Assessment of Microbiological Air Quality in Indoor Environments of Hospitals and Libraries

Ahmad Hassan

00000276552

Institute of Environmental Science & Engineering (IESE)

School of Civil & Environmental Engineering (SCEE)

National University of Sciences & Technology (NUST)

Islamabad 44000, Pakistan

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By

Ahmad Hassan

00000276552

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AHMAD HASSAN

Has been found satisfactory for partial fulfillment of the requirements of the degree of

Masters of

Science in Environmental Engineering

Supervisor: ….....

Dr. Muhammad Zeeshan Ali Khan

Assistant Professor

IESE, SCEE, NUST

GEC Member: ……

Dr. Imran Hashmi

Professor

IESE, SCEE, NUST

GEC Member: ……

Dr. Muhammad Faraz Bhatti

Associate Professor

ASAB, NUST

Annex A to NUST Letter No

/ /Exams/Thesis-Cert

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THESIS ACCEPTANCE CERTIFICATE

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Signature: _____

Name of Supervisor: Dr. Muhammad Zeeshan Ali Khan

Date: _____

Signature (HOD): _____

Date: _____

Signature (Dean/Principal): _____

Date: _____

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AHMAD HASSAN

00000276552

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ABSTRACT

Assessment and control of infectious airborne microbes is crucial due to its public health concerns in confined environments e.g. hospitals and libraries. The study aimed to assess the concentration and diversity pattern of airborne bacteria and fungi and variation of microbial indoor air quality concerning ventilation system type and microenvironmental conditions. Field measurements were performed in two hospitals and three library buildings, equipped with natural and mechanical ventilation systems. In each hospital, eight different locations were selected that includes Emergency Department (EM1), Waiting Room (WR), Operation Theater (OT1), Women Surgical Ward (WSW1), Men Surgical Ward (MSW1), Outpatient Department (OPD1), Outpatient Department (OPD2), and Laboratory (LB) in hospital A whereas Emergency Department (EM2), Women Surgical Ward (WSW2), Men Surgical Ward (MSW2), Nursery Unit (NU), Outpatient Department (OPD3), Intensive Care Unit (ICU), Burn Unit (OT2) and Operation Theater (OT3) were selected in hospital B. On the other hand, three locations were selected in the central library (CL) including ground floor (CLFG), first floor (CLF1), and second floor (CLF2) and one location in each departmental library buildings (DL1 and DL2). OT2, OT3, ICU, NU, EM2 and all three floors of CL were equipped with central "Heating, ventilation and air conditioning system" (HVAC) while rests of the locations were naturally ventilated. Microbial samples for all hospital locations were collected once per week for two months (August~September-2019). Microbial sampling in libraries was carried out in the morning between 09:00 am to 11:00 am, in the afternoon between 01:00 pm to 03:00 pm and in the evening from 07:00 pm to 09:00 pm and the study campaign lasted for three days covering the month of December-2019. In total, 560 samples from hospitals and 280 samples from library buildings were collected and analyzed. In the meanwhile, indoor and outdoor microenvironmental conditions and occupancy levels were also recorded. Results indicated higher bacterial concentration than the fungal concentration on each monitored location.

Highest bacterial concentration was found at OPD1 ranged between 2220-4980 CFU/m^3 (mean: 3415 CFU/m^3) whereas lowest on OT3 ranged 21-540 CFU/m^3 (mean: 280 CFU/m^3). Similarly, the highest fungal levels were observed on MSW1 ranging 120-920 CFU/m^3 (mean 517 CFU/m^3) whereas lowest on OT3 ranging 20-100 CFU/m3 (mean: 52 CFU/m^3). On the other hand, bacterial levels observed in libraries were ranging from 20-230 CFU/m^3 in indoor and 20-100 CFU/m^3 in the outdoor environment and fungal levels between 20-250 CFU/m^3 in the indoor and 280-510 CFU/m^3 in outdoor location. Highest bacterial and fungal levels were observed at CLFG and lowest bacterial and fungal levels were measured at DL2 and CLF2 respectively. Indoor/Outdoor (I/O) was found to be lower than 1 for fungi and higher than 1 for bacteria, indicating that the primary source of fungal pollution is outdoor (plants, soil, food waste, etc.) whereas, for bacteria, it is indoor (occupants and their activities). Genotypic identification of biological agents on different monitored locations confirmed the presence of Gram-positive cocci i.e. *Staphylococcus spp. Micrococcus spp.* and *Bacillus spp*. as the prevalent bacterial genera, whereas *Cladosporium spp., Aspergillus spp.* and *Penicillium spp*. the dominant fungal genera. The ventilation system of the building is a key factor in indoor air quality (IAQ) management; thus, central HVAC system is recommended for highly sensitive sites i.e. OT, ICU.

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List of Abbreviations

IAQ	Indoor air quality
CL	Central library
DL1	Departmental library-1
DL2	Departmental library-2
CLFG	Central library ground floor-1
CLF1	Central library first floor-2
CLF2	Central library second floor-3
CO_2	Carbon dioxide
RH	Relative humidity
T	Temperature
TSA	Tryptic soy agar
PDA	Potato dextrose agar
HVAC	Heating, Ventilation and Air-Conditioning system
MSW	Men Surgical Ward
WSW	Women Surgical Ward
ICU	Intensive Care Unit
OT	Operation Theater
WR	Waiting Room
EM	Emergency Department
OPD	Outpatient Department
LB	Laboratory
NU	Nursery unit
PCR	Polymerase Chain Reaction
LCR	Ligase Chain Reaction
DNA	Deoxyribonucleic acid

WHO	World Health Organization
PNS	Portugal national standards
CFU/m^3	Colony-forming units per cubic meter
ASHRAE	American Society of Heating, Refrigeration, and Air Conditioning Engineers
SBS	Sick building syndrome
HAI	Hospital-Acquired Infection
HEPA	High-efficiency particulate air filter
EPA	Environmental Protection Agency

Chapter 1

INTRODUCTION

1.1 Overview

This section is about the background and short introduction of preset study. In this chapter, aim and objectives of study are discussed in brief.

1.2 Research Background

Humans spend 80-90 % of their time in indoor environment and inhale 10 m^3 air daily (Awad and Farag 1999). Presence of hazardous air pollutants in indoor environment can cause detrimental effects to human health. Therefore, indoor air quality (IAQ) in confined environments is significant and one of the most concerning issues worldwide. In the past decade, humans switched their lifestyles from open-air environments to airtight energy efficient buildings (Chao, Schwartz et al. 2003). 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.

Airborne microbes are responsible approximately 5-34 % of total indoor air pollution (Mandal and Brandl 2011). Depending upon time and concentration, exposure to air pollution is associated with irritation to sensitive parts of the human body i.e. skin, eyes, respiratory or allergic problems (Madureira, Paciência et al. 2015, Mentese, Mirici et al. 2015), nosocomial infections and reduction in the routine performance (Beggs 2003, Kuhn and Ghannoum 2003, Śmiełowska, Marć et al. 2017).

The concentration of bioaerosols varies significantly in different environments (Kembel, Jones et al. 2012) depending upon several factors including Season (Frankel, Bekö et al. 2012), relative humidity (RH) (Arundel, Sterling et al. 1986), temperature (T) (Tang 2009), air

exchange rate and type of ventilation system (Jung, Wu et al. 2015), microbial content in the outdoor environment (Widder and Haselbach 2017), geographical location of the sampling area, number of "occupants and patients" in monitored location, ongoing activities (Forthomme, Joubert et al. 2014) and cleaning frequency of that area (Asif, Zeeshan et al. 2018). However, performance of ventilation system of the building is reported as the most significant factor in IAQ management (Jung, Wu et al. 2015). Proper ventilation of the building decreases the indoor dampness and the microbial content (Seppanen and Fisk 2002, Bluyssen, Cox et al. 2003, Wu, Li et al. 2005). In naturally ventilated locations quality of indoor air is affected by microbial content in outdoor air. Thus controlled ventilation is essential for healthy IAQ. Previously conducted studies compared the efficiency of different type of ventilation systems however reported contradicted results (Jung, Wu et al. 2015), that needs further investigations.

Airborne microbes i.e. bacteria and fungi are ubiquitous in the environment. Condition of ventilation system, humans, outdoor pollutants, plants, pets etc, contribute as source of indoor bacterial pollution (Widder and Haselbach 2017). On the other hand, fungal sources could be indoor as well as outdoor however, in case of natural ventilation, more fungal contribution is from outside i.e. outdoor air (Prussin and Marr 2015). Confined environments such as libraries, office buildings and public hospitals contains higher concentration of infectious pollutants establishing hazardous situation for immune-compromised patients and occupants having pre-existing health problems (Chinn and Sehulster 2003, Tsai and Macher 2005, Pasquarella, Sansebastiano et al. 2007). Thus assessment of the microbiological IAQ of libraries and hospitals is essential to control and inhibit the emerging public health issues (Napoli, Tafuri et al. 2012, Di Carlo, Chisesi et al. 2016). Few countries have established their referential standards to monitor and control airborne microbial pollution. However, to date, Pakistan like other developing countries does not have its nationally agreed standards for microbial

concentration due to limitation of available data. Thus more research studies are needed monitoring indoor bacterial and fungal levels.

1.3 Study Area

The present study was conducted in two hospitals and three library buildings equipped with different ventilation systems. Selected buildings were situated in the vicinity of metropolitan cities Rawalpindi-Islamabad. Islamabad is the capital city of Pakistan, situated at 33°41′35″N 73°03′50″E whereas Rawalpindi is the fourth largest city of Pakistan and situated at 33°36′N 73°02′E. Both Rawalpindi and Islamabad are very adjacent to each other therefore known as the twin cities of Pakistan. Eight locations were selected in each hospital building, three locations in central library (CL) and one location in each departmental library (DL1 and DL2).

1.4 Research Aim and Objectives

The present study aimed to investigate the concentration and composition of prevailing genera of fungi and bacteria in hospitals and library buildings equipped with a different type of ventilations.

The objectives of the study include,

1. Assessment of airborne microbial levels in indoor environments of public hospitals and libraries
2. Characterization of dominant airborne microbes in hospitals and libraries
3. Comparison of microbial load in natural and centralized HVAC enabled buildings.

1.5 Research significance

Determination of the concentration and composition of prevalent airborne microbes may lead to better understand the possible sources of indoor microbial pollution and their controls, thus can prevent the emerging health issues. Besides, obtained data would provide the first insight

of this problem and would assist decision makers and concerned officials to implement the proposed control measures. There is absence of nationally agreed microbial IAQ standards therefore, reported data would also be helpful in formulation of microbial IAQ standards.

Chapter 2

LITERATURE REVIEW

2.1 Indoor air pollution

The accumulation of hazardous air pollutants in the indoor air is refer to indoor air pollution. Indoor air pollution is graded among the five major risks to human health (Kotzias 2005) and reported as a major cause of approximately 4 % of global morbidities (Bruce, Perez-Padilla et al. 2000). Furthermore, indoor air is reported to be more polluted than outdoor air therefore, indoor air pollution is a more concerning issue worldwide. Most commonly occurring air pollutants are of two types; biological air pollutants i.e. molds, bacteria, viruses and chemical air pollutants i.e. CO, radon, NO_2, Lead, etc. Biological air pollutants are the colloidal suspension of the solid particles and the liquid droplets suspended in the air containing bacteria, viruses and fungal spores and accounted approximately 5-34 % for total indoor air pollution (Gizaw, Gebrehiwot et al. 2016).

2.2 Indoor air quality (IAQ)

Indoor air quality (IAQ) is the quality of air within the buildings and structures. Healthy IAQ has been linked to increase production capacity and well being of individuals. Humans are more exposed to indoor rather than outdoor air as they spend more than 80 % of their time indoors (Wichmann, Lind et al. 2010). Poor IAQ is unsafe for human health and its long-time exposure could result in several health-related illnesses. Heart disease, bronchitis, legionnaire disease(Lewtas 2007), rhinitis, keratitis and asthma are reported health issues linked with poor IAQ (Snow 1982, De Oliveira Fernandes, Jantunen et al. 2008). Therefore scientists are more concerned to IAQ than outdoor air quality.

IAQ depends on indoor as well as several outdoor sources that includes construction material and age of the Building, occupancy levels, human activities and quality of outdoor air

(Graudenz, Oliveira et al. 2005, Jurado, Bankoff et al. 2014). In addition, efficiency and type of ventilation system is also reported as the most critical factor affecting the IAQ (Batterman and Burge 1995, Wargocki and Sundell 2002, Seppanen and Fisk 2004, Toivola, Nevalainen et al. 2004, Graudenz, Oliveira et al. 2005, Wu, Li et al. 2005). Previously conducted studies reported that poorly designed ventilation systems of the building is the major cause of insufficient air exchange rates and higher microbial buildup (Batterman and Burge 1995, Seppanen and Fisk 2002, Bluyssen, Cox et al. 2003). Furthermore, indoor as well as outdoor microclimatic conditions i.e. T and RH also effect microbial growth rate thus, dependence of IAQ on outdoor air cannot be denied which requires proper control measures. According to the American Society of Heating, Refrigeration, and Air-conditioning Engineer (ASHRAE), T in range 20-25 $^{\circ}$C and RH between 40-60 % is recommended for good IAQ.

2.3 Ventilation

Ventilation is the process of providing fresh outdoor air by replacing the existing old and polluted indoor air. The efficient ventilation system of the building maintains IAQ and required thermal comfort under recommended limits. previous studies have reported that an efficient ventilation system helps in minimization of indoor air pollution by diluting the existed stale air with fresh air (free of pollutants). Globally to assess IAQ, CO_2 levels are taken as a surrogate for ventilation performance as CO_2 levels above a certain limit indicate poor ventilation which shows possibility of build-up of higher levels of other pollutants.

2.3.1 Ventilation system types

Concentration of outdoor air pollutants affecting IAQ is largely dependent on the type and condition of ventilation system of buildings. There are two main types of ventilation systems, Natural ventilation system and Mechanical ventilation system. A brief description of both systems is given below.

2.3.1.1 Natural ventilation system

Natural ventilation is a phenomenon of replacement of indoor air with fresh outdoor air naturally (without using any mechanical device/force). Fresh air enters indoor through windows and door openings and maintain required thermal comfort. A properly designed naturally ventilated system is energy efficient ventilation option that can help to maintain good IAQ without compromising indoor thermal comfort.

2.3.1.2 Mechanical ventilation system

Mechanical ventilation is the process of introducing outdoor fresh air to replace indoor air using mechanically driven forces. It is the forced ventilation option that does not rely on small openings and cracks in the buildings. It can be designed centralized as well as non-centralized. Mechanical ventilation system can help to increase IAQ by lowering indoor microbial buildup as in this system outdoor polluted air is introduced indoor through proper filtration.

