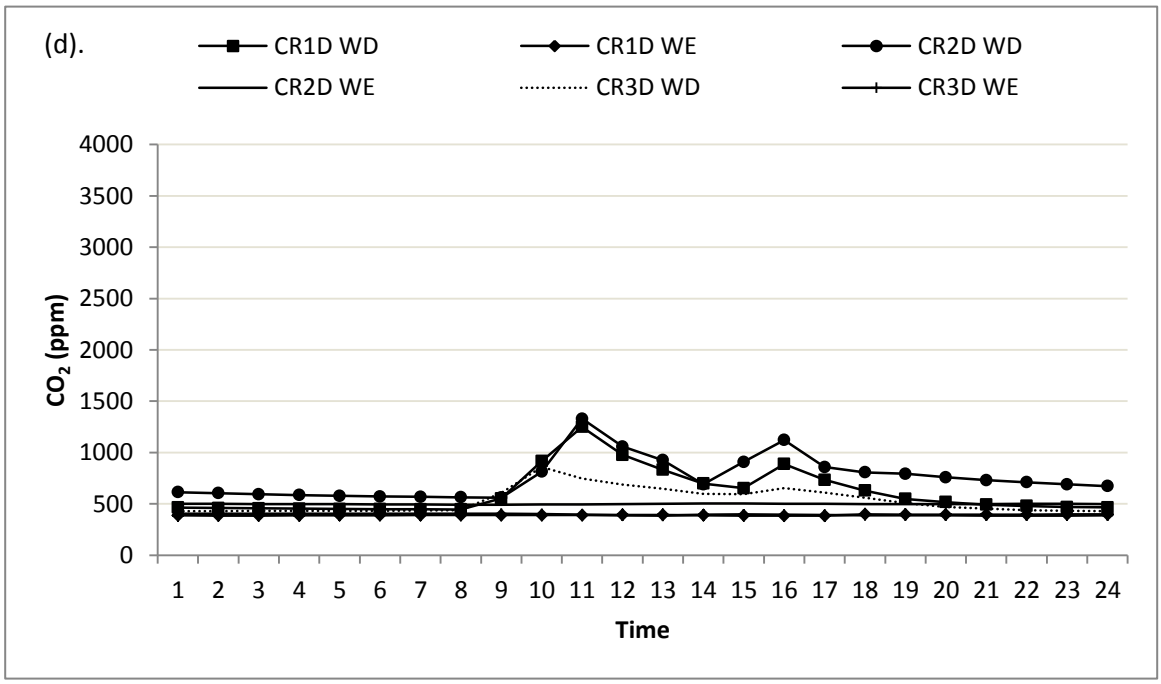
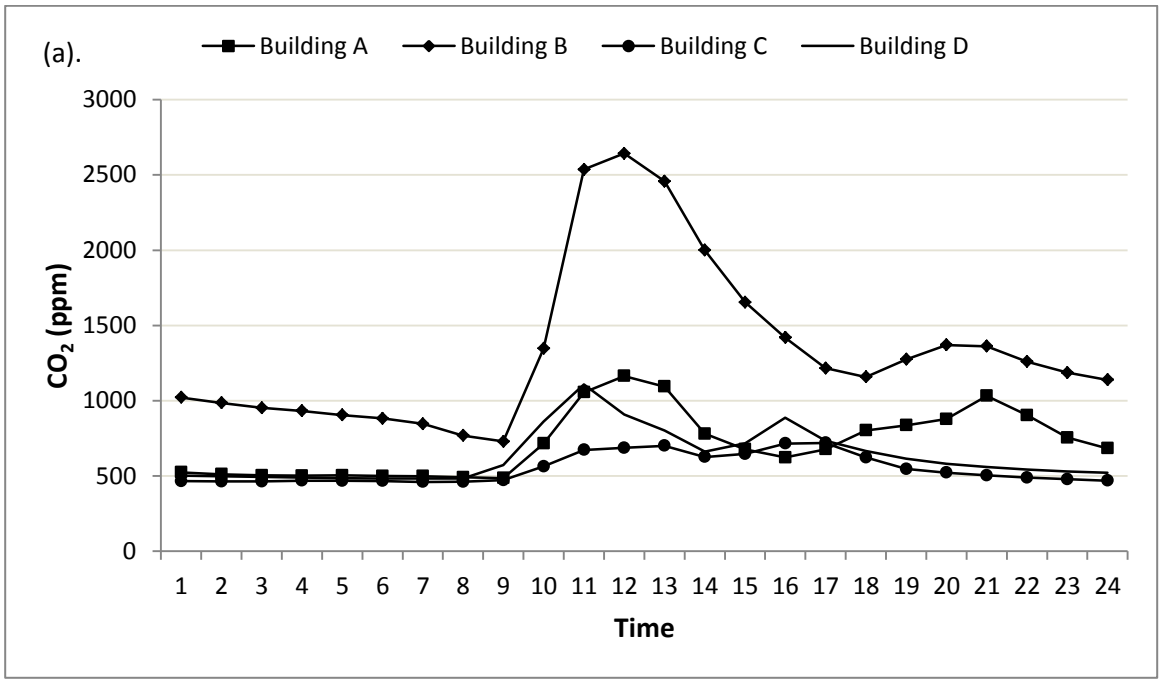
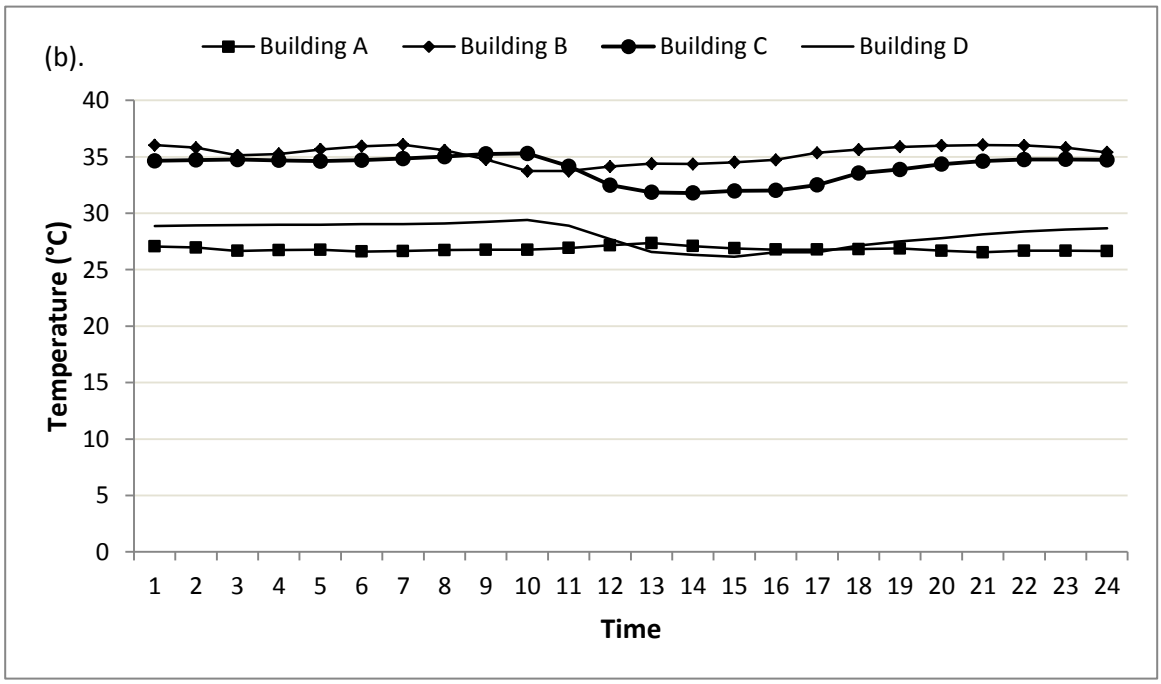


Figure 4.1: CO_2 concentration profile of classrooms in building A (a) Building B (b) Building C (c) and Building D (d)

Significant differences ($p<0.05$) in CO_2 trends of four selected buildings was observed during the WD as depicted in Fig. 4.2(a) which are likely to be due to the differences in occupation density, occupational period and type of HVAC system. Highest observed CO_2 level was 6294 ppm in CR1B of building B while lowest observed value of CO_2 was 361 ppm in CR2C of building C. Overall (as well as WD) highest mean CO_2 values were observed in classes of building B which had non-centralized HVAC system and had large occupation density as compared to other buildings. Though building A also had non-centralized HVAC system, mean CO_2 concentration of its classrooms were recorded to be lesser due to less occupation density as compared to building B. Building C and D had centralized HVAC system and showed less concentration levels as compared to those of buildings A and B but due to high occupation density of building D (comparative to C), it showed higher mean concentration.


(a).
Building A
Building B
Building C
Building D
3000
2500
2000
1500
1000
500
0
CO_2 (ppm)
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24
Time


(b).
Building A
Building B
Building C
Building D
40
35
30
25
20
15
10
5
0
Temperature (°C)
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24
Time

