ASSESSMENT OF MICROBIAL VOLATILE ORGANIC COMPOUNDS IN INDOOR ENVIRONMENTS OF DELHI

Thesis submitted to Jawaharlal Nehru University

for the award of the Degree of

DOCTOR OF PHILOSOPHY

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JULY-2016

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CERTIFICATE

The research work embodied in this thesis entitled "**Assessment of Microbial Volatile Organic Compounds in Indoor Environments of Delhi**", has been carried out at the School of Environmental Sciences, Jawaharlal Nehru University, New Delhi. The work is original and has not been submitted in part or in full, for any other degree or diploma in the university.

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Acknowledgements

At first it becomes my duty to show my heartfelt gratitude to Dr. Arun Srivastava, my supervisor, for his invaluable advices, continuous supervision, considerable concern, helpful insights and the freedom he gave me to explore the horizons of my idea. I would like to thank him profusely for his tolerant attitude. Sir, I am really lucky to have you as my guide because without your support and guidance, I am sure I could not have made this thesis in this present format.

I would also like to thank my Doctoral Committee Members, Dr. Paulraj Rajamani and Dr.Madhav Govind for their support during my work. I am thankful to Prof. S. Mukherjee (Dean) as well as Prof. I. S. Thakur (Former Dean), School of Environmental Sciences, for providing necessary facilities.

I would like to extend my deep thanks to my wife and labmate Bipasha Ghosh for understanding me during tough times as well as for directly and indirectly becoming my supporting structure throughout my research work.

I would like to thank my labmates Rajesh Kushwaha , Sumant Kumar, Sunita, Naba Hazarika, Anshul Srivasatava, Akash Kumar, Richa Verma and Dudun Mehta for their help during sampling and manuscript writing. Special thanks to Sunita and Naba Hazarika for their valuable help in editing the thesis.

I would like to extend my deep love to my loving friends namely; Pragat Singh, Bharat, Gyanendra, Shailendra, Snehashish, and Jai Prakash. A special word of thank to all these friends whose friendship is really an achievement in my life.Special thanks to Mrs. Rashmi Sinha, and Mr. Nitin Gedam for their help during my chemical analysis in CIF.I would like to also thank Mr. Anil Sharma and Mr. Amit for incessant supply of my research chemicals.Special thanks to Kaushal kishore, Alok, Shiv, Mrs Neelam, Mr. Mahendra kumar and Kashiji, for facilitating sampling at different sites.

I am thankful to Dr.T. Prameela Devi and Dr. Deeba Kamil of Indian Type Culture Collection Identification, Division of Plant Pathology, IARI for identification of fungal species.

I am spell bound while talking about my parent's role in my life. I can only say that my research would not have been possible without their blessing and love. Therefore I owe my entire existence to them. Thanks to my sisters Samiksha and Surachita, and my brothers Vishrant and Shashank for being there behind me always, whenever, wherever I needed them. The Senior

Research Fellowship provided by the Council for Scientific and Industrial Research (CSIR), New Delhi is gratefully acknowledged.

Himanshu Lal

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INTRODUCTION

Rapidly increasing population is responsible for quick urbanization and industrialization. This increasing urbanization and industrialization have been creating many problems, especially different types of pollution in the environment (Aggrawal and Maiti, 2005). Different types of pollutants introduced as contaminants into the natural environment cause an adverse change in water, soil, air and climate.

Air pollution is known to enact on all geographical as well as temporal scales that ranged from local problems (such as damage of materials, human health along with visibility), over regional phenomena (namely deforestation and acidification) to global phenomena (such as ozone depletion, climate change), which has the capability of changing nature along with conditions for man over the next centuries throughout the earth (Fenger, 1999; Adams et al., 1982). Both outdoor and indoor air pollution was found to cause 3 million premature deaths each year (Lave et al., 1979) which has risen to nearly 7 million by 2012 that is more than double of the previous estimate (WHO, 2014). Air pollution has also been estimated to cost many billions of dollars each year (Barrett et al., 1973). Among primary pollutants such as NO_x, SO_x, CO, O_3, etc., suspended particulate matter (SPM) is one of the major air pollutants that remains virtually irregulated till date (Srivastava, 2002). SPM because of its small size (with a diameter roughly one-fiftieth of the width of the human hair) can elude body's defense mechanism and can be breathed deep into the lower lung posing a serious threat to human health (Natusch et al., 1974, EPA, 1997). According to WHO initiated project "Global Burden of Disease" in conext of fine particlulates ($PM_{2.5}$) ambient air pollution is known to cause about 1% of mortality from acute respiratory infections among children under 5 yr, 3% of mortality from cardiopulmonary disease and about 5% of mortality from cancer of the trachea, bronchus, and lung worldwide. In totality such diseases were found to amount approximately 0.8 million (1.2%) premature deaths and 6.4 million (0.5%) years of life lost (YLL) [Cohen et al., 2005].

In industrialized countries, people have been found spending majority of their time in the indoor environment. Studies carried out in different countries for time budgets have found people spending 62% of their time indoors at home, 25% indoors elsewhere, while spending only 13% of their time outdoors (Jenkins et al., 1992). According to a recent study, almost 85% of the time people stay in indoor environments such as schools, homes, office and restaurants (Yassin et al.,

2012). Such studies support the WHO report that indicates one thousand times more inhalation of indoor air pollution than outdoor air pollution by the people (ICMR, 2001). According to recent studies several respiratory illnesses including asthma have close association with indoor particulate matter (Delfino, 2002; Weisel, 2002). Women and children (found spending more time indoor in comparison to others) are the most vulnerable among all to respiratory diseases due to indoor air pollution (Abt et al., 2000; Soni et al., 2013).

Indoor air pollutants includes several gases namely carbon monoxide (CO), sulfur dioxide (SO_2), nitrogen dioxide (NO_2) along with ozone (O_3); volatile organic compounds (VOCs) and microbial VOCs; particulate matter (PM); passive smoke and bioaerosol which includes suspended bacterial and fungal spores, pollen, metabolite of microorganisms, such as aflatoxin, fragments of cell wall of bacterial cells, etc. (Srikanth et al., 2008; WHO, 2009 and 2010). Outdoor infiltration along with air purifiers like ozone generator, negative ion generator and electrostatic precipitators (generally used by the public to destroy microbes and reduce odour to get relief from numerous respiratory ailments) can be attributed to be the major sources of indoor ozone. These devices were shown to accelerate indoor ozone concentrations from 16 to 453 ppb (Peden et.al., 1995; Hubbard et al., 2005). Decrements in exercise capacity and pulmonary functions along with inflammation of airways are the after effects of acute exposure to O_3 in both healthy individuals as well as those with pre-existing respiratory diseases as for example chronic obstructive pulmonary disease, asthma, etc. (Spengler et.al., 2006; Alexis et.al. 2004).

Exposure to indoor NO_2 was found to increase asthmatic reactions to inhaled allergens (Strand et. al., 1997). According to several studies, female adults along with infants and children with existing problems of atopy and/or asthma are more vulnerable to respiratory diseases due to NO_2 exposure (Belanger et al., 2006; van Strien et al., 2004).

Sulfur dioxide, a primary product of combustion fossil fuels, have been found to form a complex mixture of distinct air pollutants on coupling with acid aerosols and particles which have been found to cause a wide range of adverse human health effects that includes both short-term respiratory morbidity and mortality (Katsouyanni et al., 1997). Kerosene heaters have been attributed to be the major source of indoor SO_2 and sulfate aerosols according to certain chamber studies (Leaderer et al., 1999). A recent study reported about 10 ppb increase in SO_2 concentration due to indoor heating sources which were found to be associated with several

respiratory symptoms including chest tightness and wheezing in non-smoking women (Triche et al., 2005).

Unvented kerosene heaters, gas appliances, along with tobacco smoking, are the major sources of indoor CO concentration. Due to its capability to prevent the oxygen binding capacity of hemoglobin, an increase in CO concentration in indoor have been associated with health impacts such as headaches, breathlessness, nausea, fatigue and dizziness followed by coma and death in the case of high exposure (Weaver et al., 2002).

The particulate matter comprises a mixture of organic and inorganic substances including aromatic hydrocarbon compounds, trace metals, nitrates, and sulphates (Maroni et al., 1995). Air pollution due to suspended particulate matter has received lot of attention because of its strong association with health effects (e.g. Dockery et al., 1993; Schwartz 1994; Pope et al., 2002; Pope and Dockery, 2006). Particulate matters present in the indoor environment are generated by indoor sources as well as outdoor origin, which has infiltrated in the indoor environment (Diapouli et al., 2011). Apart from outdoor infiltration, smoking and cooking have been found to be some specific indoor sources for fine particles while mechanical movement of people (re-suspension of dust) for coarse particles (Monn, 2001). In fact activities such as movement of people and cleaning double the concentrations of particles ranging from 5 to 10 μm (Thatcher & Layton, 1995).

According to toxicological studies, in comparison to larger or coarse mode particles, ultrafine particles were found to cause greater damage to human health (Donaldson et al., 2002; Ibald-Mulli et al., 2002). Strong associations were found to exist between day-to-day air pollution due to particulates and increased risk of wide range of adverse health effects that includes cardiopulmonary mortality as well (Gehring et al., 2006). Studies carried out for long-term exposure to combustion-related fine particulate air pollution, and its impact have demonstrated fine particulate to be an important risk factor for both cardiopulmonary as well as high mortality due to lung cancer (Pope et al., 2002).

Major sources of volatile organic compounds in an indoor environment includes building materials, various types of furnishing, several chemical compounds such as paints, cosmetics, liquid and dry cleaning agents, glues and textiles (Sterling, 1985; Samfield, Ž, 1992). Xylenes

and toluene were measured in a recently renovated indoor environment having polished, and varnished furniture, wherein furnitures were found to be the major contributors of VOCs (Srivastava et.al., 2000; Talapatra and Srivastava, 2011). Long-term emissions of volatile organic compounds from building materials can be the major reason of persistent low concentrations of these pollutants in dwellings may. VOC concentrations, even though low in indoors yet, they are found to be four times higher than outdoor levels. It is higher in new and recently renovated buildings (Brown, 2002). House characteristics such as indoor smoking, type of house and age of the building are the factors which play a decisive role in the indoor VOCs level as well as their personal exposure.

Several VOCs were studied in residential indoor as well as outdoor environment at two cities namely Asan and Seoul wherein simultaneously comparative study was carried out with personal exposure for 30 participants (Son et al., 2003). Results of this study shows that the personal VOCs exposure levels, residential indoor and outdoor VOCs concentration in Seoul were significantly higher than those in Asan. In countries such as The United States, Germany and Finland almost similar VOC exposure studies were carried out as well (Edwards et al., 2001; Hoffman et al., 2000; Wallace, 1991).

Adverse health responses due to VOCs exposure in non-industrial indoor environments include irritant effects along with systemic effects as for example difficulty in concentration and fatigue as well as carcinogenicity a form of toxic effect (Cometto et al., 2004; Pouli et al., 2003). The strongest association has been with VOCs causing mucous membrane irritation (Wolkoff et al., 2006).

Microorganisms are universally found in the earth or more precisely in the biosphere where they are usually present in large quantities as well as in diverse compositions. Air contains a significant number of microbes which vary in size and composition. In recent years, several studies were conducted focusing on characterization of naturally occurring microbiological material in the atmosphere, so-called Bioaerosol (Bauer et al., 2008). Natural sources, such as soils, lakes, animals and humans are major sources of the bioaerosol (Li and Kendrick, 1994). Bioaerosol are defined as airborne particles consisting of living organisms such as microorganisms or originating from living organisms, such as metabolites, toxins (aflatoxins) or

fragments of microorganisms (Douwes et al, 2003). Components of bioaerosol may include fungal spores, byproducts of fungal metabolism, cellular fragments and bacterial cells present in the form of a liquid, particulate or volatile organic compounds (Stetzenbach et. al., 2004).

It is well known that biogenic aerosols have a significant effect on human health, the environment, climate, atmosphere and other phenomenon (Poschl, 2005; Jaenicke et al., 2007; Ho and Duncan, 2005; Huffman et al., 2010). Research groups such as Franc and Demott (1998) and Qi and Gao (2006) and Bauer et al., (2003) have found bioaerosol to act as a cloud condensation nuclei (CCN) as well as ice nuclei (IN) as well as indirectly impacting atmospheric processes and global climate.

Majority of bioaerosol fall in the respirable size ranges, namely; 0.003 μm for viruses (Taylor, 1988), 1 to 30 μm for fungi (Gregory, 1973), 0.25 to 20 μm for bacteria (Thompson, 1981) and from 17 to 58 μm for plant pollens (Stanley and Linskin, 1974). The size distributions of bioaerosol were studied and found that it varied with regions. Particles of different sizes has been distributed into four categories according to their diameter by the US Environmental Protection Agency (EPA) such as ultrafine <0.1 μm, fine 0.1 -2.5 μm, coarse 2.5 -10 μm and super coarse > 10 μm (EPA 2004). According to studies carried out in different countries such as Sweden (Bovallius et al., 1978), The United States (Lighthat and Shaffer, 1995) as well as in Asian country such as China (Fang et al., 2004; Liu et al., 2008; Xu et al., 2011) majority of bacteria exist in coarse particles (>2.5 μm). Both density and shape of the particle plays a key role in its aerodynamic diameter. Thus along with filtration and separation, respiratory deposition is also characterized by the aerodynamic diameter of the particle (Hassan & Lau, 2009). The American Conference of Governmental Industrial Hygienists (ACGIH), the International Organization for Standardization (ISO), and the European Standards Organization (CEN) have reached agreement on definitions of the inhalable, thoracic and respirable fractions of dust particle (ACGIH, 1999; ISO, 1995; CEN, 1993; ICRP, 1994). So in occupational hygiene terms such as “inhalable”, “thoracic” and “respiratory” particles are mostly used rather than using the terms “fine” and “coarse” particles. Particles have the tendency to deposit in various parts of the human respiratory system, wherein the principal factor that determines the deposition location is the particle size. In relative to deposition concerns, ISO (1995) uses the following particle size classifications:

(a) Inhalable fraction – mass fraction of total airborne particles that can be inhaled through the nose and mouth.

(b) Thoracic fraction – mass fraction of inhaled particles that can penetrate beyond the larynx.

(c) Respirable fraction – mass fraction of inhaled particles that can reach the gas exchange region of the lung.

According to European Standardization Committee the 50% cut-off diameter for both respirable fraction and thoracic fraction are 4 μm and 10 μm respectively (CEN 1993). Out of the total airborne mass fraction, the inhalable fraction is made up of particles that are easily inhaled via both mouth and nose. Out of the particles with diameter >50 μm that enters the nose and mouth, particles with diameter >10 μm are found to be deposited on the ventilation pathway surfaces just above the trachea. Fine particles gain entry to the alveolar region of the lungs. In case of fungi, although the particle size was found to be different in various regions yet their size distribution exhibited a single peak distribution. Airborne bacteria presented a skewed distribution pattern, whereas a single-peak distribution was exhibited by the number of total airborne microbes. In addition, the airborne microbes were found primarily in coarse particles with diameters larger than 2.5 μm (Li et al., 2011).

Sources of bioaerosol in the indoor environment include floor cavities, ceiling, wall contaminated with fungus; building materials and furnishings as well as the movement of spores, cells and cell fragments through gaps in structural joints and wall openings (Srikanth et al., 2008; Ghosh et al., 2015). Although poorly ventilated indoor environment, poorly regulated temperature and higher level of relative humidity contribute to the presence as well as multiplication of airborne microbes (Hurst, 2007), yet other factors like substratum, mechanical movements, and human activities are also important factors of bioaerosol generation (Ghosh et al., 2013). Occupancy levels along with human activities within an indoor environment have also been responsible for high bioaerosol concentration (Ghosh et al., 2013). Studies have been done for microbial concentration in hospital indoor air and found that apart from number of occupants and their activity, ventilation system is also highly influencing (Ayliffe et al., 1999). Study in indoor environment reveals that indoor fungal concentration was found to be unaffected by the number of occupants, but the elevated indoor bacterial concentration in comparison to outdoor is

found to be much higher especially in the library which is due to occupancy level in indoor environment (Goh et al., 2000). Apart from human perspiration acting as a substrate source for microorganisms, generation of airborne microorganisms can also occur from body through mucus and exhalation pathways (Tekonda, 1987).

Assessment of bioaerosol solely depends upon the collection of air sample onto agar media (or any other solid) or into liquid followed by microbiological, microscopical, immunochemical or biochemical analysis (Eduard and Heederik, 1998). For the characterization and counting of bioaerosol load, two different approaches used are 'Culture-based methods' and 'non-culture methods'. Sampling of culturable bioaerosol is based on impactor (microorganism are collected directly on a culture medium), liquid impinge (collection of microorganisms are in liquid collection fluid) or air filtration methods (collection of microorganisms on a filter). After sampling bacterial and fungal colonies are grown for period of 3-7 days at defined temperature over culture media. Colonies are usually counted either by the application of image analysis techniques or manually. Proper counting of microorganisms tha can be cultured are associated with drawbacks such as selection of certain fungal or bacterial species mainly because of the use of specific culture media, poor reproducibility, temperature etc. along with the fact that cell debris, dead microorganisms as well as different components of microbes are not detected, even though they are known to possess toxic and/or allergenic properties (Fogelmark et al., 1992, 2001). Traditionally applied methods of culturable techniques have been found with little use for assesing bioaerosol exposure quantitatively. Thus, qualitative outputs are mostly produced by such techniques instead of quantitative data (Ghosh et al., 2015).

Non-culture-based methods enumerate organisms irrespective of their viability. Sampling of non-culturable bioaerosol is generally collected using liquid impinger or air filtration techniques. Fluorochrome such as acridine orange are used to stain microorganisms collected followed by counting with an epifluorescence microscope (Thorne et al., 1994). For better determination, Electron microscopy (EM) or scanning EM can also be used (Eduard et al., 1988; Karlsson and Malmberg, 1989). Simple light microscopy can also be used for counting microorganisms, wherein morphological recognition is the only basis of counting. Flow cytometric technique may be used for counting bacteria collected with liquid impingers or filters either after staining with 4′,6-diamino-2- phenylindole (DAPI) or by the application of fluorescent in situ hybridization

(FISH) [(Lange et al., 1997; Porter et al., 1997)]. The use of advanced techniques, such as immunoassays and polymerase chain reaction (PCR)-based method, have evolved into new avenues for both detection as well as speciation of organisms regardless of the fact whether they are culturable or not (An et al., 2006; Zeng et al., 2006; West et al., 2008) . For assessment of fungal biomass measurement of markers such as ergosterol can also be done using gas chromatography–mass spectrometry (GC-MS) [(Miller and Young, 1997)].

It is well known that microorganisms produce a diversity of natural compounds (usually small molecular mass substances) which were interestingly often overlooked. Lack of appropriate absorption as well as detection technologies may be the partial reason of their ignorance. Most of these small molecules (<300 Da) exhibit low boiling points, high-vapour pressures and are lipophilic in character, thereby supporting volatility. Thus with due course of time volatile organic compounds produced by fungi have also been recognsed as suitable markers of fungal growth that could be measured using chromatographic techniques (Polizzi et al., 2012; Ruzsanyi et al., 2003; Meruva et al., 2004; Dillon et al., 1996).