2.3.2 Pros and cons of each ventilation system type

Each ventilation system has its own advantages and disadvantages. Mechanical ventilation system provides heat recovery, stable airflow and good IAQ. However, mechanical ventilation systems are complex having less life and requiring large space, high energy consumption and high operational cost. On the other hand, natural ventilation is an energy efficient ventilation option but at the same time it is also responsible for issues like compromising noise. Besides, inefficiently design natural ventilation does not incorporate changes in levels of thermal comforts (T and RH) due to changes in outdoor climatic conditions. Previously conducted studies compared the efficiency of different type of ventilation systems however reported contradicted results about the effectiveness and adequacy of ventilation systems (Jung, Wu et al. 2015). Few studies concluded, natural ventilation as a suitable option for reduction and control of transmission of infectious airborne microbes (Escombe, Oeser et al. 2007, Qian, Li et al. 2010, Gilkeson, Camargo-Valero et al. 2013) and reported Mechanical system as indoor

contamination source (Seppanen and Fisk 2002). On the other hand, several studies contradicted the previous findings and reported that outdoor air is the main source of indoor fungal buildup (Jain 2000, El-Sharkawy and Noweir 2014, Jung, Wu et al. 2015, Wallner, Munoz et al. 2015) and natural ventilation allows outdoor air to indoor directly without pre filtration. Thus they suggested installation of HVAC system due to the inability of a natural ventilation system in maintaining required thermal comfort and microbial IAQ under severe outdoor microclimatic conditions.

2.4 Nature of airborne microbes

Biological agents, bioaerosols and airborne microbes are the terms used for indoor microbial pollutants. Airborne microbes exist in a wide variety of range in size and composition in air. Fungi and bacteria are the common microbial damaging factors of IAQ, thus are a great concern for environment and human health. Long-term exposure of pathogenic microbes could leads to several health related illness (Dharmage, Bailey et al. 2001, Douwes, Thorne et al. 2003, Peccia, Milton et al. 2008).

2.4.1 Fungus

Fungi are ubiquitous in the environment and some of the pathogenic fungi are a potential risk to public health (Samet and Spengler 2003). Indoor as well as outdoor sources contribute to indoor fungal pollution however outdoor air is the main source of indoor fungal contamination (Beggs 2003, Lee, Grinshpun et al. 2006, Abbasi and Samaei 2019). Indoor sources contributing to indoor fungal contamination may be human activities, indoor plants, etc (Awad, Saeed et al. 2018). Several indoor elements support fungal colonization on their surface i.e. polyethylene composites, ducts in ventilators, air filters (Noris, Siegel et al. 2011), wood furniture (Thacker 2004, Sailer, van Nieuwenhuijzen et al. 2010) and building material e.g. gypsum, ceiling tiles (Erkara, Asan et al. 2008). Fungal species grow on painted walls and ultimately destroy wall paintings (Shirakawa, Gaylarde et al. 2002). Favorable microclimatic

conditions support fungal growth thus to control fungal growth T between 20-25 ºC and RH in range 50-60 % is recommended (Rajasekar and Balasubramanian 2011).

2.4.2 Bacteria

Bacterial species are prokaryotic unicellular organisms. Humans are the major source of indoor bacterial contamination as bacterial species e.g. *Micrococcus actinobacteria*, *Phyla proteobacteria* and *Firmicutes* are reported to be originate from the human skin or the intestinal tract (Prussin and Marr 2015). Water-damaged buildings and the rainy seasons support bacterial growth (Perkins, Mayfield et al. 2009). Outdoor bacterial species may also enter indoor due to human movement as outdoor species get attached to shoes and dressing of the visitors. High bacterial buildup may be due to high RH and T, high CO_2 concentration or inadequate ventilation and higher occupants density (Kembel, Jones et al. 2012, Robertson, Baumgartner et al. 2013, Triadó-Margarit, Veillette et al. 2017).

2.5 Suggested control measures

Control strategies e.g. closing the doors and windows openings, maintaining the environmental condition in the optimum range and removing the indoor pollution sources could help in indoor microbial pollution reduction (Ayoko, Morawska et al. 2004). Plants requiring regular watering should not be indoors as regular water usage increases the indoor dampness which supports the fungal growth. Besides, use of dehumidifiers is also recommended to keep the moisture in range (RH< 50%) (Cole and Cook 1998, Khan and Karuppayil 2010). Outdoor polluted air directly enters in naturally ventilated buildings without filtration thus installation of mechanical filteration/ventilation systems could help to overcome microbial contamination coming from outside the building (Buckmaster 2008). Carpets should be cleaned regularly as indoor carpets have been reported as a source of indoor bacterial contamination (Ferguson, Bursac et al. 2009).

2.6 Effects of bacteria and fungi

2.6.1 Effects on human health

Exposure to infectious air pollutants is linked with several health related illness depending upon the pathogenicity, exposure time and individual immune system (Frontczak and Wargocki 2011, Loupa, Fotopoulou et al. 2017, Liu, Wang et al. 2018). Hypersensitivity, tiredness, headaches, irritations to skins and infection to human sensitive parts i.e. throat, eyes are caused by airborne microbes (El-Sharkawy and Noweir 2014, Jurado, Bankoff et al. 2014, Wallner, Tappler et al. 2017). Airborne microbes are also associated with several other health problems i.e. respiratory problems (Cox-Ganser, Rao et al. 2009), pneumonia, building-related illness (BRI) and sick building syndrome (SBS) (Srikanth, Sudharsanam et al. 2008, Kim, Kabir et al. 2018). Various diseases like allergic sinusitis, allergic rhinitis and Bronchopulmonary mycoses are reported to be caused by pathogenic fungi (Piecková and Wilkins 2004). In particular, *Aspergillus* species causes serious respiratory, Chronic obstructive pulmonary disease and cystic fibrosis (Haliki-Uztan, Ateş et al. 2010, Baxter, Jones et al. 2011). *Aspergillus spp.* may cause nosocomial infections, carcinogenicity, aspergillosis, skin irritation and allergic problems (VandenBergh, Verweij et al. 1999, Morris, Kokki et al. 2000, Viegas and Rosado 2011). *Aspergillus fumigatus* and *Aspergillus flavus* spread invasive aspergillosis (Pasqualotto 2009, Tang 2009). *Penicillium spp.* are well known to cause asthma-related problems to children (Ostro, Lipsett et al. 2001, Liu, Li et al. 2014). *Staphylococcus spp.* are reported to cause Skin, blood, urinary and respiratory infection *Staphylococcus aureus* is infectious to human skin and causes abscesses, pneumonia, cellulitis and furuncles**.** Exposure to *Micrococcus spp.* is linked with peritonitis in continuous ambulatory peritoneal dialysis (CAPD) (Bannerman 2007).

2.6.2 Effects on cultural heritage

The libraries are vulnerable to airborne microbes due to excess of nutrient enrich material i.e. papers, cardboards, etc. that provides suitable growth surfaces for bacteria and fungi activities (De Paolis and Lippi 2008). Microbes starts the process of biodegradation under favorable microclimatic conditions (Allsopp, Seal et al. 2004). Bacterial species from the biofilm on paper and damage the paper structure (Lavin, Gómez de Saravia et al. 2014. However fungi species are reported as the major cause of biodeterioration (Montanari, Melloni et al. 2012).

2.7 Monitoring/sampling of airborne microbes

Scheduled monitoring of airborne microbes is indispensable due to growing frequency of infections caused by airborne micro-organisms. Two sampling techniques are reported to examine indoor bioaerosols: surface sampling and air sampling. Surface samples are collected using adhesive tape, agar contact (contact sampling) and surface wash sampling technique (Yamamoto, Schmechel et al. 2011). Whereas air sampling is a technique used to assess the concentration of airborne microbes accumulated/suspended in air. Air sampling technique includes liquid impinger, impactor and air filtration methods. Anderson, SAS, multistage and single-stage sampler are used in impactor air sampling technique (Khan and Karuppayil 2012). Commonly used air sampling methodologies are discussed below.

2.7.1 Active sampling

Active sampling is a technique in which a known volume of air sample is drawn by using mechanical energy, pump. Air samples are collected on filter paper that is placed on nutrient media or air is directly drawn on petri plate. After the sample collection, petri plates are incubated for a specific temperature range which is 37 $^{\circ}$C for bacterial isolates and 27 $^{\circ}$C for fungal isolates. Recovered colonies then counted using microbial colony counter and their concentration is reported in colony forming unit (CFU/m^3).

2.7.2 Passive sampling

Passive sampling is a technique in which Petri plates containing nutrient media are directly exposed to air (at some specific height i.e. 1-1.5 meter to incorporate normal breathing zone) it is also known as a settled plate method (Yamamoto, Schmechel et al. 2011). After sampling, plates are sealed and placed in an incubator. However, in case of passive sampling recovered colonies are expressed in CFU/plate.

2.7.3 Pros and cons of each sampling technique

Recommended use of specific air sampler or air sampling technique is still under discussion as each sampling technique has its own pros and cons thus it is open choice for the researcher (Pasquarella, Albertini et al. 2008). Previous studies have experimentally compared both techniques however reported contradicted results as some researcher found inconsistent results using two different methodologies in the same sampling area (Sayer, MacKnight et al. 1972, Petti, Iannazzo et al. 2003) whereas few of them have also reported similar results using two different sampling methods (Perdelli, Sartini et al. 2000).

The active sampling technique requires a standardized air sampler that is too much costly and needs periodic calibration. Energy requirement, noise during sampling, and disinfection of sampler before taking every next sample are also the disadvantages of active sampling technique. In study of site surgical infections only concentration of free-falling microbes on the wound is calculated. However in active sampling all the suspended airborne microbes get collected on filter paper and we are unable to determine settled microbes' concentrations. Some previously conducted studies supports passive sampling because it gives real-time microbial concentration falling on the wound during surgical operation (Abedraboo 2015). However, using passive sampling technique actual microbial exposure cannot be determined. Thus in most of the cases passive sampling is adopted only for qualitative analysis.

Concluding here, passive sampling is better when someone is interested in concentration of actual falling microorganism for site contamination risk assessment (Whyte 1996) contrarily when concentration of inhalable microbial particles is required then active sampling gives better results (Napoli, Marcotrigiano et al. 2012).

2.8 Identification of biological strains

Airborne bacterial and fungal colonies are characterized by phenotypic or genotypic identification methods. Both methods are discussed below in detail.

2.8.1 Phenotype identification

Phenotypic identification of airborne microbes is based on the physical appearance of colonies front and back sides, characteristics e.g. shape, size, and colour. Similarly, different tests i.e. gram staining, Voges-Proskauer (VP) Test, oxidase-catalyze test, etc. and some other features like susceptibility to antimicrobial agents and cell protein profile are also utilized to distinguish each other.

2.8.2 Genotype identification

It is the advanced technique that is employed for the identification of bacterial and fungal strains up to their genus level. Different reactions have been reported in the literature for genotypic identification i.e. Ligase chain reaction (LCR), polymerase chain reaction (PCR), multilocus sequence typing (MLST), ribotyping and analysis of plasmids polymorphism. PCR is the amplification of deoxyribonucleic acid (DNA). It is a more accurate, sensitive, and rapid method for identification of airborne microbes therefore it is a worldwide adopted technique.

2.9 Indoor air quality of hospitals

Health care facilities are vulnerable to pathogenic airborne microbes more due to presence of indoor pollution sources i.e. infectious patients (Qian, Li et al. 2008). Exposure to infectious bioaerosols typically resulted in hospital-acquired infections (HAI). HAIs are the infections acquired by visitors or patients during his stay in a hospital. There are different facilities in the

hospital i.e. ICU, OPD, OT etc. with different working environment. Visitors, health staff and patients with different immune systems and health status are present in hospitals. Diversification of working conditions and occupant's immune system makes hospitals a very complex environment. In hospitals, most of the patients in the hospital have a very narrow thermal comfort zone and cannot tolerate slightly warm or cold condition. Therefore special attention should pay in maintaining T and RH in recommended range as normal indoor microenvironmental conditions in healthcare facilities are more correlated with the patient recovery state. Besides, in OT whole body of the patient is exposed to contaminated air (Buettcher and Heininger 2010, Farr and Jarvis 2010) Totaro, Porretta et al. reported that 15 % of nosocomial infections are caused during surgery (Totaro, Porretta et al. 2018). Therefore strict maintenance of levels of airborne micro-organisms and control measures are needed according to the sensitivity of patients present there.

2.10 Indoor air quality of libraries

Libraries are complex and dynamic environments and vulnerable to microbial exposure due to the presence of nutrient enrich material i.e. documents, books etc. Previous studies have observed that most of the fungal and bacterial activities are associated with the phenomena of biodeterioration (Grabek-Lejko, Tekiela et al. 2017). Previously conducted studies in libraries reported that in addition to biodeterioration, presence of airborne microbes in library buildings is also a potential threat to the health of students and visitors (Karbowska-Berent, Górny et al. 2011, Kalwasinska, Burkowska et al. 2012, Micheluz, Manente et al. 2018, Wu, Zhang et al. 2020). Therefore monitoring and control of the airborne microbes in libraries is essential to preserve precious paper heritage and public health. Monitoring the IAQ in libraries would also be helpful in the better determination of microbial composition, possible originating sources and controls. Libraries are confined environments mostly equipped with poor ventilation

systems. Poor ventilation gives rise to abnormal microenvironmental conditions i.e. high RH and T, offer more favorable conditions for the growth of biological agents.

2.11 Biological air quality standards

International standards and guidelines for microbial IAQ are not available till date (Mandal and Brandl 2011). However, healthcare organizations and research groups have proposed environment-specific exposure limits for biological pollutants. World health organization (WHO) professionals (Organization 1990) and Portugal national standards (PNS) (Verde, Almeida et al. 2015) proposed a concentration limit of 1000 CFU/m^3 for total bioaerosols (Heseltine and Rosen 2009) limiting fungal levels should not exceed from 500 CFU/m^3. Korea proposed 800 CFU/m^3 as maximum allowable limits for airborne bacteria (Kim, Kabir et al. 2018). American Conference of Governmental Industrial Hygiene (ACGIH) and National institute for occupational safety and health (NIOSH) proposed a threshold limit of 1000 CFU/m^3 for the total no of bioaerosols whereas for bacteria, it should not exceed the upper limit of 500 CFU/m^3 (Cox 1995, Jensen and Schafer 1998, Gębarowska, Pusz et al. 2018).

According to Netherland national standards sites having fungal and bacterial concentration more than 500 CFU/m^3 will be considered as contaminated (Association 1986, Heida, Bartman et al. 1995, Kim, Kabir et al. 2018). Bielawska-Drózd, *et al.* proposed 5000 CFU/m^3 as the maximum allowable limit for total bioaerosols (Bielawska-Drózd, Cieślik et al. 2018). However, to date like other developing countries, Pakistan has not established their national standards due to a lack of available data.

Chapter 3

MATERIALS AND METHODS

3.1 Overview

This chapter includes a detail description of methodology adopted during the research work. In this chapter site selection and sample collection procedures are discussed in detail. The graphical layout of the methodology is presented in Figure 3.1.