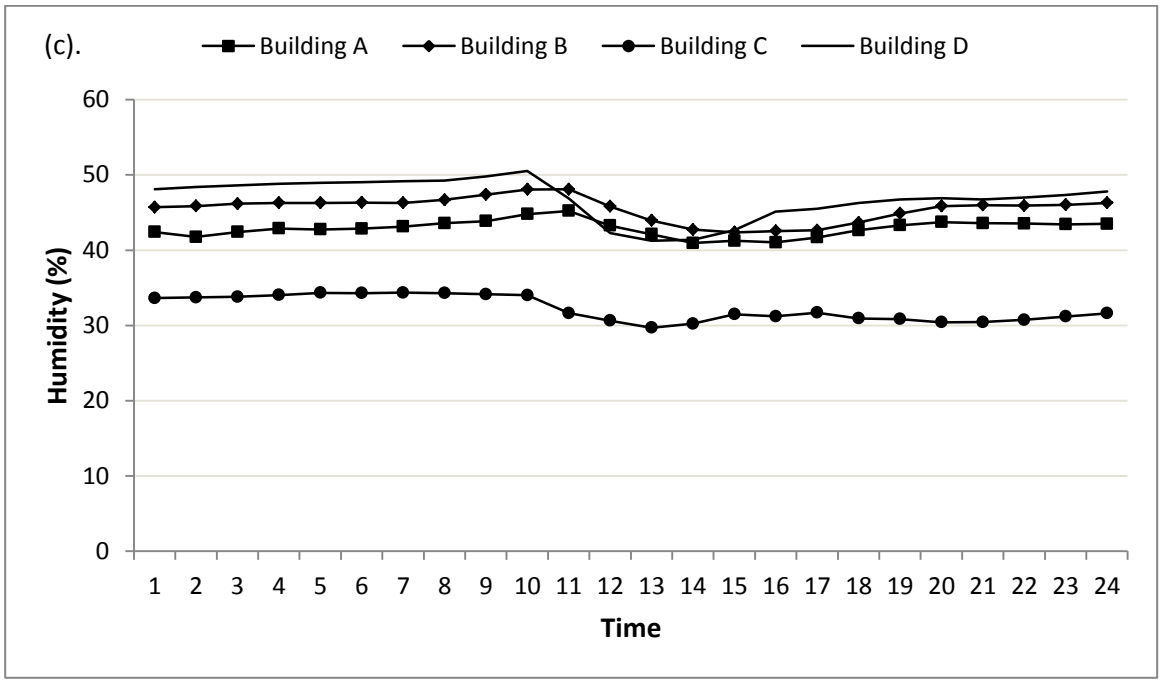
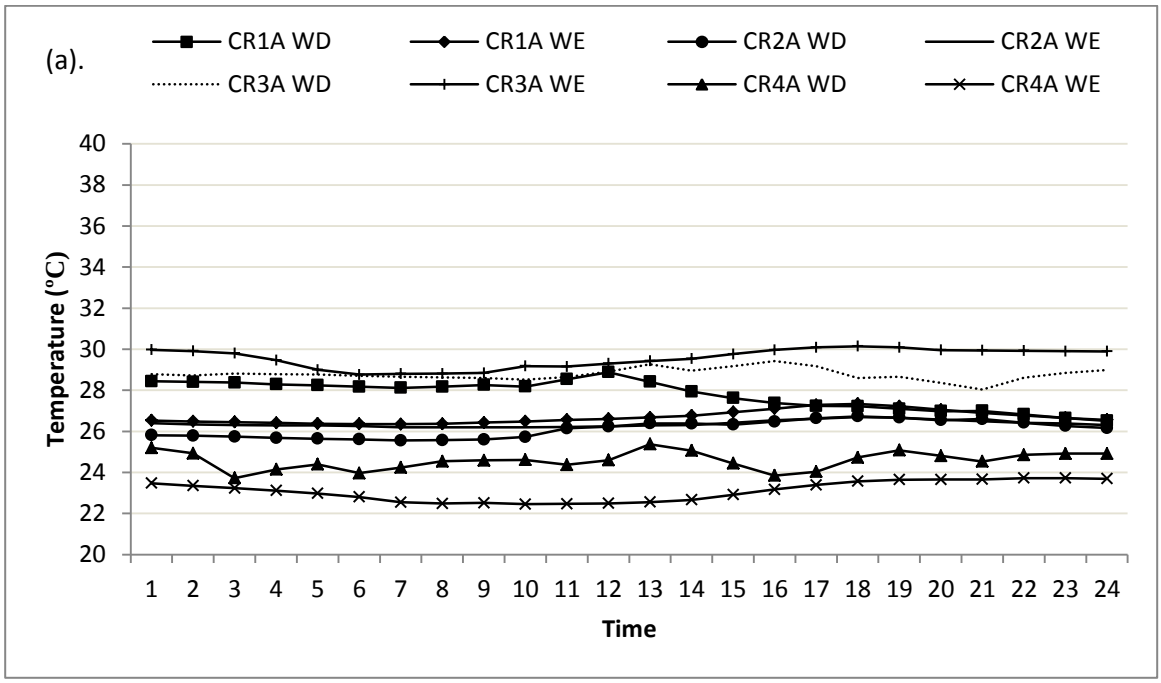
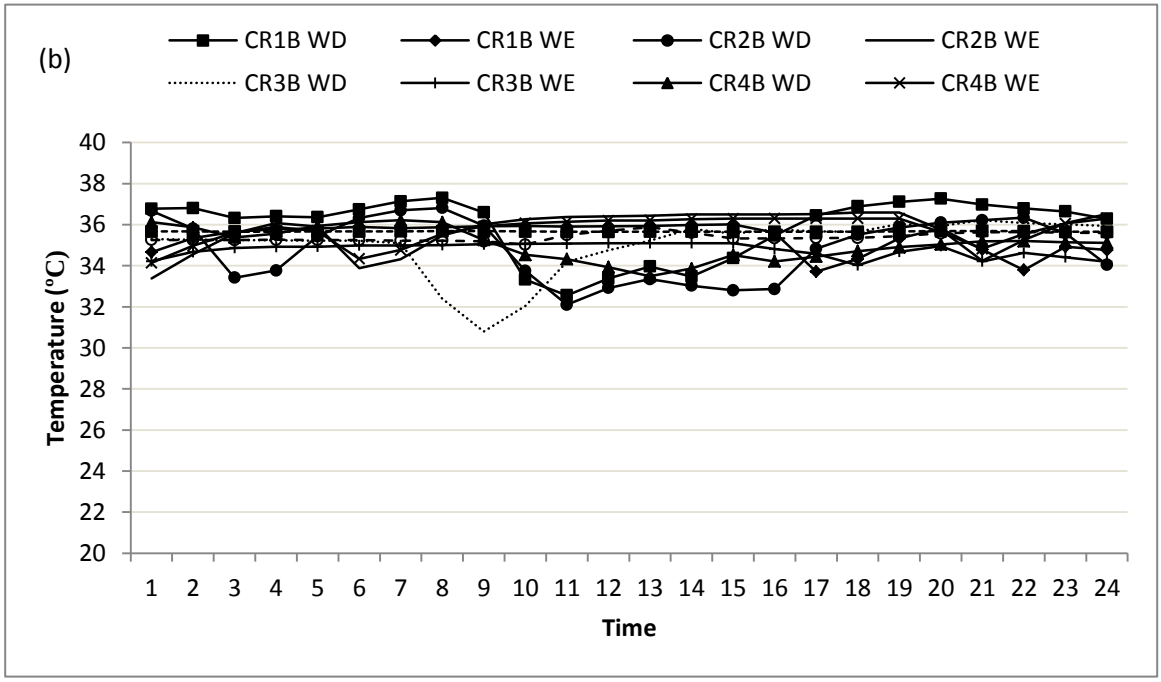


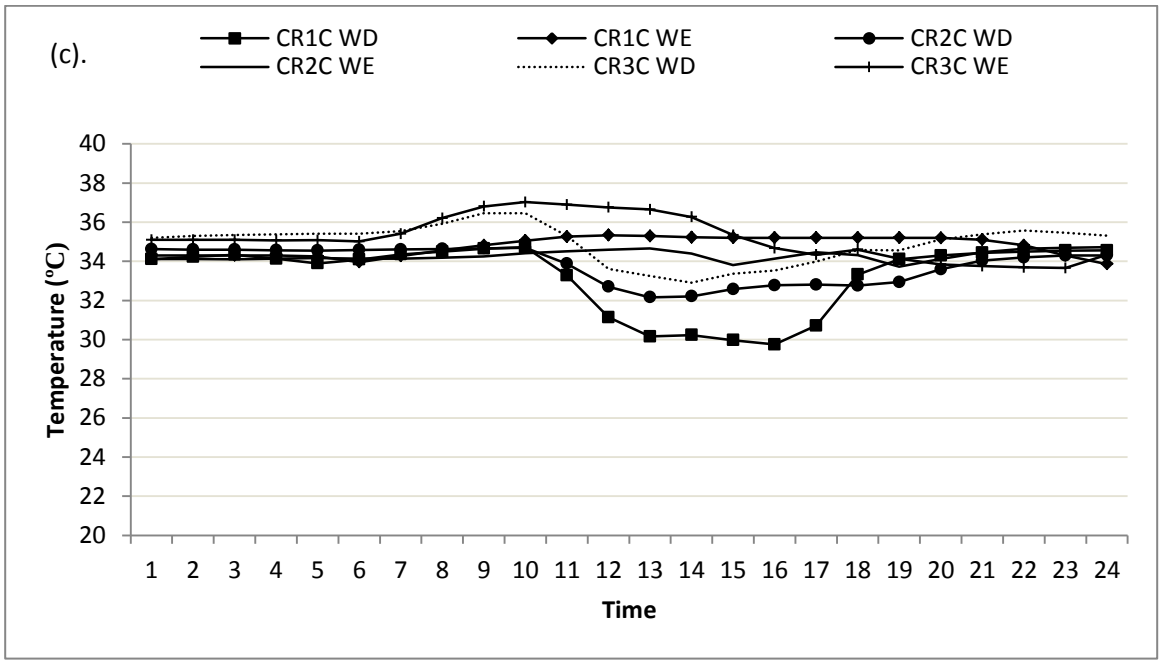
Figure 4.2: Comparative CO_2 (a), temperature (b) and relative humidity (c) profiles of selected buildings

4.1.2.2. Temperature and Relative Humidity

Mean hourly variation in indoor T and RH over the day are shown in Fig. 4.3 and Fig. 4.4 respectively. Multiple factors contribute towards the variation of these parameters including outdoor conditions, orientation of rooms, occupation density and type of HVAC system. Observed trends during WE of each building for temperature and humidity showed no significant variation ($p>0.05$) over the day.


(a).
CR1A WD
CR1A WE
CR2A WD
CR2A WE
CR3A WD
CR3A WE
CR4A WD
CR4A WE
Temperature (°C)
Time


(b)
CR1B WD
CR1B WE
CR2B WD
CR2B WE
CR3B WD
CR3B WE
CR4B WD
CR4B WE
Temperature (°C)
Time



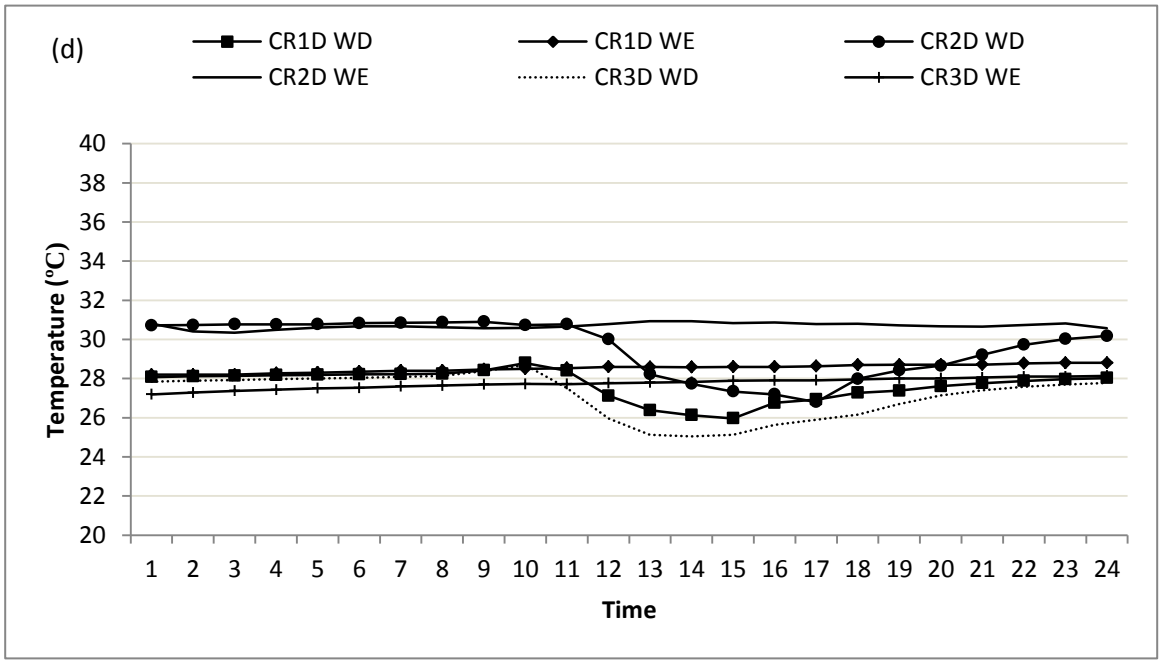
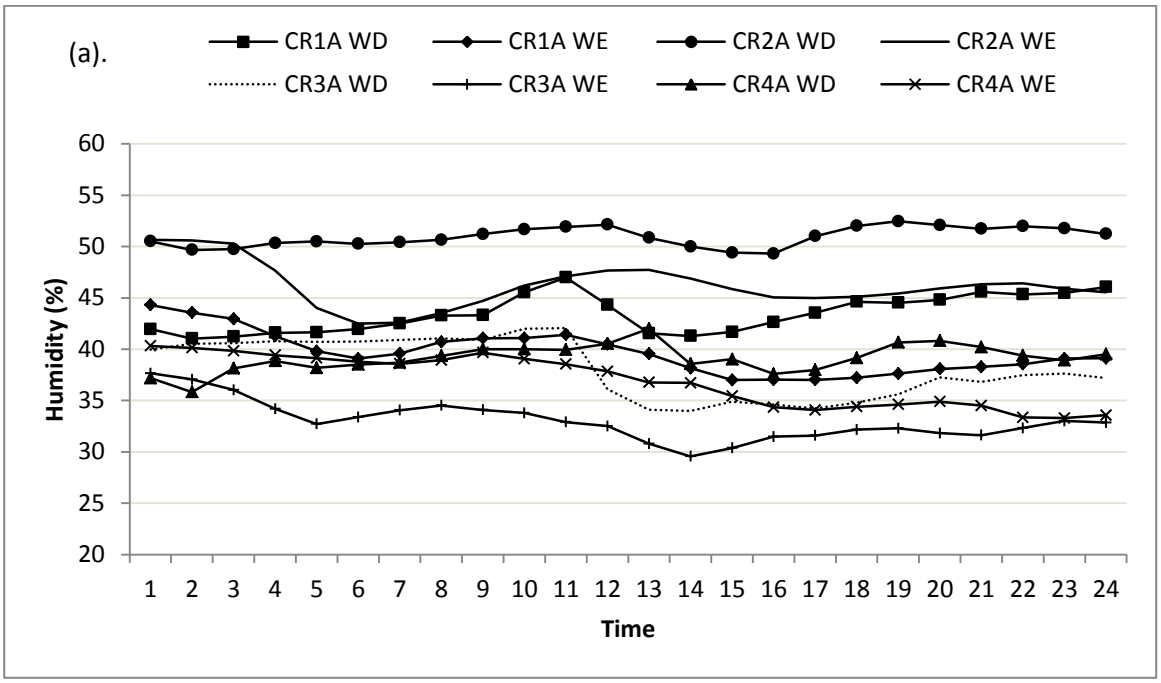
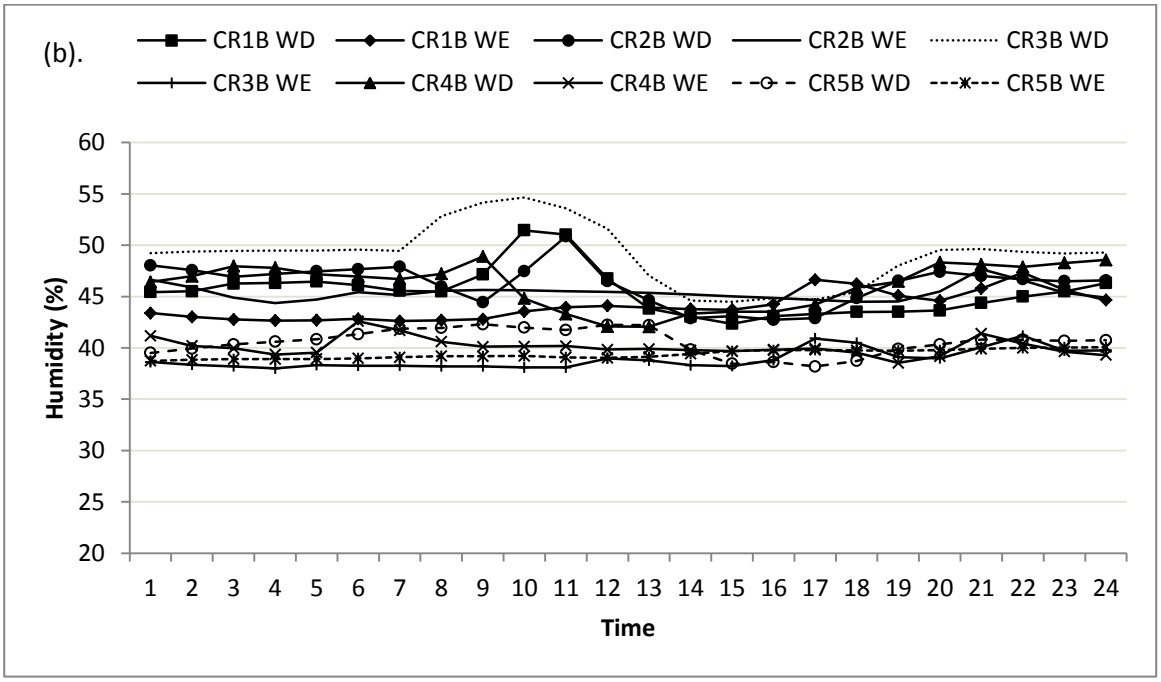
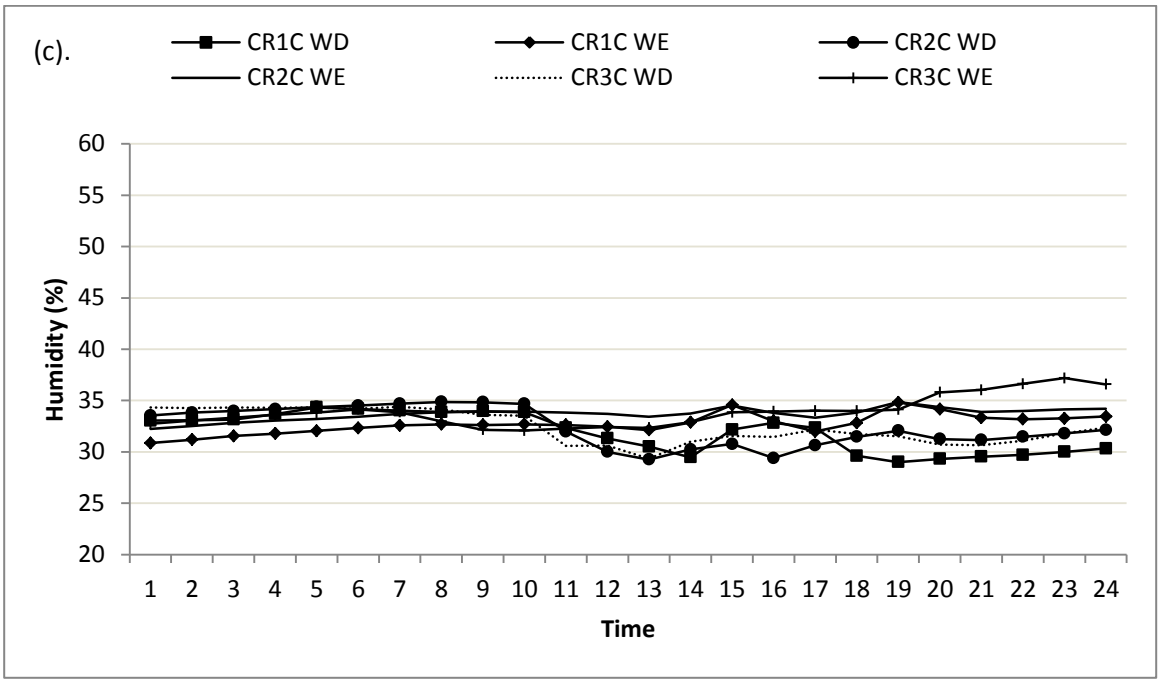