Studies have been done on size distribution and seasonal variation of bioaerosol concentration. Various environmental parameters have been found to affect bioaerosol load in different environments. Different environmental conditions (including temperature, relative humidity, and wind speed) influenced the different microbial categories (Qi, et al., 2014; Li, et al., 2011). Distinct seasonal variations in bioaerosol concentration have been revealed by many studies. For example, fungal bioaerosol measured at six different parts of a sewage treatment plant for four different seasons (such as monsoon, post-monsoon, winter, and pre-monsoon) of India, revealed maximum concentration in post monsoon while minimum in winter season (Maharia and Srivastava, 2015). According to a study in an occupational environment of two sanitary landfills in Finland concentrations of airborne microbes were higher in summer (Rahkonen et al., 1990), which resembled the result exhibited by other studies as well (Nielsen et al., 1997; 2000). Therefore seasonal and temporal effects on the distribution of bioaerosol should be considered for environmental exposures. Size segregated seasonal variation of bioaerosol was studied in commuting trains in northern Taiwan and found that the bacterial concentration reached its highest level in autumn, and was at the lowest level in winter whereas, the fungal concentration was highest in spring and lowest in winter (Wang et al., 2010). Many researchers revealed

highest load of airborne microbes in summer season in countries such as Canada (Montreal), Moscow, Saudi Arabia Shoubra and Siberia mainly because of weather condition which is hot (Kelly and Pady, 1954; Vlodavets and Mats, 1958; Mahdy & El-Sehrawi, 1997 and Matthias-Maser et al., 2000) . In Dublin, Ireland almost similar seasonal trend could be seen (Gorman and Fuller, 2008). However, researches have shown that with different geographical environments the seasonal patternal also varies (Lighthat and Shaffer, 1994; Jones & Harrison, 2004; Jun et al., 2006; Wei et al., 2016). As for example, although fungal airborne microbes were found at their peak concentration in the autumn at Ohio, USA, yet during the same season both bacterial and fungal bioaerosol were found at their minimum levels in the outdoor environments of Guangzhou, China (Adhikari et al. 2006; Ouyang et al. 2006). On the other hand, higher level of bacterial concentration were found in both summer and fall in Beijing, China (Fang et al. 2007).

Among the indoor air pollutants, the contribution of bioaerosol is to about 5-34% (Wanner et al., 1994; Srikanth et al., 2008). Seasonal variability of airborne bacteria were studied in different indoor environments, and most of the sites revealed significantly less bioaerosol concentration in winter than in summer except inside the room which has indoor air conditions by air conditioning system artificially in both summer and winter (Lee et al., 2012). Studies carried out in high rise residential apartments in Korea reveals that bioaerosol exposure within an apartment were highly associated with different characteristics such as apartment floor, room location, seasonal variation and summer survey period (seasonal rain-front period (SRFP) or no rain-front period (NRFP) [(Lee and Jo, 2006)].

Other than fungi and bacteria, some secondary metabolites such as endotoxins (produced by bacteria only), mycotoxins (produced by fungus only), β (1→3)-glucans and peptidoglycans are studied limited to occupational environment and less studied in residential indoor environment (Douwes et al., 1996). For the first time, exsistnce of fungal mycotoxins along with the co-occurrence of toxic bacterial metabolites was shown for indoor air samples by Taubel et al., (2011). Among the various bacterial compounds that are highly bioactive chloramphenicol was found in house dust that included settled airborne dust while nonactin, staurosporin, valinomycin and monactin were particularly detected from moist structures in building materials. These bacterial metabolites usually produced by Streptomyces sp., a group of microbes that is

eventually considered as an indicator of moisture damage in indoor environments (Täubel et al., 2011).

β (1→3)-glucans are mostly released by most higher and lower plants along with some bacteria and most of the fungi (Stone and Clarke, 1992). They are types of glucose polymers that have variable degree of branching and molecular weight (Williams, 1997). In accordance with several studies, exposure to airborne β (1→3)-glucans play a very important role in bioaerosol-induced inflammatory responses as well as other respiratory symptoms (Rylander et al., 1992; Rylander, 1996) especially when the concentration is as high as 28.1 ng/m^3 (Beijer et al., 2002). Chamber exposure studies have also reported irritant effects of dust laden with glucans (Bodin et al., 2009). In fact within nasal mucosa very little inflammatory responses were found by Sigsgaard et al. (2000) after nasal installation of β (1→3)-glucans when studied upon few garbage workers. Several researchers have reported relationship between atopy, lung function, respiratory symptoms and airway inflammation among β (1→3)-glucans exposed individuals such as paper mill workers, waste collectors along with those present in indoor environments such as day care centers, schools, office buildings etc. (Rylander et al., 1994; Rylander, 1997a,b; Wan and Li, 1999). Studies carried out upon animals by Fogelmark et al.,(1992 and 1994) revealed synergistic effect of endotoxin along with β(1→3)-glucan in causing airway inflammation thereby suggesting an enhancement in the specific IgE production due to β(1→3)-glucan.

Secondary metabolites of low molecular weight that are produced specifically by fungi and are highly toxic to both humans and animals are called fungal toxins or Mycotoxins (Bennett, 1987). Mycotoxins produced by diverse array of molds (D'Mello, 1997) possess specific and distinct chemical structures and functional groups such as hydroxyl or phenolic groups, carboxylic acids, primary and secondary amines and amides and accordingly have been classified into different groups (Nelson et al., 1983; Krough, 1984). Different fungal genera may produce the same mycotoxin while on the other hand different mycotoxins can be produced by a single fungal species. For example, ochratoxin A is known to be produced by both *Penicillium* and *Aspergillus* species while several trichothecene have been found to be produced by *S. chartarum* only (Ciegler and Bennet, 1980; D'Mello, 1997) Such variability highly depends on the species growth conditions such as temperature, humidity, etc. (Jarvis, 1990; Pasanen et al., 1993). Several studies carried out on animals have revealed that when inhaled mycotoxins such as

aflatoxins can be both immunosuppressive as well as carcinogenic (Lauria et al., 1974; Zarba et al., 1992; Jakab et al., 1994). Apart from causing liver cancer with hepatitis B acting as a co-risk factor in human with the ingestion of aflatoxins (Bruce, 1990), epidemiological study have also shown higher probability of both lung disease and cancer rates among workers of peanut processing plants on inhalation of aflatoxins as well (Burge et al., 1981). According to an another study, women exposed to mycotoxins while working in farms have found to suffer from late abortions or pre-mature delivery mainly due to both hormone like and immunotoxic effect (Kristensen et al., 2000). Although mycotoxins produced by *Penicillium, Fusarium* and *Aspergillus* genera have been reported to be associated with the respirable fraction of airborne cotton dust (Salvaggio et al., 1986) as well as corn dust (Sorenson, 1990) yet the contribution of these components to the reported respiratory symptoms among the workers are yet to be deciphered.

Endotoxin which is composed of lipopolysaccharides (LPS) a non-allergic component of cell wall of gram negative bacteria are commonly found in many outdoor and indoor environments, both residential and occupational exhibiting strong pro-inflammatory properties (Peterson et al., 1964; Douwes et al., 2000; Gehring et al., 2008). Several studies have revealed inhalation of endotoxin to be the causative agent of fever, shivering, asthma symptoms like chest tightness and dyspnea, neutrophilic airways inflammation as well as reduction in the capacity of lung diffusion (Castellan *et al.,* 1987; Clapp *et al.,* 1994; Michel *et al.,* 1996; Thorn and Rylander *et. al.,* 1998; Janssen et al., 2013). Inhalation of endotoxins at higher concentration have been found to cause organic dust toxic syndrome (ODTS) within few hours of exposure (Von Essen et al., 1990) eventually leading to chronic obstructive pulmonary disease (COPD) [Vogelzang et al., 1998].

"Respiratory diseases", "infectious diseases" and "cancer" are the three distinctive groups of diseases that are associated with airborne microbial exposure. Infectious diseases usually originate from fungi, bacteria, viruses, helminthes and protozoa that are transmitted to a susceptible host from their respective reservoirs either through direct contact or through airborne transmission. Most of the airborne-transmitted infectious diseases are occupation specific or cluster specific as for example swine influenza, Q-fever and anthrax among farmers, veterinarians; tuberculosis and measles among health workers; tularaemia among forestry workers (Driver et al., 1994) as well as winter stomach flu and influenza among aviation and

military workers (Van den Ende et al., 1998). Pontiac fever and Legionnaires disease are few other bioaerosol transmitted related infectious diseases due to both occupational and non occupational exposure to a specific gram negative bacteria legionellae that inhabit in both the natural and man-made water bodies like cooling towers, air conditioning system from which they become airborne due to active aerosolization process (Brown et al., 1999; Kool et al., 1999; Den Boer et al., 2002).

Respiratory disease symptoms usually result from occupationally related airway inflammation due to exposure to bioaerosol toxins or allergens. Non-allergic work-related asthma symptoms referred to as "asthma-like disorder or syndrome" (Chan-Yeung *et al.,* 1999) are usually found among farmers or other farm related workers mainly due to bioaerosol exposures, particularly endotoxins (Anonymous, 1998) wherein apart from cross-shift reversible decrease in lung function, chronic obstructive pulmonary disease (COPD) symptoms are also associated herewith (Vogelzang et al., 1998).

Variety of factors such as oncogenic virus, other biological agents and secondary metabolic products like mycotoxins can cause cancer. Aflatoxin and Ochratoxin are myctoxins that are considered to be human carcinogens whose exposure route is both the ingestion and inhalation as studied in industries such as animal feed processing plant or peanut processing (Sorenson et al., 1984; National Toxicology Program, 1991; Autrup et al., 1993) wherein increased risk of cancers of salivary gland, biliary tract and liver have been found (Olsen et al., 1988; Bray and Ryan, 1991).

Sick building syndrome is described as "a group of symptoms of unclear aetiology" by both European as well as United States terminology which includes mucus membrane symptoms commonly found in population related to eyes, throat, nose, dry skin, headache etc which have temporal relationship with the occupancy of any particular building and tend to improve after moving out of the building within a few hours (Burton, 1993; Burge, 2004). Increased rate of insulated buildings along with poor ventilation and building dampness resulting in elevated indoor mould exposure have been found to be associated with rhinitis and other symptoms of building related illness (Walinder *et al.,* 2001; Website for WHO Regional office for Europe, 2009; Jaakkola et al., 2013; Mendell et al., 2011). A study carried out by Skov et al. (1990) revealed that indoor climate factors highly influence the symptoms of sick building syndrome in the office environments of Denmark. According to this study factors associated with the

prevalence of SBS symptoms are number of work places in the office, the floor covering, macromolecular organic floor dust, the type of ventilation as well as the age of the building. Apart from the factors such as age of buildings (Skov & Valbjørn, 1987) and type of ventilation (Berglund, & Lindvall, 1986), other factors like amount of fleecy material in the building (Valbjorn, & Skov, 1987), temperature within the room (Jaakkola et al., 1989) as well as passive smoking (Jaakkola et al., 1989) also influence SBS symptoms.

Few epidemiological studies carried out in schools in different part of the world have shown the presence of moulds and bacterial markers within the building to be associated with rhinitis and SBS among the school children. According to a study carried out in a school in Denmark, high concentration of moulds prevalent in floor dust was found to be associated with eye and throat irritation, dizziness, headache and concentration problems among the school children (Meyer et al., 2004). Almost similar problems were found in a school building in Stockholm, Sweden, which eventually reduced (especially the eye symptoms) mainly due to the intervention to reduce dampness exposure by the installation of ventilated floors (Åhman et al., 2000). The relationship between viable airborne moulds and fungal DNA fragments present in school dust with rhinitis among school going children was deciphered by a multi-centre study carried out in five different countries in Europe (Simoni et al., 2011).

Apart from the mould and bacterial concentration, prevalence of building related illness symptoms have also been found to be associated with the concentration of volatile organic compounds (VOCs) along with the presence of carpet (from wall to wall) in buildings (Norbäck et al., 1990; Norbäck 1989). A recent study carried out in the schools of Malaysia have revealed endotoxin, ergosterol, muramic acid and fungal DNA was found in dust to be closely associated with both rhinitis and other SBS symptoms such as headache, fatigue, ocular symptoms, dermal and throat symptoms (Norbäck et. al., 2016).

Several studies have clearly revealed the release of volatile compounds by a wide range of organisms starting from microbes to higher plants with the purpose of influencing populations and communities of other organisms during interactions (Schulz and Dickschat, 2007; Baldwin 2010; Herrmann, 2010; Morath et al., 2012; Effmert et al., 2012; Kramer and Abraham, 2012). Common physical and chemical properties of such biogenic VOCs are that they are mostly

lipophilic compounds usually belonging to thiols, esters, alcohols, terpenois, aldehydes and fatty acid derivatives exhibiting high vapour pressure (0.01 kPa or higher at 20°C) and low molecular weight (<300 Da) facilitating evaporation easily at normal temperature and pressure (Pagans et al., 2006; Schulz and Dickschat, 2007). Plants are one of the most important producers of VOCs releasing up to 30,000 different VOCs that perform a wide range of functions such as mediating communication with other organisms (Baldwin, 2010; Wenke et al., 2010). There are several pieces of evidence that point out to the fact that microbial VOC play cognate roles in modifying their surrounding environment, mediating antagonism, inter and intraspecies regulation of both the cellular as well as developmental processes (Bitas et al., 2013).

Zoller and Clark (1921) were one of the earliest researchers who demonstrated the production of organic volatiles such as formic acid and butyric acid by bacteria. Several laboratory trials have demonstrated the emission of microbial volatile compounds (MVOCs) by both bacteria and fungi into their corresponding environments (Wilkins and Larsen, 1995; Sunesson et al., 1995, 1996; Korpi et al., 1998a; Kiviranta et al., 1998; Wilkins et al., 2000; Magan and Evans, 2000; Gao et al., 2002; Claeson et al., 2002). Fungi alone have been found to release approximately 250 MVOCs as a mixture of hydrocarbons such as heterocycles, alcohols, aldehydes, phenols, ketones, thioesters, benzene derivatives along with cyclohexanes (Chiron and Michelot, 2005; Ortiz-Castro et al., 2009). Both the primary and secondary metabolic pathways lead to the production of MVOCs in microorganisms (Korpi et al., 2009).

As both bacteria and fungi has been detected in several indoor environments (Ayliffe et al., 1999; Ghosh et al., 2013), although a major challenge, yet detection of certain MVOCs were proposed by several researchers in order to predict indoor moulds, specially the hidden ones (Ström et al., 1994; Wessen and Schoeps, 1996; Pasanen et al., 1997; Lorenz, 200; Keller, 2001). Ström et al., (1994) alone had investigated as many as fifteen VOCs (such as 2-methyl-1-propanol, 3-octanone, 2-octen-1-ol, 3-methylfuran, 2-methylisoborneol, 2-isopropyl-3-methoxypyrazine, 2-pentanol etc.) in several microbial infested houses, reference houses as well as outdoors produced by both bacteria and fungi with significantly higher concentration in houses complaining microbial odour. Along several ketones, MVOCs such as 3-octanol and 1-octen-3-ol have also been suggested as mould indicators by several research groups (Wilkins et al., 1997; Bjurman et al., 1997). According to the study of Elke et al. (1999) instead of 1-octen-

3-ol, alcohol such as 3-methylbutan-1-ol and ketones like heptan-2-one and hexan-2-one can act as reliable concealed mould indicators, especially in damp and moldy residential sites. Although most of the studies suggest MVOCs as mould indicators, yet researchers such as Pasanen et al. (1998) have found that instead of indicating exclusive mould infestation, presence or absence of excess moisture in building walls and materials can be precisely indicated by common MVOCs as well. Since it is well known that microbial growth rate is related to the moisture content of materials, hence MVOCs can be used as an indicator for both hidden biocontamination along with the excess moisture problems in building with the absence or presence of odour complaint.

However, there are few groups of researchers who have also raised the question as to whether detection of MVOCs can actually indicate hidden moulds or not mainly due to two problems such as presence of MVOCs in very low concentration (< 1µg/m^3) as well as the interference with similar VOCs released from other indoor sources such as furniture, paints etc. (Schleibinger et al., 2005; Kim et al., 2007). Moreover, reports also depict dependency of MVOC patterns on substratum over which moulds grow that can be any usual indoor materials apart from building materials such a wall paper, gypsum board and textile fibres (Wilkins et al., 2000; Claeson et al., 2002; Wady et al., 2003). Thus, slower growth on different indoor materials in comparison to laboratory substrates such as agar medium can be attributed to being the major reason of low emission rate of MVOCs resulting into very trace concentration in indoor air. In fact several invitro studies of MVOCs have shown that under laboratory conditions commonly found indoor moulds generate highly complex and variable profiles of VOCs that are dependent upon and vary according to the substratum, duration of incubation, species as well as other environmental parameters (Claeson and Sunesson, 2005; Matysik et al., 2008).

A very sensitive sampling and analytical methods are necessary for the determination of MVOCs as they vary in polarity and volatility and are also found at very low concentrations (µg/m^3) in air. Both the active and passive sampling of MVOCs have been carried out by several research groups using adsorption materials such as porous polymers that are usually chemically inert, thermally stable, can adsorb and desorb polar as well as non polar compounds quantitatively even at very low concentrations in various indoor and outdoor environments (Sunesson et al., 1995; Claeson et al., 2002; Matysik et al., 2008; Matysik et al., 2009; Ryan and Catherine, 2013). In an attempt to identify the best among eight the commercially available adsorbents (namely;

Chromosorb 102, Tenax TA, Carbopack B, Tenax GR, Porasil C/n-octane, Anasorb 747, Carbotrap C and Anasorb 727), Sunesson et al. (1995) studied the above mentioned characteristics by creating a test atmosphere similar to a "sick building" containing very low concentrations of compounds such as 3-octanol, 1-octen-3-ol, 2-isopropyl-3-methoxypyrazine, furfural, 2-propanol, 2- methylisoborneol, geosmin etc., each differing in polarity and volatility. Considering the amount of compound obtained at the end of sampling/analyses, the standard deviation as well as the breakthrough volume, Tenax TA was found to be the best adsorbent among all. According to Matysik et al. (2009) activated charcoal filters when used reactive and low volatile compounds (such as phenols, aldehydes, amines, etc.) due to their strong adsorption could not be recovered efficiently while MVOCs belonging to alcohols, esters, glycol ether, ether and ketone groups were adsorbed proficiently.

Solvent extraction and thermal desorption are the two most commonly used desorption technique of MVOCs from the adsorbent, where after the extracts are mostly added unto a gas chromatograph column for identification. Carbon disulfide (1ml) is mostly used as a solvent in solvent extraction technique (Elke et al., 1999; Matysik et al., 2009). Simultaneous distillation extraction (SDE) a combination of both solvent extraction and volatile distillation is one of the traditional methods was found to be inadequate in examining full MVOC profile released by *Penicillium vulpinum* in comparison to other methods sampling headspace (Larsen and Frisvad, 1995). Thermal desorption is a highly sensitive extraction technique employed so that MVOCs of trace concentrations (as low as 250 pg L^{-1}) could be sampled (Dillon et al., 2005). In this technique, for sample processing the adsorbent tubes are placed in a thermal desorber through which for 1 min 50ml of ultra high purity helium gas (>99.999%) is flushed so as to remove both air and water present within the tubes followed by 10 min of desorption purge at a high temperature of 300^0C resulting the compounds present to be carried onto a cryotrap (-10^0C) focusing all volatile organics present in the tube. Desorption purge is followed by ballistical heating of the trap to 300^0C wherein it is held for 5 min so as to load or transfer volatile organic compounds onto a gas chromatograph column (Ryan et al., 2013). In comparison to other extraction methods, such as solvent extraction wherein due to low sensitivity large volumes of sample air (approx 720L) pre concentrated is needed for lower limit detection, thermal desorption technique even with a small sample volume (approx 2.64L) delivers the best recovery

as well as detection and quantification of all target volatile organic compounds yet (Ramírez et al.,2010).

Solid phase microextraction (SPME) introduced in early nineties (Arthur and Pawliszyn, 1990) is a portable method for both adsorption and desorption of VOCs present specially in culture headspace is adsorbed onto a fibre with desorption occurring in GC injector (Jelén, 2003). In recent years, this technique have become increasingly popular because of highly reliable as well as rapid analytical capability due to combination of three into one step (i.e., extraction, concentration and introduction) in comparison to other extraction methods (Zhang and Li, 2010). Due to high sensitivity, this technique has been applied by several researchers for analyzing low dose of MVOCs (Nilsson et al., 1996; Fiedler et al., 2001; Wady et al., 2003; Wady and Larsson, 2005).