3.2 Site selection

Two different environments were selected for the purpose of study, hospitals and libraries. Demographic information of each sampling site is discussed below.

3.2.1 Hospital sites description

The research was planned in two public hospitals named Hospital A and Hospitals B (the real name of hospitals is not mentioned due to privacy concerns). Hospital A was founded in 1974 and surrounded on an area of 4500 m^2 and has beds capacity of 100 beds whereas Hospital B was established in 1962 and is extended on 50,000 m^2 of the area and has 700 beds capacity for patients. Both hospitals are situated in the vicinity of Rawalpindi district, Pakistan. Eight different locations were selected in each hospital depending upon the diversification of working environment. Emergency Department (EM1), Waiting Room (WR), Operation Theater (OT1), Women Surgical Ward (WSW1), Men Surgical Ward (MSW1), Outpatient Department (OPD1), Outpatient Department (OPD2), and Laboratory (LB) in Hospital A whereas Emergency Department (EM2), Women Surgical Ward (WSW2), Men surgical ward (MSW2), Nursery Unit (NU), Outpatient Department (OPD3), Intensive care unit (ICU), Burn Unit (OT2) and Operation Theater (OT3) were selected in Hospital B. Both hospitals are situated within 2 km distance to each. All the sampling sites in Hospital A were naturally ventilated while mechanically ventilated (equipped with centralize HVAC system) in Hospital B.

Detailed description of sampling locations in Hospital A and Hospital B is shown in Table 3.1 and Table 3.2 respectively.

3.2.2 Library sites description

Assessment of microbial IAQ was performed in three library buildings of National University of Science and Technology (NUST) Islamabad, Pakistan (33.73° N, 73.09° E). It is a semi-government university of Pakistan founded in 1991. Two departmental libraries (DL1 and DL2) and one central library (CL) were selected. DL1 and DL2 were the library buildings of Institute of Environmental Science and Engineering (IESE) and NUST Institute of Civil Engineering (NICE), respectively. CL was a three-story building including ground floor (CLFG), first floor (CLF1) and second floor (CLF2) and all three floors were selected for microbial IAQ monitoring. On the other hand, DL1 and DL2 were single-floor buildings and one sampling location was selected in each building. CL was facilitated with centralize HVAC system while DL1 and DL2 were naturally ventilated. In summer, thermal comforts in DL1 and DL2 were maintained through split air conditioning units (AC). However no heating system was available during winter. There are two entrances to CL on CLFG; the main entrance designated for students and staff and the side entrance, occasionally opened for delegates/individuals on official visit to university. Ground floor of CL (CLFG) is designated for scientific publications, newspapers and magazines. First floor (CLF1) houses reference books whereas Second floor (CLF2) is designated for Science and Engineering books, university archives, proceedings and reports There is no entrance from outside to CLF1 and CLF2. Students use stairs (located on CLFG) to access CLF1 and CLF2. All the three selected library buildings were situated within 1 kilometer radius. For outdoor air sampling central point of the three libraries was selected as the sampling location. Detail description of each site is enlisted in Table 3.3.

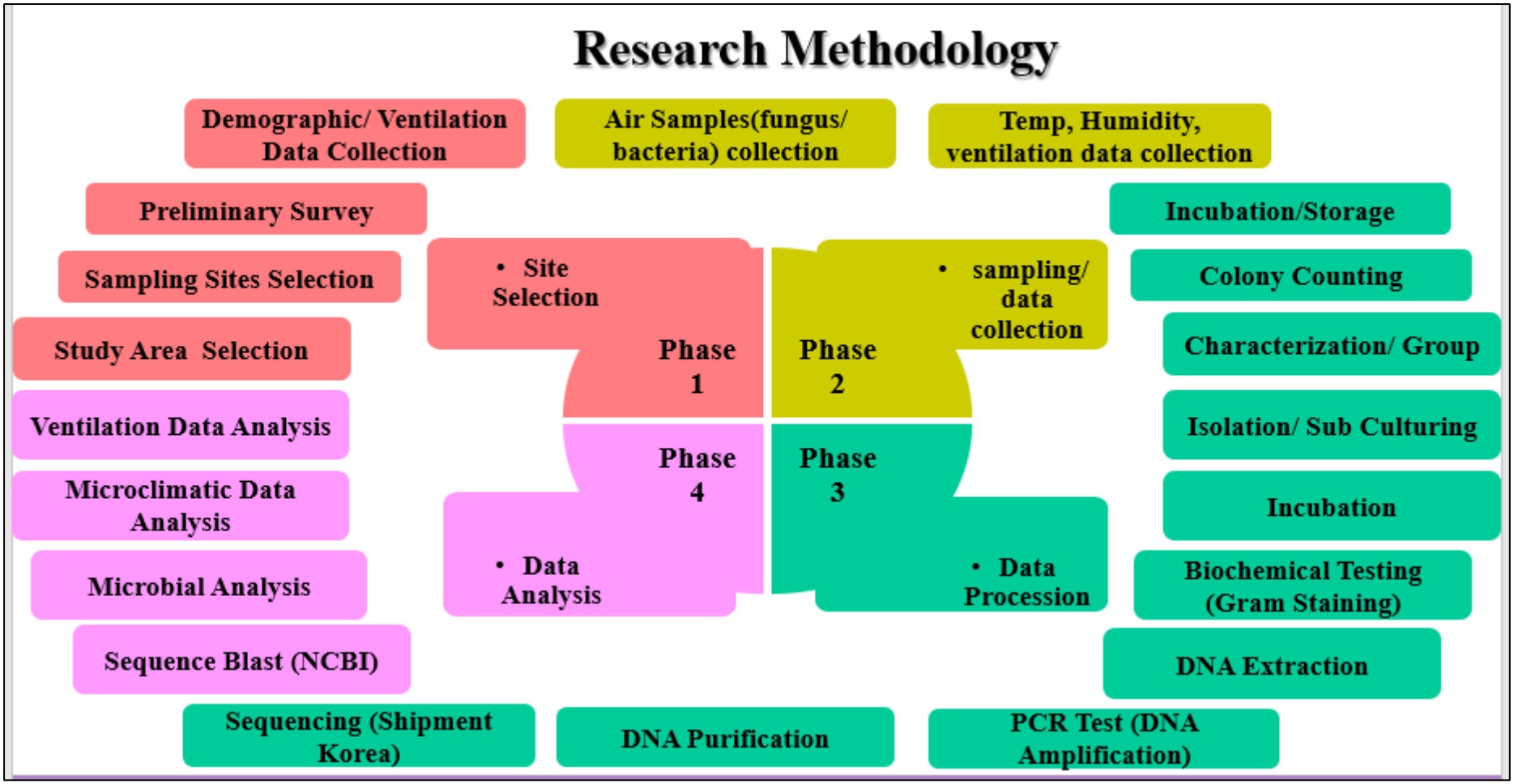


Figure 3. 1: Layout of research methodology

Table 3. 1: Detailed description of selected locations in Hospital-A

Hospital-A									
Sr. NO	**Sampling location**	**Floor**	**Building type**	**Ventilation System**	**Facility area (m^2)**	**Mean occupancy (persons)**	**Operational time (h/day)**	**Number of windows**	**Number of doors**
1	EM1	Ground floor	Closed	Natural	10	10	24	2	1
2	WR	Ground floor	Semi closed	Natural	30	40	6	-	1
3	OT1	Ground floor	Closed	Natural[b]	15	2[a]	6	2	1
4	WSW1	Ground floor	Closed	Natural	50	30	24	4	1
5	OPD1	Ground floor	Semi closed	Natural	40	70	6	--	--
6	OPD2	Ground floor	Semi closed	Natural	40	60	6	--	--
7	MSW1	Ground floor	Closed	Natural	40	20	24	4	1
8	LB	Ground floor	Closed	Natural[b]	10	5	8	1	1

[a] Average frequency of surgical operations per day

[b] Split air conditioning units (ACs) were being used for maintaining acceptable/comfortable temperature levels.

Table 3. 2: A detailed description of selected locations in Hospital-B

Hospital-B									
Sr. No	**Sampling location**	**Floor**	**Building type**	**Ventilation type**	**Facility area (m^2)**	**Mean occupancy (persons)**	**Operational time (h/day)**	**No of windows**	**No of doors**
1.	EM2	Ground floor	Closed	Mechanical	100	40	24	2	1
2.	WSW2	First floor	Closed	Natural	70	40	24	4	2
3.	OT2	Ground floor	Closed	Natural[b]	15	6[a]	10[c]	1	1
4.	NU	Ground floor	Closed	Mechanical	50	25	24	4	1
5.	OPD3	Ground floor	Semi closed	Natural	30	40	8	--	--
6.	MSW2	First floor	Closed	Natural	70	30	24	6	2
7.	ICU	First floor	Closed	Mechanical	50	18	24	4	1
8.	OT3	First floor	Closed	Mechanical	20	4[a]	10	0	1

[a] Average frequency of surgical operations per day

[b] OT2 was equipped with centralize HVAC, however, due to maintenance problem, HVAC was not operational during the sampling campaign therefore window type split AC was installed.

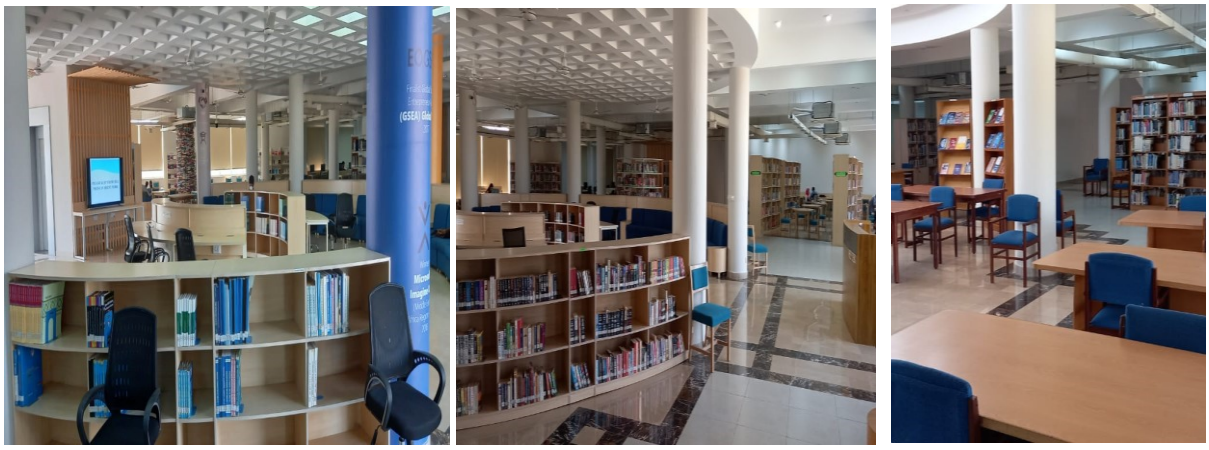
[c] OT2 is opened for 2 days per week only.

Table 3. 3: A detailed description of sampling locations in library buildings

Sampling Location	Floor area (Ft^2)	Ventilation type	Collections of books, thesis	Seating capacity	Occupancy mean (range)[a]	Number of window and doors	Floor-type
DL1	1100	Natural	5000	50	10 (6-19)	5+1	Carpeted
DL2	1500	Natural	9000	50	13 (5-20)	5+1	Ceramic tiles
CLG	14000	Mechanical	50000	250	49 (26-82)	4+1	Ceramic tiles
CLF1	14000	Mechanical	20000	150	65 (24-121)	4+0	Ceramic tiles
CLF2	14000	Mechanical	25000	150	66 (24-106)	4+0	Ceramic tiles

[a] Number of occupants present in indoor premises at the sampling time.

(a) (b)



(c) (d) (e)

Figure 3. 2: Monitored library locations (a) Departmental library-1, (b) Departmental library-2 (c) CL ground floor (d) CL first floor (e) CL second floor

3.3 Study design

3.3.1 Study design for libraries

Duplicate samples for bacteria and Fungi were collected from five indoor and one outdoor location. Sampling was conducted in December 2019 during normal working days; in the morning between 9:00 am to 11:00 am, in the afternoon between 1:00 pm to 3:00 pm and in the evening between 7:00 pm to 9:00 pm to assess temporal variation of microbial pollution. Airborne microbial samples were collected from height of one-meter above the ground and one-meter distance away from the walls.

3.3.2 Study Design for hospitals

The study in hospital buildings was lasted for two months that was started from august 2019 to September 2019. Air samples were collected once per week from each sampling location. Air sampler was operated for 5 min with airflow rate of 10 L/min to get 50 L of air volume. Cellulose nitrate filter paper (Sartorius, 13107-47-CAN), pore size of 0.45 μm was used for the collection of bacterial and fungal samples. The filter paper was subsequently placed on their respective nutrient media.

3.3.3 Nutrient Media

Tryptic soya agar (TSA) (OXOID CM0131) and potato dextrose agar (PDA) (OXOID CM0139) were utilized as nutrient growth media for the growth of bacterial and fungal isolates, respectively (Asif, Zeeshan et al. 2018). Petri plates containing air samples were sealed with parafilm, transported to the laboratory, and stored in the incubator. Bacterial samples were incubated at 37 $^{\circ}$C±1 $^{\circ}$C for 24 to 48 h while fungal samples at 27 $^{\circ}$C± 2°C for 5 to 7 days depending upon the growth. Fungal and bacterial species were counted by using the microbial colony counter and the concentration was expressed in CFU/m^3 (Asif, Zeeshan et al. 2019)

3.3.4 Contamination control

After sampling, petri plates were quickly wrapped and sealed with cellophane paper. Sampler and related items used during sampling were cleaned with 70 % ethanol before every next sample. Field blanks were used as controls during sampling, storage and transportation. To ensure reproducibility of results, duplicate air samples were collected for both bacteria and fungi and their concentration mean was used for further statistical analysis.

3.4 Identification of dominant Strains

3.4.1 Phenotypic identification of Bacteria and Fungi

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. Bacterial strains were initially characterized according to the recommended methods based on the phenotypical characteristics i.e. morphology (shape, color, texture), gram staining and other biochemical tests i.e. oxidase and catalase tests (Cowan and Steel 1965, Holt, Krieg et al. 1994). Based on gram staining, strains were classified into gram-positive and gram-negative rods and cocci. Initially fungal colonies were classified on their physical appearance and spores' characteristics (Barnett and Hunter 1972).

3.4.2 Genotypic identification of bacteria and fungi

Most frequently occurring bacterial and fungal species were isolated and identified genotypically. Genotypic identification incorporated DNA extraction, PCR amplification and purification of extracted genomic DNAs. Purified DNA samples were sent to Macrogen Korea for sequencing. Resultant sequenced were blast in National Center for Biotechnology Information (NCBI) and compared with the existing database.