Figure 4.3: Temperature profile of classrooms in building A (a), building B (b), Building C (c) and Building D (d)


(a).
CR1A WD
CR1A WE
CR2A WD
CR2A WE
CR3A WD
CR3A WE
CR4A WD
CR4A WE
Humidity (%)
60
55
50
45
40
35
30
25
20
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24
Time


(b).
CR1B WD
CR1B WE
CR2B WD
CR2B WE
CR3B WD
CR3B WE
CR4B WD
CR4B WE
CR5B WD
CR5B WE
Humidity (%)
60
55
50
45
40
35
30
25
20
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24
Time



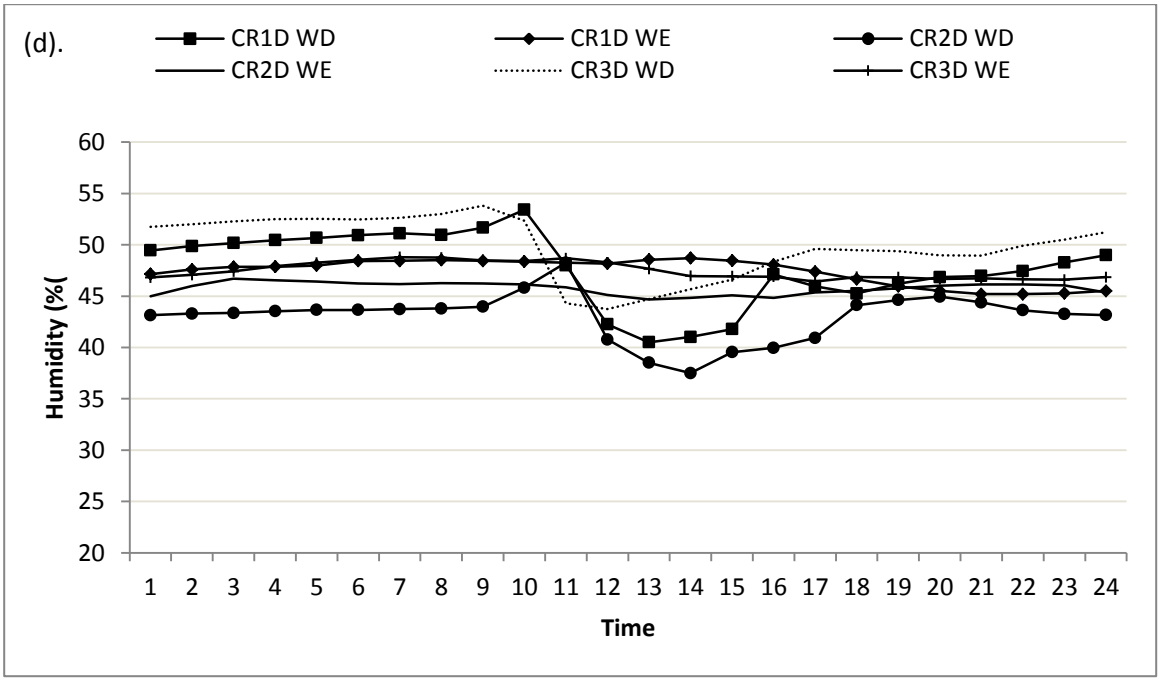


Figure 4.4: Relative humidity profiles of classrooms in building A (a), building B (b), Building C (c) and Building D (d)

Mean outdoor air temperature was 24.8°C (min:14.8 and max:33.75°C) during monitoring of building A. Air-conditioners were not switched on. No significant difference ($p>0.05$) in temperature was observed along the day on both WE and WD as shown in Fig. 4.3(a). Mean observed temperature values in CR1A, CR2A, CR3A and CR4A were 27.7, 26.1, 28.8 and 24.6°C respectively. Maximum observed values were 32.9, 27.4, 30.7, and 26.5°C while minimum values were 25.6, 25.2, 25.8 and 21.8°C respectively. Mean outdoor humidity level during the sampling period was 41.3% (min:7.25 and max:77%). No clear pattern over day or difference in trends between WD and WE were observed (Fig. 4.4(a)) which may be attributed to the fact that rooms were exposed to ambience (with open windows). Mean observed values in CR1A, CR2A, CR3A and CR4A were 43.4, 50.9, 38.1 and 39.1% respectively. Maximum observed humidity levels were 52.5, 58.6, 48.4 and 50.5% while minimum levels were 34.3, 41.9, 20.9 and 27.9% respectively.

Mean outdoor air temperature during the sampling period of building B was 27.3°C (min:12 and max:39°C). Air-conditioners were switched on during the occupational period of sampling days resulting in decrease in indoor air temperature with the start till the end of class sessions, representing significant difference ($p<0.05$) between temperature values of occupational and non-occupational period as depicted in Fig. 4.3(b). CR1B and CR3B were exposed to direct sunlight due to their orientation resulting in high mean temperature values as compared to other rooms. Mean indoor air temperatures of CR1B, CR2B, CR3B, CR4B and CR5B were 35.9, 25.7, 32, 27.7 and 29.5°C respectively. Maximum observed levels were 38.5, 36.6, 34.7, 31.9 and

30.2°C while minimum values were 31.4, 24.5, 24.1, 24.8 and 28.6°C respectively. Mean outdoor humidity levels during sampling period of building B was 36.5% (min:4.4 and max:72.2%) . The indoor humidity trend of building B showed increase in levels at the start of occupational period and then gradual decrease (Fig. 4.4(b)) as the temperature visibly decreased (Fig. 4.3(b)). Mean indoor humidity values in CR1B, CR2B, CR3B, CR4B and CR5B were 27.4, 46.2, 31.1, 46.1 and 40.6% respectively. Maximum levels were 45.5, 56.9, 49.1, 52.2 and 47.7% while minimum values were 21.2, 26.2, 19.5, 32 and 31.7% respectively.

During sampling period of building C and D, mean outdoor air temperature values were 31.3 and 32.2°C and mean outdoor humidity levels were 38.7 and 36.8% respectively. Both buildings had a controlled thermal environment due to the presence of centralized HVAC system which was only switched on during occupational hours of WD, thus showing no noticeable variation ($p>0.05$) in trends of temperature and humidity during non-occupational hours. A visible decrease in the values of T and RH with the start of occupational period at 09:00 AM may be observed in Fig. 4.3(c) and Fig. 4.4(c) for building C and Fig. 4.3(d) and Fig. 4.4(d) for building D respectively. Switching off of the centralized HVAC system at 05:00 PM, results in increase in values of two parameters. Mean observed values of indoor temperature in CR1C, CR2C, CR3C, CR1D, CR2D and CR3D were 33.2, 33.8, 34.9, 27.7, 29.6 and 27.1°C respectively. Maximum observed values were 36.2, 35.8, 37.8, 30.5, 32.2 and 29.5°C while minimum values observed were 25.2, 27.1, 27.9, 23.8, 24.7 and 21.3°C respectively. Similarly, mean recorded indoor humidity levels of the six classrooms

were 31.9, 32.4, 32.4, 47.7, 42.9 and 49.9%, maximum values were 40, 40.7, 41.1, 62.2, 55.1 and 66.7% while minimum values were 25.8, 24.8, 23.8, 35.9, 32.9 and 37 respectively.

As mentioned earlier indoor thermal comfort parameters are linked to building thermal insulation, its orientation and outdoor conditions in addition to occupation density and present HVAC system. Among four buildings under study, significant variation ($p<0.05$) of T and RH trends was found between any two buildings, which is also visible in Fig. 4.2(b) and Fig. 4.2(c). The highest observed T value was 38.5°C in CR1B of building B while minimum observed value was 21.3°C in CR3D of building D. Highest humidity level was observed in CR3D of building D as 66.7% while minimum value was observed in CR3B of building B as 19.5%. Overall, during occupational hours, highest mean temperature values were observed in building B, followed by building A, C and D respectively. While highest mean humidity level was observed in building D followed by building A, B and C respectively.