With the significant development in "separation science" gas chromatographic technique have become increasingly popular wherein gas chromatography along with mass spectrometry (with significantly high sensitive detection capability) have been used for detecting several MVOCs (Wesse´n and Schoeps, 1996; Keller et al., 1997; Matysik et al., 2009; Booth et al., 2011). MVOCs released by fungal species such as Aspergillus, Cladosporium and Penicillium were successfully detected by combining passive sampling with GC-MS after solvent extraction method using carbon disulphide by Matysik et al., (2009). Gas chromatography coupled with time of flight mass spectrometry (GC-TOFMS) was used by Wihlborg et al., (2008) to evaluate the culture headspace MVOC emission profile of *Penicillium roqueforti; Penicillium camemberti and Penicillium italicum*. Gas chromatography along with flame ionization detector (GC-FID) had been knowingly used for analysis and detection of "normal" VOCs such as toluene, benzene, xylenes, etc. (Portsmann et al., 1994; Begerow et al., 1996). Elke et al., (1996) was the first user of GC-FID to successfully analyse and detect both actively and passively sampled MVOCs (3-methylbutan-2-ol, 3-methylbutan-1-ol, pentan-2-ol, hexan-2-one, octan-3-ol, etc.) from children's room of 132 dwellings.

Although highly used, yet GC-MS has disadvantages such as the requirement of pre-concentration and chromatography as well as their incapability in determining the fine temporal variations in the profiles of the MVOCs emitted during the various stages of microbial growth.

To overcome such difficulties researchers have used proton transfer reaction-mass spectrometry (PTR-MS) which has the potential of highly sensitive detection of MVOCs without the requirements of preparing of sample or presence of chromatography (Lindinger et al., 1998). In this technique organic molecules (M) ionizes to form MH^+ due to their reactions with H_3O^+ in the gas phase, where after standard quadrupole/multiplier mass analyzer is used for detection. Although, several researchers have pointed out the potential of PTR-MS for MVOC analysis (Mayr et al., 2003; Critchey et al., 2004; Ezra et al., 2004; Mayrhofer et al., 2006) still temporal changes in the MVOC emission profiles were not addressed by the majority of these studies. Bunge et al. (2008) coupled PTR-MS with a recently developed sampling system so as to facilitate on-line monitoring of the temporal changes occurring in the MVOC profile culture headspaces of four organisms namely *Salmonella enterica, Escherichia coli, Candida tropicalis and Shigella flexneri*.

Selected Ion Flow Tube-Mass Spectrometry (SIFT-MS) is one of the recently developed rapid and sensitive technique with the potential of both real-time detection as well as quantification of trace volatile organic compounds to the lower levels of part-per-billion (ppb) without the requirement of pre-concentration (Senthilmohan et al., 2001). To characterize and identify the VOCs emitted by fungi, chemical ionization reactions of mass-selected ions are used. Scotter et al., (2005) used this technique to study MVOCs produced by five fungal species namely; *Mucor, Aspergillus, Cryptococcus, Candida and Fusarium.*

Several research groups have reported relationship between health complaints and increasing rate of dampness found in indoor environments (Bornehag et al., 2001; Engvall et al., 2002; Saijo et al., 2004) wherein exposure to microbial growth, dust mite allergens and degraded building materials emitting chemical compounds have been suggested as causative agents as in which dampness- related exposures actually cause the health problems are not known (Bornehag et al., 2004).

Association between health issues and MVOC concentrations have been sought soon after in the late nineties when Wessen and Schoeps (1996) reported higher concentration of 26 MVOCs in indoor air in comparison to outdoor released by certain microbes. Although few studies have revealed MVOCs may form a link between building related illness symptoms and dampness due to the presence of their higher concentration in buildings with damp related problems in comparison to control buildings (Wieslander et al., 2007), still such suggestions have been

countered by several other studies wherein these so-called MVOCs have been found to be released from other sources apart from microbes such as furniture, paints etc. hereby failing to strongly associate between MVOCs found and indoor mould (Konig et al., 1995; Pasanen et al., 1998; Korpi et al., 1999; Schleibinger et al., 2004; Schleibinger et al., 2005, 2008). However, in the later years researchers have successfully linked between damp related building illness symptoms and fugal VOCs (Takigawa e al., 2009; Araki et al., 2010, 2012).

Very limited field studies have been carried out linking MVOCs and human health problems in different indoor environment. Smedje et al. (1996) carried out a study among school employees and reported a significant relationship between the history of asthma complained by the employees and the presence of MVOCs such as 2-heptanone, 2 methyl-iso-borneol, 1-octen-3-ol and 2-methylfuran even though their concentrations were much lower than the recommended levels. Studies carried out in other school environments have also reported nocturnal breathlessness among the school going students (Kim et al., 2007). In the residential settings Elke et al., (1999) were the first to report a higher prevalence of wheezing, asthma, eye irritation and hay fever due to high levels of MVOCs among the children residing significantly.

Several human and animal experimental studies have been carried out in order to understand the toxicological effects of MVOCs produced by moulds such as 1-octen-3-ol; 1-pentanol and 3-methyl-1-butanol. In an animal study 50% reduction in the respiratory rate was observed along with some synergistic effects for mice due to 1-octen-3-ol as well as mild changes in liver structure and functions in rats because of 1-pentanol mainly due to the presence of the hydroxyl group (Wakabayashi et al., 1991; Korpi et al., 1999; Wålinder et al., 2005). Wålinder et al., (2005, 2008) carried out human chamber studies where MVOCs were found to cause acute effects on eyes and airways with signs of mild irritation. In the above two mentioned studies, the MVOCs and their concentration used were 1-octen-3-ol (1 mg/m^3) and 3-methylfuran (10 mg/m^3) on human study for 2 hrs while higher concentration ranging from 58 mg/m^3 to >10,000 mg/m^3 for 3- octanol; 1-octen-3-ol and 3-octanone on animal studies which were usually much higher than those measured in real indoor environments. In another animal study, necrotizing suppurative rhinitis, damage of airway epithelium with pneumonitis along with epithelial metaplasia and fibrosis in airways, was reported among rats exposed to 1000 mg/m^3 of 3-methylfuran for a duration of 1 hr (Haschek et al., 1984) wherein airway reactions were found

due to its presence in much lower concentration in epidemiological studies (Smedje et al. 1996). In fact, the median values of MVOC levels measured within residential sites usually ranged between 1-5 ng/m^3 (Elke et al., 1999; Keller et al., 2006; Matysik et al., 2009) with obvious health effects of upper airway and eye irritation even in less concentration (Korpi et al., 2009). However, the inflammatory effect due to the low level of MVOCs is highly suspicious in indoor environments (Korpi et al., 2009). Continuous exposure to a mixture of multiple MVOCs for a repeated and longer period may be attributed to being the reason behind the health effects of residential inhabitants even at lower concentrations.

Few cytotoxic studies were also carried out by research groups with few MVOCs through tissue culture assays wherein 1-octen-3-ol even at very low concentration (0.6 mM) was reported to be toxic (Kreja and Seidel, 2002a, b). In an another laboratory experiment carried out on human embryonic stem cells as well as Drosophila melanogaster model, very low concentration of 1-octen-3-ol in gas phase was also found to be neurotoxic (Inamdar et al., 2010; Inamdar et al., 2011).

Padhy and Varshney (2005) were the first to study plant VOCs among the biogenic VOCs released by numerous local plant species found in Delhi, India wherein they reported both interspecies variation along with diel ad seasonal fluctuations among the VOCs released. Total annual biogenic VOC emission from India was eventually prepared based on this report. As of indoor air pollution, different indoor environments such as central library at Bhabha Atomic Research Centre, Mumbai, a laboratory, a kitchen where a kerosene stove was used for cooking, office rooms where the employees smoke and rooms without any apparently visual source of VOCs were selected where study of VOC profile were carried out by Srivastava et al. (2000). As of now no single study has been reported to be carried out in indoor environment.

In the light of the above discussions and literatures reviews which have been carried out by various researchers and/or organizations so far, the present investigation was attempted to observe the characterization of MVOC's, which has not been carried out by this time on Delhi environment. Keeping in mind, the importance of the role of MVOC's in the sensitive environments with annual variation, the present study has been undertaken with the following objectives:

- Identification, quantification and seasonal variation of microbial volatile organic compounds present in different indoor environments.
- Identification of fungal and bacterial VOCs from surface dust and building material.
- Identification of species specific fungal VOCs from Surface dust and Building materials.
- Characterization and seasonal variation of bioaerosol in different indoor environments.
- Relationship of MVOCs and bioaerosol with meteorological parameters.

MATERIALS AND METHODS

2.1. Study Area

Delhi (also known as the National Capital Territory of India), is a metropolitan region of India. The capital is known as India's second-most-populous city after Mumbai, and the largest city in terms of the total area. The city occupies a population of 22 million in according to the census 2011. Meanwhile, the city is also known as the fourth largest city in the world. Delhi is located at 28.61°N 77.23°E, and it lies in Northern India. The National Capital Territory of Delhi covers an area of 1,484 km^2; where 783 km^2 is designated rural and 700 km^2 urban therefore making it the largest city in terms of area in the country. It has a length of approximately 52 km and a width of 49 km. It borders the Indian states of Haryana in the north, west and south of Uttar Pradesh (UP) in the east. Delhi features can be characterized as humid subtropical climate. The warm season lasts from April 9 to July 8 with daily average higher temperature above 36°C. The average annual rainfall is approximately 714 mm (28.1 inch), most of which falls during the monsoon in July and August.

2.2. Sampling sites

Sampling was carried out at six different sites in Delhi (Figure 2.1). Sampling sites were selected on the basis of on site survey carried out previously. The six indoor sampling sites are located in Delhi. Six different indoor environments were selected on the basis of different characteristics such as ventilation, age of building, location of building, moisture affected buildings and different types of surrounding environments such as commercial, residential, industrial, institutional and semi urban.

The six sites are as follows:

- Central Library, JNU (Institutional)
- Ghaziabad (Residential)
- Loknayakpuram (Semi urban)
- Mukherjee Nagar (Residential)
- Palam (Residential)

- Okhla (Industrial)

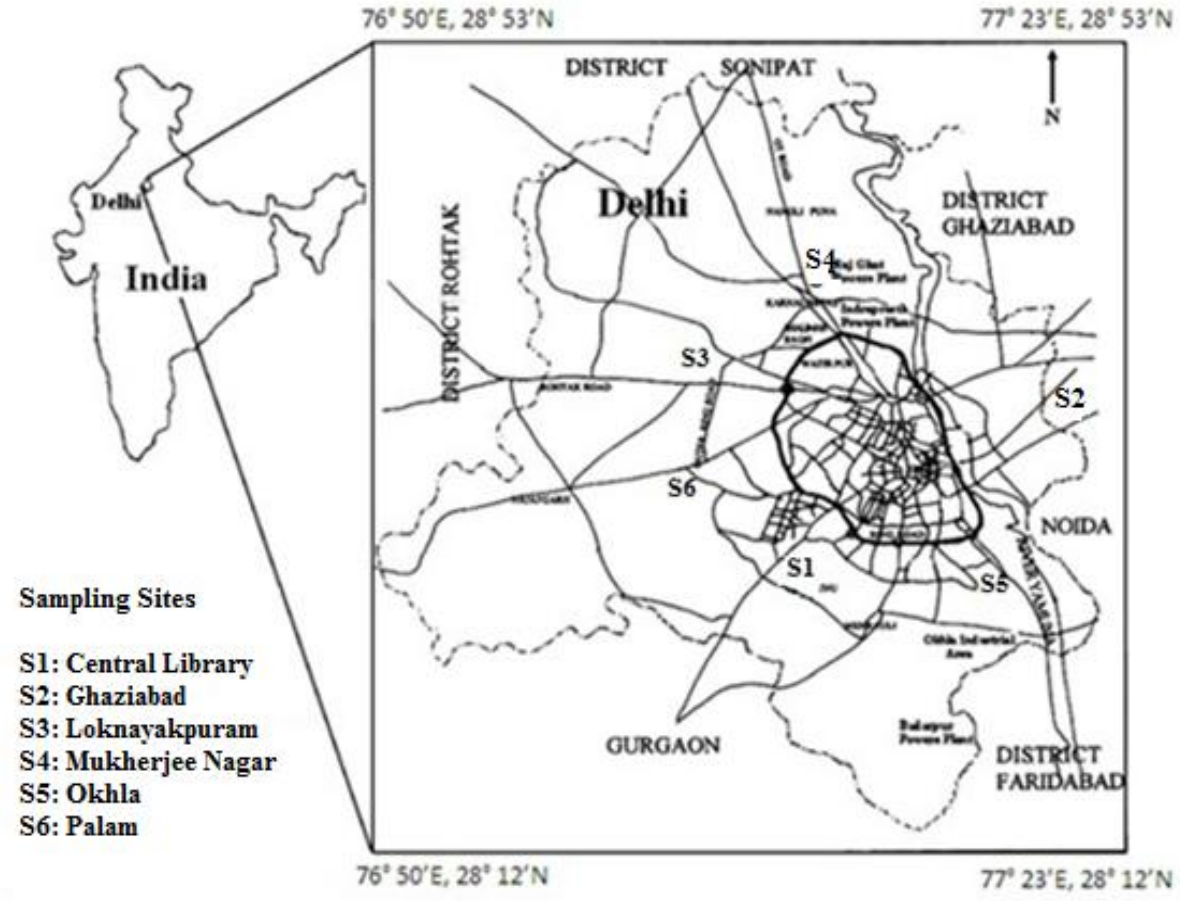


Figure 2.1: Diagrammatic representation of the sampling sites in Delhi.

2.2.1. JNU (Institutional site)

Central Library (Site 1): Central Library of JNU is situated in the campus. The Campus is situated in the bush forest on the Aravalli Hill ridge in the southern part of Delhi. The site is also surrounded by dense and/or scrubby vegetation. The central Library of JNU is a nine story building of 43 years old with a wider basement below and a huge partitioning hall with two floors which is made of metal frames and wooden planks. The area occupies about 12 small windows, out of which 5 remain open during working hours of the day. Ventilation of basement is natural, but the ventilation rate is very poor. It consists of wooden and several metal racks storing books related to the subjects of Social Sciences as well as

collection of Periodicals etc. Settled dust on books, periodicals and corners of wooden book racks and building material from moisture damaged wall were collected for analysis.

2.2.2. Ghaziabad (Residential site)

Residential Site (Site 4): This residential site is a part of a colony named, "Siddharth Niketan" in Kaushambi, Ghaziabad, is situated in extreme east of the Delhi, with a close vicinity of a huge dumping site around 400m as well as a busy highway (NH-24). Samples were collected from ground floor of the building which is 28 years old. The building is comparatively well ventilated in comparison to other sites. The wall of the building is made up of concrete, covered with plaster with no use of bricks. Common wall of bathroom and hall is affected by moisture facilitating mold growth on the plaster. Building material sample from the mold infected wall and surface dust was collected for analysis.

2.2.3. Loknayakpuram (Semi-urban site)

Residential Site (Site 3): This residential site is also a part of Loknayakpuram, a planned residential colony build by DDA (Delhi Development Authority) in 2006 in the western outskirts of Delhi. Samples were collected from third floor of the building which is 9 years old. The building is moderate to low ventilated with very less feasibility of cross ventilation. The wall of the building is made up of concrete, covered with plaster with no use of bricks. Colony is surrounded by agricultural field from two sides. Dust that accumulated for a long period at the corners of wooden furniture was sampled for analysis.

2.2.4. Mukherjee Nagar (Residential site)

Men's Hostel (Site 2): This site is a privately built men's hostel in a congested residential area in Mukherjee Nagar in North of Delhi. The room of this newly build building (approximately 4 years) is occupied by only one person. Samples were collected from ground floor of the building. No sunlight reaches the room. Humidity of the room is unusually high due to attached bathroom and kitchen with very poor ventilation. The only source of ventilation is a small exhaust fan fitted in the bathroom. Common wall of bathroom and room is affected by moisture coming from bathroom. Undisturbed dust accumulated at the corners of the wooden table and building material from moisture laden wall was collected for analysis.

2.2.5. Okhla (Industrial area)

Residential (Site 5): Okhla is a Suburban colony in South Delhi district located at Delhi border. It is more commonly known as Okhla Industrial Area (OIA) or Okhla Industrial Estate, an industrial suburb in South Delhi. Sampling site is a residential house near the busy main road. Indoor sampling site is occupied by only one person with attached bathroom and kitchen.

2.2.6. Palam (Residential site)

Residential (Site 6): It is a major suburb (residential colony) in southwest Delhi. The residential indoor environment of Palam is less ventilated. Dust and building materials were collected from third floor of the building. Room is equipped with balcony and a window. In summers an air cooler was used on window and in winters it remains closed for two to three months. In other seasons window remain open. The room is naturally ventilated. The wall of two sides of this room has direct contact with atmosphere and affected by rain in monsoon season.

2.3. Sampling of bioaerosol

Bioaerosol samples were collected using Anderson six stage viable cascade impactor, manufactured by Tisch Environmental USA (figure 2.2 and 2.3). Samplings were carried out for two different fractions of bioaerosols viz. bacteria and fungi during the period July 2014 to June 2015. According to Indian Meteorological Department (IMD), these twelve months (July-June) covers all the four seasons of India i.e. Winter season: January- February, Pre-Monsoon Season: March-May (Summer), Southwest Monsoon Season: June-September (monsoon) Post-Monsoon Season: October-December.

Figure 2.2: Anderson six-stage viable bioaerosol sampler

Figure 2.3: Six stages of the bioaerosol sampler

The sampling was carried out by a cascade impactor which consists of several impaction stages arranged in a stack. Each stage contains one or more nozzles and a target and/or substrate. Air stream passes through the nozzles wherein particles greater in size than an aerodynamic diameter is impacted onto a collection surface, while smaller size range of particle proceed through other stages (figure 2.4). Growth media such as Potato Dextrose Agar (PDA) for fungi and Tryptone Soya Agar (TSA) contained in petridishes can act as the target. Each succeeding stage collects smaller particles. The details of nozzle diameter and particle diameter for six stage impactor are given in Table 2.1.