Genomic DNA of frequently observed bacterial isolates was extracted and 16S rDNA gene region of bacterial genomes was amplified using universal primer set, 9F and 1429R. On the other hand using universal primers set, ITS1F and ITS4R, ITS region was amplified of each fungal genome. PCR reaction mixture and thermal condition for bacterial and fungal identification are listed in Table 3.4 and Table 3.5, respectively.

Table 3. 4: Description of PCR mixture and thermal conditions for bacterial identification

PCR Reaction Mixture			**Thermal conditions**		
Entity	**Conc.**	**Quantity**	**Cycle**	**T (°C)**	**Time (min)**
Forward primer	0.4 μM	2 μl	Initial Denaturation	95	3
Reverse primer	0.4 μM	2 μl	Denaturation (32 Cycles)	95	0.75
PCR taq buffer	1X	5 μl	Annealing	57.3	0.75
$MgCl_2$	1.5mM	1.5 μl	Extension	72	1
dNTPs	0.1 mM	2.5 μl	Final Extension	72	15
Taq DNA polymerase		0.2 μl	Cooling	4	
DNA Template		7.9 ng			

Table 3. 5: Description of PCR mixture and thermal conditions for fungal identification

PCR Reaction Mixture			Thermal conditions		
Entity	**Conc.**	**Quantity**	**Cycle**	**T (°C)**	**Time (min)**
Forward Primer	0.4 μM	2 μl	Initial Denaturation	94	3
			Denaturation (35		
Reverse Primer	0.4 μM	2 μl	Cycles)	94	0.75
PCR taq Buffer	1X	5 μl	Annealing	58	0.75
$MgCl_2$	2.5 mM	3 μl	Extension	72	0.75
dNTPs	2 Mm	2 μl	Final Extension	72	7
Taq DNA Polymerase		0.5 μl	Cooling	4	
DNA Template		2 μl			
Nuclease Free (NF)					
Water		23.5 μl			

3.5 Data Collection

Sampling was performed in two different types of environments: hospitals and libraries. Data collected from selected location is discussed below.

3.5.1 Hospitals sites

Duplicate samples were collected for both fungi and bacteria. The air sampler was operated to draw a volume of 50 L. Filter paper was subsequently placed on sterile TSA and PDA media for bacteria and fungus, respectively. The study campaign lasted for seven weeks with sample collection frequency of once per week.

Hospital-A = 8 Sites

Hospital-B = 8 Sites

Total sites = 8+8=16 sites

Total fungal samples collection= Total sites* no of sampling days* samples collected per day

=16*7*2=224 samples

Total bacterial samples collection= Total sites* Number of sampling days* samples collected per day

=16*7*2=224 samples

Total samples collected for bacteria and fungi= 224+224 = 448 Samples

Besides, approximately 60 blanks were used as control. Related data e.g. number of occupants, patients and staff members were also recorded simultaneously.

3.5.2 Library buildings

3.5.2.1 Data collection of microclimatic factors

Study performed in libraries assessed the microenvironmental data (RH and T) in indoor locations of library buildings. HT-2000 equipped with a non-dispersive infrared (NDIR) CO_2 sensor was utilized to measure the CO_2, RH and T on each sampling location. The mean average of RH and T was used in further statistical analysis. Characteristic of HT-2000 were (Range ±Accuracy) 0-9999 ppm ±5%, -10 - 70ºC ±1.2ºC, 0.1-99.9% ±3%, for CO_2, T and RH respectively. The number of students and library staff as well as the number of books were also noted to check their influence on microbial concentration.

3.5.2.2 Data collection for microbial load

To assess the microbial load in libraries three locations in CL, one location in each DL1 and DL2 and one outdoor location was selected. The sample collection frequency was thrice per day for both fungus and bacteria.

Central library = 3 Sites

Department libraries = 2 Sites

Outdoor location = 1 site

Total sites = 3+2+1= 6 sites

Total fungal samples collected = Total sites* Number of sampling days* Samples collected per day

=6*3*6 =108 samples

Total bacterial samples collected = Total sites* Number of sampling days* Samples collected per day

=6*3*6=108 samples

Total number of samples collected = 216 samples

3.6 Data Analysis

3.6.1 Microclimatic factors

T, RH and CO_2 data measured on each sampling site was analyzed in MS Excel (Microsoft Corporation, USA) and SPSS 14 (IBM Corp., USA). Difference of the three parameters (T, RH and CO_2) along the day and between the monitored locations was analyzed using One-way ANOVA test.

3.6.2 Microbial levels

The concentration of fungal and bacterial communities was counted using microbial colony counter and reported in CFU/m^3. The shape, Color, Elevation, Texture and Margins of each colony were noted in the excel sheet. Later, dominant strains with higher frequency of occurrence were isolated and streaked on their respective growth media. As discussed in section 3.4, DNA of dominant strains was extracted, amplified and subsequently purified. Later, DNA samples were sent to Macrogen Korea. The resultant sequences were analyzed in Bio edit tool and then blast in the gene bank of the NCBI where we found our query sequences by comparing the similarity with the published database.

3.7 Statistical analysis

Microsoft Excel (Microsoft Corporation 365, USA) and SPSS 14 (IBM Corp., USA) were used to analyze the measured data. T-test was used to analyse the significant difference of indoor and outdoor microbial load while one-way ANOVA test was used to find the significant difference among the different selected sampling locations. Spearman rank correlation

coefficient test was used to analyze the correlation between microenvironmental factors and microbial levels (to evaluate the effect of outdoor and indoor climatic conditions on indoor bacterial and fungal load). The statistical significance was set at $P \leq 0.05$.

Chapter 4

RESULTS AND DISCUSSION

4.1 Microbial analysis in hospitals

4.1.1 Bacterial and fungal load in Hospital-A

Concentration of airborne bacteria and fungi was assessed in eight different facilities in Hospital-A. significant variation ($P < 0.05$) was observed in concentration of bacteria and fungi on monitored locations of both hospitals. The concentration of bioaerosols are reported to be influenced by number of occupants and their activities (Täubel, Rintala et al. 2009, Verde, Almeida et al. 2015), functionality and working condition i.e. emergency or normal (Fonseca, Abreu et al. 2019), type of ventilation system and staff dressing (Flannigan, Samson et al. 2016). As these factors varied across the monitored locations, variation was observed in microbial levels accordingly. Highest bacterial load was found in OPD1 (mean: 3415 CFU/m^3). Highest number of patients with diverse nature of diseases was the possible reason for higher bacterial load. This assertion is supported by previous findings (Park, Yeom et al. 2013, Bomala, Saramanda et al. 2016). Higher microbial levels observed in wards may be due to the attached toilets as reported in previous findings (Gizaw, Gebrehiwot et al. 2016, Kunwar, Tamrakar et al. 2019). OPD1 was found more polluted than OPD2 and WSW1 was found more polluted than MSW1 regarding bacterial load. The difference in the observed values may be attributed to the "occupant's density" difference at the time of sampling (Table 3.1). Statistical results also confirmed our present finding as a significant correlation ($r = 0.65$, $P \leq 0.05$) was found between the number of occupants and bacterial load. Lowest bacterial load was observed in OT1 (mean: 384 CFU/m^3) that can be supported by the fact that it was the site having lowest occupancy levels and highest cleaning frequency. Restricted personal access limits airborne microbes (Tselebonis, Nena et al. 2020), Thus, lowest bacterial and fungal levels were observed

in OTs. Highest fungal load was observed in WSW1 (mean: 517 CFU/m^3). WSW1 was surrounded with high "plantation and greenery" and thermal Comfort and ventilation rates were being achieved through doors and windows openings. Outdoor air was the possible reason for higher fungal load in Wards as when outdoor air entered inside the building it carries the plants-based fungal spores with it (Lee, Grinshpun et al. 2006, Sautour, Sixt et al. 2009, Adams, Miletto et al. 2013). The fungal load was also lowest in OT1 which confirms that high cleaning frequency, lower occupancy level and recommended dressing by health care staff reduces the fungal load inside the medical facility area. Bacterial and fungal load observed on each monitored location of in Hospital-A is shown in Table 4.1.

4.1.2 Bacterial and fungal load in Hospital-B

Highest bacterial (mean: 1996 CFU/m^3) and fungal load (mean: 269 CFU/m^3) was observed in OPD3. As discussed above in section 4.1.1, the concentration of bacterial communities is highly influenced by the occupancy levels that was the reason of more bacterial load in OPD3. Lowest bacterial and fungal concentration was found in OT3 having bacterial load (mean: 280 CFU/m^3) and fungal load (mean: 52 CFU/m^3). Prior studies have also observed higher microbial load in OPD and the lowest in OT (Qudiesat, Abu-Elteen et al. 2009, Nasir, Mula et al. 2015, Sivagnanasundaram, Amarasekara et al. 2019) due to effective ventilation, lower occupants density and appropriate disinfection procedures. Though a very low concentration of airborne microbes was observed in three OTs and ICU as compared to other sites, however more efforts are required regarding IAQ management and air pollution engineering controls, as these are susceptible sites for opportunistic pathogenic airborne microbes which may facilitates the transmission of surgical site infections (SSI). Bacterial and fungal load observed on each monitored location of Hospital-B is shown in Table 4.2.

Table 4. 1: Descriptive statistics of microbial load in Hospital-A

Hospital-A								
Hospital monitored Location	**Fungal Load (CFU/m^3)**				**Bacterial Load (CFU/m^3)**			
	Min	**Max**	**Median**	**St. Dev**	**Min**	**Max**	**Median**	**St. Dev**
Emergency Department **(EM_1)**	100	500	272	120	800	1870	1258	330
Waiting Room **(WR)**	130	410	270	88	360	2720	1850	755
Operation Theater **(OT_1)**	64	160	80	30	280	490	380	77
Women Surgical Ward **(WSW_1)**	120	920	500	256	480	3600	1938	959
Outpatient Department **(OPD_1)**	180	520	370	113	2220	4980	3220	835
Outpatient Department **(OPD_2)**	200	660	243	155	580	4220	2500	1044
Men Surgical Ward **(MSW_1)**	140	860	260	266	230	2020	1140	557
Laboratory (**LB**)	114	250	180	51	180	1340	860	335

Table 4. 2: Descriptive statistics of microbial load in Hospital-B

Hospital-B								
Hospital monitored Location	**Fungal Load (CFU/m^3)**				**Bacterial Load (CFU/m^3)**			
	Min	**Max**	**Median**	**StDev**	**Min**	**Max**	**Median**	**StDev**
Emergency Department **(EM_2)**	80	260	164	55	360	1520	830	350
Women Surgical Ward **(WSW_2)**	90	430	250	101	100	1190	690	293
Burn Unit **(OT_2)**	29	580	170	104	100	470	260	113
Nursery Unit **(NU)**	60	300	90	73	130	490	310	98
Outpatient Department **(OPD_3)**	90	530	250	115	829	3370	2080	678
Men Surgical Ward (MSW_2)	110	520	140	150	420	1400	645	326
Intensive Care Unit **(ICU)**	20	310	50	102	380	780	510	132
Operation Theater **(OT_3)**	20	100	40	28	21	540	300	191

4.1.3 Microbial composition in Hospital-A

Assessment of the bacterial and fungal composition accumulated in the indoor air is essential for better determination of sources, possible health risks and controls. The composition of dominant fungal and bacterial strains identified in Hospital A is shown in Figure 4.1 and Figure 4.3, respectively. In hopsital-A, the frequently occurring fungal species were distributed as *Aspergillus spp.* (59 %) and *Penicillium spp.* (34 %). Genotypic identification showed the dominance of *Aspergillus niger* (35 %), *Talaromyces macrosporus* (21 %), *Aspergillus flavus* (12 %), *Aspergillus fumigatus* (11 %), *Penicillium chrysogenum* (9 %), *Penicillium oxalicum* (2 %), *Talaromyces funiculosus* (2 %) and *Aspergillus nodulous* (1 %). The composition of dominant bacterial strains identified in Hospital A and Hospital B are shown in Figure 4.3 and Figure 4.4, respectively. All the frequently occurring bacterial species were gram-positive distributed as gram-positive cocci (83%), gram-positive rods (10 %). Gram-positive cocci include *Staphylococcus spp.* (50 %) and *Micrococcus spp.* (33 %) whereas gram-positive rods include *Bacillus spp.* (10 %). In Hospital A, most frequently occurring bacterial strains were identified genotypically which includes *Staphylococcus haemolyticus* (49 %), *Micrococcus aloeverae* (17 %), *Micrococcus luteus* (14 %), *Bacillus subtilis* (4 %), *Bacillus pumilus* (4 %), *Micrococcus yunnanensis* (2 %), *Lysinibacillus macroides* (2 %) and *Staphylococcus aureus* (1 %).

4.1.4 Microbial composition in Hospital-B

The composition of dominant fungal and bacterial strains identified in Hospital B is shown in Figure 4.2 and Figure 4.4, respectively. Microbial composition in Hospital-B was more alike the same as identified in Hospital-B due to similar geographical location. Gram-positive cocci (84 %) and gram-positive rods (11 %) were the most dominant bacterial group identified in Hospital-A, distributed as *Staphylococcus* (56 %) and *Micrococcus* (28 %) and *Bacillus spp.* (11%). Genotypic identification showed the dominance of *Staphylococcus haemolyticus* (54

%), *Micrococcus luteus* (15%), *Micrococcus aloeverae* (9%), *Lysin bacillus macroides* (7%), *Micrococcus yunnanensis* (4%), *Bacillus subtilis* (2%), *Bacillus Pumilus* (2 %) and *Staphylococcus aureus* (2%).