4.1.3. Percentage Exceedance from Standards

According to ASHRAE Standards 62-1989, indoor CO_2 concentration should be less than 1000 ppm whereas, for T and RH, the ranges are 22.8-26.1ºC and 30-60% to be maintained respectively. Table 4.3 shows the percentage exceedance of the monitored mean hourly concentrations of CO_2, T and RH during WD, WE and the occupational period from reference standards mentioned above. No exceedance of indoor CO_2 levels was observed during the WE in any building. However, buildings with natural

ventilation (buildings A and B) showed frequent cases of exceedance on WD and particularly during the occupational hours, the exceedance frequency was high. This was in spite of the fact that the occupation density of these buildings was in the same range as that of buildings C and D (Table 3.1), indicating inadequacy of the natural ventilation system. Indoor T appeared to exceed the upper allowable limit (26.1ºC) during most of the monitoring period for all buildings with an exception of CR4A. It is to be noted that during monitoring of CR4A, ambient temperature was comparatively low (Table 4.2). This indicates that all buildings were inefficiently insulated and thus outdoor climatic conditions significantly affected the indoor thermal conditions.

Table 4.3: Exceedance (%) to ASHRAE Standards 62.1-2010 of mean hourly values of CO_2, T and RH

Buildings	Rooms	ASHRAE Standards													
		CO_2 (>1000 ppm)			Temperature (22.8-26.1°C)						Relative Humidity (30-60%)					
		Weekdays	Weekends	Occupational Period	Weekdays		Weekends		Occupational Period		Weekdays		Weekends		Occupational Period	
					<22.8	>26.1	<22.8	>26.1	<22.8	>26.1	<30 %	>60 %	<30 %	>60 %	<30 %	>60 %
A	CR1A	9.7	0	19.4	0	84.7	0	100	0	100	4.2	0	0	0	0	0
	CR2A	30.6	0	52.8	0	48.6	0	100	0	75	4.2	0	0	0	0	0
	CR3A	8.3	0	13.9	0	100	0	100	0	100	17.7	0	20.8	0	20.8	0
	CR4A	16.7	0	20.8	2.1	4.2	35.4	0	0	8.3	8.3	0	0	0	8.3	0
B	CR1B	39.6	0	64.6	0	100	0	100	0	100	86.5	0	100	0	83.3	0
	CR2B	62.5	0	70	0	31.7	0	100	0	38.3	4.2	0	100	0	0	0
	CR3B	80.2	0	85.4	0	99	0	100	0	100	38.5	0	0	0	58.3	0
	CR4B	32.3	0	52.1	0	88.9	0	100	0	77.8	4.2	0	0	0	0	0
	CR5B	62.5	0	80.6	0	100	0	100	0	100	4.2	0	0	0	0	0

Buildings	Rooms	ASHRAE Standards														
		CO_2 (>1000 ppm)			Temperature (22.8-26.1°C)						Relative Humidity (30-60%)					
		Weekdays	Weekends	Occupational Period	Weekdays		Weekends		Occupational Period		Weekdays		Weekends		Occupational Period	
					<22.8	>26.1	<22.8	>26.1	<22.8	>26.1	<30%	>60%	<30%	>60%	<30%	>60 %
C	CR1C	2.1	0	2.1	0	99	0	100	0	100	34.4	0	91.7	0	16.7	0
	CR2C	0	0	0	0	100	0	100	0	100	23.9	0	0	0	20.8	0
	CR3C	0	0	0	0	100	0	100	0	100	30.2	0	0	0	25	0
D	CR1D	8.3	0	16.7	0	88.5	0	100	0	77.1	4.2	0	0	0	0	0
	CR2D	12.5	0	25	0	91.7	0	100	0	83.3	4.2	0	0	0	0	0
	CR3D	2.1	0	4.2	3.13	91.7	0	100	3.13	64.6	4.2	0	0	0	0	0

4.2. Hospital Sites

4.2.1. Microbial Analysis

4.2.1.1. Airborne Bacterial and Fungal Concentrations

Indoor bacterial concentration in a hospital is supposed to be affected by the type and number of patients in that area. Moreover indoor fungal concentration depends on the indoor moisture conditions, cleaning frequency and outdoor atmospheric conditions of that particular area. Table 4.4 shows descriptive statistics of indoor concentration of airborne bacteria and fungus in six sites of hospital under study.

Table 4.4: Descriptive statistics of indoor bacteria and fungus

Locations		**Bacteria (CFU/m^3)**	**Fungi (CFU/m^3)**
OT1	Mean	221	58.4
	Median	200	40
	StDev	136.7	68.3
	Range	60-466.7	0-266.7
OT2	Mean	236.2	41.1
	Median	220	20
	StDev	100.2	46.6
	Range	60-460	0-153.8

Locations		Bacteria (CFU/m^3)	Fungi (CFU/m^3)
OPD	Mean	1649.7	176.5
	Median	1600	140
	StDev	795.9	124.6
	Range	380-3577	20-500
ES	Mean	1028.9	166.4
	Median	1060	192.3
	StDev	575.3	126.3
	Range	280-2280	19.2-384.6
SW	Mean	369.9	135.2
	Median	340	100
	StDev	317.3	96.5
	Range	20-1038.5	20-307.7
GMW	Mean	383.9	193.4
	Median	340	180
	StDev	231.1	117.1
	Range	100-840	60-433.3

Results showed higher range and mean values of airborne bacteria as compared to fungal levels in all the studied sites. Highest concentration of bacteria was found in OPD (mean: 1649.7 CFU/m^3) supported by the fact that it was the site having maximum number of patients with variable disease types. Moreover, lowest

concentration was found in OT1 (mean: 221 CFU/m^3). Highest fungal load on the other hand was found in GMW (mean: 193.4 CFU/m^3) while lowest in OT2 (mean: 41.1 CFU/m^3).

Due to the difference in nature of activities and conditions at sites, large variation in concentrations of airborne micro-organisms was observed among the sites. Microbial concentrations observed at each site were compared with other sites and results showed significant statistical difference ($p<0.05$) in 73% of bacterial and 53% of fungal concentrations. As operation theaters are supposed to be controlled environments with less number of occupants and more cleaning frequency in comparison with other monitored locations, no significant difference ($p>0.05$) was observed in airborne bacterial and fungal concentrations of two operation theatres. Similarly, both studied wards had similar level of occupation, with same ambient conditions and showed no significant difference ($p>0.05$) in microbial concentrations. . Moreover, bacterial concentration of OPD and ES showed significant difference ($p<0.05$) while fungal concentrations didn't show any significant difference.

4.2.1.2. Seasonal Variations

Seasonal (winter and spring) variation of airborne bacteria and fungus has been shown in Fig. 4.5 (a) and Fig. 4.5 (b) respectively. No significant variation ($p>0.05$) in concentration levels of airborne bacteria has been observed between the two studied seasons for any of monitored locations except OPD, showing various other factors (discussed below) to be more significant contributors towards buildup of bacterial load than change in ambient conditions over the two seasons. However, significant

variation ($p<0.05$) in concentration levels of airborne fungal concentrations of OPD, ES, GMW and SW has been observed showing seasonal variation as significant contributor. Similar results have been reported earlier for effect of seasonal factors on fungal and bacterial indoor levels in hospitals (Kim et al., 2009).

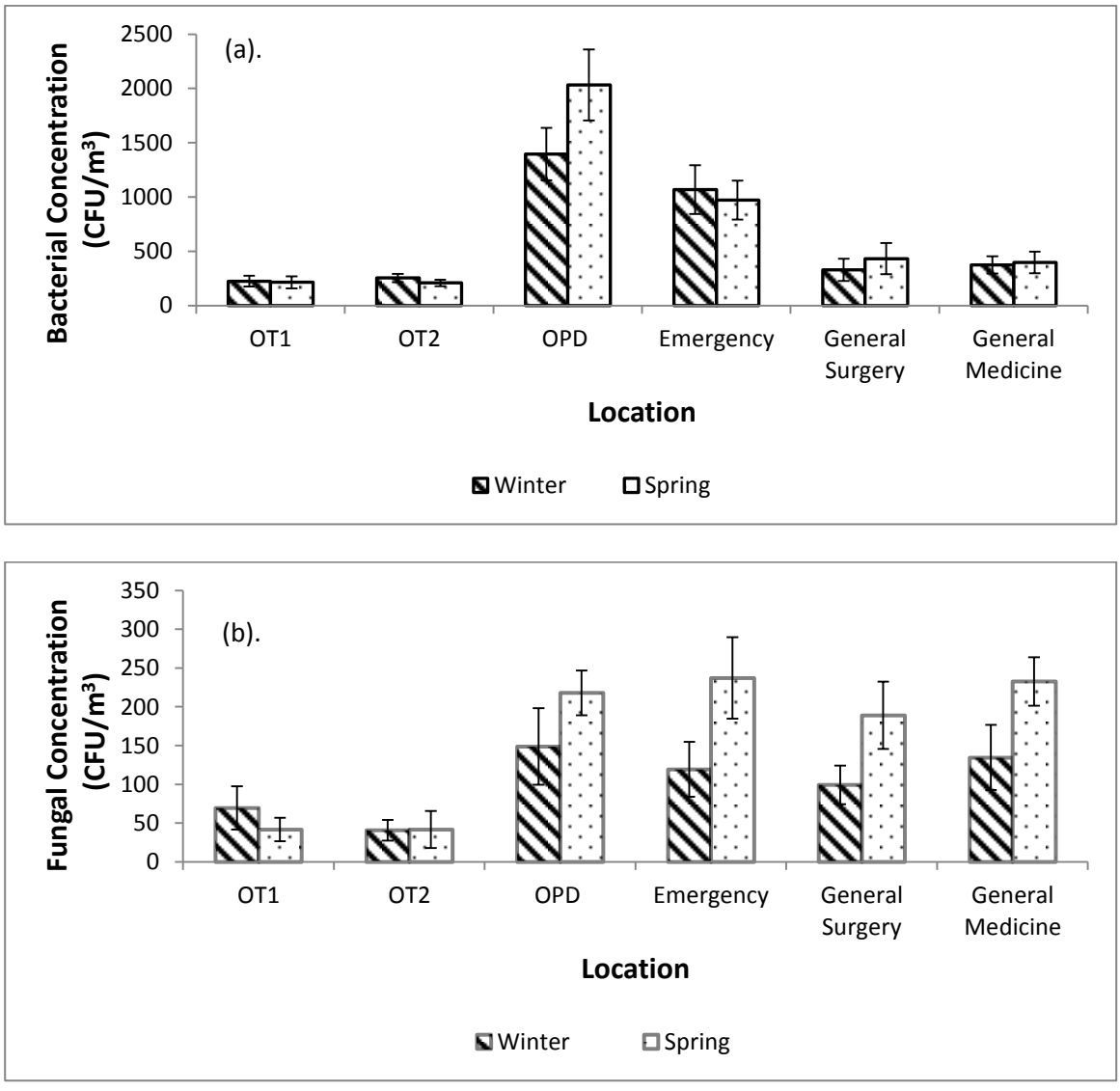


Figure 4.5: Seasonal variation in airborne microbial concentrations (a). Bacterial concentration (b). Fungal concentration

As discussed earlier, airborne bacterial concentration depends on type and utilization of the studied area. OPD and ES are similar facilities, both having large number of attendants and patients with diversity in disease types. Thus, airborne microbial counts in both the areas were found to be highest among monitored sites. Highest microbial counts in both the seasons were recorded in OPD ranging from 380-2480 CFU/m^3 in winter and 1308-3577 CFU/m^3 in spring. Moreover, airborne bacterial concentration of ES ranged from 280-2280 CFU/m^3 in winter and 481-1519 CFU/m^3 in spring. Fungal load of OPD was also highest during winter season ranging from 20-500 CFU/m^3 while that of ES ranged from 20-333 CFU/m^3. However, during spring season, ES showed the highest fungal load ranging from 19-385 CFU/m^3. Descriptive statistical analysis of seasonal changes is depicted in Table 4.5.