Table 2.1: Dimensions of jet orifice and range of particles for each stage

Stages	Orifice diameter (in. mm)	Particle size range (in. µm)
1	1.18	>7.0
2	0.91	4.7-7.0
3	0.71	3.3-4.7
4	0.53	2.1-3.3
5	0.34	1.1-2.1
6	0.25	0.65-1.1

[Source: operations manual of Cascade Impactor Series 10-8XX Viable (Microbial) Particle Sizing Instruments, Tisch Environmental, Inc]

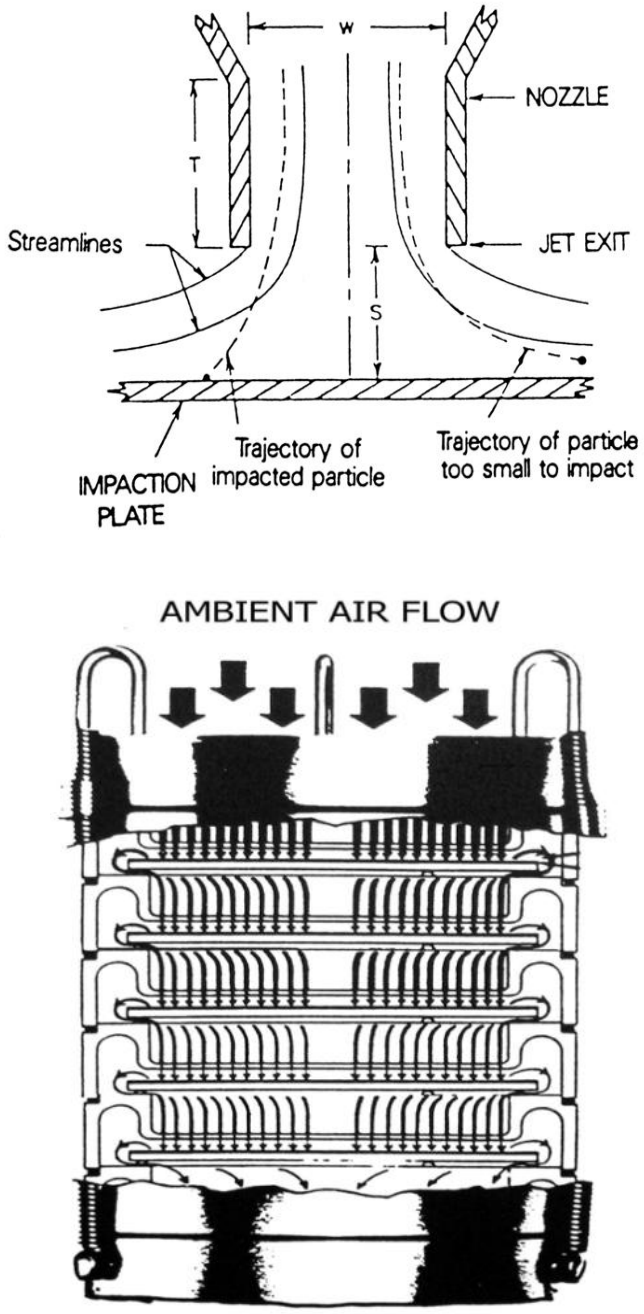


Figure 2.4: Schematic of impactor stages of bioaerosol sampler

(Source: operations manual of Cascade Impactor Series 10-8XX Viable (Microbial) Particle Sizing Instruments, Tisch Environmental, Inc)

Design of the sampler, “Tisch Viable” simulated the human respiratory system which is designed in accordance to aerodynamic system of airborne particulates. The device can also be used for the respiratory tract as a substitute and as a collector of airborne particles; as such, it might reproduce to a reasonable degree of the lung penetration through these particles. The fractions of particles which are inhaled retained in our respiratory system and the deposition site varies with other physical properties (e.g., density, size, shape) of particles. Due to the lung penetrability nature of particles of unit density is known and since the sizes of particles that are collected on “Tisch Viable Sampler” of each stage have been determined, if an authentic model of these types of samplers is used in accordance to recommended operating procedures, then the extent to which the collected materials would penetrate the human respiratory system could be easily indicated. Deposition of large particles usually occur in the nasal-pharyngeal area, while sub-micrometer sized particles mostly deposit in the pulmonary area (Figure 2.5 and 2.6).

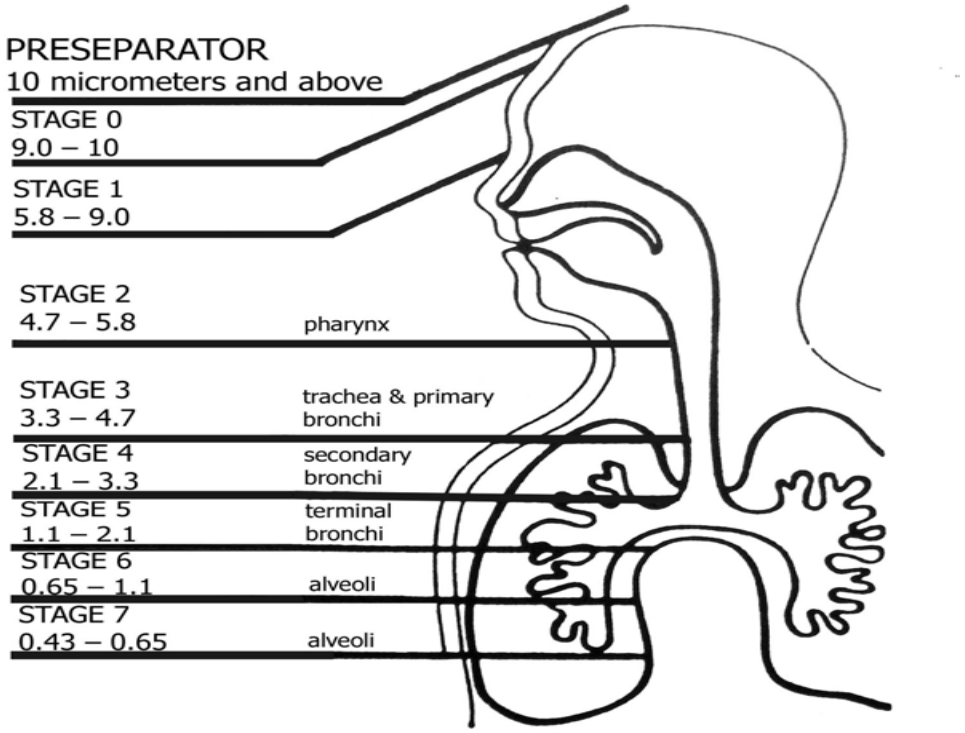


Figure 2.5: Bioaerosol sampler simulating the human respiratory system

(Source: operations manual of Cascade Impactor Series 10-8XX Viable (Microbial) Particle Sizing Instruments, Tisch Environmental, Inc

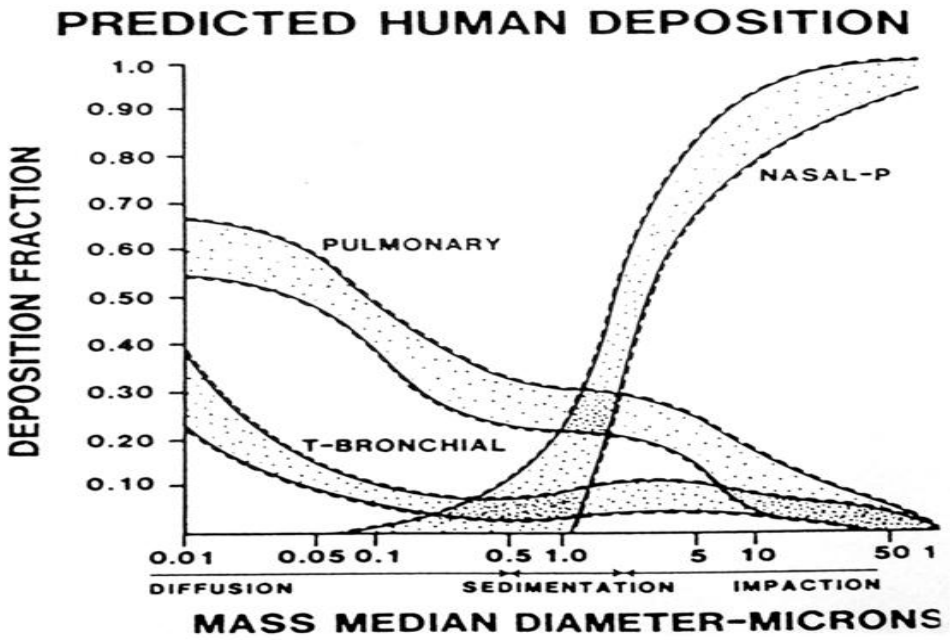


Figure 2.6: Graphical representation of predicted deposition in human respiratory system

(Source: operations manual of Cascade Impactor Series 10-8XX Viable (Microbial) Particle Sizing Instruments, Tisch Environmental, Inc)

Each bioaerosols samples were collected at the rate of 28.3l/min for two minutes with the help of a pump. Fungal fraction was collected over the Potato dextrose agar media (Figure 2.7), which is a common media of microbiological made of potato infusion, and the dextrose (sugar of corn). PDA is known to promote fungal growth due to the presence of carbohydrate and Potato infusion, while presence of antibiotic and low pH restricts the bacterial growth. Over TSA Agar, bacterial fraction of bioaerosol was sampled (Figure 2.8).

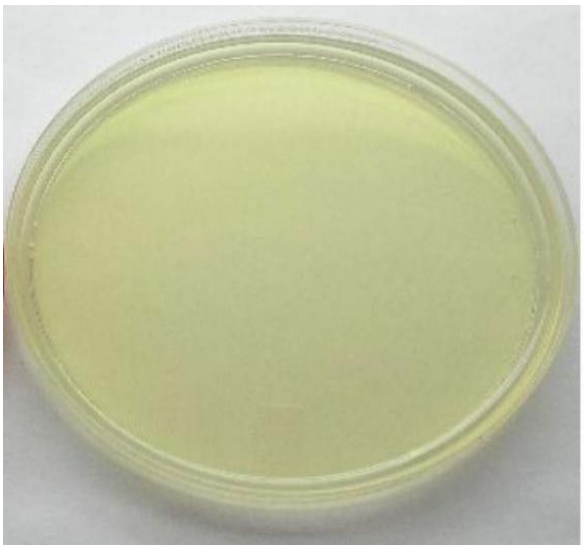


Figure 2.7: Potato dextrose agar media.

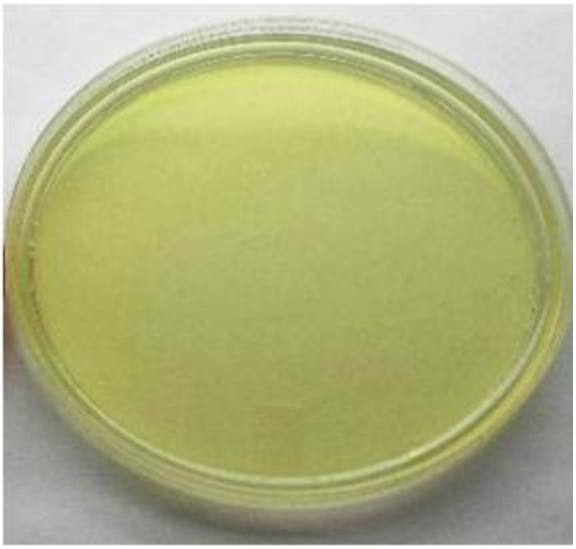


Figure 2.8: TSA agar media

2.4. Characterization of bioaerosol

After sampling inoculated agar plates of were placed in incubator at appropriate range of temperature (for fungus 25-28^{0}C and about 30-35^{0}C for bacteria) to develop into a visible colony. After three to five days visible colonies of fungus and bacteria were observed

macroscopically on a solid culture medium of PDA and TSA respectively. A digital colony counter was used for counting of the visible colonies.

Concentration (in cfu/m^3) of culturable microorganisms is being calculated by dividing the volume of air sampled from the colonies (total number considered) which were observed onto the plate. Normally, concentrations of culturable bioaerosol are reported as colony forming unit (cfu) per unit air sampled volume. Cfu represents the microorganism's number that can replicate so as to form colonies, as is determined by the number of colonies which is developed.

$$Bioaerosol\ Concentration\ (cfu/m^3) = \frac{\text{No. of colonies}}{\text{Flow rate} \times \text{sampling duration (minutes)}}$$

For identification microscopic examination will be done. Identification will be done comparing the fungal spore of the samples with the existing result viz. published papers, available literature and images available on the internet. For identification till species level pure cultures will be sent to the Plant Pathology Department, IARI, New Delhi.

2.4.1. Positive Hole Correction

Anderson provided a positive hole correction or conversion table for use with 400 hole inpactor. The table was based on principle that as the number of viable particle impinging on a plate increased, the probability of particle entering unoccupied hole decreased. Values shown in the table were calculated from the following formula:

$$Pr = N[\frac{1}{\text{N}} + \frac{1}{N-1} + \frac{1}{N-2} + \cdots + \frac{1}{N-r+1}$$

Where Pr is the expected number of viable particle required to produce r positive holes and N is the total number of holes per stage. Positive hole correction is not needed for stage 1and 2 because the deposition of particle does not follow the jet pattern (Macher 1989).

2.4.2. Laboratory Media Blanks

Unexposed PDA and TSA blank media petriplates were prepared in the laboratory. These petridishes were not carried out into the field for sampling. In order to check the sterility of the media, incubation of blank culture plates was done under the same conditions as prepared for the field samples before the sampling of bioaerosol.

2.4.3. Field Blanks

Field blanks were simply unopened plates of fresh media that included labeling similar to those used in the fields without drawing air unto them through the sampler. A field blank was prepared for both bacterial and fungal bioaerosol for each site.

2.4.4. Limitations of bioaerosol study

In problematic atmospheres, methods of bioaerosol collection are "grab sample" technique which represents an approxinate transient microbial concentration. It is not possible to ascertain the involvement of bioaerosol due to the nature of time dependency of cultivated samples and enumeration of colonies in the subsequent. This method, thus pertain far to culturable microorganisms. Microorganisms, that are injured or stressed either by the bioaerosol sampling procedures or environmental conditions may be viable, but may not be culturable (McFeters et al. 1982). In laboratory culture, certain species may be too fastidious to grow. As for examples, bioaerosols of some species (e.g., Legionella pneumophila, Pneumocystis carinii or Histoplasma capsulatum) if not impossible, are generally very difficult to collect and culture (Ibach et al. 1954; Dennis 1990).

2.5. Sampling and analytical detection of MVOCs

MVOC measurement was conducted using active sampling method for indoor environment. Air samples were collected with the help of a pump at flow rate of 200 ml/min on activated charcoal tubes ORBO™ 90 Carboxen® 564 (Figure 2.9). After sampling of MVOCs on adsorbent material further analysis were proceed by desorption of MVOCs from adsorbing material by method of solvent extraction. After sampling, the sorbent is extracted with 1 mL of carbon disulfide and then 1 μL of the extract is analysed in GC–FID (Elke et al., 1999). Six MVOCs (1-

pentanol, 1-octen-3-ol, 2- Hexanone, 1-Heptanone, 3-Octanone, 1-Hexene) was identified and quantified using Gas chromatography- Flame Ionisation Detector (GC-FID).

Figure 2.9: ORBO™ 90 Carboxen® 564 charcoal tube used for sampling of MVOCs

2.5.1. Surface dust and building material samples

Settled Surface dust samples in indoor environment were collected with the help of sterilized cotton buds and small portion of wall material from moisture damage wall were collected as building material sample. Dust samples were often taken from shelves, windows, books and wooden furniture. Building samples were collected from walls with special emphasis on the location of visible growth of molds.

2.5.2. Fungal species isolated from surface dust and building materials

Fungal strains extraction from surface dust and building material was carried out by stirring the collected samples for one hour in 50 ml physiological sterile solution (i.e., 0.85 % NaCl, 0.5 % Tween 80) followed by inoculation on the PDA media with 0.5 ml of this solution.

This process helps in the detection of contaminations of fungi which are hidden on the inner-side of the building material, which may be the hidden source of MVOCs.

2.5.3. Passive sampling above pure cultures for MVOCs

For passive sampling of MVOCs, charcoal tubes ORBO™ 90 Carboxen® 564 from Sigma-Aldrich was used. Mixed culture of microorganism present in dust and building material were prepared by stirring the samples for an hour in 100 ml sterile physiological solution (0.85 % NaCl, 0.5 % Tween 80) that was diluted five times by a factor of 10 consecutively and 200 µl of each of them were used to inoculate PDA and TSA media for fungus and bacteria respectively for all samples of dust and building material. The charcoal tubes ORBO™ 90 tube were exposed to the mould culture inside the flask just few cm above the culture (Figure 2.10). To prevent contamination the flasks were closed with sterile cotton plugs which allowed air exchange. Flasks were stored at room temperature. In the same way, one flask was prepared, without inoculation that represented a blank sample. The charcoal tubes were removed after eight days from the flasks which were then closed tightly and stored as well at -20^0C till the analysis (Matysik et al., 2009).

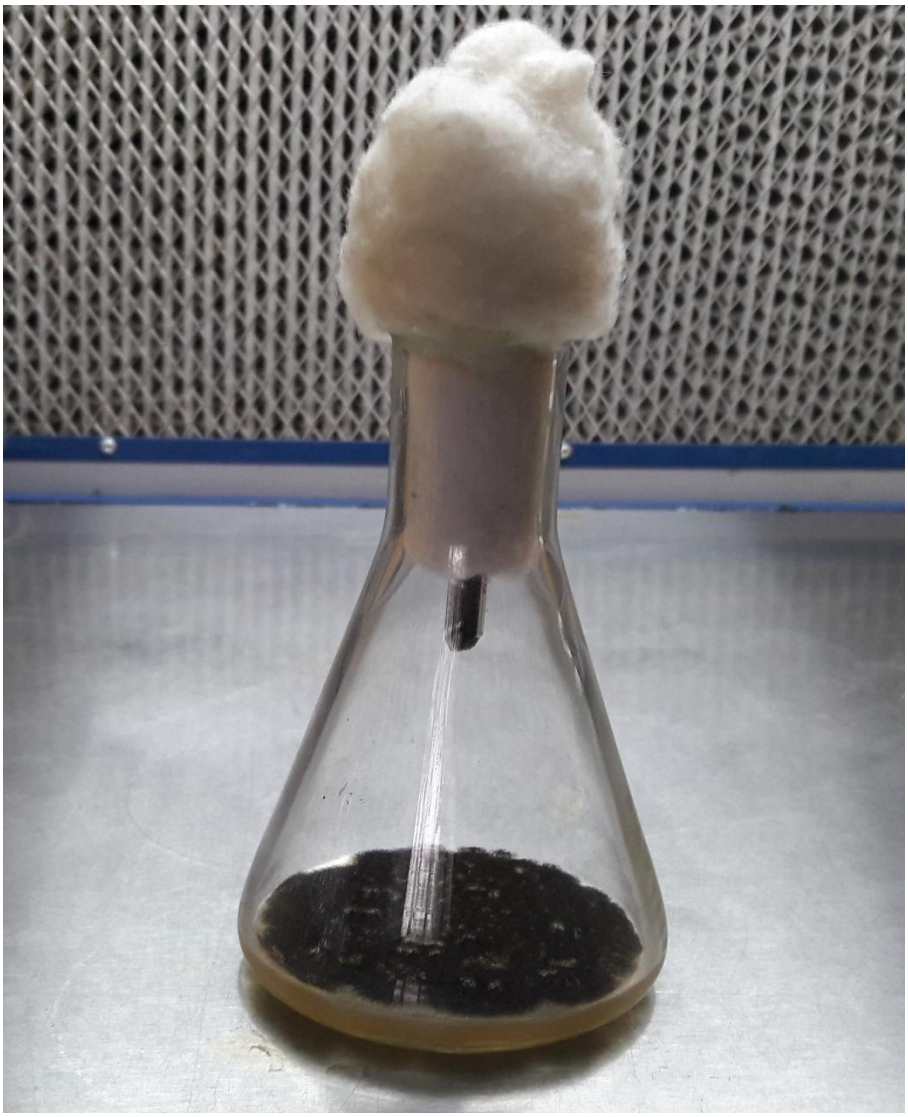


Figure 2.10: Experimental setup for passive sampling of MVOCs released from pure fungal culture

2.5.4. Preparation of sample for MVOC analysis

From the charcoal tubes, MVOCs were desorbed (activated charcoal pads) using 1 ml carbon disulfide (CS_2) in a vial. The vials were mechanically shaken on vortex shaker during the extraction period of 30 min (Matysik et al., 2009). Afterwards, for analysis through the Gas Chromatography-Flame Ionization Detector (GC–FID) technique, into the GC vials, the extract was decanted.

2.5.5. Analyses through GC–FID

MVOC Analysis was carried out with "SHIMADZU GC-2010 Plus High-end Gas Chromatograph". For separation a *SUPELCO* Equity®-5 Capillary fused silica GC Column (Equity®-5 Capillary GC Column L × I.D. 30 m × 0.20 mm, df 0.50 µm Bonded; poly (5% diphenyl / 95% dimethyl siloxane) phase, non-polar) with nitrogen as carrier gas was used. Column was connected to a Flame Ionization Detector (FID) which was held at 330 ºC. The following temperature program was used for column: 38 ºC for 5 min, ramp at 4 ºCmin^{-1} to 200 ºC, which was held for 30 min (Elke et al., 1999).

2.5.6. Data analysis and calibration

The six selected MVOCs standards: 1-Octen-3-ol, 3-Octanone, 1-Hexene, 3-Methyl-1-butanol, Cyclohexanone, 1-pentanol, were purchased from TCI Chemicals (India) Pvt. Ltd. Calibration was performed with a set of four standard solutions of concentration 0.1, 0.5, 1.0, 1.5µg/ml of each MVOC. A blank solution (CS_2) was run before and after every five samples (figure 2.11). Retention time of six selected MVOCs is given in table 2.2 and their chromatogram presented in figure 2.12. Chromatograms of blank tube as well as blank PDA and TSA media are also shown in figures 2.13, 2.14 and 2.15 respectively.