On the other hand, *Aspergillus spp.* (75%) and *Penicillium spp.* (21%) were predominant in Hospital-B. Genotypic identification showed the dominance of *Aspergillus Niger (42 %), Talaromyces macrosporus (14 %), Aspergillus fumigatus (15 %), Aspergillus flavus (13 %), Aspergillus nidulaus (5 %), Penicillium chrysogenum (5 %) and Talaromyces funiculosus (2 %).*

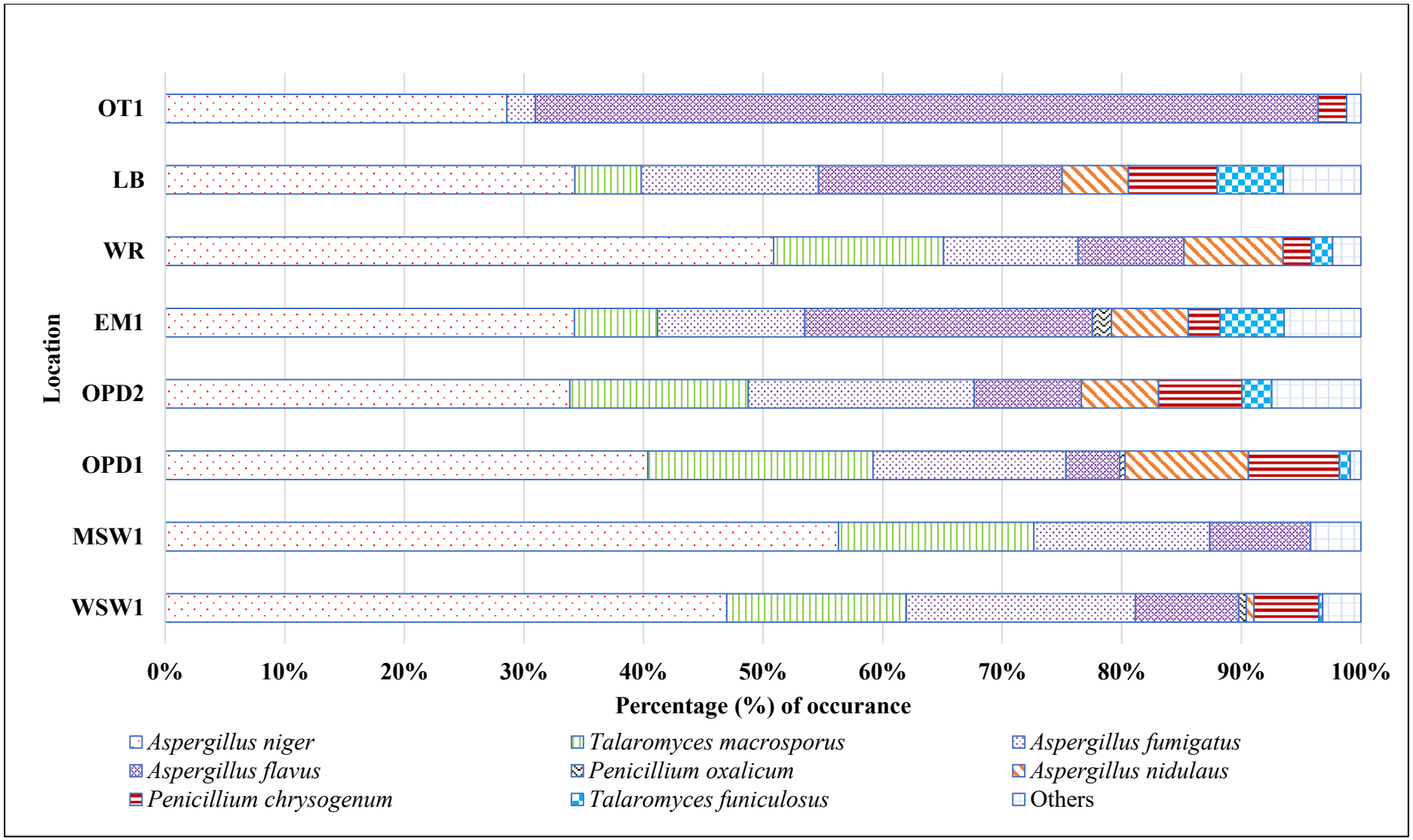


Figure 4. 1: Fungal composition identified at Hospital-A

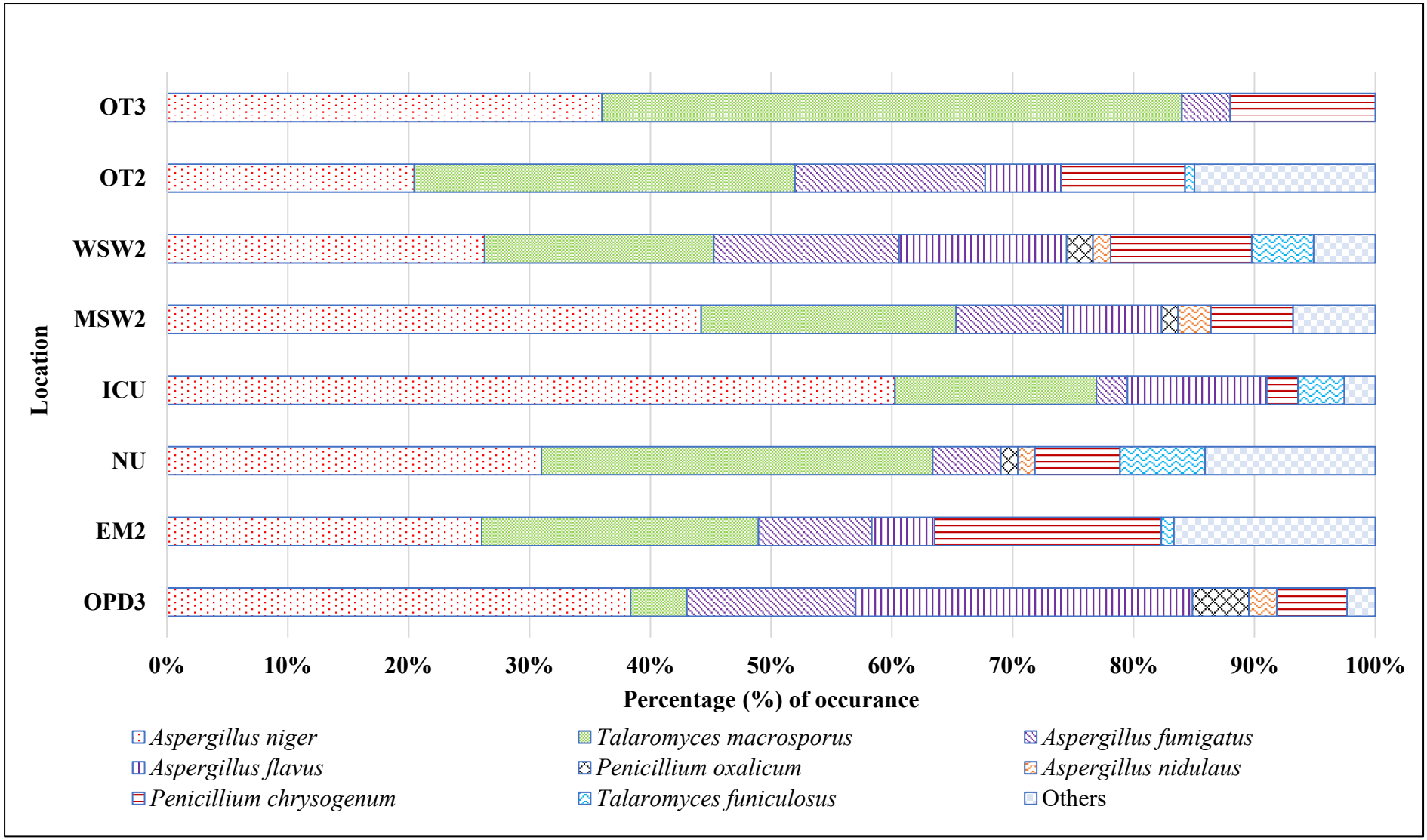


Figure 4. 2: Fungal composition (%) identified in Hospital-B

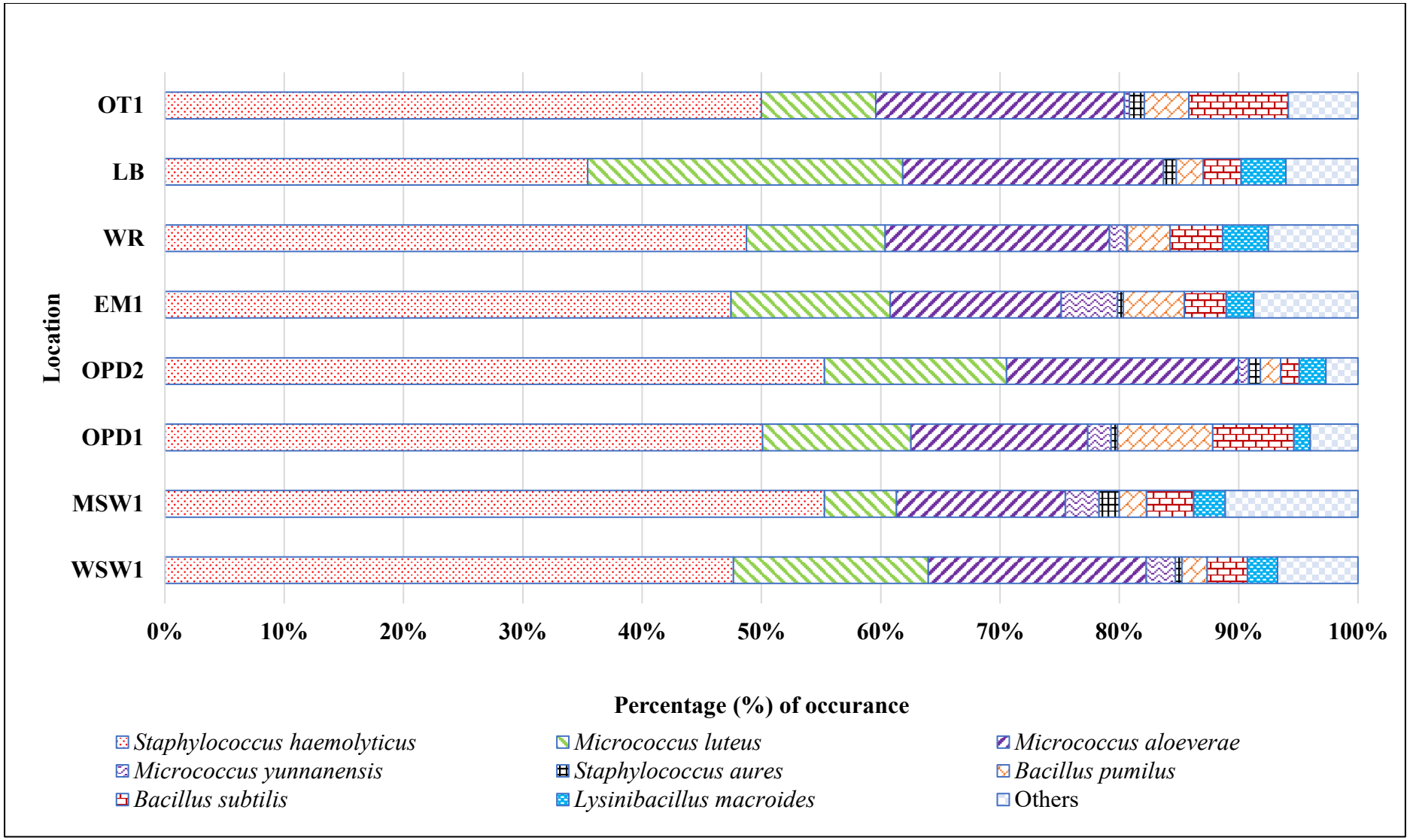


Figure 4. 3: Bacterial composition (%) identified in Hospital-A

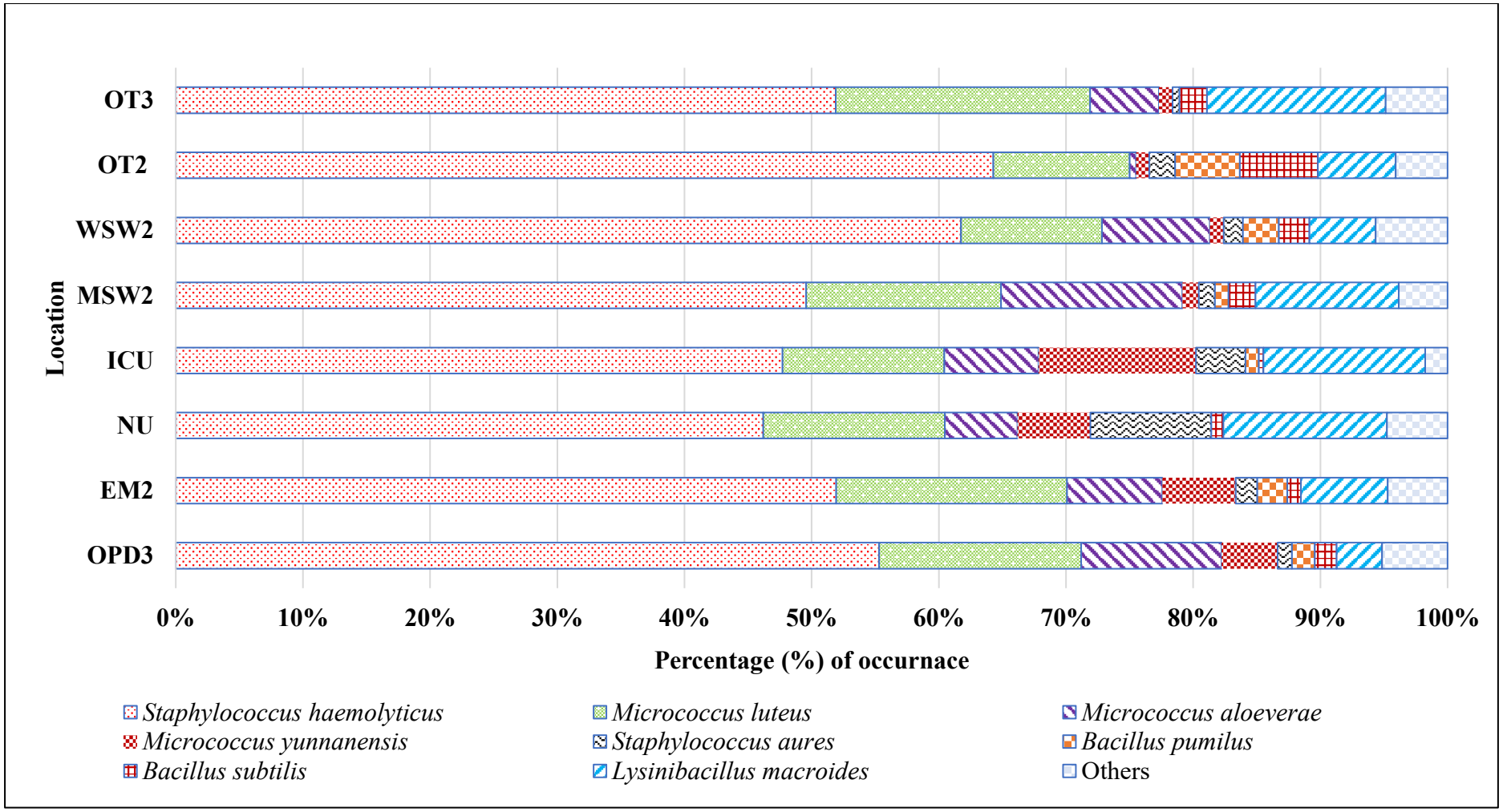


Figure 4. 4: Bacterial composition (%) identified in Hospital-B

4.1.5 Comparison of hospitals

4.1.5.1 Comparison of Ventilation System

Performance of ventilation system of the building has great impact on microbial IAQ (Douwes, Thorne et al. 2003, Li, Leung et al. 2007, Naruka, Gaur et al. 2014). However experience and suggestion about the type of ventilation system is different according to different research studies as some studies supports natural ventilation (Escombe, Oeser et al. 2007, Qian, Li et al. 2010, Gilkeson, Camargo-Valero et al. 2013) whereas more studies recommend the installation of mechanical ventilation system (Gilkeson, Camargo-Valero et al. 2013, Yang, Liao et al. 2014, Wallner, Munoz et al. 2015). However, in the present study, bacterial and fungal levels were found higher in natural ventilated locations as compared to those equipped with centralize HVAC system that can be supported by the fact that natural ventilation system could not inhibit and control the outdoor microbial load (Qian, Li et al. 2010, Wallner, Tappler et al. 2017, Stanley, Onwuna et al. 2019). Although microbial load observed at central HVAC ventilated locations was comparatively lower than naturally ventilated but it was higher than the recommended limits. The removal efficiency of the centralize HVAC ventilated system was compared for fungus and bacteria. Results showed that centralize HVAC system has more removal efficiency for fungi as compared to bacteria as previously conducted by Wu, *et al.* and Ortiz, *et al.* reported that HEPA filters installed in HVAC system remove fungi more efficiently than bacteria (Wu, Li et al. 2005, Ortiz, Yagüe et al. 2009).