Table 4.5: Seasonal Descriptive Statistical Analysis of Airborne Micro-organisms

Locations		**Bacteria (CFU/m^3)**		**Fungus (CFU/m^3)**	
		Winter	**Spring**	**Winter**	**Spring**
OT1	Mean	225.18	214.74	69.63	41.67
	Median	200	192.31	40	28.85
	StDev	145.52	135.66	83.27	37.32
	Range	60-466.67	96.15-461.54	0-266.67	0-96.15
OT2	Mean	254.81	208.33	40.74	41.67
	Median	220	221.15	20	19.23
	StDev	115.79	71.35	40.47	58.85
	Range	60-460	76.92-269.23	0-120	0-153.85

Locations		Bacteria (CFU/m^3)		Fungus (CFU/m^3)	
		Winter	**Spring**	**Winter**	**Spring**
OPD	Mean	1394.81	2032.05	148.89	217.95
	Median	1380	1875	120	230.77
	StDev	726.34	798.17	148.02	70.57
	Range	380-2480	1307.69-3576.92	20-500	96.15-307.69
ES	Mean	1067.41	971.15	119.26	237.18
	Median	1060	942.31	60	250
	StDev	674.58	438.15	106.48	128.52
	Range	280-2280	480.77-1519.23	20-333.33	19.23-384.61
SW	Mean	328.15	432.69	99.26	189.1
	Median	340	375	80	173.08
	StDev	307.02	350.98	74.87	106.32
	Range	20-980	57.69-1038.46	20-260	76.92-307.69
GMW	Mean	374.81	397.43	232.59	134.61
	Median	340	336.54	180	105.77
	StDev	237.99	241.93	126.33	76.92
	Range	100-840	115.38-769.23	60-433.33	76.92-269.23

Wards, on the other hand, had less number of patients and attendants as compared to OPD and ES facilities, thus comparatively less microbial load was observed. Airborne bacterial concentration in SW during winter and spring ranged from 20-980 CFU/m^3 and 58-1038 CFU/m^3 respectively. However, concentration levels in GMW ranged from 100-840 CFU/m^3 in winter and 115-769 CFU/m^3 in spring season. Airborne fungal levels in SW during winter and spring ranged from 20-260and 77-308 CFU/m^3 while in GMW from 60-433 and 77-269 CFU/m^3 respectively.

OTs are the most sensitive places of a hospital, thus requiring proper control of airborne microbes. Bacterial concentration in OT1 was found between 60-467 CFU/m^3 during winter and 96-461 CFU/m^3 during spring season. However, in OT2 range was 60-460 CFU/m^3 during winter and 77-269 CFU/m^3 during spring. Fungal load in OT1 during winter and spring was recorded as 0-267 and 0-120 CFU/m^3 while in OT2 as 0-96 and 0-154 CFU/m^3 respectively.

- **Exceedance from Standards**

Pakistan has no national standards for airborne microbial concentrations and there is no such proposed limit available which is accepted by all relevant scientific communities. Upper limit for indoor fungal levels as suggested by World Health Organization (WHO) is 500 CFU/m^3. According to American Conference of Governmental Industrial Hygienists (ACGIH), for persons with immune deficiencies, limiting value of bacterial concentration is 100 CFU/m^3. A similar study conducted earlier in Portugal (Cabo Verde et al., 2015) reported limiting value for both airborne bacteria and fungus according to Portuguese National Standards (PNS) as 500 CFU/m^3 for winter season. Same standards are used in this study as reference. No location exceeded WHO/Portuguese National Standards for fungal concentrations during monitoring period. However, for the bacterial loads, OPD, ES and GMW showed 100% observations beyond ACGIH limits for both seasons and 77 and 100% (OPD) and 77 and 89% (ES) observations exceeds PNS limits for winter and spring seasons respectively. Table 4.6 shows detailed exceedance occurrences of airborne bacterial and fungal levels with standard limits for all monitored locations during both seasons.

Table 4.6: Exceedance (%) to Standards

Location	Bacterial Standards				Fungal Standards	
	ACGIH (100 CFU/m^3)		Portuguese (500 CFU/m^3)		WHO/ Portuguese (500 CFU/m^3)	
	Winter	Spring	Winter	Spring	Winter	Spring
OT1	89	78	0	0	0	0
OT2	89	89	0	0	0	0
OPD	100	100	77	100	0	0
ES	100	100	77	89	0	0
SW	66	83	22	50	0	0
GMW	100	100	22	16	0	0

4.2.1.3. Identification of Airborne Micro-organisms

I. Bacteria

Morphological characteristics of pre-dominant bacterial strains are given in Table 4.7.

Table 4.7: Morphological characteristics of bacteria

Identified	Form/ Shape	Color	Elevation	Texture	Margins
Staphylococcus Haemolyticus	Punctiform	Yellowish White	Raised	Smooth	Entire
Kocuria Rosea	Punctiform	Orange	Raised	Smooth	Entire
Kocuria Rhizophila	Circular	Orange	Raised	Smooth	Entire
Bacillus Cereus	Circular	White	Flat	Smooth	Entire
Kytococcus sedentarius	Circular	Cream	Raised	Smooth	Entire
Micrococcus terreus	Circular	Yellow	Raised	Smooth	Entire
Micrococcus luteus	Punctiform	Yellow	Raised	Smooth	Entire
Aerococcus viridans	Circular	Yellow	Flat	Smooth	Entire
Kocuria Kristinae	Circular	Reddish Orange	Raised	Smooth	Entire
Bacillus Subtilis	Circular	Light Orange	Flat	Dry	Irregular

Identified	Form/ Shape	Color	Elevation	Texture	Margins
Pseudomonas stutzeri	Circular	Creamy White	Raised	Smooth	Entire
Staphylococcus Aureus	Punctiform	Yellow	Flat	Smooth	Entire
Staphylococcus Cohnii	Circular	White	Flat	Smooth	Entire

- **Phenotypic Identification**

Bacterial phenotypic identification through biochemical tests (gram-straining, oxidase and catalase tests) indicated presence of three types of airborne micro-organisms (gram-positive cocci, gram-positive rods and gram negative rods) in both monitored seasons details of which is given in Table 4.8. Gram-positive cocci was the most dominant group of genera (89.8%) identified followed by gram-positive rods (7.2%) and gram-negative rods (3%). Seasonal assessment of recovered airborne bacterial colonies also showed dominancy of gram-positive cocci (winter: 87.12%, spring: 93.22%) in both seasons.

Table 4.8: Biochemical characteristics of bacterial strains

Colonies	Gram Reaction	Oxidase Test	Catalase Test
Staphylococcus Haemolyticus	Gram Positive Cocci	Negative	Positive
Kocuria Rosea	Gram Positive Cocci	Positive	Positive
Kocuria Rhizophila	Gram Positive Cocci	Positive	Positive
Bacillus Cereus	Gram Positive rods	Negative	Positive
Kytococcus sedentarius	Gram Positive Cocci	Negative	Positive
Micrococcus terreus	Gram Positive Cocci	Positive	Positive
Micrococcus luteus	Gram Positive Cocci	Positive	Positive
Aerococcus viridans	Gram Positive Cocci	Positive	Positive
Kocuria Kristinae	Gram Positive Cocci	Positive	Positive
bacillus subtilis	Gram Positive rods	Negative	Positive

Colonies	Gram Reaction	Oxidase Test	Catalase Test
Pseudomonas stutzeri	Gram negative rods	Positive	Positive
Staphylococcus Aureus	Gram Positive Cocci	Negative	Positive
Staphylococcus Cohnii	Gram Positive Cocci	Negative	Positive

- **Genotypic Identification**
  - ✓ **Bacterial DNA Extraction**

Identification of most frequently observed bacterial colonies was made up-to the genus level by extracting genomic DNA of isolated airborne bacterial colonies. The extracting was made by using manual method. Extraction process of DNA was confirmed by subjecting it to agarose gel electrophoresis using 1 kb ladder and and observed under UV transluminator. The gel picture saved by using Dolphin-Doc plus Image System is given in Figure 4.6.

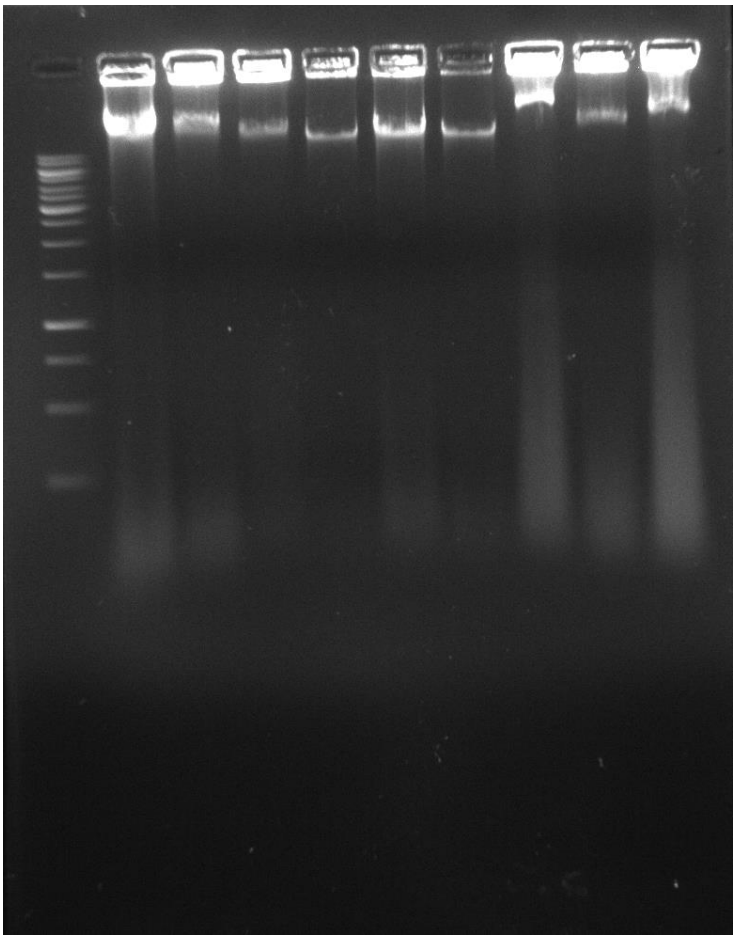


Figure 4.6: Gel picture for Genomic DNA extraction

- ✓ **Bacterial DNA Amplification**

Genomic DNA extracted was then amplified by polymerase chain reaction (PCR) with the help of paired universal primers following an optimized protocol. Amplified was purified after confirmation of 1500 bp PCR bands on agarose gel and sequenced. Gel picture for amplified DNA is given in Figure 4.7

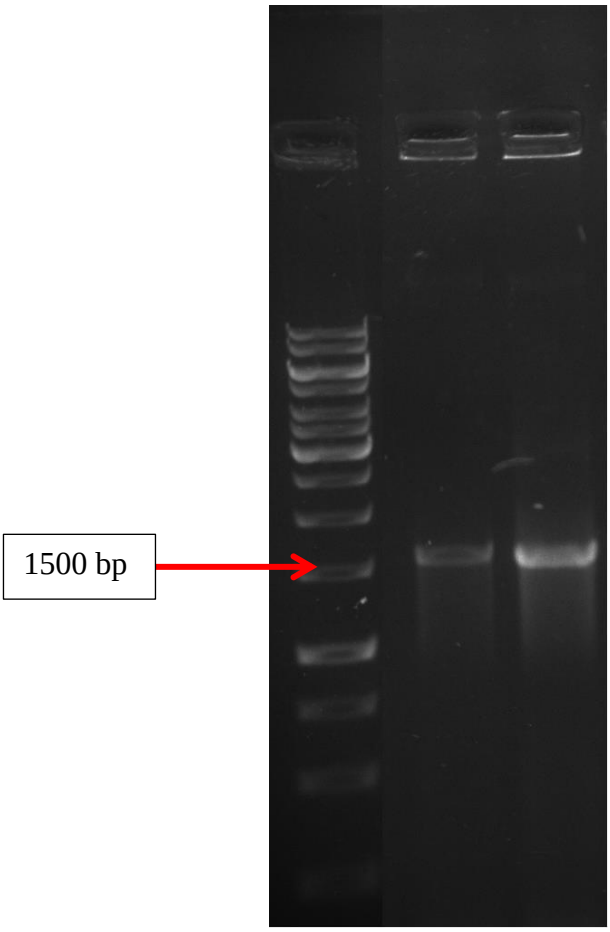


Figure 4.7: Gel Picture for Amplified DNA

- ✓ **Identified Bacterial Colonies**

Bacterial genotypic identification of frequently observed colonies characterized gram positive cocci into staphylococcus such as staphylococcus haemolyticus, staphylococcus aureus and staphylococcus cohnii, micrococcus such as micrococcus luteus and micrococcus terreus, kocuria such as kocuria rosea, kocuria rhizophila and kocuria kristinae, Aerococcus viridans and Kytococcus sedentarius. However, identified gram positive rods include bacillus cereus and

bacillus subtilis and gram negative rods include pseudomonas stutzeri. The detailed overview of the identified airborne bacterial isolates is given in Table 4.9.