Table 2.2: List of selected Microbial Volatile Organic Compounds and their Retention time (Rt)

MVOCs	Retention time (Rt)	Codes
1-Octen-3-ol	22.512	1
3-Octanone	22.7	2
1-Hexene,	5.3	3
3-Methyl-1-butanol	10.576	4
Cyclohexanone	18.367	5
1-Pentanol	11	6

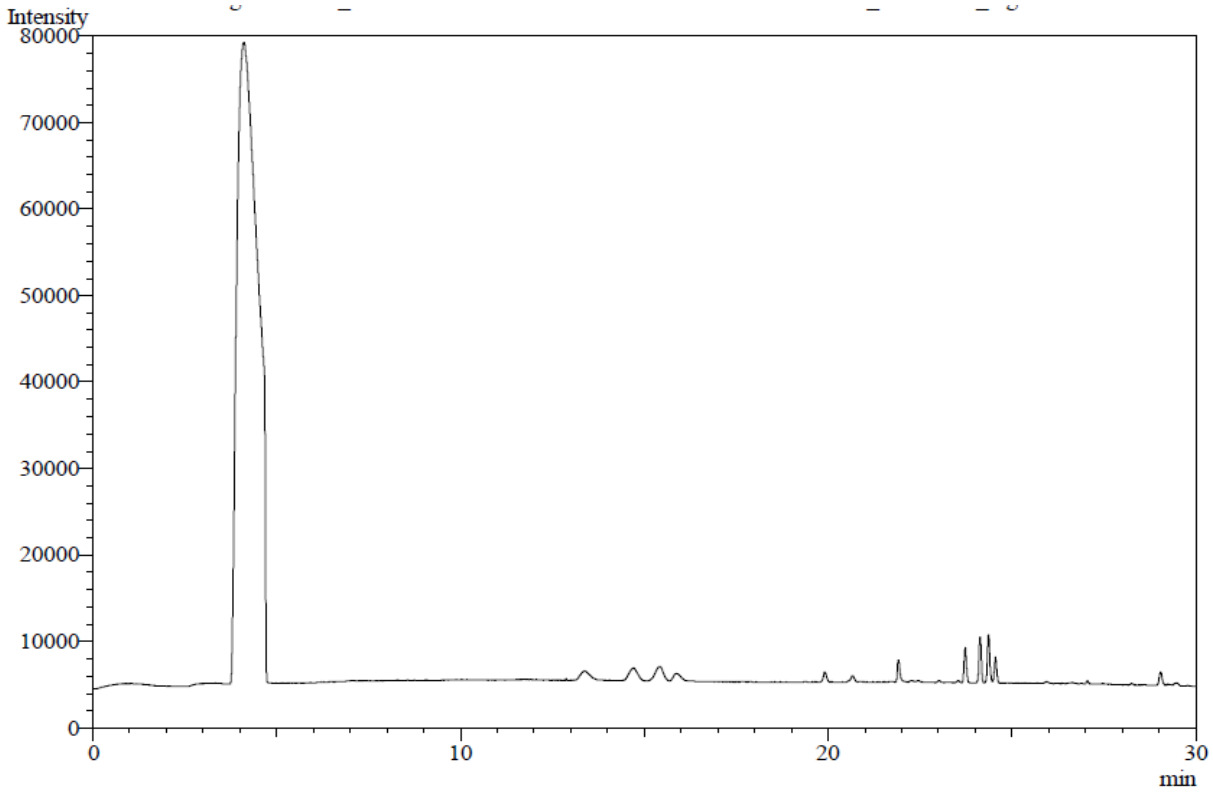


Figure 2.11: Chromatogram of blank carbon disulfide (CS_2)

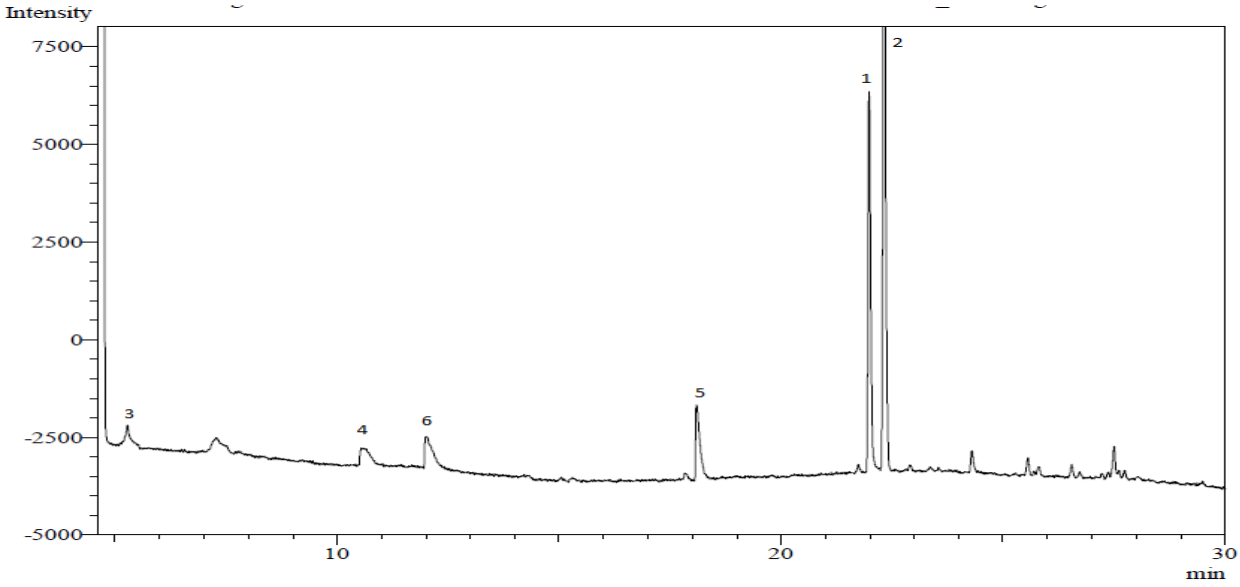


Figure 2.12: Chromatogram of all the six MVOCs namely (1) 1-octen-3-ol, (2) 3-octanone, (3) 1-hexene, (4) 3-methyl-1-butanol, (5) cyclohexanone and (6) 1-pentanol

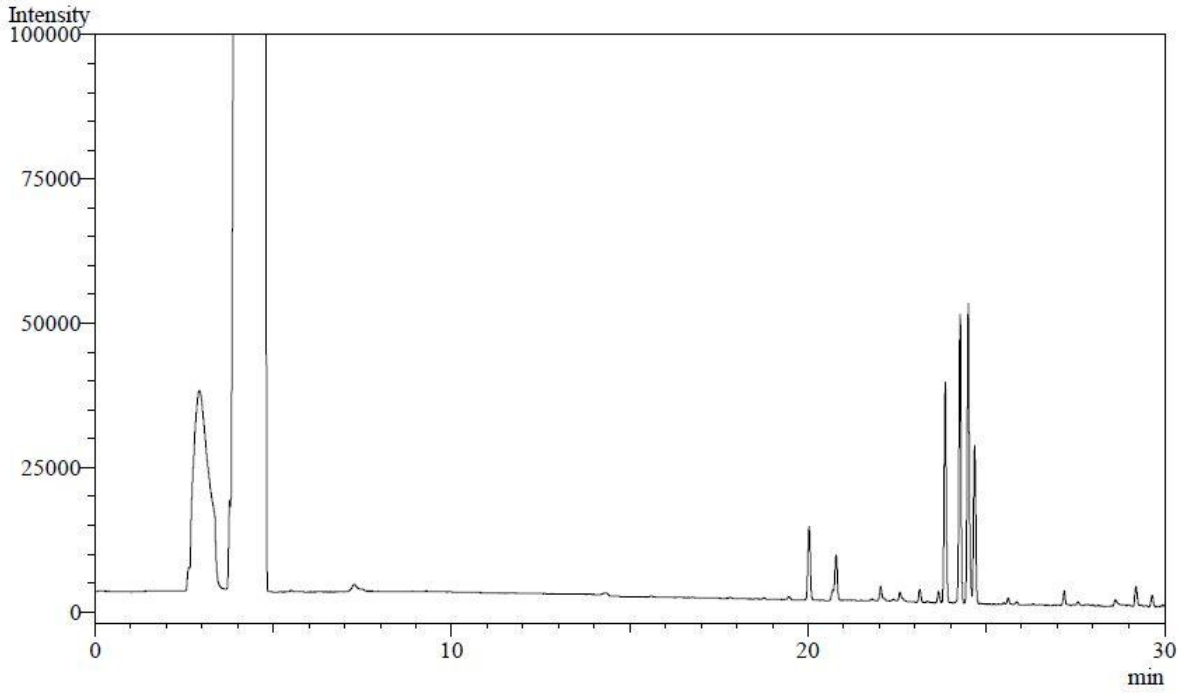


Figure 2.13: Chromatogram of blank tube

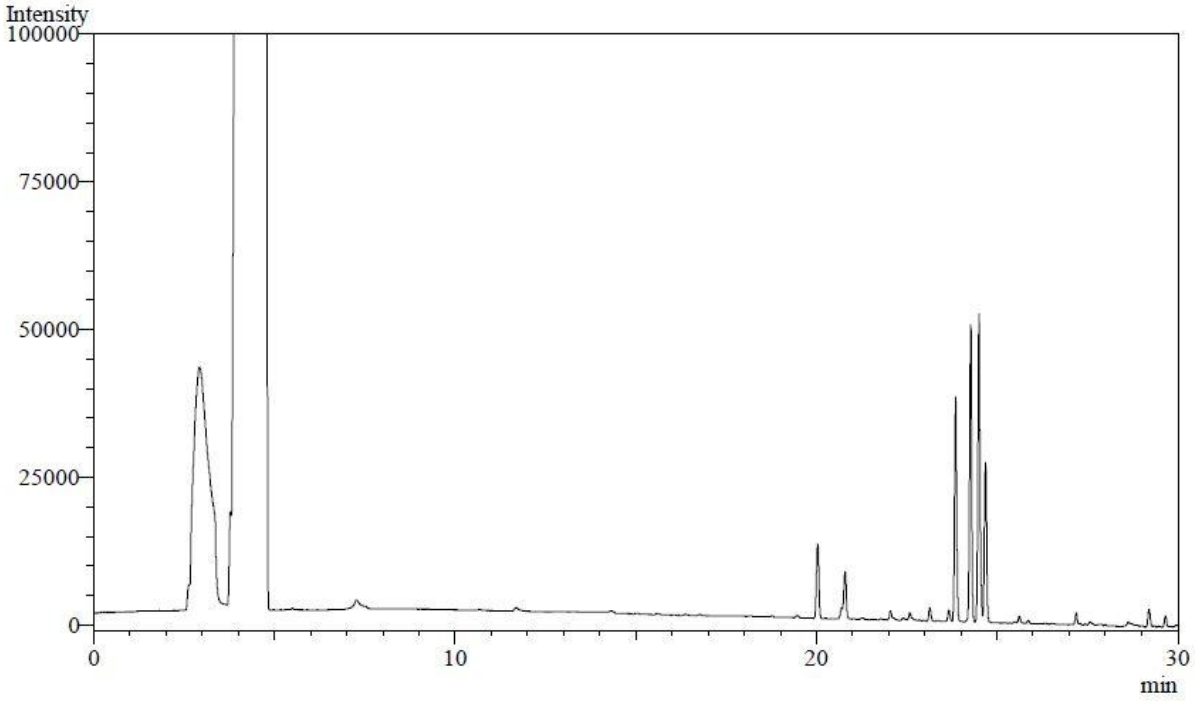


Figure 2.14: Chromatogram of blank PDA media

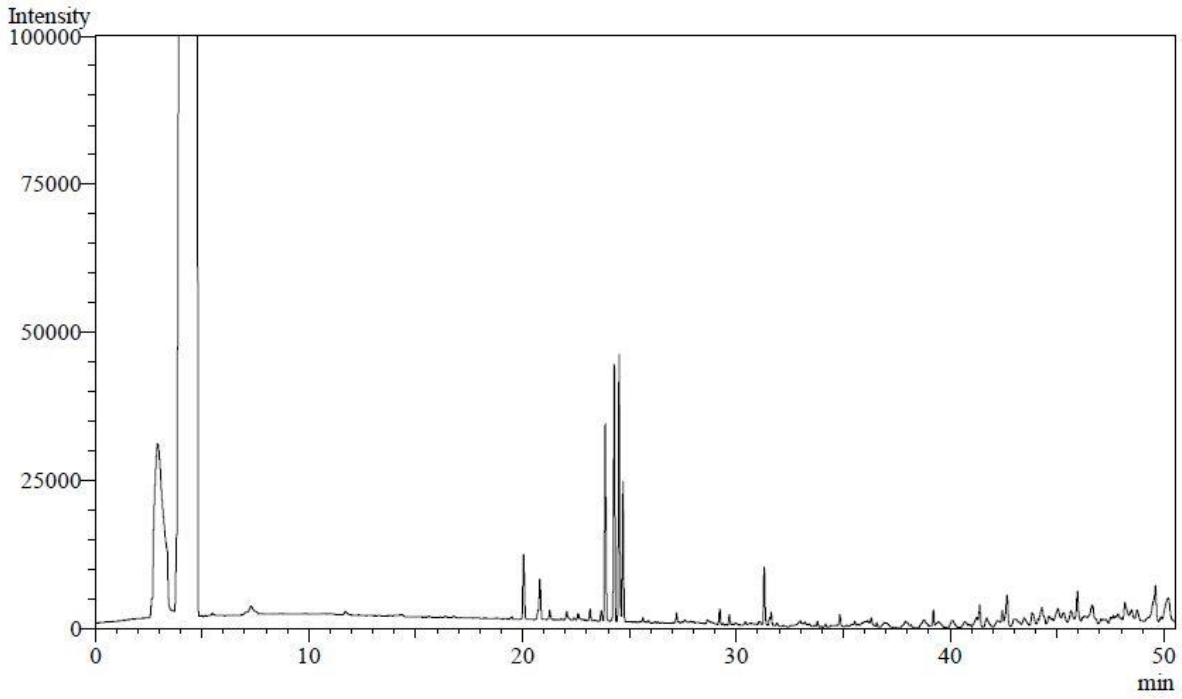


Figure 2.15: Chromatogram of blank TSA media

2.5.7. Statistical Analysis

Normality test for data was carried out using two tests namely; "Kolmogorov-Smirnov" and "Shapiro-Wilk" test. Analysis of Variance (ANOVA) mainly compares the means from two or more groups with the null hypothesis that the mean, i.e., the average result of the dependent variable remains same for all the groups. In this study one way analysis of variance (ANOVA I) was used to compare the mean values of different MVOCs concentration in different seasons. It was also used to compare the mean values of both fungal and bacterial bioaerosol concentration in different seasons as well as at different sites. In order to identify whether the MVOC concentration vary significantly among visibily moldy and non-moldy indoor environments, ANOVA was also carried out. F values and significance value in ANOVA test does not tell about which groups vary significantly from each others. To find relationship between groups (significant difference or similarity), two test was performed in ANOVA analysis namely "Sheffee" and "Bonferroni" test (a stricter test) which has an increased possibility of finding significant relationships since multiple tests are performed. Pearson's correlation coefficient and multiple stepwise linear regression was used to estimate the impact and degree of effectiveness of meteorological parameters such as temperature, relative humidity as well as bioaerosol

concentration (fungal and bacterial concentration) on different MVOCs that were found in abundance such as 1-hexene, cyclohexanone and 1-pentanol. The tests were performed with the software SPSS 16.0 and Excel 2007.

RESULT

3.1. Identification, quantification and seasonal variation of microbial volatile organic compounds present in different indoor environments.

3.1.1. Identification and quantification of microbial volatile organic compounds present in different indoor environments

As seen in table 3.1 among all the six MVOCs 1-pentanol and cyclohexanone are maximally found MVOCs followed by 1-hexene. Both 1-pentanol and cyclohexanone have been detected at almost all the six sites in all the four season with few exceptions such as absence of cyclohexanone in the pre-monsoon season as well as absence of both in post-monsoon season at site 6. Similar to cyclohexanone, 1-hexene has been detected in winter season at all the sites but was absent in pre-monsoon season. 3-octanone is the minimally detected one among all the six MVOCs at all the six sites. Among all the four seasons, 3-octanone was absent at all the six sites in both winter and pre-monsoon seasons.

It is again clear from table 3.2 among all the season and at all the sites 1-pentanol have quantified to be the highest with maximum concentration of 29.23 μgm^{-3} at temperature and relative humidity range of 25.3-36.2^{0}C and 54.3 - 79.3% respectively. 1-pentanol was followed by 1-hexene and cyclohexanone with maximum concentration of 13.40 μgm^{-3} and 5.50 μgm^{-3} respectively. Both the MVOCs were found maximally at the temperature and relative humidity similar to 1-pentanol i.e., 25.3-36.2^{0}C and 54.3 - 79.3% respectively.

Table 3.1: Identified Microbial Volatile Organic Compounds at six indoor environments:

Seasons	MVOCs	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6
Monsoon	1-Octen-3-ol	+	-	+	+	-	+
	3-Octanone	+	-	+	+	-	+
	1-Hexene	-	+	-	+	+	+
	3-Methyl-1-butanol	-	+	-	-	+	-
	Cylohexanone	+	+	+	+	+	+
	1-Pentanol	+	+	+	+	+	+
Post-monsoon	1-Octen-3-ol	-	+	-	-	-	-
	3-Octanone	-	-	+	-	-	-
	1-Hexene	-	+	-	+	+	-
	3-Methyl-1-butanol	-	-	-	+	-	-
	Cylohexanone	+	+	+	+	+	-
	1-Pentanol	+	+	+	+	+	-
Winter	1-Octen-3-ol	+	+	+	+	-	-
	3-Octanone	-	-	-	-	-	-
	1-Hexene	+	+	+	+	+	+
	3-Methyl-1-butanol	+	+	+	+	-	-
	Cylohexanone	+	+	+	+	+	+
	1-Pentanol	+	+	+	+	+	+
Pre-monsoon	1-Octen-3-ol	+	+	-	-	-	-
	3-Octanone	-	-	-	-	-	-
	1-Hexene	-	-	-	-	-	-
	3-Methyl-1-butanol	-	-	-	-	-	-
	Cylohexanone	-	-	-	-	-	-
	1-Pentanol	+	+	+	+	+	+

Table 3.2: Microbial volatile organic compounds concentration in µgm^{-3} in four different seasons

Seasons	MVOCs	Mean	SD	Minimum	Maximum	Temperature (ºC)	Relative Humidity (%)
Monsoon	1-Octen-3-ol	0.482	0.154	0.18	0.67	25.3 - 36.2	54.3 - 79.3
	3-Octanone	0.619	0.489	0.34	1.49		
	1-Hexene	4.392	5.440	0.00	13.40		
	3-Methyl-1-butanol	1.255	1.408	0.26	2.25		
	Cylohexanone	1.952	1.839	0.15	5.50		
	1-Pentanol	6.504	7.072	1.70	29.23		
Post-monsoon	1-Octen-3-ol	1.171	.	0.00	1.17	26.2 - 32.1	53,2 – 61.1
	3-Octanone	2.744	.	0.00	2.74		
	1-Hexene	0.871	0.264	0.33	0.99		
	3-Methyl-1-butanol	0.188	.0003	0.19	0.19		
	Cylohexanone	0.970	1.838	0.07	5.46		
	1-Pentanol	1.496	1.375	0.10	4.50		
Winter	1-Octen-3-ol	1.641	0.778	0.52	2.32	14.0 – 26.1	50.9 – 72.1
	3-Octanone						
	1-Hexene	5.715	4.444	0.16	11.26		
	3-Methyl-1-butanol	0.335	0.421	0.09	1.22		
	Cylohexanone	1.039	0.873	0.04	2.55		
	1-Pentanol	4.384	4.852	0.58	17.98		
Summer	1-Octen-3-ol	0.157	0.159	0.04	0.27	26.7 – 37.2	30.7 – 42.1
	3-Octanone						
	1-Hexene						
	3-Methyl-1-butanol						
	Cylohexanone						
	1-Pentanol	1.287	1.565	0.07	5.30		

3.1.2. Seasonal variation of microbial volatile organic compounds present in different indoor environments

It is indent from figure 3.1, 1-octen-3-ol was detected and quantified in very low average concentration at four sites namely site 1 (0.50 µgm^{-3}), 3 (0.32 µgm^{-3}), 4 (0.48 µgm^{-3}) and 5 (0.56 µgm^{-3}) in monsoon season. In post-monsoon season, 1-octen-3-ol was totally absent from all the air samples collected from all the different indoor environments. In winter season, comparatively moderate average level of 1-octen-3-ol was detected at site 2 (1.87 µgm^{-3}), 3 (2.32 µgm^{-3}) and 4 (1.84 µgm^{-3}). Almost similar average concentration was detected at site 1 as in monsoon i.e., 0.52 µgm^{-3}. However, the average concentration

eventually decreased to 0.04 μgm^{-3} and 0.27 μgm^{-3} at sites 1 and 2 respectively and was totally undetected at other sites in pre-monsoon season. Thus, it can be seen that among all the four seasons, 1-octen-3-ol was found at its maximum average concentration in winter season followed by monsoon, and was almost negligible and absent in pre-monsoon and post-monsoon season.