4.1.5.2 Frequency of exceedance from microbial standards

Pakistan does not have national standards for microbial IAQ therefore results for microbial load were compared with the proposed standards of WHO and PNS reported <500 CFU/m^3 for bacteria and <500 CFU/m^3 for fungus and 1000 CFU/m^3 for total bioaerosols. Comparison showed that most of the monitored sites have meetup the fungal criteria set by the WHO and PNS. On the other hand, nearly all sites exceeded the conformity criteria set for bacterial load. Furthermore, only OT1, OT2, OT3, ICU and NU were under the allowable limits for total bioaerosols concentration limit. Frequency of exceedance (%) from the proposed limits of WHO and PNS is shown in Table 4.3 and Table 4.4 for Hospital A and Hospital B, respectively.

Table 4. 3: Frequency (%) of exceedance from the established standards in Hospital-A

Hospital A			
Location	**>1000 CFU/m^3**	**> 500 CFU/m^3**	
	Total bioaerosols	**Fungi**	**Bacteria**
Emergency Department **(EM_1)**	86	0	100
Waiting Room **(WR)**	86	0	86
Operation Theater **(OT_1)**	0	0	0
Women Surgical Ward **(WSW_1)**	100	43	86
Outpatient Department **(OPD_1)**	100	14	100
Outpatient Department **(OPD_2)**	86	14	100
Men Surgical Ward **(MSW_1)**	86	29	86
Laboratory (**LB**)	57	0	86

Table 4. 4: Frequency (%) of exceedance from the established standards in Hospital-B

Hospital B			
Location	**>1000 CFU/m^3**	**>500 CFU/m^3**	
	Total bioaerosols	**Fungi**	**Bacteria**
Emergency Department **(EM_2)**	43	0	71
Women Surgical Ward **(WSW_2)**	43	0	71
Burn Unit **(OT_2)**	0	0	0

Location	>1000 CFU/m^3	>500 CFU/m^3	
	Total bioaerosols	Fungi	Bacteria
Nursery Unit **(NU)**	0	0	0
Outpatient Department **(OPD_3)**	100	14	100
Men Surgical Ward (M**SW_2**)	43	14	86
Intensive Care Unit **(ICU)**	0	0	57
Operation Theater **(OT_3)**	0	0	14

4.2 Microbial analysis in libraries

4.2.1 Bacterial load in libraries

In libraries temporal variation of the microbial levels over the day was assessed for winter season. Bacterial concentrations recorded indoor was ranging from 20-230 CFU/m^3 whereas from 20-100 CFU/m^3 in an outdoor environment. The highest bacterial was observed at CLFG (mean: 102 CFU/m^3) whereas lowest bacterial levels were measured at DL2 (mean: 66 CFU/m^3). Although both DL1 and DL2 have similar ventilation and occupancy load. However higher bacterial value at DLI as compared to DL2 may be attributed to the carpeted floor and lower cleaning frequency as reported in previous studies (Bholah and Subratty 2002, Bouillard, Michel et al. 2005, Ramachandran, Adgate et al. 2005) observed comparatively higher microbial load on sampling sites with the carpeted floor. Similarly, CLFG, CLF1 and CLF2 were the three floors of the same building and were equipped with centralize HVAC system however CLFG was found slightly more contaminated as compared to CLF1 and CLF2. It is already mentioned, there was no exit and entrance to CLF1 and CLF2 from outside, thus students/visitors first entered CLFG and use stairs to move CLF1 or CLF2. Higher microbial levels at CLFG may be justified due to more visitors' movements as well as resuspension of settled microbial spores into the air a spores attached with visitors' shoes and dress, get amassed in the indoor air (Kim and Kim 2007, Ghosh, Lal et al. 2013).

Bacterial concentration in the indoor environment was higher than the outdoor environment (in most cases), which is supported by the fact that bacterial load in the indoor environment did not influence by the outdoor air rather the presence of indoor bacterial source (occupants). Previous studies have confirmed that humans and their activities i.e. walking, talking etc. are indoor bacterial pollution sources. Although we find no significant correlation between the number of occupants and the microbial buildup however it was clear that bacterial levels increase in the presence of students and vice versa. In the morning, higher bacterial levels were observed at CLF2 (mean: 30 CFU/m^3) and CLF1 (mean: 113 CFU/m^3). In the afternoon, students attend their classes, therefore, the occupancy load in afternoon was found lower than morning and evening. Thus we found lower bacterial load in afternoon as compared to other sampling time.

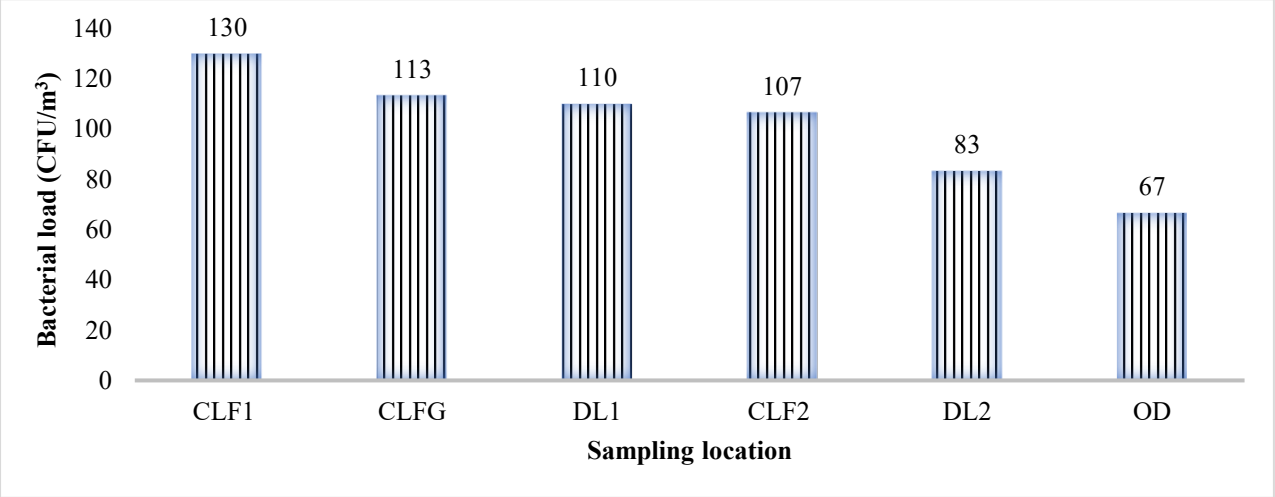


Figure 4. 5: Mean bacterial load in morning on sampling locations of libraries

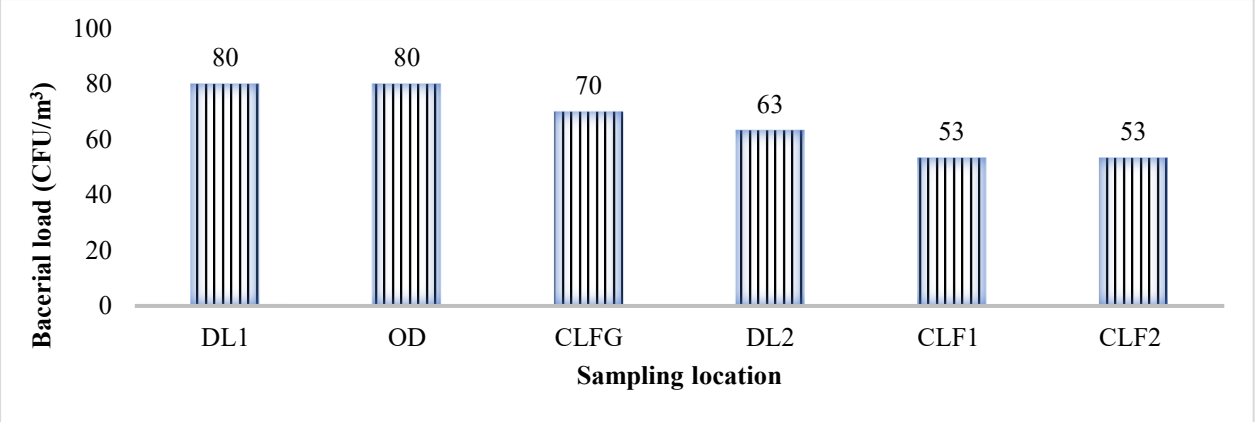


Figure 4. 6: Mean bacterial load in afternoon on sampling locations of libraries.

Outdoor microbial load was found higher in the afternoon than another sampling period, the reason may be the optimum microclimatic conditions in afternoon. In winter, outdoor T was found higher in the afternoon as compared to morning and evening, higher T supports the microbial growth.

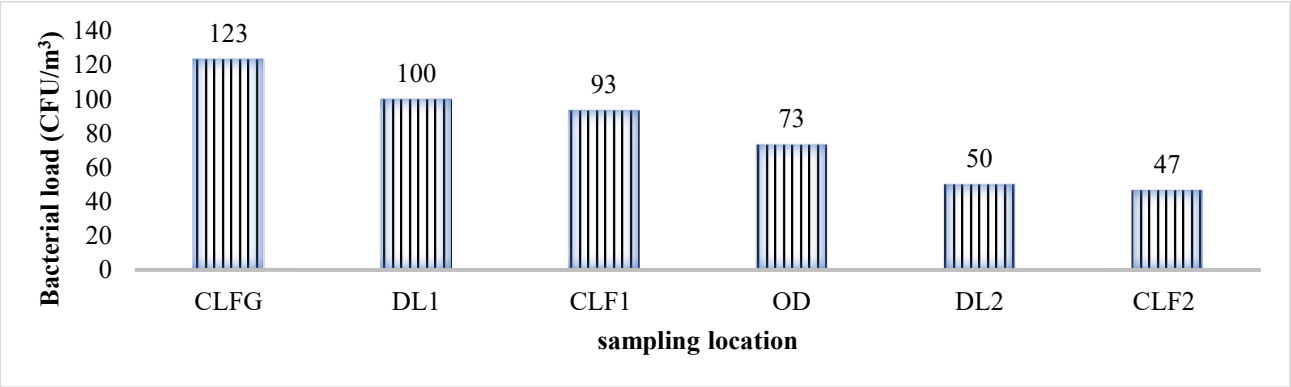


Figure 4. 7: Mean bacterial load in evening on sampling locations of libraries

Overall CLF2 and DL2 were observed as less polluted whereas CLFG and DL1 as most polluted monitored locations.

4.2.2 Fungal load in libraries

The fungal load was also assessed in the morning, afternoon and evening to investigate the temporal variation over the day. The fungal load was ranging between 20-250 CFU/m^3 indoor and 280 to 510 CFU/m^3 in the outdoor environment. The highest fungal load was observed at CLFG (mean: 149 CFU/m^3) and lowest fungal levels were found at CLF2 (mean: 69 CFU/m^3).

A possible explanation of higher fungal load at CLFG maybe the infiltration of untreated outdoor air through the entrance door that remained almost opened (due to higher opening frequency) (Kim and Kim 2007). Higher number of books at CLFG than the other sampling places could also be the possible reason for higher fungal load. A significant difference ($P < 0.05$) in fungal load was observed between indoor and outdoor locations with multiple times higher fungal load outdoor. Overall CLFG was observed as the highest contaminated site regarding fungal load whereas CLF2 was the most cleaned place.