Table 4.9: Identified airborne bacterial colonies from hospital sites

Bacterial Colonies	OT1		OT2		OPD		ES		SW		GMW	
	Winter	**Spring**	**Winter**	**Spring**	**Winter**	**Spring**	**Winter**	**Spring**	**Winter**	**Spring**	**Winter**	**Spring**
Gram positive cocci	76 (83.5)	64 (96.9)	89 (89.9)	57 (98.3)	391 (87.5)	330 (92.9)	274 (86.4)	282 (93.1)	150 (86.7)	86 (89.6)	138 (88.5)	89 (92.7)
Staphylococcus Haemolyticus	38 (50)	33 (51.6)	48 (53.9)	31 (54.4)	164 (41.9)	75 (22.7)	143 (52.2)	79 (28)	64 (42.7)	32 (37.2)	73 (52.9)	36 (40.5)
Micrococcus luteus	17 (22.4)	8 (12.5)	13 (14.6)	7 (12.3)	124 (31.7)	83 (25.2)	60 (21.9)	52 (18.4)	49 (32.7)	14 (16.3)	27 (19.6)	11 (12.4)
Micrococcus terreus	6 (7.9)	3 (4.7)	13 (14.6)	4 (7)	28 (7.2)	37 (11.2)	9 (3.3)	15 (5.3)	12 (8)	10 (11.6)	13 (9.4)	10 (11.2)
Kocuria Rosea	4 (5.3)	2 (3.1)	2 (2.5)	2 (3.5)	11 (2.8)	49 (14.8)	7 (2.6)	59 (20.9)	5 (3.3)	5 (5.8)	6 (4.4)	5 (5.6)
Staphylococcus Aureus	0 (0)	7 (10.9)	1 (1.1)	7 (12.3)	4 (1)	26 (7.9)	9 (3.3)	24 (8.5)	2 (1.3)	12 (13.9)	3 (2.2)	15 (16.8)
Kocuria Rhizophila	1 (1.3)	3 (4.7)	3 (3.4)	2 (3.5)	28 (7.2)	14 (4.2)	16 (5.8)	10 (3.5)	3 (2)	3 (3.5)	3 (2.2)	3 (3.4)
Kocuria Kristinae	4 (5.3)	1 (1.5)	1 (1.1)	1 (1.7)	8 (2)	18 (5.4)	14 (5.1)	9 (3.2)	7 (4.7)	6 (6.9)	4 (2.9)	0 (0)
Aerococcus viridans	0 (0)	3 (4.7)	1 (1.1)	2 (3.5)	6 (1.5)	8 (2.4)	4 (1.5)	7 (2.5)	0 (0)	3 (3.5)	1 (0.7)	3 (3.4)
Kytococcus sedentarius	1 (1.3)	0 (0)	3 (3.4)	0 (0)	4 (1)	5 (1.5)	0 (0)	7 (2.5)	4 (2.7)	0 (0)	0 (0)	0 (0)
Staphylococcus Cohnii	0 (0)	2 (3.1)	0 (0)	0 (0)	4 (1)	2 (0.6)	0 (0)	2 (0.7)	0 (0)	0 (0)	1 (0.7)	0 (0)
Others	5 (6.6)	2 (3.1)	4 (4.5)	1 (1.7)	10 (2.6)	13 (3.9)	12 (4.4)	18 (6.4)	4 (2.7)	1 (1.2)	7 (5.1)	6 (6.7)

Bacterial Colonies	OT1		OT2		OPD		ES		SW		GMW	
	Winter	**Spring**	**Winter**	**Spring**	**Winter**	**Spring**	**Winter**	**Spring**	**Winter**	**Spring**	**Winter**	**Spring**
Gram positive rod	3 (3.3)	2 (3)	9 (9.1)	1 (1.7)	50 (11.2)	12 (3.4)	34 (10.7)	10 (3.3)	19 (10.9)	0 (0)	16 (10.3)	7 (7.3)
Bacillus Cereus	2 (66.7)	0 (0)	5 (55.5)	0 (0)	21 (42)	0 (0)	17 (50)	0 (0)	8 (42.1)	0 (0)	10 (62.5)	0 (0)
Bacillus Subtilis	0 (0)	0 (0)	4 (4.4)	1 (100)	17 (34)	2 (16.7)	3 (8.8)	0 (0)	8 (42.1)	0 (0)	4 (25)	4 (57.1)
Gram negative rod	12 (13.2)	0 (0)	1 (1)	0 (0)	6 (1.3)	13 (3.7)	9 (2.8)	11 (3.6)	4 (2.3)	10 (10.4)	2 (1.3)	0 (0)
Pseudomonas stutzeri	12 (13.2)	0 (0)	1 (1)	0 (0)	6 (1.3)	13 (3.7)	9 (2.8)	11 (3.6)	4 (2.3)	10 (10.4)	2 (1.3)	0 (0)

*Colony counts (percentage)

II. Fungus

Presence of fungal spores in the indoor air of buildings can't be denied. In normal cases, fungal species are not considered as a risk factor from health perspective. However, in the sensitive places like hospitals, presence of these spores is a concern due to their ability to cause respiratory diseases in immune- suppressed patients of hospitals. The most frequently observed airborne fungal genera in the seasonal assessment of all the six monitored sites were identified as Cladosporium (47%), Aspergillus (17.05%), Penicillium (7.14%), Alterneria (6.22%), Geotrichium (3.68%) and Ulocladium (3.22%). Cladosporium spp. was found to be the most dominant fungal isolate in both the seasons (winter: 44.25%, spring: 50.25%). Abundance of cladosporium spp. in all the monitored sites may be related to the higher concentration levels of propagules in the outdoor environment due to presence of forests around the monitored sites as reported earlier (Sautour et al., 2009; Medrela-Kuder, 2003). Three species of Aspergillus (Aspergillus Fumigatus, Aspergillus Niger and Aspergillus Flavus) were identified from the recovered isolates. In previous studies, presence of Aspergillus species in indoor air of hospitals was considered as a risk factor for patients due to their ability to cause nosocomial infections and allergies (Cabo Verde et al., 2015) . Among the dominant species of Alterneria, the most frequently observed species was Alterneria Alternata and that for the case of Ulocladium, Ulocladium Chartarum was the most dominant observed species. Overview of the identified airborne fungal isolates is given in Table 4.10.

Table 4.10: Detailed overview of isolated airborne fungal colonies from hospital sites

Fungal Colonies	OT1		OT2		OPD		ES		SW		GMW	
	Winter	**Spring**	**Winter**	**Spring**	**Winter**	**Spring**	**Winter**	**Spring**	**Winter**	**Spring**	**Winter**	**Spring**
Cladosporium Spp.	9 (45%)	7 (70%)	5 (38.5%)	6 (60%)	23 (52.3%)	28 (49.1%)	15 (48.4%)	33 (62.3%)	18 (40.9%)	16 (40%)	34 (40.9%)	10 (34.5%)
Aspergillus Fumigatus	7 (35%)	0	3(23.1%)	0	3 (6.8%)	3 (5.3%)	2 (6.5%)	0	10 (22.7%)	2 (5%)	20 (24.1%)	1 (3.5%)
Penicillium Spp.	0	0	0	0	6 (13.6%)	2 (3.5%)	3 (9.7%)	1 (1.9%)	6 (13.6%)	4 (10%)	3 (3.6%)	6 (20.7%)
Alterneria Alternata	0	0	0	0	1 (2.3%)	7 (12.3%)	2 (6.5%)	3 (5.7%)	2(4.6%)	8 (20%)	3 (3.6%)	1 (3.5%)
Geotrichum Spp.	0	0	0	2 (20%)	0	2 (3.5%)	0	2 (3.8%)	2(4.6%)	1 (2.5%)	5 (6%)	2 (6.9%)
Ulocladium Chartarum	1 (5%)	2 (20%)	0	0	2 (4.5%)	3 (5.3%)	1 (3.2%)	1 (1.9%)	1 (2.3%)	1 (2.5%)	2 (2.4%)	0
Aspergillus Niger	0	0	2 (15.4%)	0	0	3 (5.3%)	2 (6.5%)	0	1 (2.3%)	1 (2.5%)	2 (2.4%)	0
Aspergillus Flavus	0	0	1 (7.7%)	0	4(9.1%)	0	1 (3.2%)	0	0	0	6 (7.2%)	0
Others	3 (15%)	1 (10%)	2 (15.4%)	2 (20%)	5 (11.4%)	9 (15.8%)	5 (16.1%)	13 (24.5%)	4 (9.1%)	7 (17.5%)	8 (9.6%)	9 (31%)

*Colony counts (percentage)

4.2.2. Ventilation Analysis

Descriptive statistical parameters (mean, median, minimum, maximum and standard deviation) for the hourly mean indoor CO_2, T and RH levels of monitored hospital sites have been summarized in Table 4.11.

Table 4.11: Descriptive statistics of CO_2, temperature and relative humidity of monitored hospital sites

		OT		ICU	
		WD	**WE**	**WD**	**WE**
CO2	Mean	993.97	508.64	1177.21	954.35
	Median	763	502	1040.5	964
	Mode	496	491	1025	975
	Max	2970	628	2430	1081
	Min	421	475	624	776
	StDev	600.46	23.45	365.15	71.73
Temperature	Mean	22.87	22.67	24.63	25.70
	Median	22.9	22.7	24.6	25.3
	Mode	22.3	22.7	24.7	24.8
	Max	24.7	23	28.9	29.2
	Min	20.8	22.4	21.7	23.5
	StDev	0.59	0.16	1.66	1.56

		OT		ICU	
		WD	**WE**	**WD**	**WE**
	Mean	40.93	36.57	40.81	35.87
	Median	41.4	36.8	40.3	35.95
Relative	Mode	29.7	37.3	40.1	37.5
Humidity	Max	56.6	37.7	52.9	40.3
	Min	29.6	34.8	33.9	32.2
	StDev	6.49	0.80	3.78	1.82

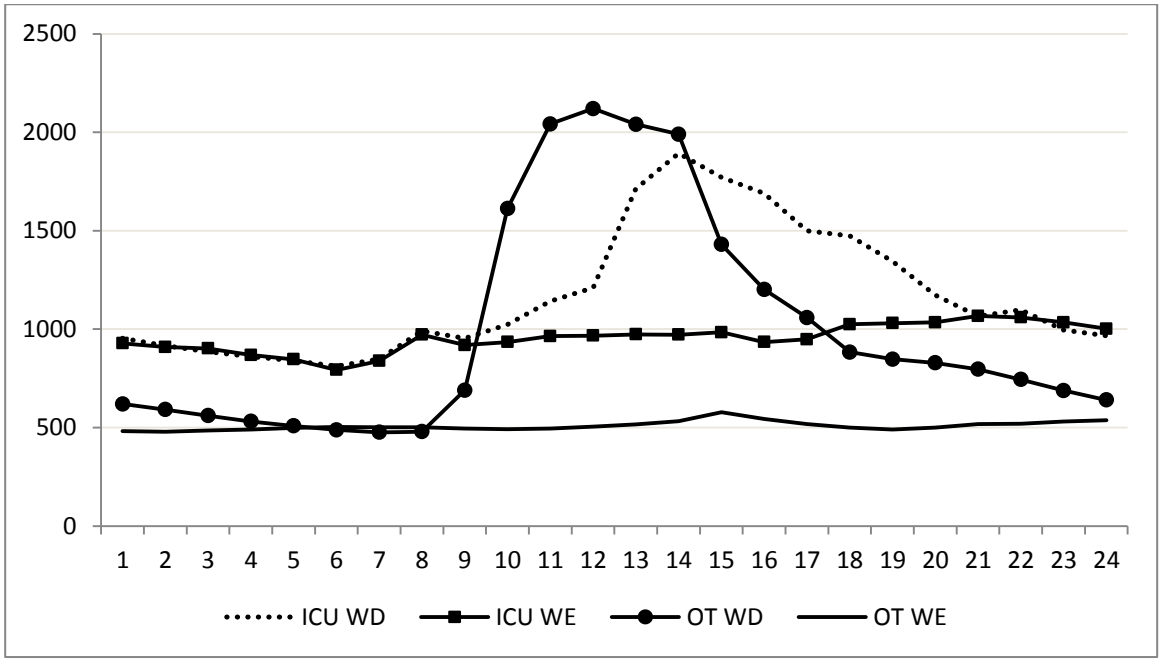
4.2.2.1 Variation of CO_2 and Comfort Parameters

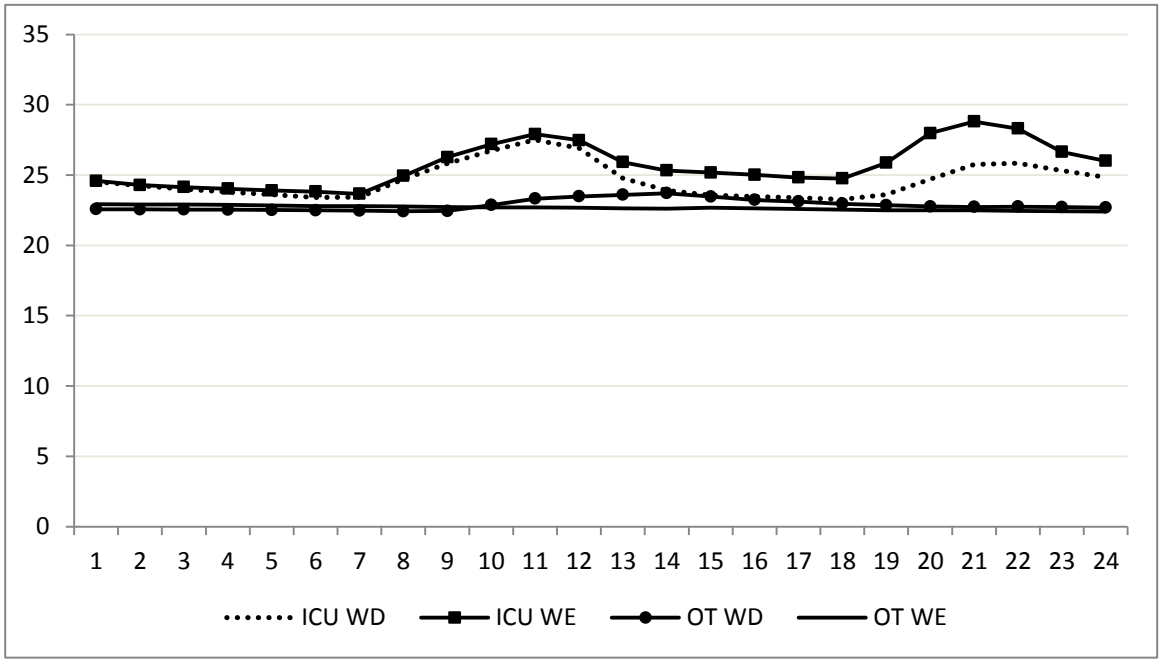
- **CO_2 Variation**

Monitoring of OT3 and ICU of selected hospital showed significant variation ($p<0.05$) in CO_2 levels along the day while during WE no significant variation ($p>0.05$) along the day was observed. 24-hour mean hourly CO_2 concentration profiles are shown in Fig. 4.6(a) for both monitored sites.