As seen in figure 3.2; similar to 1-octen-3-ol, 3-octanone was detected and quantified in low average concentration at four sites namely; site 1 (0.50 μgm^{-3}), 3 (0.32 μgm^{-3}), 4 (0.48 μgm^{-3}) and 6 (0.56 μgm^{-3}) in monsoon season. Unlike 1-octen-3-ol, 3-octanone was detected and quantified at only one site but with a comparatively moderate average concentration i.e., site 3 (2.74 μgm^{-3}) in post-monsoon season. 3-octanone was totally absent in all the air samples collected in all the six sites both in winter and pre-monsoon seasons. Thus, it can be said that among all the four seasons, 3-octanone was found in most of the sites in monsoon season only while it was quantified at a higher average level only at one site in post-monsoon season.

As seen in figure 3.3; unlike 1-octen-3-ol and 3-octanone, 1-hexene was detected and quantified at a higher average concentration level at two sites namely; site 4 (11.17 μgm^{-3}) and 5 (10.54 μgm^{-3}) while at site 2 and 6 the average concentration was at the lower side similar to that of 1-octen 3-ol and 3-octaone i.e., 1.38 μgm^{-3} and 0.65 μgm^{-3} respectively in monsoon season. In post-monsoon season, 1-hexene was detected and quantified at a very low average concentration level at site 2 (0.33 μgm^{-3}), 4 (0.97 μgm^{-3}) and 5 (0.98 μgm^{-3}). Unlike the above two mentioned MVOCs, 1-hexene was detected and quantified at all the six sites in winter season. On an average higher concentration was found at site 1 (9.09 μgm^{-3}), 5 (9.8 μgm^{-3}) and 6 (9.7 μgm^{-3}) while lower level at site 2 (0.73 μgm^{-3}) and 3 (0.17 μgm^{-3}). Similar to 3-octanone, 1-hexene was totally absent in all the air samples collected from all the six sites in pre-monsoon season. Thus, it can be seen that among all the four seasons, 1-hexene was found at its maximum average concentration in monsoon season at two sites followed by winter season, and was almost negligible and absent in post-monsoon and pre-monsoon season.

As seen in figure 3.4, in monsoon season 3-methyl-1-butanol was detected and quantified at two sites only namely site 2 (2.25 μgm^{-3}) and 5 (0.26 μgm^{-3}). 3-methyl-1-butanol was detected and quantified at very low average concentration 0.19 μgm^{-3} at the site 4 only in post-monsoon season. In winter, it was detected at four sites with comparatively low average

concentration such as 0.09 μgm^{-3}, 0.8 μgm^{-3}, 0.18 μgm^{-3} and 0.11 μgm^{-3} at sites 1, 2, 3 and 4 respectively. Similar to 3-octanone and 1-hexene, 3-methyl-1-butanol was totally absent in all the air samples collected from all the six sites in pre-monsoon season. Thus, it can be seen that among all the four seasons, 3-methyl-1-butanol was found at its maximum average concentration in monsoon season at one site followed by winter season, and was almost negligible and absent in post-monsoon and pre-monsoon season.

As seen in figure 3.5; unlike all the above mentioned MVOCs, cyclohexanone was detected and quantified at all the six sites in monsoon season with a comparatively moderate average level at sites 2 (2.42 μgm^{-3}) and 6 (3.74 μgm^{-3}) while lower average concentration at sites 1 (0.67 μgm^{-3}), 3 (0.94 μgm^{-3}), 4 (1.29 μgm^{-3}) and 5 (0.65 μgm^{-3}). In post-monsoon season, cyclohexanone was detected and quantified at five sites namely sites 1 (0.26 μgm^{-3}), 2 (0.17 μgm^{-3}), 3 (1.87 μgm^{-3}), 4(0.06 μgm^{-3}) and 5 (0.11 μgm^{-3}). Similar to monsoon season, cyclohexanone was detected and quantified at all the six sites in winter season but at a comparatively low average concentration such as 0.28 μgm^{-3}, 1.14 μgm^{-3}, 0.69 μgm^{-3}, 0.41 μgm^{-3}, 0.92 μgm^{-3} and 1.16 μgm^{-3} at sites 1, 2, 3, 4, 5 and Thus, it can be seen that among all the four seasons, cyclohexanone was found at its maximum average concentration in monsoon season at sites 1, 2, 4 and 6 followed by winter season with an exception at site 3 and 5, and was almost negligible and absent in post-monsoon and pre-monsoon seasons at site 6, respectively. Similar to 3-octanone, 1-hexene and 3-methyl-1-butanol, cyclohexanone was totally absent in all the air samples collected from all the six sites in pre-monsoon season.

As seen in figure 3.6, in monsoon season 1-pentanol was observed at all the six sites with higher average concentration at sites 2 (5.04 μgm^{-3}), 5 (9.48 μgm^{-3}) and 6 (6.56 μgm^{-3}), while moderate average concentration at sites 1 (3.97 μgm^{-3}), 3 (2.39 μgm^{-3}) and 4 (3.67 μgm^{-3}). Similar to cyclohexanone, 1-pentanol was detected at five sites with moderate average concentration at site 2 (2.28 μgm^{-3}) and 3 (3.54 μgm^{-3}) while low average concentration at sites 1 (0.57 μgm^{-3}), 4 (0.38 μgm^{-3}) and 5 (0.32 μgm^{-3}) in post-monsoon season. In winter 1-pentanol was detected at all the six sites and was quantified at a higher average concentration at site 2 only (5.65 μgm^{-3}) while in the rest of the five sites it depicted moderate average concentration such as 1.71 μgm^{-3}, 1.9 μgm^{-3}, 2.24 μgm^{-3}, 3.25 μgm^{-3} and 3.24 μgm^{-3} at sites 1, 3, 4, 5 and 6, respectively. Unlike all the other MVOCs, 1-pentanol was detected and quantified at all the six sites in pre-monsoon season with a moderate average concentration at site 3 (2.12 μgm^{-3}), while low average concentration at rest of the five sites such as site 1

(0.43 µgm^{-3}), 2 (0.87 µgm^{-3}), 4 (0.89 µgm^{-3}), 5 (0.41 µgm^{-3}) and 6 (0.39 µgm^{-3}). Thus, it can be seen that among all the four seasons, 1-pentanol was found at its maximum average concentration in monsoon season at sites 1, 4, 5 and 6 followed by winter, post-monsoon and pre-monsoon seasons with an exception at sites 2 and 3.

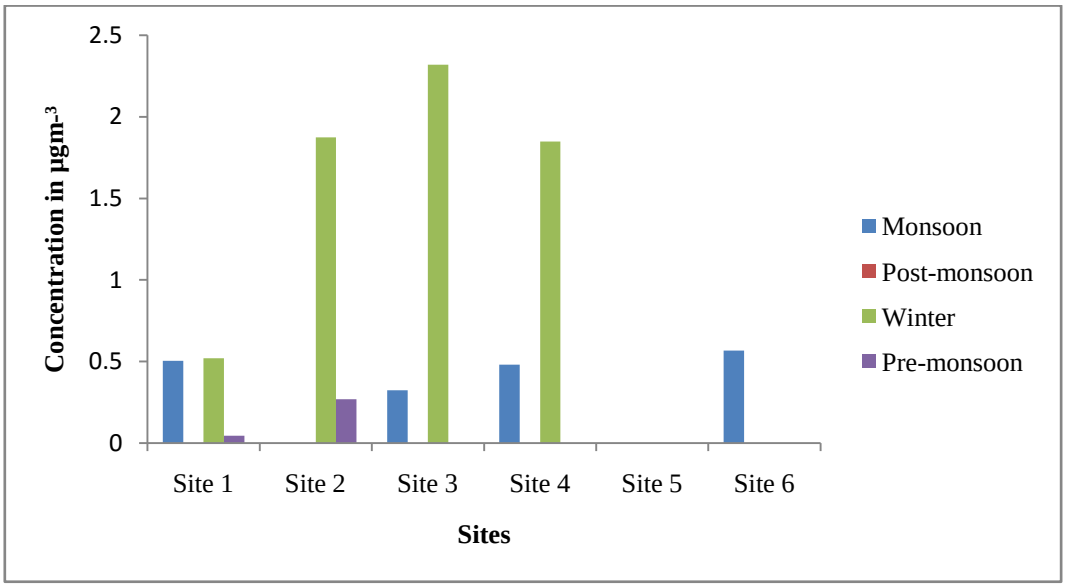


Figure 3.1: Seasonal variation of 1-octen-3-ol at different indoor sites

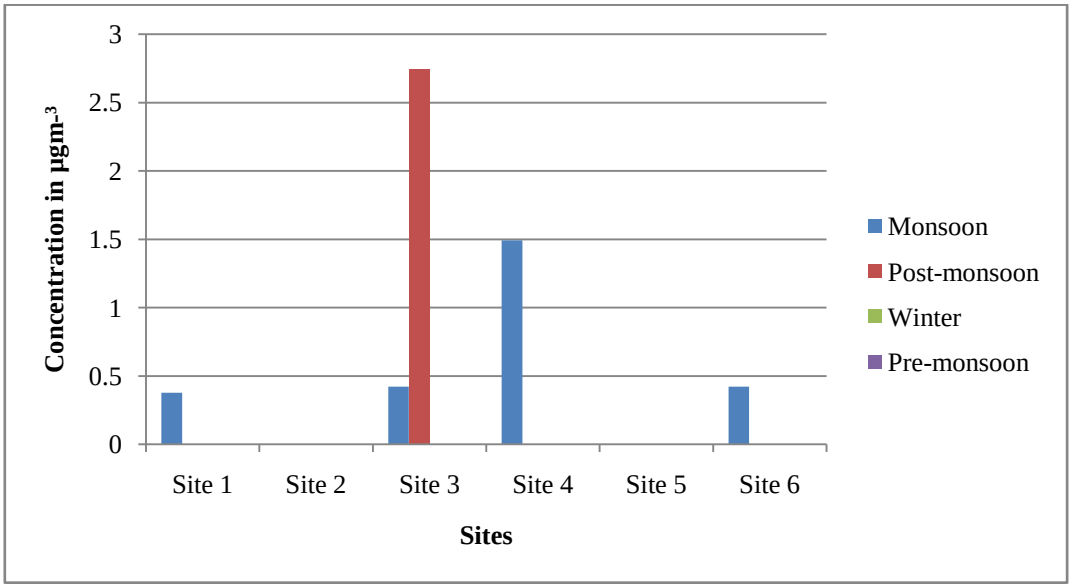


Figure 3.2: Seasonal variation of 3-octanone at different indoor sites

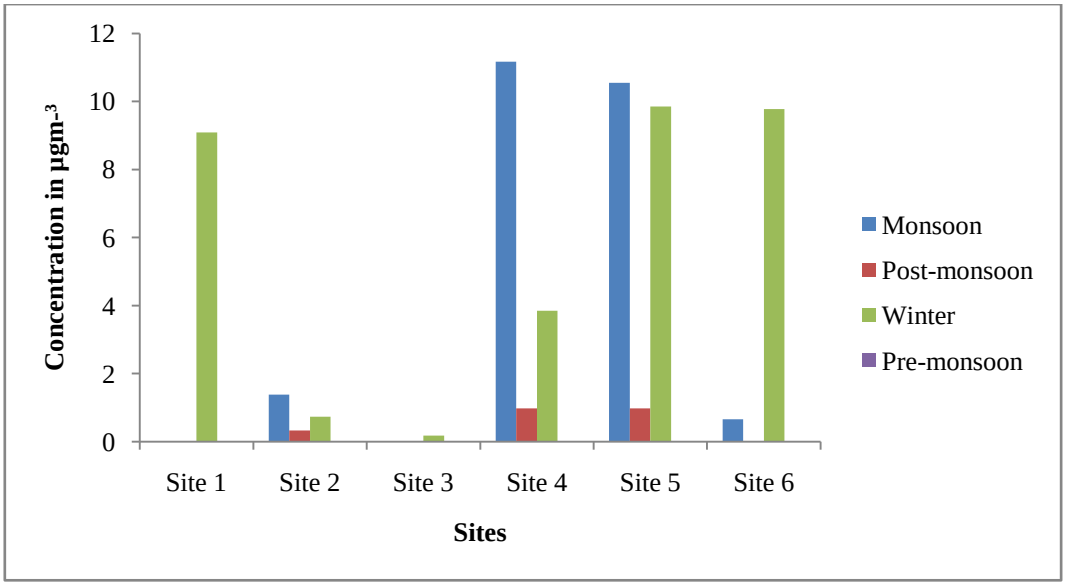


Figure 3.3: Seasonal variation of 1-hexene at different indoor sites

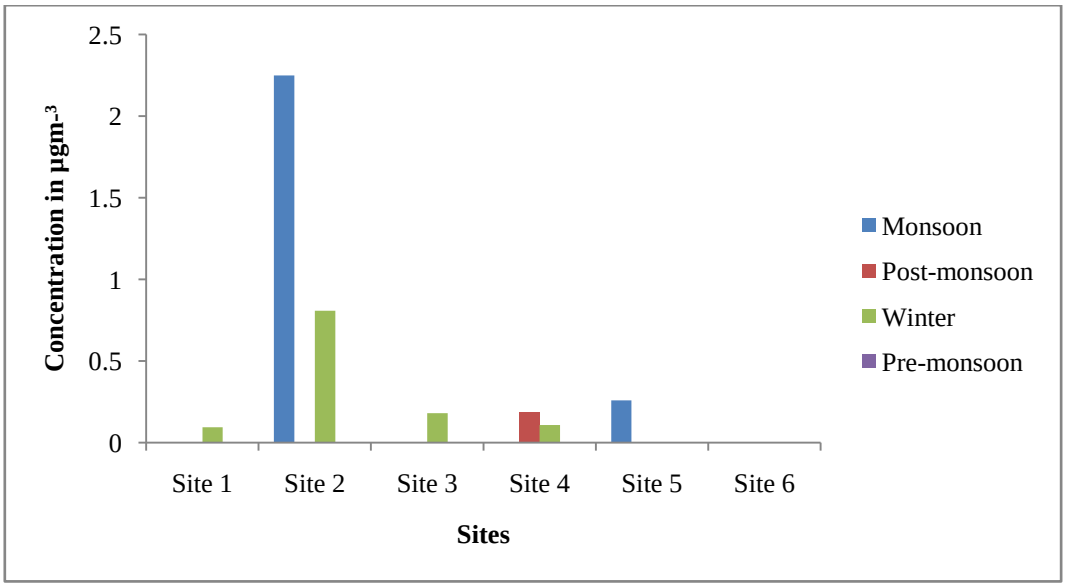


Figure 3.4: Seasonal variation of 3-methyl-1-butanol at different indoor sites

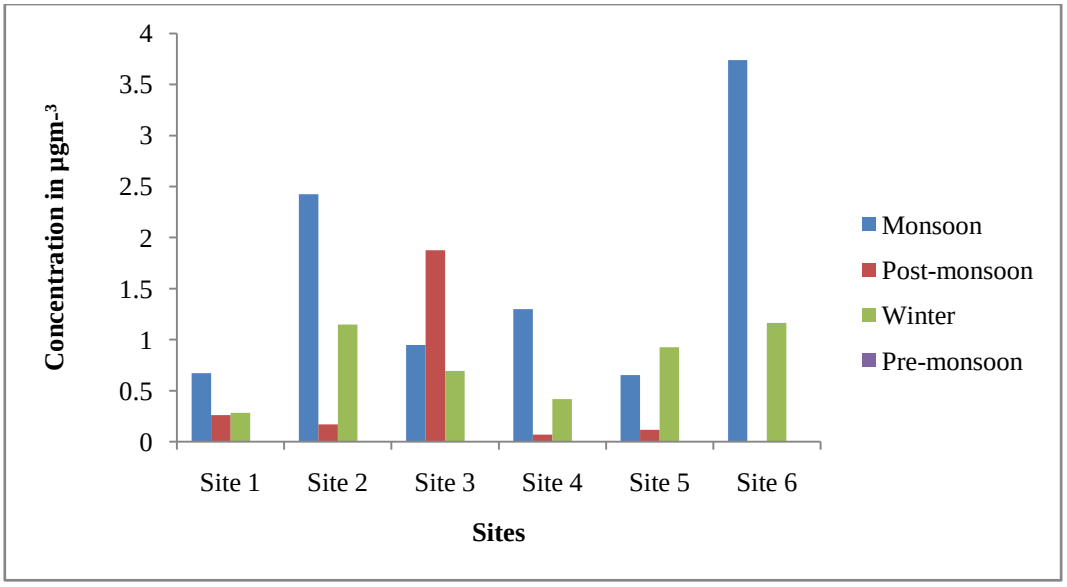


Figure 3.5: Seasonal variation of cyclohexanone at different indoor sites

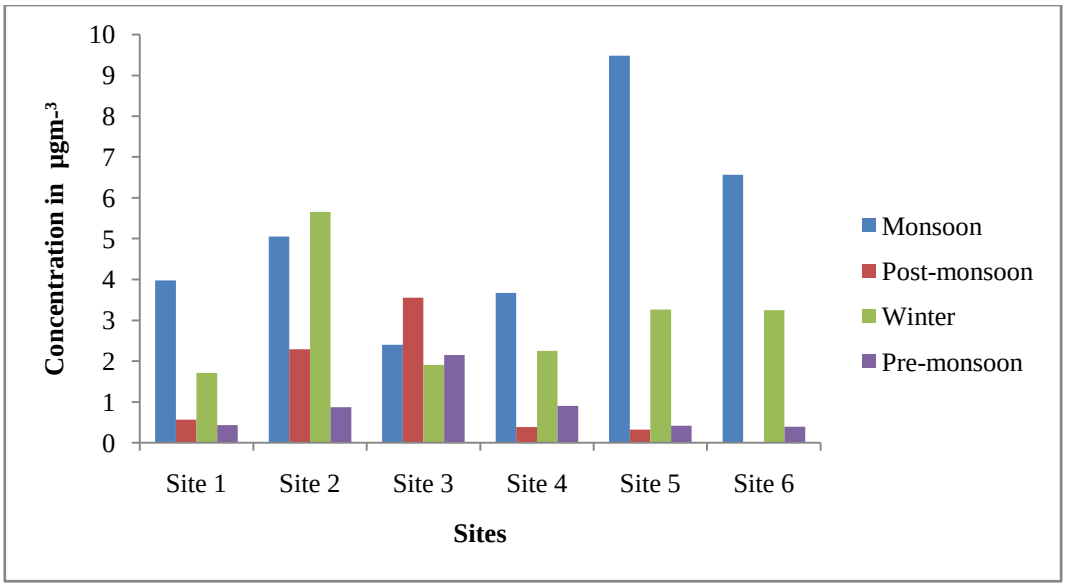


Figure 3.6: Seasonal variation of 1-pentanol at different indoor sites

3.2. Identification of Microbial VOCs from surface dust and building material

3.2.1. MVOCs from dust samples

Identification of the microbial volatile compounds was confirmed using the reference chemicals described earlier and whose chromatogram is seen in figure 2.12. As seen in table 3.3 and in figure 3.7; among six MVOCs, 1-hexene, 3-methyl-1-butanol and 1-pentanol were detected in the dust samples from almost all the sites with few exceptions. 1-hexene has been detected in the dust samples of sites 1, 4, 5 and 6 in terms of both overall fungal and bacterial community out of which it was quantifiable only in case of dust sample from site 4 in terms of overall fungal community to be 0.22 µg/ml as well as from sites 5 and 6 in terms overall bacterial community only (0.20 µg/ml and 0.19 µg/ml, respectively). In sites 2 and 3, 1-hexene was detected from the dust samples in terms of overall bacterial community only. Similar to 1-hexene, 3-methyl-1-butanol was also detected in the dust samples from sites 1, 4, 5 and 6 in terms of both overall fungal and bacterial community and in site 3 in terms of overall bacterial community only. However, unlike 1-hexene, apart from detection 3-methyl-1-butanol was also quantifiable. Maximum concentration of 3-methyl-1-butanol in dust samples in terms of overall fungal community was quantified to be 1.25 µg/ml in site 6 followed by 1.08 µg/ml from site 1 whereas in case of dust samples in terms of overall bacterial community the descending order of 3-methyl-1-butanol concentration were 12.20 µg/ml, 9.82 µg/ml, 4.93 µg/ml and 1.03 µg/ml from sites 6, 1, 4 and 3, respectively. Unlike 1-hexene and 3-methyl-1-butanol, 1-pentanol was detected in the dust samples from all the six sites. 1-pentanol was only detected and not quantified due to very low concentration in the dust samples of sites 1 and 2 in terms of overall fungal and bacterial community. In the remaining sites maximum concentration of 1-pentanol in terms of overall fungal community was quantified to be 0.55µg/ml in site 5 followed by 0.42µg/ml in site 4 whereas in case of dust samples in terms of overall bacterial community the descending order of its concentration were 0.30µg/ml, 0.28µg/ml and 0.15µg/ml from sites 5, 3 and 6, respectively. Apart from the above three MVOCs, cyclohexanone was also detected in the surface dust sample collected from site 5 in terms of bacterial community only.