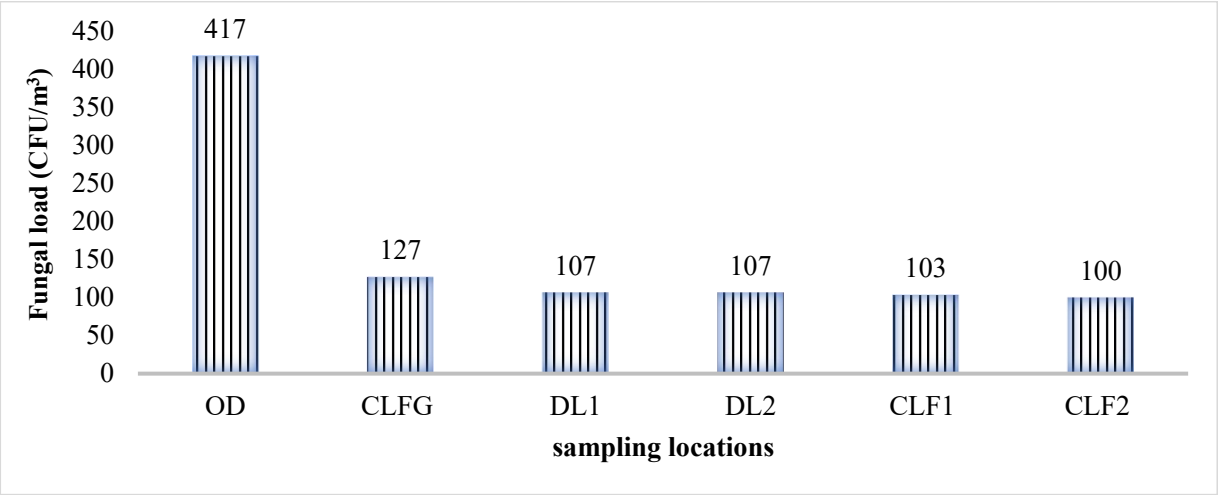


Figure 4. 8: Mean fungal load in morning on sampling locations of libraries

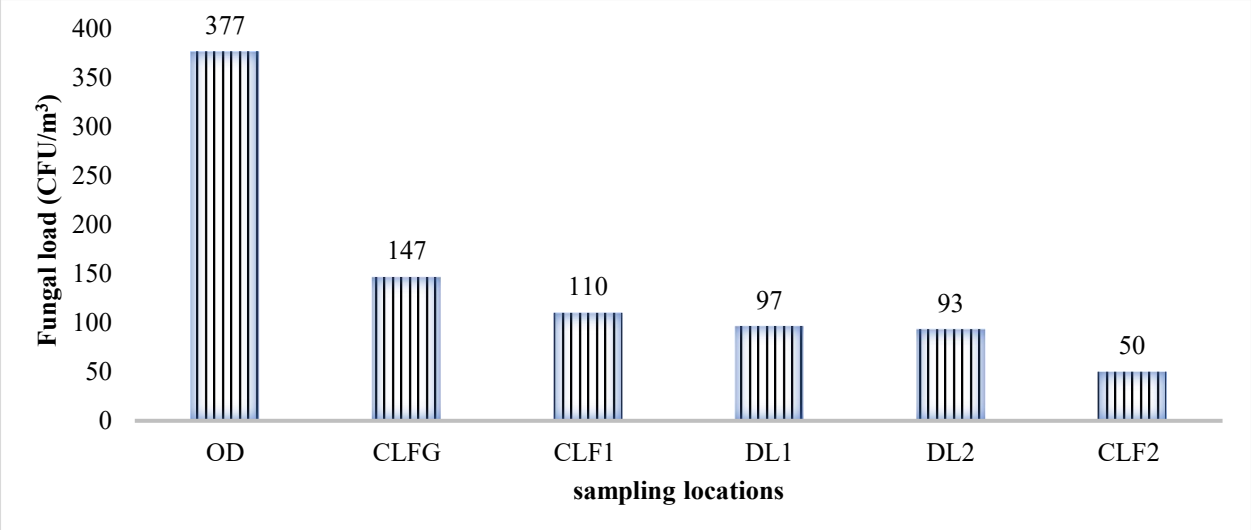


Figure 4. 9: Mean fungal load in afternoon on sampling locations of libraries

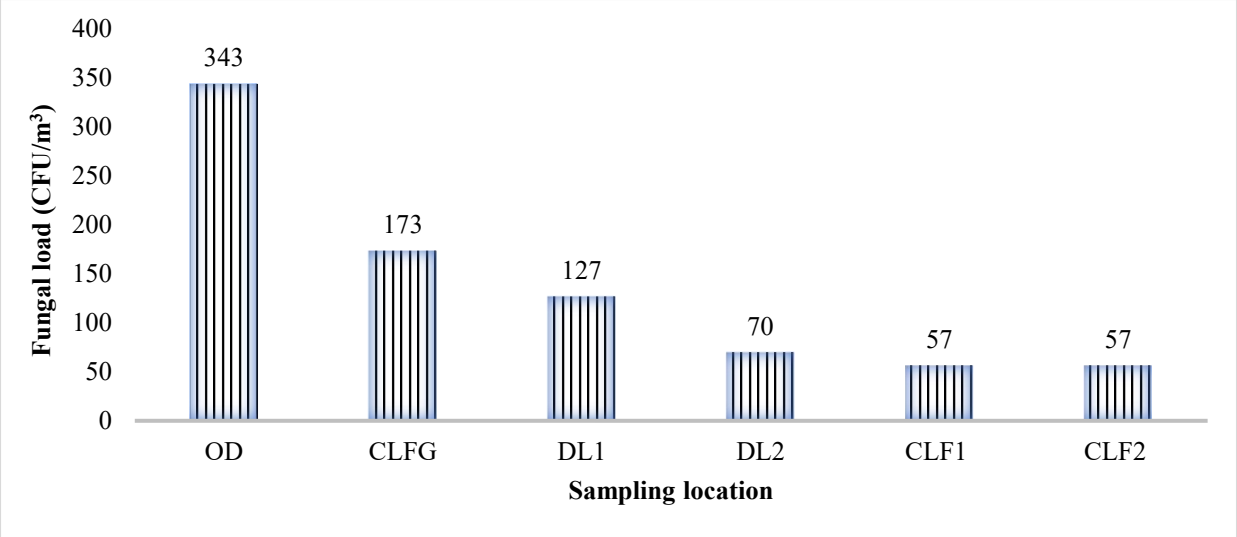


Figure 4. 10: Mean fungal load in evening on sampling locations of libraries

4.2.3 Bacterial composition in libraries

Overall seven abundantly occurring bacterial strains were isolated and their DNA was extracted (DNA Extraction protocol is described in chapter 3). Composition (%) of bacterial communities identified on respective monitored location is shown in Figure 4.11. Phenotypical identification showed gram-positive bacteria as dominant bacterial genera in library environment. Frequently occurring bacterial strains were *Micrococcus luteus*, *Micrococcus aloeverae, Bacillus Pumilus, Lysin bacillus macroides, Staphylococcus haemolyticus, Bacillus cereus, Bacillus paralicheniformis*. Dominance of *Bacillus spp.* has been reported in previous studies (Borrego and Perdomo 2012, Hayleeyesus and Manaye 2014, Okpalanozie, Adebusoye et al. 2018). Higher levels of *Bacillus spp.* in the indoor environment could be due to water-damaged buildings although we have not observed this phenomenon. Another study conducted in Korea, find out *Staphylococcus spp.* and *Micrococcus spp.* as dominant species indoor (Kim, Kim et al. 2009).

4.2.4 Fungal composition in libraries

Overall *Cladosporium asperulatum, Penicillium oxalicum, Talaromyces macrosporus, Aspergillus niger, Penicillium chrysogenum, Talaromyces funiculosus, Aspergillus fumigatus,*

Penicillium expansum, Penicillium mallochii were found as the abundantly occurring fungal strains. Dominance of *Cladosporium* in the indoor as well as the outdoor environment is supported by the findings of a study conducted in Pakistan (Asif, Zeeshan et al. 2019).

Library's buildings were surrounded by the green parks, plants and trees, that may be the possible reason for high levels of *Cladosporium*, as *Cladosporium* is reported as plant pathogen. A study conducted in Korea (Kim, Kim et al. 2009), Portugal (Madureira, Paciência et al. 2015) and Italy (Valeriani, Cianfanelli et al. 2017) have also reported the dominance of *Penicillium spp.* and *Aspergillus spp* in indoor . Composition (%) of fungal communities identified on respective monitored location is shown in Figure 4.12.

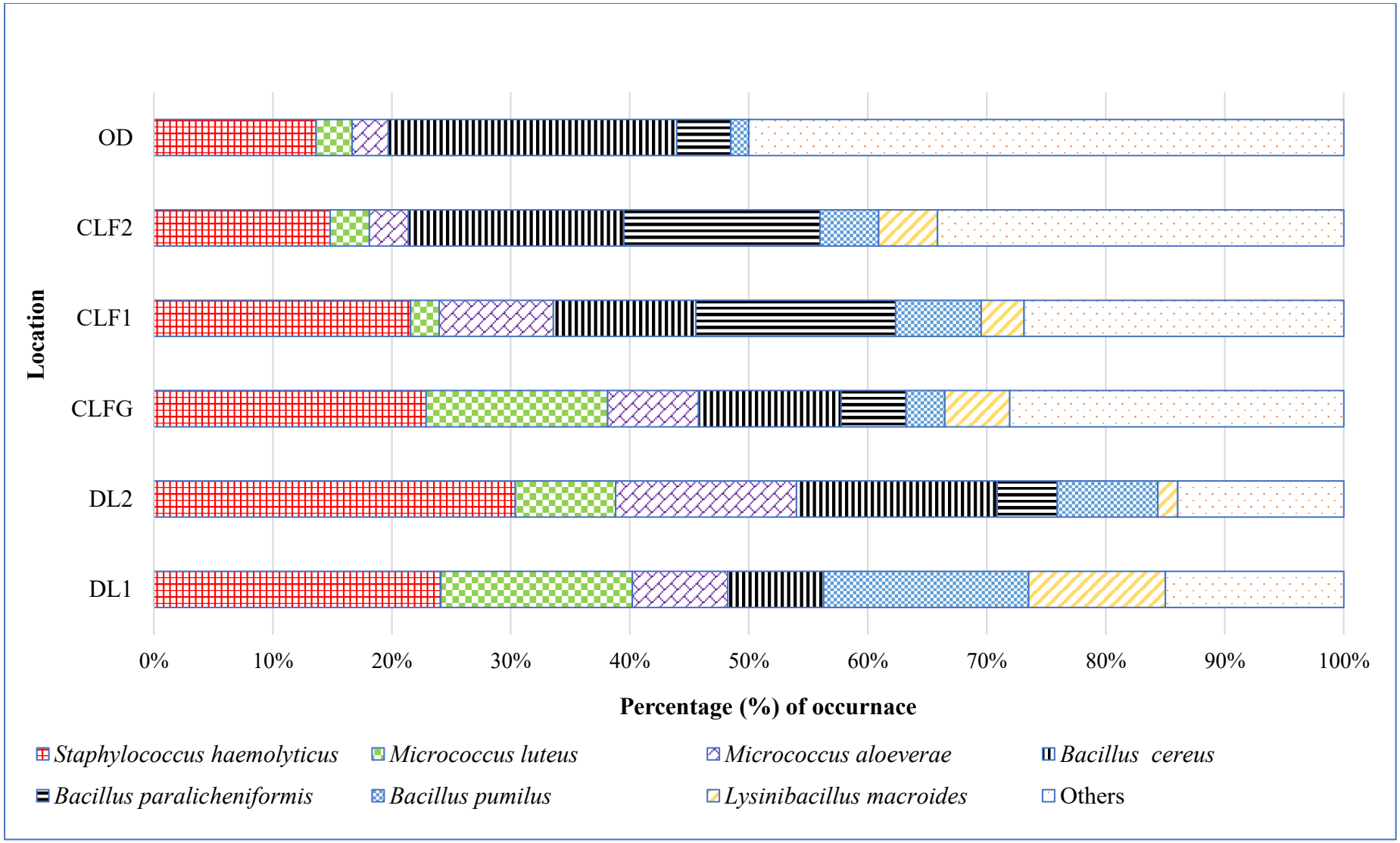


Figure 4. 11: Bacterial composition on sampling locations of libraries

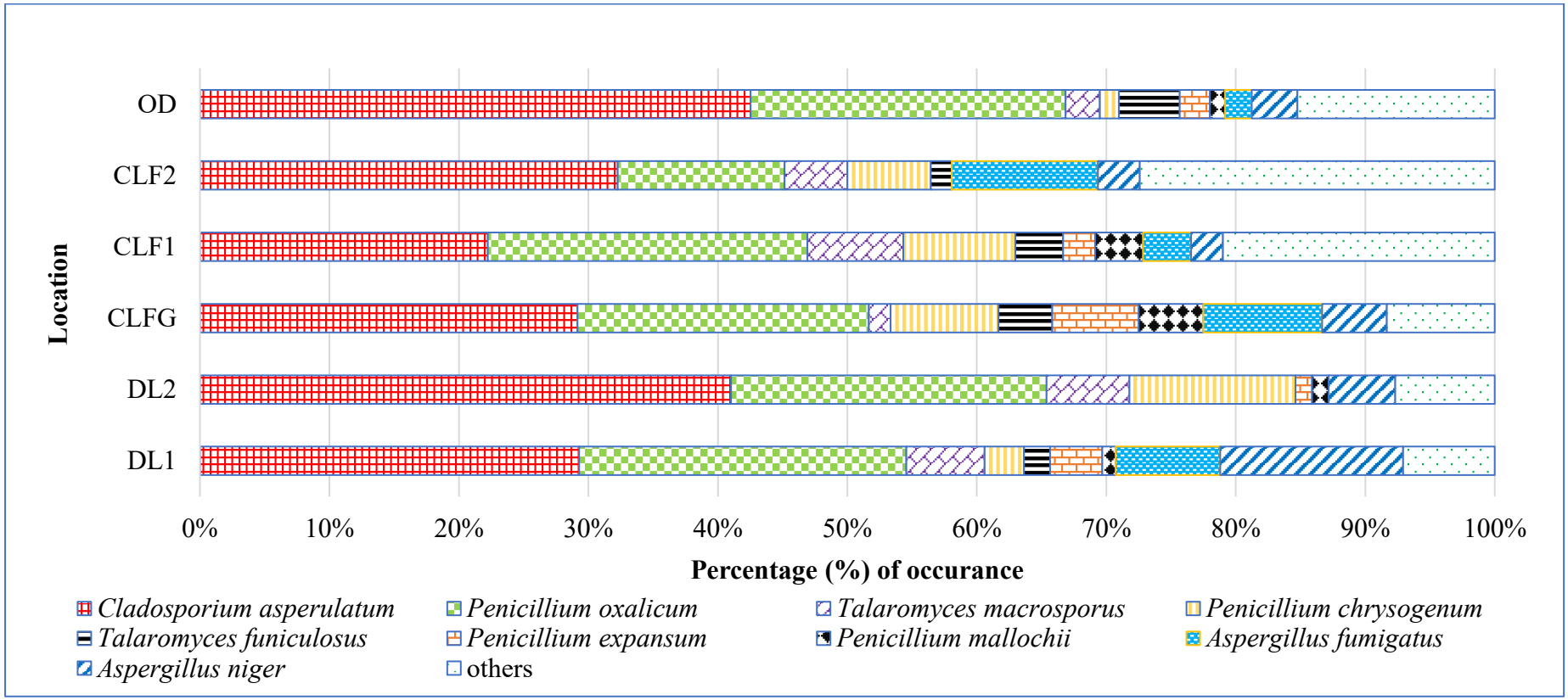


Figure 4. 12: Fungal composition on sampling locations of libraries

The similarity in fungal composition found indoor and outdoor, further confirms that outdoor air was the major source of indoor fungi (Madureira, Paciência et al. 2015). High *Aspergillus spp.* and *Penicillium spp.* may be due to the handling of books and resuspension of the fungal spores as reported by (Pyrri, Tripyla et al. 2020). The difference in observed species and their concentration levels relative to other studies, geographical locations, season of sampling and indoor microenvironmental conditions (Sen and Asan 2009, Viegas, Almeida-Silva et al. 2014). Human's occupancy levels and their health condition at sampling time also influences the load as well as the composition of microbial communities (Hospodsky, Qian et al. 2012).

4.3 Microenvironmental factors analysis

4.3.1 CO_2, T, RH

The indoor concentration of CO_2 was used as the surrogate for ventilation rate or ventilation system efficiency (Asif, Zeeshan et al. 2018). Similar increasing trend was observed in CO_2 levels over the day on all the sites (morning < afternoon < evening). However we observed weak correlation ($r = 0.17$, $P>0.05$) between the CO_2 and the number of occupants. The highest CO_2 concentration was observed at DL1 (mean: 1438 ppm). whereas lowest at CLFG (mean: 922 ppm). Although CLFG was mechanical ventilated location like CLF1 and CLF2 however CO_2 levels at CLFG were lower as compared to other monitored locations. It has been mentioned earlier that CLFG was being operated with integrated ventilation as fresh air entered indoor through door opening (that remained opened most of the time due to excessive movements), which was the major reason for lower CO_2 levels on CLFG. The number of students at CLFG, CLF1 and CLF2 were ten times higher as compared to DL1 and DL2, but lower concentrations at CL indicates the proficiency of mechanical ventilation system. Though building DL1 and DL2 both had non-centralized HVAC system, lower mean CO_2 concentration at DL2 were recorded to be lesser (as compared to DL1) due to less occupation density as compared to DL1.