Surgical activities in OT3 start from 8:00 AM and lasts till 2:00 PM which results in increase in CO_2 levels from 8:00 AM. Maximum values were observed at 11:00 AM and maximum recorded value was 2970 ppm while minimum was 421 ppm. Moreover, in ICU there were 8 patients with 8-10 hospital staff members present during 24-hour monitoring period which do not allow CO_2 concentration levels to fall below even

during night time. During the time between 8:00 AM and 2:00 PM concentration rises due to increase in number of doctors and hospital staff. Maximum observed value in the monitored ICU was 2430 ppm while minimum was 624 ppm.





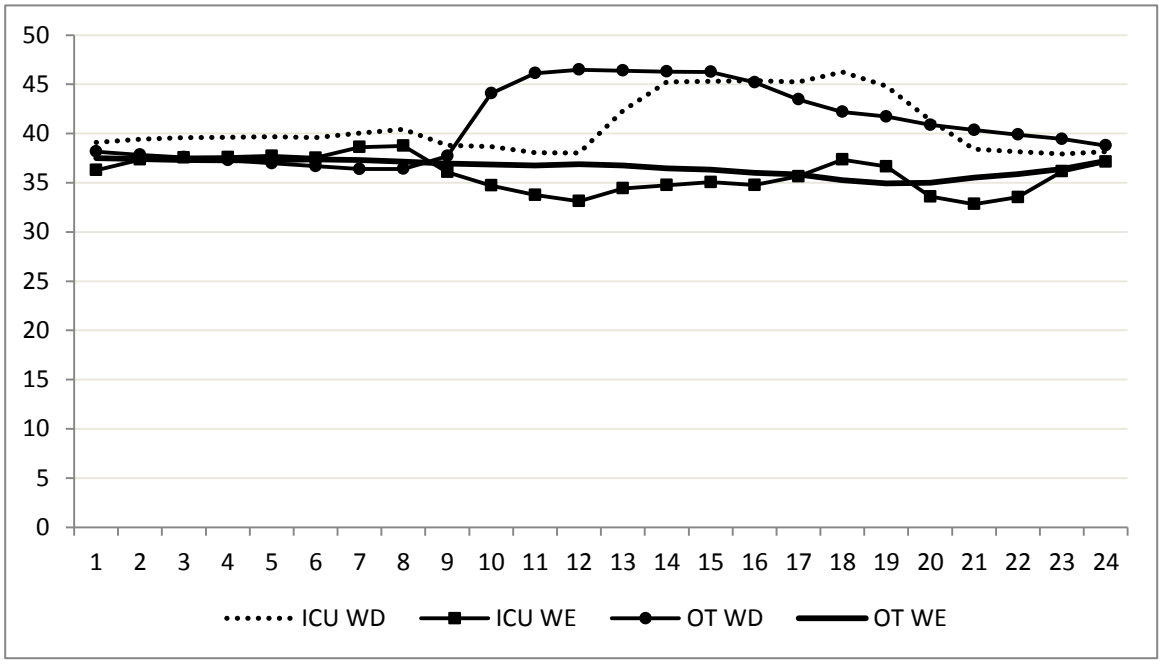


Figure 4.8: Indoor CO_2 (a), temperature (b) and relative humidity (c) profile for hospital sites

- **Temperature and Relative Humidity**

Mean hourly variation in indoor T and RH has been shown in Fig 4.6(b) and Fig 4.6(c) respectively. Mean outdoor air temperature during the sampling period of both the sites was found as 10.15ºC (max: 21ºC, min: 2ºC) while mean outdoor relative humidity level was 72.9% (max: 100%, min: 22%). Significant variation ($p<0.05$) in T and RH values have been observed over the day during WD while no significant variation ($p>0.05$) have been observed during WE of both the monitored sites. As mentioned earlier, multiple factors may result in increasing T and RH values in addition to number of occupant e.g. outdoor climatic conditions, building orientation

and type of HVAC system. Maximum observed T value in OT3 and ICU were 24.7ºC and 28.9ºC while minimum were 20.8ºC and 21.7ºC respectively. Moreover, maximum observed RH values were 56.6 and 52.9% while minimum were 29.6 and 32.2% respectively.

4.3. Cafeteria

4.3.1. Airborne Microbial Load in Cafeterias

Overall (outdoor as well as indoor) fungal load was found to be 2.6 times of that of bacteria during three-day monitoring of each cafeteria. In indoor samples, this trend is same where fungal load was observed to be 2.2 times higher than bacterial loads. Overall highest bacterial count was observed at $CafeB_C$ (mean: 214.7 CFU/m^3) and lowest at $CafeC_{S2}$ (mean: 70.5 CFU/m^3). Moreover, highest fungal count was found at $CafeA_{SC}$ (mean: 525.6 CFU/m^3) and lowest at $cafeC_{S2}$ (mean: 44.87 CFU/m^3). Mean concentrations of airborne micro-organisms for each site are given in Table 4.12

Table 4.12: Airborne microbial concentrations

Locations	Sites	Concentration (CFU/m^3)		Temperature (°C)	Relative Humidity (%)
		Bacteria	Fungus		
Cafeteria A	$CafeA_C$	211.5	467.9	31.2	28.8
	$CafeA_{SC}$	144.2	525.6	31.8	28.9
	Ambient	109.0	609.0	32	27.2

Location	Site	Concentration (CFU/m^3)		Temperature (°C)	Relative Humidity (%)
		Bacteria	Fungus		
Cafeteria B	$CafeB_C$	269.2	400.6	35.3	29.1
	$CafeB_{SC}$	182.7	493.6	32.8	28.6
	Ambient	192.3	775.6	31.0	28.8
Cafeteria C	$CafeC_{C1}$	128.2	83.3	29.5	36.2
	$CafeC_{C2}$	70.5	44.9	30.1	37.1
	Ambient	96.2	288.5	37	22.1

Indoor bacterial concentrations were found to be 6.2 times higher than that of outdoor. Among the cafeterias, highest indoor bacterial concentration was found in cafeteria B as 1.9 times, followed by A, as 1.8 times of that of cafeteria C. Higher bacterial loads in cafeterias A and B are explained by longer operational hours as well as inhouse cooking and dish washing activities at these sites. Similar trends have been reported for bacterial levels in a previous study focused on food courts (Rajasekar & Balasubramanian, 2011). Figure 4.7 shows the detailed trends of bacterial concentrations in all the monitored sites during three day monitoring period.

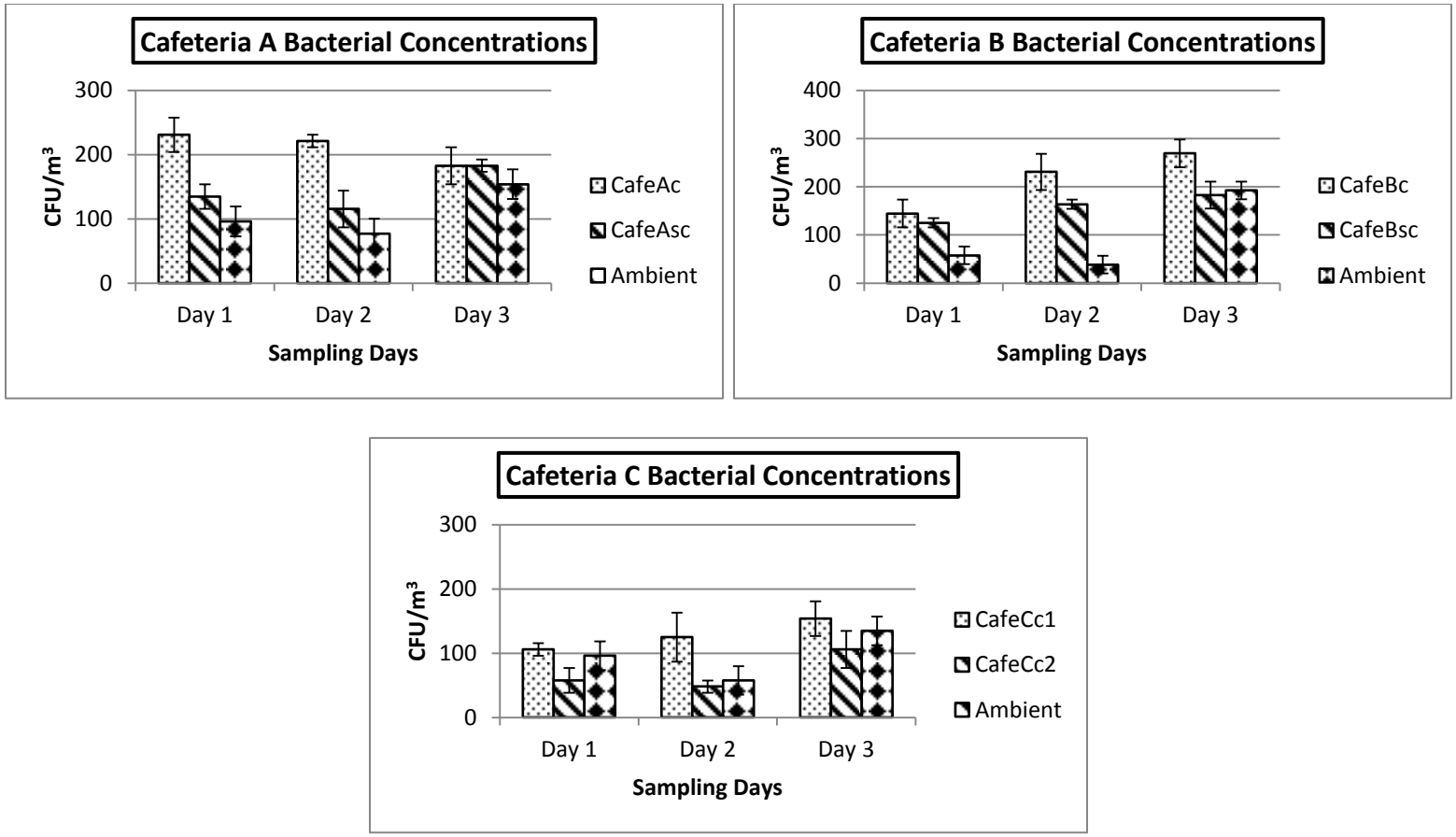


Fig. 4.9: Airborne bacterial concentrations in monitored cafeterias

Airborne bacterial concentrations in cafeteria C were lower as compared to other two due to less occupant density and operational hours, higher cleaning frequency and maintenance.

Contrary to bacterial concentrations, outdoor fungal concentration was found to be 2.5 times higher than that in indoor averaged for all the monitored sites. Lowest indoor fungal concentration was found in cafeteria C followed by cafeteria B (6.9 times of C) and A (7.7 times of C). Figure 4.8 showed the detailed trends of fungal concentrations in all the monitored sites during three day monitoring period.