3.2.2. MVOCs from building materials

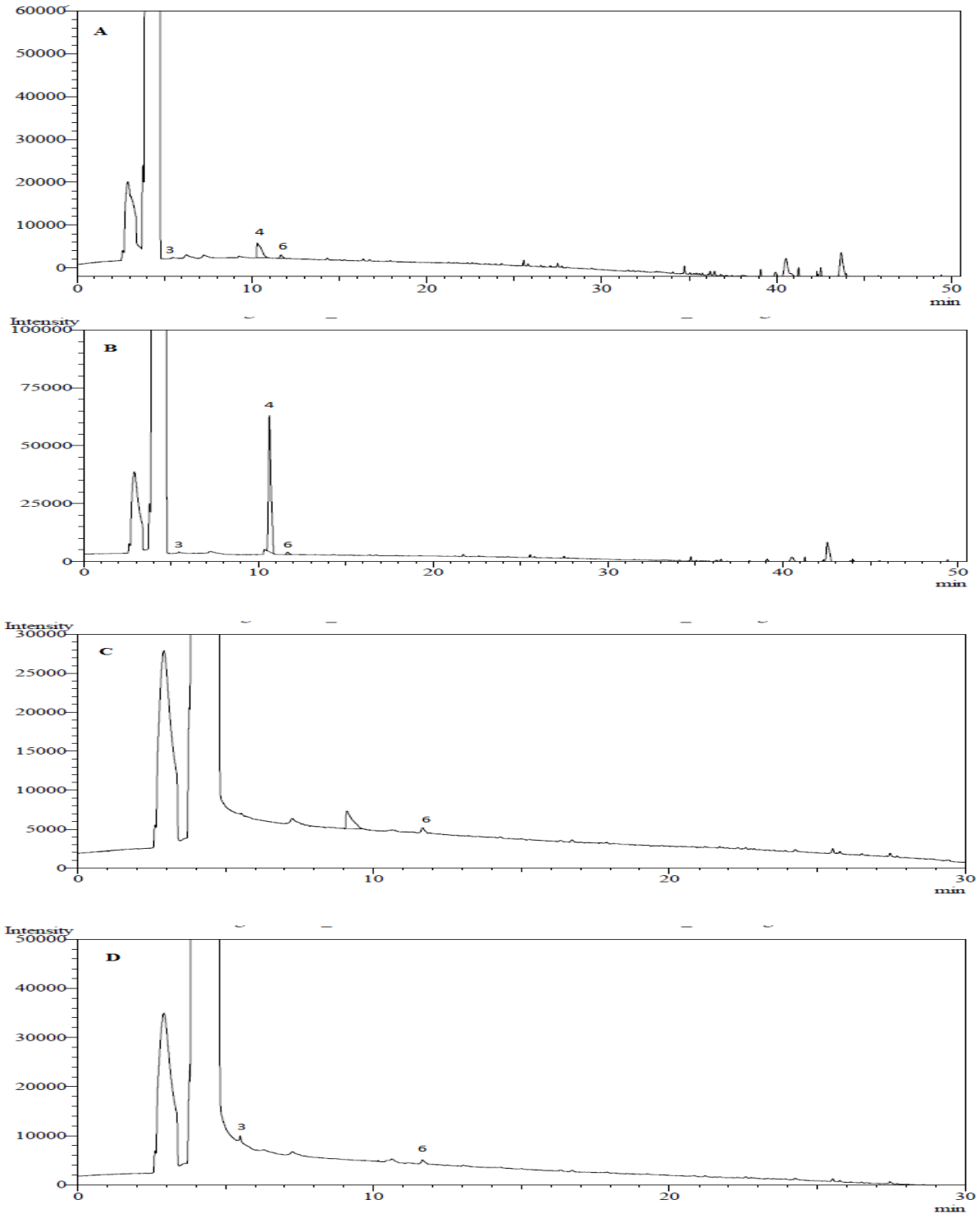
As seen in table 3.3 and figure 3.8, among the preselected six MVOCs, 1-hexene, 3-methyl-1-butanol and 1-pentanol were detected in the building material samples from almost all the

sites with few exceptions. 1-hexene was detected in building material of site 1 in terms of overall fungal community while in sites 2, 3, 4 and 6 in terms of bacterial communities only. Being very low in concentration, none of the samples could be quantified for 1-hexene. Site 5 is the exceptional one wherein 1-hexene was quantified from overall fungal and bacterial community with 0.11 µg/ml and 0.25 µg/ml concentration, respectively. Similar to 1-hexene, 3-methyl-1-butanol was detected in the building material of sites 3, 4, 5 and 6 in terms of overall bacterial community only. 3-methyl-1-butanol on the other hand was quantified as well in the building materials of almost all the sites with an exceptional to site 2 where it was detected in terms of overall fungal community but could not be quantified due to very low concentration. Highest amount of 3-methyl-1-butanol was found in the building material of site 5 with 7.35 µg/ml followed by site 4 and 1 with 2.73 µg/ml and 2.29 µg/ml concentration in terms of overall bacterial community. 1-pentanol is the only MVOC that was detected in the building materials of site 1, 3, 4, 5 and 6 in terms of both overall fungal and bacterial community. Among the 11 detected samples 10 could be quantified with very low variation in the concentration range with the highest being 0.46 µg/ml (site 5) and lowest being 0.20 µg/ml (sites 2 and 6) in terms of overall bacterial community respectively. Apart from the above three MVOCs, cyclohexanone was also detected in the building material sample collected from sites 2 and 5 in terms of bacterial community and fungal community, respectively.

Table 3.3: MVOCs from dust and MB after 8th day of passive sampling (µg/ml)

Sites	Site 1				Site 2				Site 3				Site 4				Site 5				Site 6			
	Dust		BM		Dust		BM		Dust		BM		Dust		BM		Dust		BM		Dust		BM	
MVOCs	FC	BC	FC	BC	FC	BC	FC	BC	FC	BC	FC	BC	FC	BC	FC	BC	FC	BC	FC	BC	FC	BC	FC	BC
1-Octen-3-ol	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3-Octanone	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1-Hexene	+ NQ	+ NQ	+ NQ	-	-	+ NQ	-	+ NQ	-	+ NQ	-	+ NQ	+ 0.22	+ NQ	-	+ NQ	+ 0.04	+ 0.02	+ 0.11	+ 0.25	+ 0.07	+ 0.19	-	+ 0.1
3-Methyl-1-butanol	+ 1.08	+ 9.82	+ 0.28	+ 2.29	-	-	+ NQ	+ 0.05	-	+ 1.03	-	+ 0.37	+ 0.07	+ 4.93	-	+ 2.73	+ 0.83	+ 0.16	-	+ 7.35	+ 1.25	+ 12.20	-	+ 0.18
Cylohexanone	-	-	-	-	-	-	-	+ NQ	-	-	-	-	-	-	-	-	+ 0.07	+ 0.07	+ 0.07	-	-	-	-	+ NQ
1-Pentanol	+ NQ	+ NQ	+ 0.36	+ NQ	+ NQ	+ NQ	-	+ 0.20	+ NQ	+ 0.30	+ 0.34	+ 0.37	+ 0.42	-	+ 0.42	+ 0.44	+ 0.55	+ 0.28	+ 0.22	+ 0.46	+ 0.22	+ 0.15	+ 0.21	+ 0.20

Note: (FC) Fungal Community grown over PDA; BC Bacterial Community grown over TSA; (+) Detectable; (-) Not Detectable; (NQ) Not Quantifiable; (BM) Building Material.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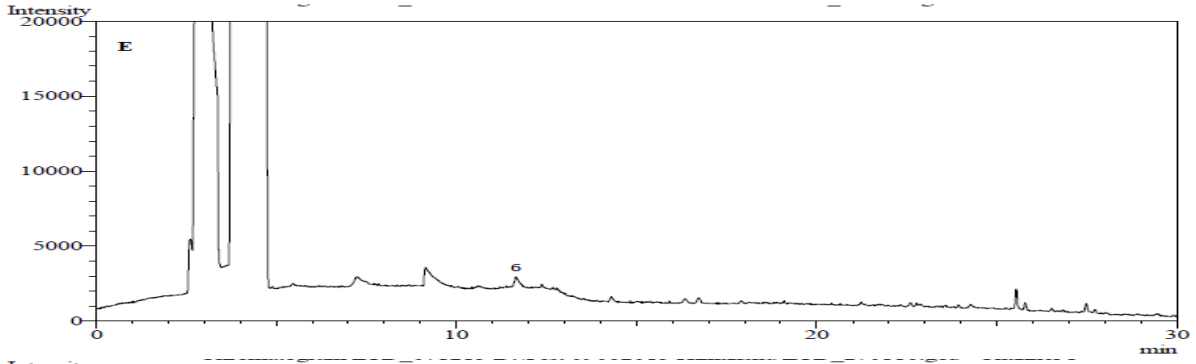
B
C
D
Intensity
min
3
4
6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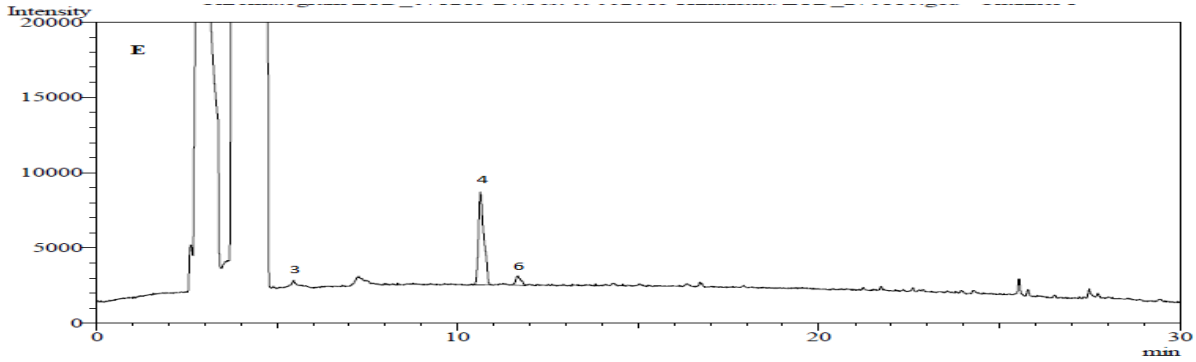
30
min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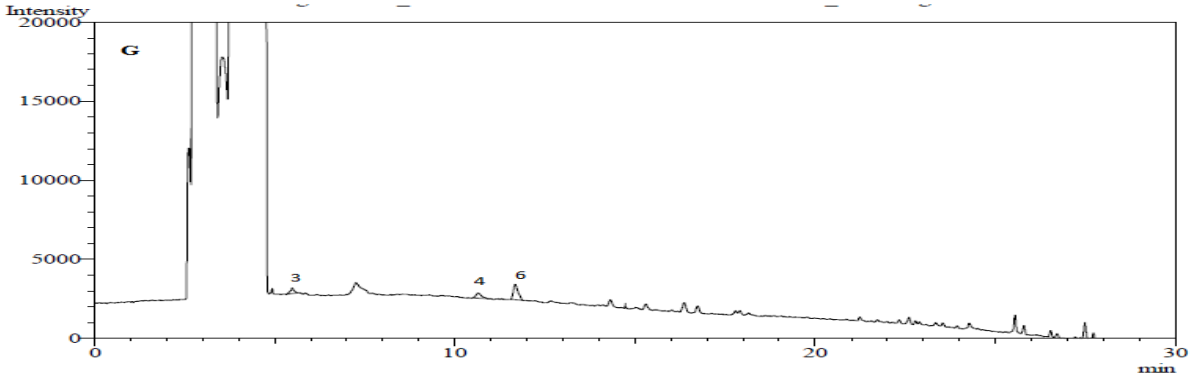
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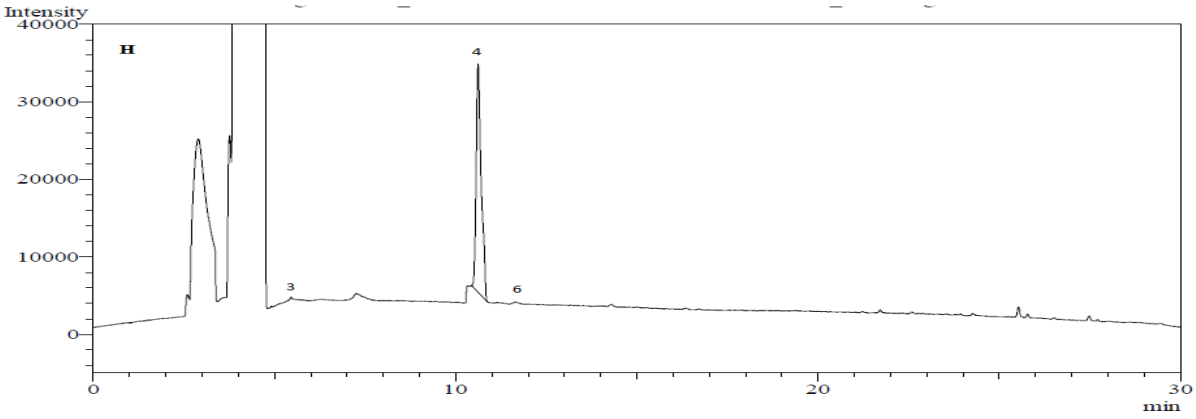
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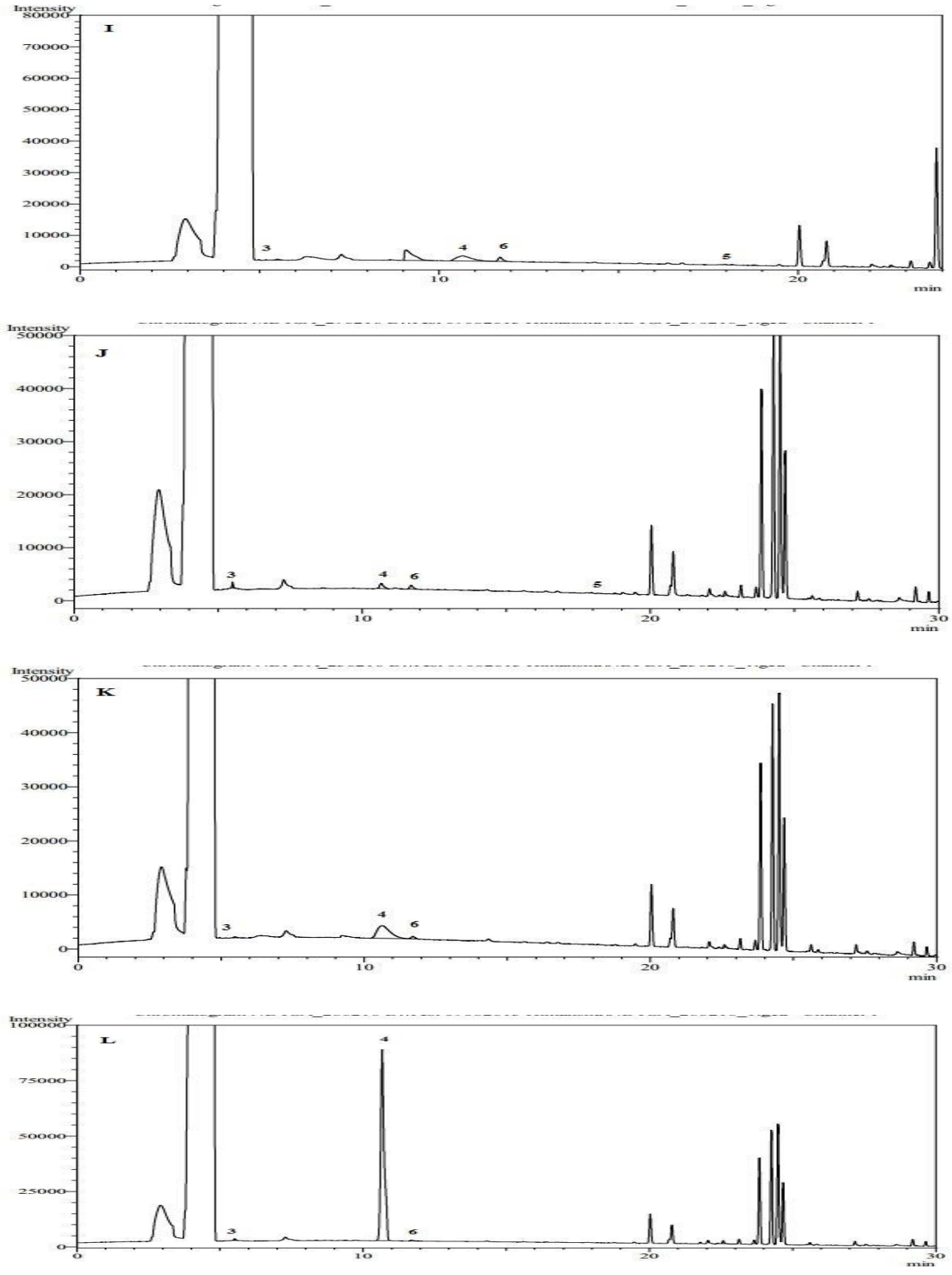
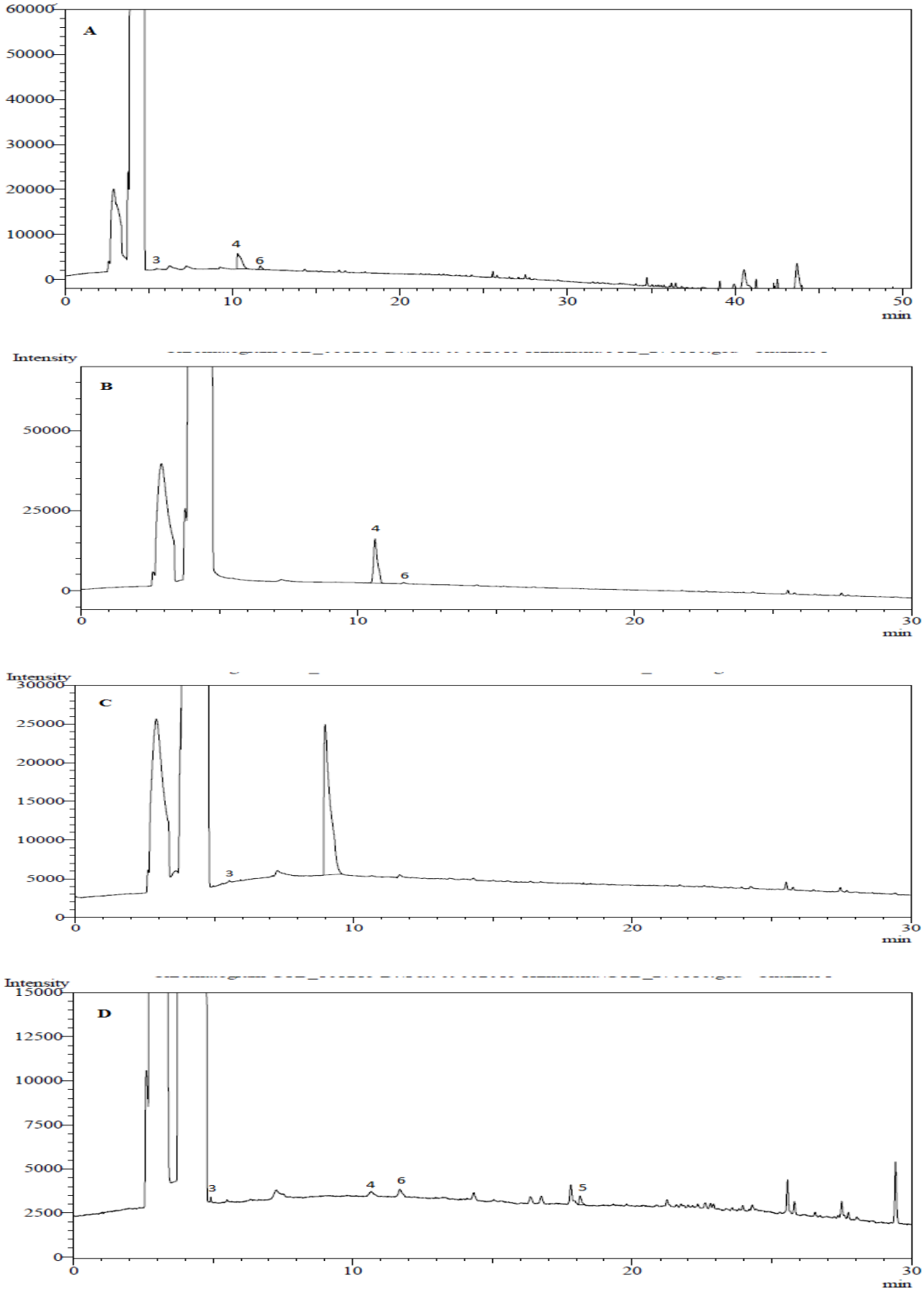
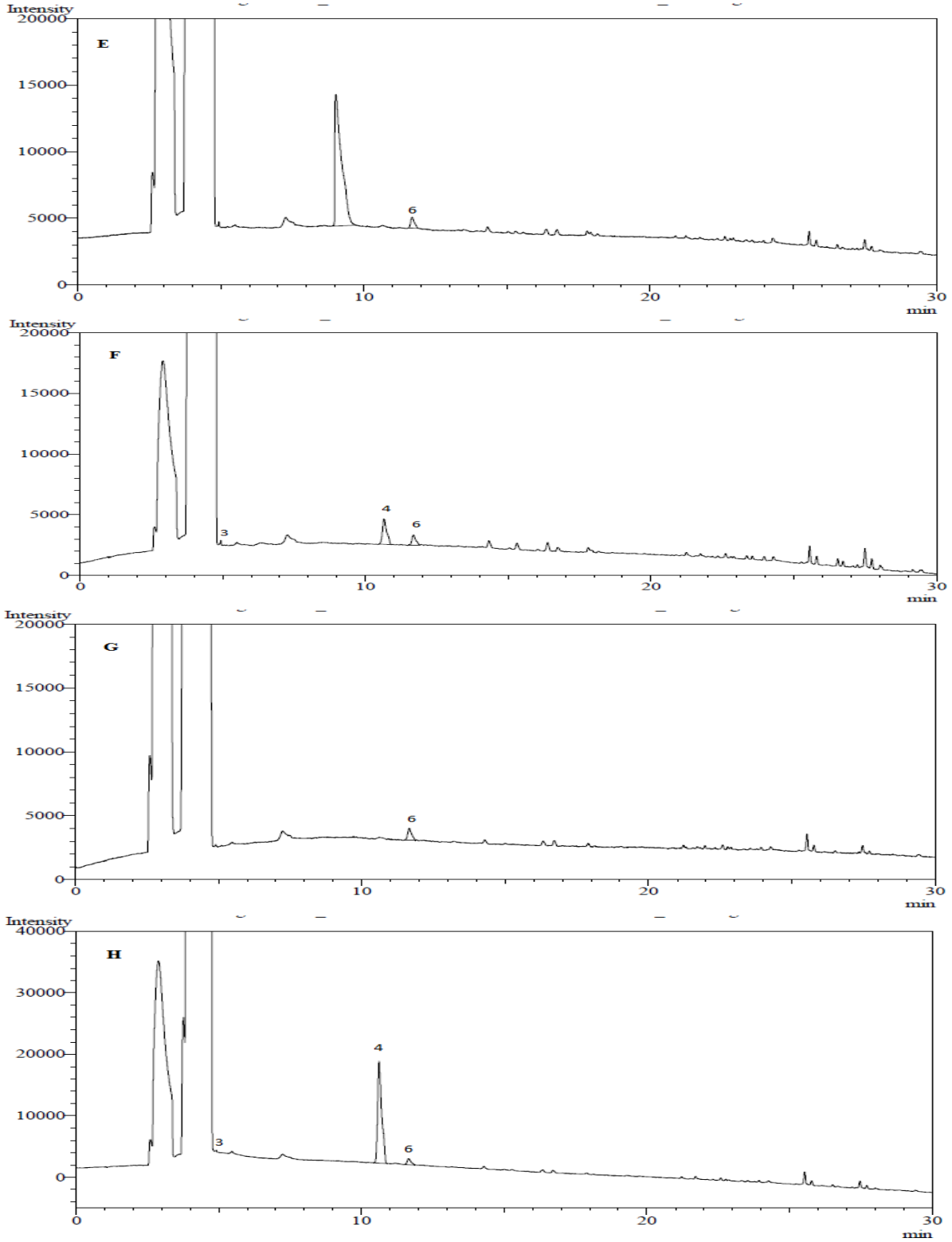