Mean concentrations of T and RH measured on different sampling locations are shown in Figure 4.14 and Figure 4.15, respectively. We found a significant difference ($P < 0.05$) between the outdoor and indoor T. One way ANOVA showed no significant difference between the sites having similar ventilation system however significant difference ($P < 0.05$) of T was observed between the sites with different types of ventilation. A slightly lower T at CLFG was due to the opened doors. RH was found lower indoor in afternoon as compared to morning and evening. Similarly significant difference ($P < 0.05$) was observed over the day (in three different times of the day) in T and RH naturally ventilated buildings however in CL, RH and T showed no significant variation ($P > 0.05$) over the day. As mentioned earlier, multiple factors may result in increasing T and RH values in addition to number of occupants e.g., outdoor climatic conditions, building orientation etc. Microenvironmental data analysis revealed that thermal comfort of CL were in the recommended range (most of the times) proposed by ASHRAE standards. On the other hand, high T and RH on naturally ventilated locations shows the inefficiency of the natural ventilation system to maintain indoor thermal comfort.

4.3.2 Effect of microenvironmental factors on microbial load

Indoor and outdoor microenvironmental conditions greatly affect the microbial growth (Frankel, Bekö et al. 2012, Alghamdi, Shamy et al. 2014). Microbes start biodeterioration in suitable environmental conditions (Pinzari, Pasquariello et al. 2006). Spearman's rank correlation coefficient test was performed between environmental conditions and microbial load. CO_2 was strongly correlated ($r = 0.7$, $P < 0.05$) with bacterial concentrations at DL2. Similarly, bacterial concentration was correlated with indoor T at CLFG ($r = 0.39$, $P < 0.05$) and CLF1 ($r = 0.22$, $P < 0.05$). A weak correlation between RH and fungal levels ($r = 0.17$, $P < 0.05$) was observed at CLF1. Wang, Liu *et al.* had also reported similar findings in residential buildings (Wang, Liu et al. 2016). Overall, we did not find significant correlations between

the number of occupants and fungal load except at CLF2. Present finding is in line with previous studies reporting no significant correlation between fungal load and number of occupants (Heo, Lim et al. 2017, Wu, Zhang et al. 2020). Similarly most of the sites did not show significant correlation between indoor bacterial levels and number of occupants. Besides, significant correlation was not observed between indoor and outdoor bacterial levels. Absence of significant correlation between indoor and outdoor bacterial levels indicate that outdoor air was not the significant contributor of indoor bacterial buildup rather any indoor source e.g. humans and their dressings (Hospodsky, Qian et al. 2012, Madureira, Pereira et al. 2014, Adams, Bhangar et al. 2015). Awad *et al.* also reported that humans are the main source of indoor bacterial buildup (Awad, Saeed et al. 2018). I/O bacterial ratio for all locations was found >1, it also confirmed that outdoor air was not the significant contributor of indoor bacterial buildup rather any indoor source i.e humans and their activities. On the other hand, I/O ratios for fungal flora were ≤ 1, indicating main source of indoor fungal contamination is outdoor i.e. outdoor air (Heseltine and Rosen 2009, Madureira, Paciência et al. 2015). Similar results have been reported in previous studies (Zhai, Li et al. 2018, Wu, Zhang et al. 2020).

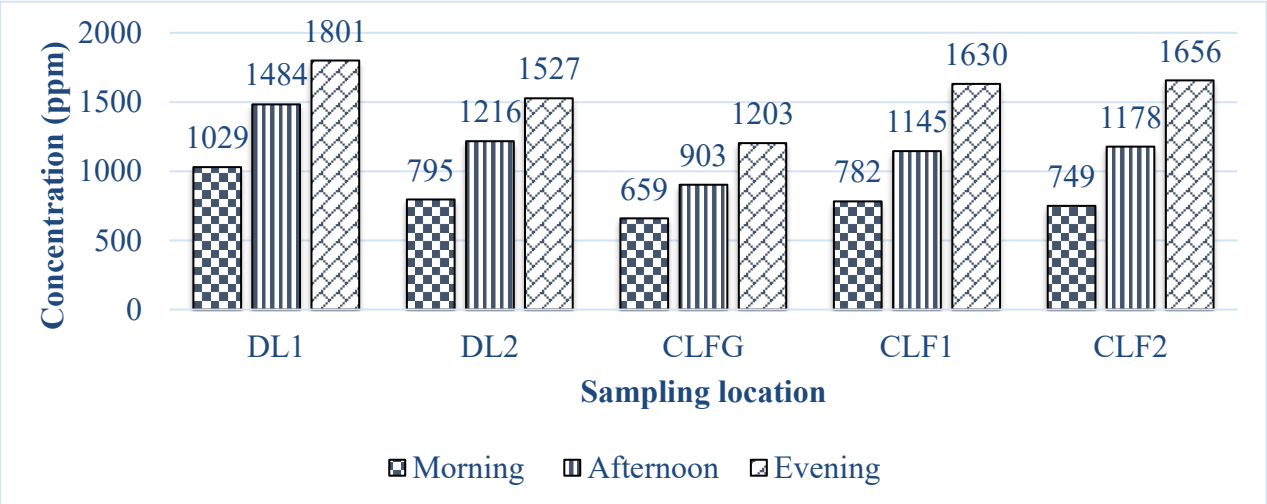


Figure 4. 13: Variation of carbon dioxide measured over the day in selected libraries

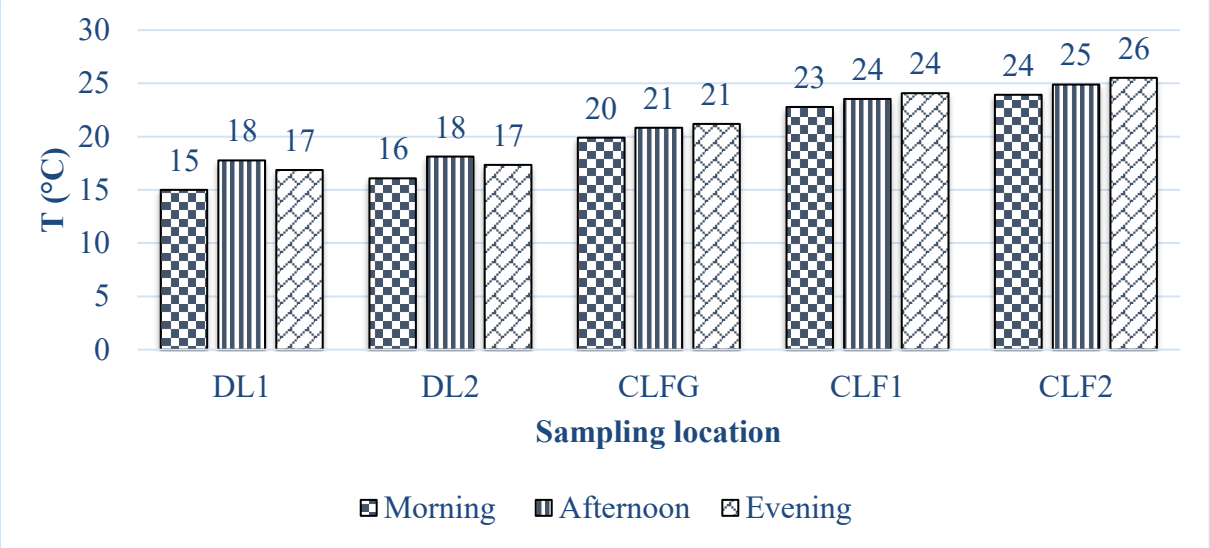


Figure 4. 14: Variation of Temperature measured over the day in selected libraries

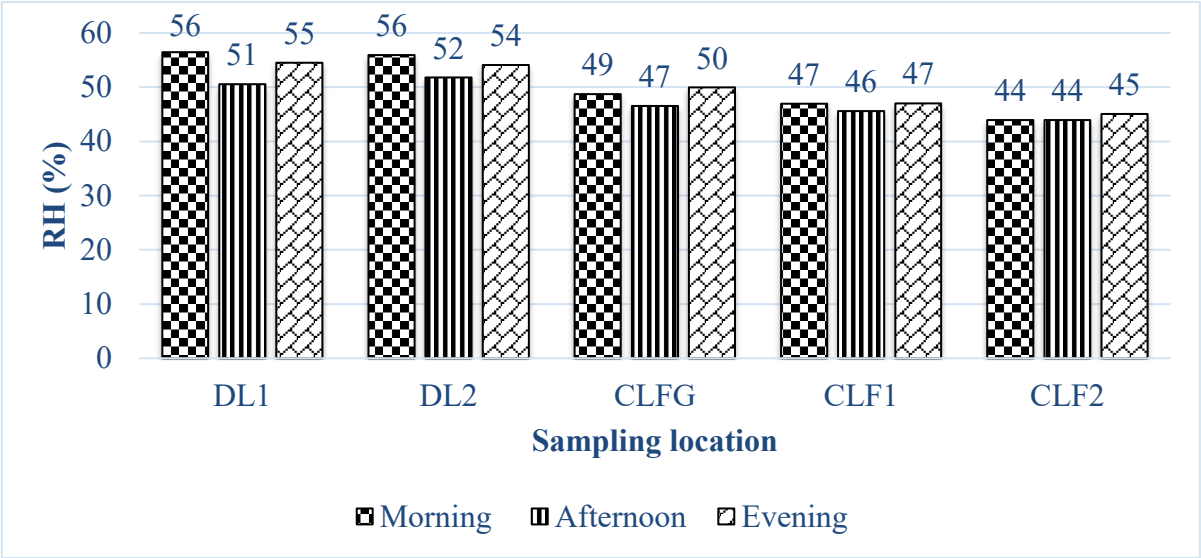


Figure 4. 15: Variation of relative humidity (RH) measured over the day in selected libraries

4.4 Comparison of libraries

4.4.1 Comparison of the ventilation systems

Ventilation of the building has a significant role in microbial IAQ management (Lugauskas and Krikŝtaponis 2004). Prefiltration of outdoor air prohibits the direct intrusion of outdoor fungal spores indoor (Borrego, Guiamet et al. 2010, Awad, Saeed et al. 2018). CL building designed with central mechanical HVAC system is supposed to be less polluted than DL1 and DL2 (naturally ventilated buildings). Thus, to assess the influence of mechanical ventilation

system, microbial levels measured in both types of environments were compared. Comparing the fungal load it was observed that fungal load was higher at CLFG than CLF1 and CLF2. It is mentioned earlier that CLFG has HVAC ventilated location, however due to excessive in/out movement entrance door almost remained opened door at CLFG allows direct infiltration of fungal spores (Kim and Kim 2007). In addition, no significant difference (p-value > 0.05) was observed in bacterial and fungal levels between naturally and mechanical ventilated locations. However, approximately 15 times higher occupancy levels were also observed in CL as compared to DL1 and DL2. Thus observed microbial levels in CL can be justified by the higher number of occupants as reported earlier (Kim and Kim 2007, Mentese, Arisoy et al. 2009, Madureira, Paciência et al. 2015, Pyrri, Tripyla et al. 2020).

4.4.2 Frequency of exceedance from microbial standards

To date, Pakistan does not have its nationally agreed standards for airborne bacteria and fungi concentrations. Thus microbial levels in libraries were compared with sanitary standards for non-industrial premises designed by the European Commission in 1993. The frequency of exceedance (%) for bacterial and fungal load observed in the specified range is enlisted in Table 4.5. Bacterial observations show that DL1 and CLFG were the most contaminated sampling locations. However, sample collected for fungi indicates that fungal load at CLF1 and CLF2 fall more in smaller range whereas at DL1 and DL2 more in the higher range. Trend observed at CLFG was unique as it was mechanical ventilated location however, it was more contaminated as compared to other locations in the same building.

Table 4. 5: Evaluation of IAQ on monitored library locations according to the sanitary standards for non-industrial premises designed by the European Commission in 1993.

	Range	DL1	DL2	CLFG	CLF1	CLF2
Bacterial Pollution	**<50**	22	22	22	11	56
	50-100	44	67	44	67	22
	100-500	33	11	33	22	22
	500-2000					
	>2000					
Fungal Pollution	**<25**					
	25-100	56	56	22	67	78
	100-500	44	44	78	33	22
	500-2000					
	>2000					

Chapter 5

Conclusion and Recommendations

▪ Hospitals Locations

A significant variation ($P < 0.05$) was observed in fungal and bacterial concentrations over sixteen different hospital locations. High variation of microbial levels in different hospital facilities indicates that indoor air quality (IAQ) mainly depends on occupancy levels, cleaning frequency and existing ventilation system of the building. OPDs and OTs were found highest and lowest polluted locations, respectively. Overall locations facilitated with centralized HVAC system having higher cleaning frequency were found less contaminated as compared to naturally ventilated locations suggesting installation of mechanical ventilation system rather than natural ventilation. *Staphylococcus* (50 %), *Micrococcus* (33 %) and *Bacillus* (10 %) were found as dominant bacterial genera whereas *Aspergillus* (75 %) and *Penicillium* (21 %) were the most dominant fungal genera. Long term exposure to these microbial species may cause the respiratory and allergic diseases, particularly *Micrococcus luteus* and *Aspergillus fumigatus* are pathogenic and hazardous for the people of risk groups i.e. patients in intensive care unit (ICU) and OTs. Thus special care is needed in sensitive locations of hospitals by increasing their hygienic condition and improving their ventilation. Regular air monitoring is recommended to ensure the healthy IAQ to avoid hospital-acquired infections and other nosocomial diseases.

- **Libraries Locations**

Outdoor fungal levels were found significantly different ($P < 0.05$) from all indoor locations with multiple times higher concentrations in outdoor however, no significant difference was observed in bacterial levels. The highest microbial levels were measured at CLFG whereas the lowest bacterial and fungal levels were observed at DL2. Compositional analysis revealed the dominancy of *Penicillium* (43.6 %), *Cladosporium* (30.5 %), and *Aspergillus* (13 %) fungal genera while *Bacillus* (35 %) *Staphylococcus* (23 %), and *Micrococcus* (18 %) were found as dominant bacterial genera. I/O ratio was found >1 for bacteria and <1 for fungi respectively, indicating source for bacterial contamination is mainly indoor whereas outdoor as the major contributor of indoor fungal buildup. Multiple times higher occupancy levels were observed in CL as compared to DL1 and DL2, however similar microbial levels on monitored locations show the proficiency of centralize HVAC system to deal with high occupancy. Present study was conducted in winter season only so picture for the rest of the seasons is unclear and seasonal effect on IAQ further needs to be evaluated.

REFRENCES