Indoor fungal concentrations may be strongly linked to outdoor fungal load a as depicted in Figure 4.8. As mentioned earlier, the selected monitored sites have been surrounded by plants which are the probable reason of indoor higher fungal load. As cafeteria A and B have natural ventilation, each having a semi-closed area, they are more exposed to ambience and fungal spores as observed in these areas resulting in higher fungal load percentages (49.28 and 44.36% respectively) as compared to cafeteria C.

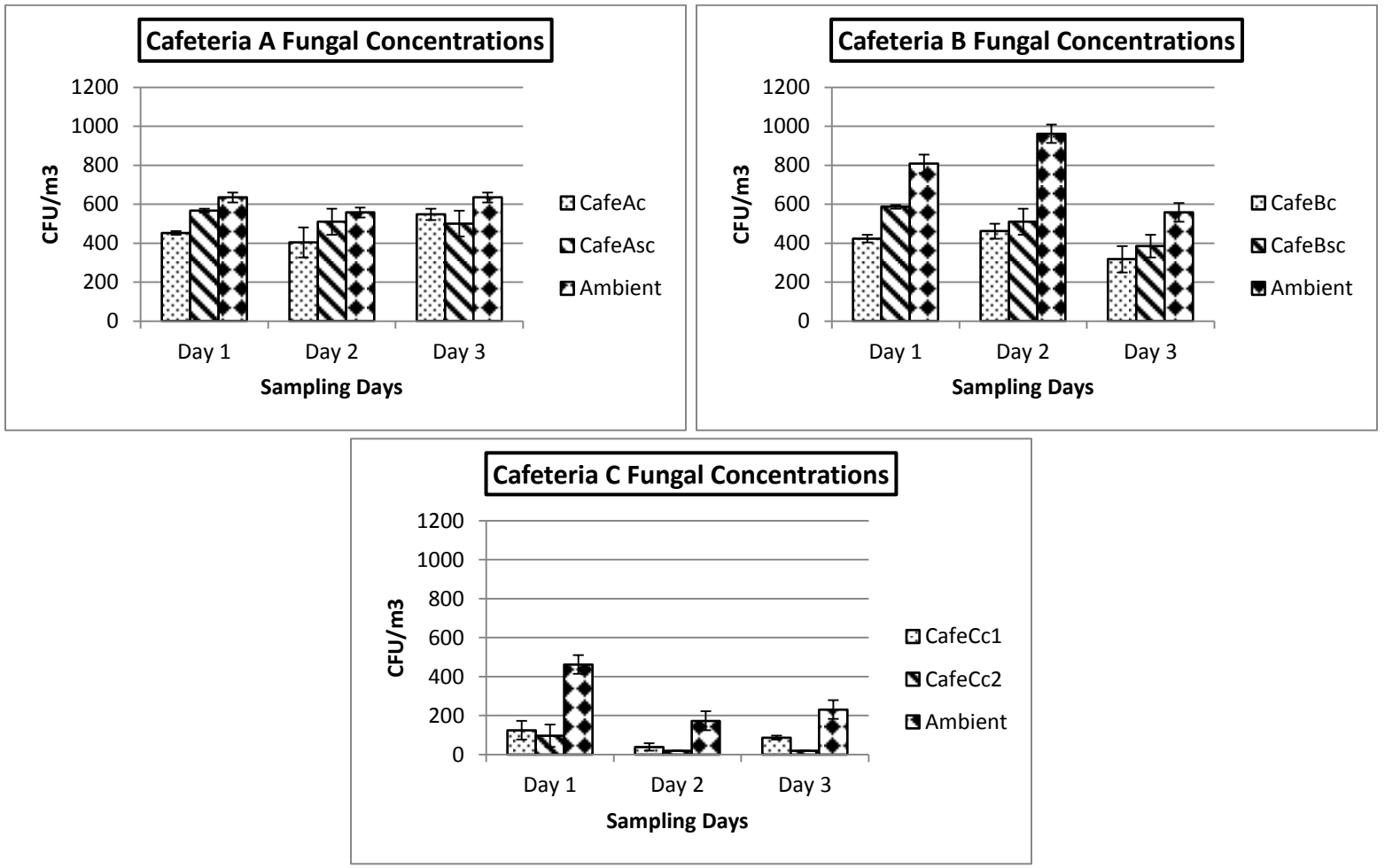


Fig. 4.10: Airborne fungal concentrations in monitored cafeterias

4.3.2. Indoor and Outdoor Airborne Micro-organisms

Indoor levels of airborne micro-organisms are largely affected by their outdoor concentration levels, thus making investigation of their impacts on indoor concentration levels imperative. Correlation of indoor and outdoor microbial levels for the two indoor locations of each cafeteria was established using spearman's correlation test. Indoor sampling locations of all the monitored cafeterias showed strong positive correlation with outdoor fungal load. However, indoor bacterial observations were found to be poorly correlated with their ambient observations. Correlation coefficients of indoor fungal levels with that of outdoor, are shown in Table 4.13.

Table 4.13: Correlation of indoor airborne microbial levels with outdoor

Locations	**Sites**	**Correlation of Fungus with ambient concentration**
Cafeteria A	$CafeA_C$	0.76
	$CafeA_{SC}$	0.38
Cafeteria B	$CafeB_C$	0.99
	$CafeB_{SC}$	0.72
Cafeteria C	$CafeC_{C1}$	0.92
	$CafeC_{C2}$	0.98

It is perceived that I/O ratio of microbial concentrations greater than 1 is the indication of more contribution of indoor sources towards the buildup of indoor microbial levels.

Moreover, I/O ratio less than 1 is an indication of a higher contribution of outdoor sources (Teixeira & Oliveira, 2015).

Findings of all the monitored sites showed indoor sources as defining factors for indoor bacterial levels (average I/O ratio>1). respectively. However, findings for fungal levels showed outdoor sources dominancy in buildup of indoor fugal levels as I/O ratio for all locations was less than 1.

4.3.3. Isolation and Identification of Airborne Micro-organisms

The most dominantly occurring isolated fungal colonies in the three monitored cafeterias, identified using the optical microscope as cladosporium spp. (45.97%), geotrichium spp. (8.56%), ulocladium chartarum (8.5%), alterneria alternata (7.93%), fuserium spp. (6.55%), curvularia lunata (3.02%), aspergillus spp. (2.39%) and penicillum spp. (1.26%). As mentioned earlier, I/O ratio of fungal concentrations in all monitored cafeterias was less than 1, thus showing the contribution of outdoor sources in raising the indoor levels more as compared to indoor sources. In outdoor environment of all the monitored cafeterias, cladosporium spp. was found as a predominant fungal isolate (cafeteria A: 67.39%, cafeteria B: 60% and cafeteria C: 26.83%), which resulted in high concentration of cladosporium spp. in indoor environment (cafeteria A: 44.78%, cafeteria B: 39.62% and cafeteria C: 41.38%). Details of each isolated fungal colony is given in Table 4.13.

Table 4.14: Detailed overview of isolated airborne fungal colonies from cafeterias

Fungal Colonies	Cafeteria A (%)			Cafeteria B (%)			Cafeteria C (%)		
	$CafeA_C$	CafeAsc	Ambient	$CafeB_C$	$CafeB_{SC}$	Ambient	$CafeC_{c1}$	$CafeC_{C2}$	Ambient
Cladosporium Spp.	41.67	47.71	67.39	44.26	35.66	60	37.50	46.15	26.83
Alterneria Alternata	9.03	9.80	3.26	4.92	14.69	7.14	0	0	0
Geotrichum spp.	4.86	12.42	5.43	7.37	8.39	18.57	0	0	7.32
Ulocladium chartarum	9.72	4.58	4.35	7.37	9.09	5.71	31.25	15.38	24.39
Fuserium spp.	6.94	1.96	4.35	9.84	12.59	4.29	6.25	7.69	0
Curvularia lunata	2.78	0.65	6.52	3.28	4.20	0	6.25	0	4.88
Aspergillus Spp.	3.47	1.31	0	8.19	0.69	0	6.25	0	0
Penicillium Spp.	0	0	0	0	0	0	6.25	23.08	14.63
Other	21.53	21.57	8.69	14.75	14.69	4.29	6.25	7.69	21.95

Microscopic morphological appearance of each recovered colony showed dominancy of gram positive cocci (90.03%) followed by gram positive rods (7.01%) and gram negative rods (2.96%). In cafeteria A, B and C this trend is same where gram positive cocci contribute 88.8, 87.72 and 94.67% respectively. These results are supported by many previously reported studies which also showed dominancy of gram positive cocci in indoor environment (Cabo Verde et al., 2015; Rajasekar & Balasubramanian, 2011; Kim et al., 2009). The most frequently occurring colonies were identified up-to their species level by DNA sequencing with the help of paired primers. Details of each isolated bacterial colony is given in Table 4.14.

Table 4.15: Identified airborne bacterial colonies from cafeterias

Bacterial Colonies	Cafeteria A			Cafeteria B			Cafeteria C		
	L1	L2	A*	L1	L2	A	L1	L2	A
Staphylococcus Aureus	35.2	44.4	33.3	35.6	30.0	31.2	45.5	38.7	28.6
Kocuria Rosea	9.9	6.4	11.1	15.5	13.3	18.7	9.1	9.7	35.7
Kocuria Rhizophila	9.9	15.9	16.7	8.5	5.0	18.7	2.3	0.0	7.1
Bacillus Subtilis	2.8	3.2	0.0	1.7	5.0	0.0	2.3	6.5	7.1
Micrococcus Terreus	1.4	3.2	0.0	8.5	5.0	0.0	6.8	22.58	0.0
Micrococcus Luteus	12.7	11.1	11.1	15.3	15.0	6.3	15.9	22.6	0.0
Kocuria Kristinae	4.2	0.0	11.1	8.5	0.0	0.0	13.6	0.0	21.4
Staphylococcus cohnii	12.7	4.8	0.0	1.7	1.7	12.5	0.0	0.0	0.0
Others	11.3	11.1	16.7	5.1	25.0	12.5	4.6	0.0	0.0

*A=Ambient

Chapter 5

CONCLUSIONS

- **NUST Classrooms**

Comparative analysis of ventilation system performance through CO_2 levels and thermal comfort through T and RH of four different educational buildings showed that in addition to occupation density, quality of indoor air is largely dependent on type of ventilation system present. Besides, it was found that thermal comfort is highly linked to outdoor thermal conditions and orientation of the building in addition to the above-mentioned factors for indoor air. CO_2 concentration levels were found highest in the buildings with non-centralized HVAC system. Comparison with standards also showed exceeding levels of CO_2 concentration from ASHRAE standards frequently during the occupational period of study duration. In buildings with centralized HVAC system, CO_2 levels were found to be much lower than with buildings without centralized system consequently, showing lesser exceedance beyond safe limits during the occupational period. Thermal comfort parameters (T and RH) were found to be more affected by external factors like outdoor temperature and relative humidity conditions, orientation of buildings due to which T values were found to be exceeding

from standards with high frequency. RH values, on the other hand, were found to be lower than 60% as recommended in ASHRAE standards.

It is recommended that during the building design phase, the orientation should be given due consideration in order to maintain required level of thermal comfort at optimum level of energy consumption. Moreover, the extent of occupation density should also be foreseen more accurately while building design phase in general and building HVAC system design phase in particular. Further studies should explore the effect of type of ventilation system on indoor air quality as well as thermal comfort and their link with outdoor climatic conditions should be conducted to ensure health safety and enhanced learning performance of the students.

- **Hospital Sites**

Airborne microbial assessment of six selected sites of a government run hospital showed dependency of microbial levels on type of area as well as activities and types of patients in those areas. Occupancy level showed significantly affecting the bacterial levels as two of the busiest locations (OPD and ES) showed maximum bacterial concentrations during both seasons. However, outdoor climatic conditions appeared to affect indoor fungal concentrations more significantly. Cleaning frequency, on the other hand showed significant impact on both, bacterial as well as fungal concentration levels as both OTs, having higher cleaning frequencies, were observed to have lesser microbial loads. Gram positive cocci was found most dominant bacterial group in all

monitored sites followed by gram positive rods and gram negative rods. Outdoor conditions appeared to affect indoor fungal concentrations as cladosporium sp. was found to be the most frequently observed fungal genera in indoor air. While other observed fungal genera included aspergillus, penicillium, alterneria and ulocladium. Prevalence of alarmingly higher bacterial concentrations necessitate the provision of improved control and monitoring arrangements in sensitive areas such as hospitals.

- **NUST Cafeterias**

Indoor bacterial levels were found to be correlated with indoor sources while fungal levels with outdoor sources. Cladosporium spp. was found to be the most dominant fungal genera in all the monitored sites due to its abundance in outdoor environment. Moreover, for the case of bacterial colonies, gram positive cocci were the most frequently occurring airborne bacteria followed by gram positive and gram negative rods.

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