Figure 3.7: MVOCs identified in surface dust samples collected from Site 1 to Site 6 from overall fungal community (A, C, E ,G, I and K, respectively) and overall bacterial community (B, D, F, H, J and L, respectively). Compounds detected: (3) 1-Hexene, (4) 3-methyl-1-butanol and (6) 1-pentanol.


A
B
C
D
Intensity
min
3
4
5
6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G
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Intensity
H
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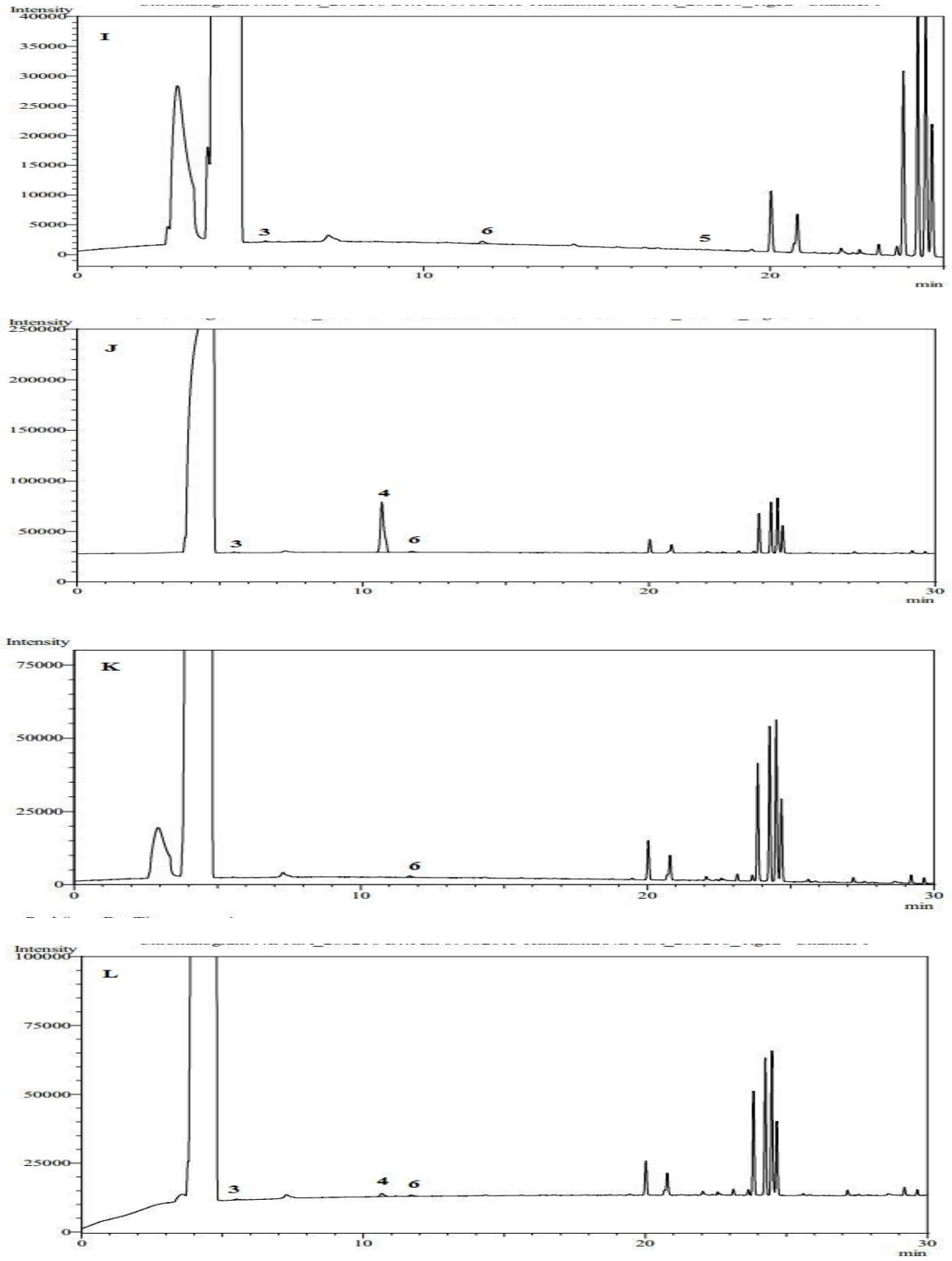


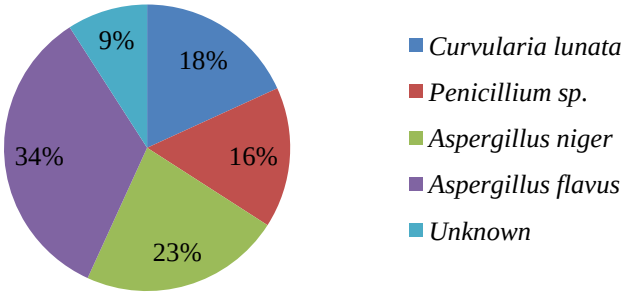
Figure 3.8: MVOCs identified in building materials collected from Site 1 to Site 6 from overall fungal community (A, C, E G, I and K ,respectively) and overall bacterial community (B, D, F, H, J and L, respectively). Compounds detected: (3) 1-Hexene, (4) 3-methyl-1-butanol, (5) Cyclohexanone, (6) 1-pentanol

3.3. Identification of species specific fungal VOCs from Surface dust and Building materials

3.3.1. Fungus isolated from indoor surface dust and building materials

Figure 3.9 contain the microbiological data in terms of fungal strains from all the dust samples and building material collected from all the four sites. As seen in Figure 3.9A, *Aspergillus flavus* is the dominant fungal species followed by *Aspergillus niger* in the dust samples collected from site 1, Central Library whereas in the building material the later dominated followed by *Aspergillus nidulans* and *Cladosporium cladosporioides* with *Penicillium sp.* being the minimum (Figure 3.9B). Among the remaining sites, *Aspergillus flavus* was found maximally in the dust sample collected from site 2, Ghaziabad residence (Figure 3.9C) as well as in both in the dust and building material collected from site 4 (Figure. 3.9G and 3.9H), site 5 (Figure. 3.9I and 3.9J) and site 6 (Figure. 3.9K and 3.9L). *Aspergillus niger*, *Aspergillus nidulans* and *Cladosporium cladosporioides* are the next maximally found fungal species in both dust and building material collected from site 4 (Figure. 3.9G and 3.9H). *Aspergillus nidulans* and *Aspergillus niger* were the next maximally found fungal species in both dust and building material collected from site 5 and 6 (3.9I, 3.9J, 3.9K and 3.9L). Among all the 6 sites, the fungal community in both dust and building material collected from site 3 reflected a different composition. As seen in figure. 3.9E and 3.9F, *Curvuleria lunata* was the dominant fungal species followed *Penicillium sp.* and *Aspergillus flavus* in the dust samples while *Aspergillus niger* dominated the fungal community in the building material sample followed by *Aspergillus flavus* and *Aspergillus nidulans*. Very few unidentifiable hyphal structures were found in both dust and building material samples collected from sites 3 and 4 and were termed as “Unknown” as seen in figure. 3.9E to 3.9H, while these “Unknown” species were found at a comparatively higher concentration in both dust and building materials at sites 5 and 6 as seen in figure 3.9I, 3.9J, 3.9K and 3.9L.

A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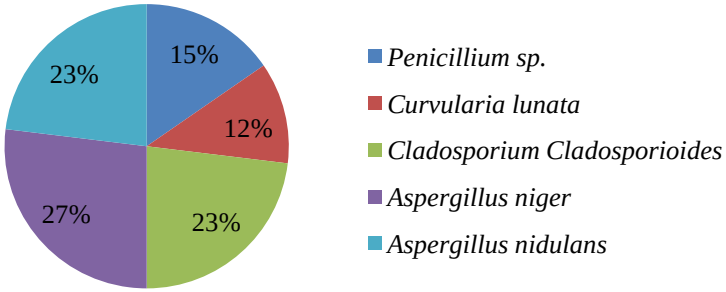
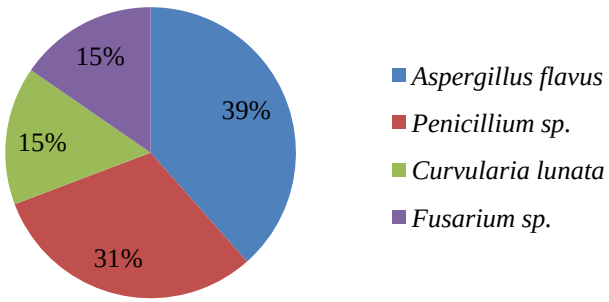
34%
16%
23%
Curvularia lunata
Penicillium sp.
Aspergillus niger
Aspergillus flavus
Unknown

B


15%
23%
12%
27%
23%
Penicillium sp.
Curvularia lunata
Cladosporium Cladosporioides
Aspergillus niger
Aspergillus nidulans

C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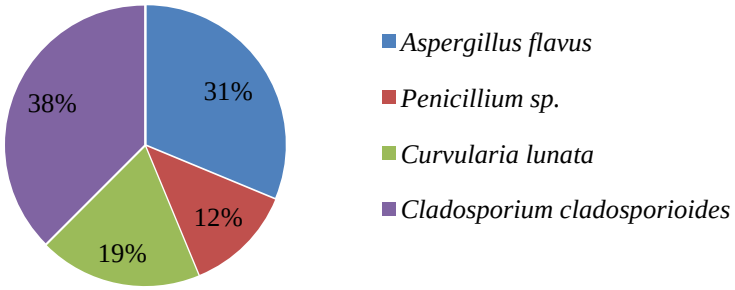
39%
15%
31%
Aspergillus flavus
Penicillium sp.
Curvularia lunata
Fusarium sp.

D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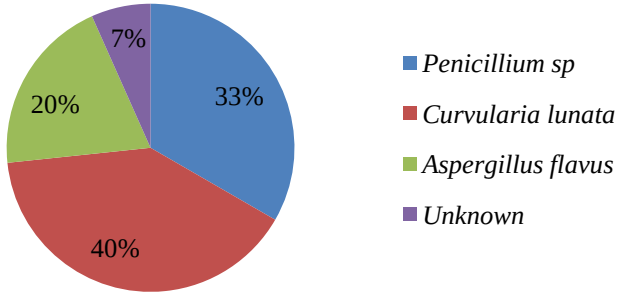
19%
38%
Aspergillus flavus
Penicillium sp.
Curvularia lunata
Cladosporium cladosporioides

E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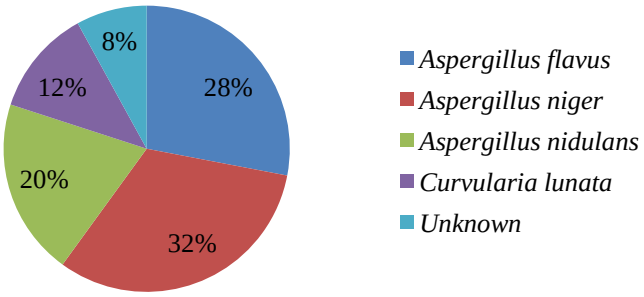
40%
20%
7%
Penicillium sp
Curvularia lunata
Aspergillus flavus
Unknown

F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32%
20%
12%
8%
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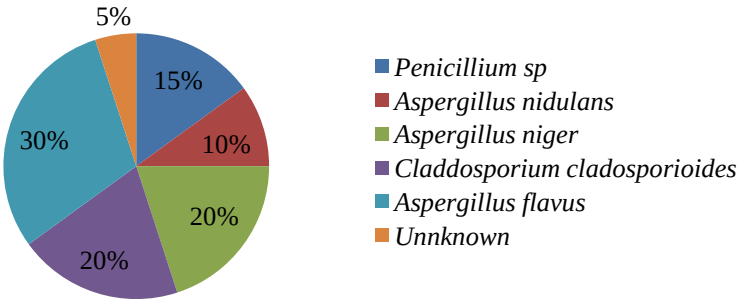
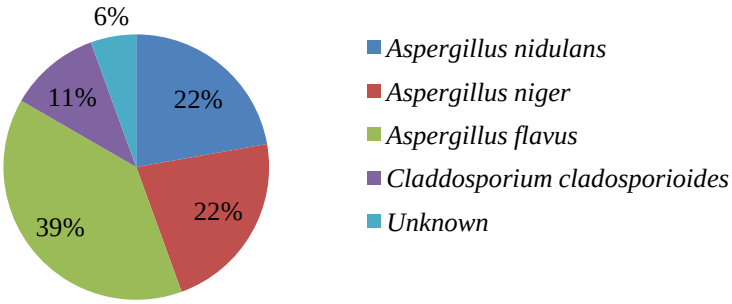
Aspergillus niger
Aspergillus nidulans
Curvularia lunata
Unknown

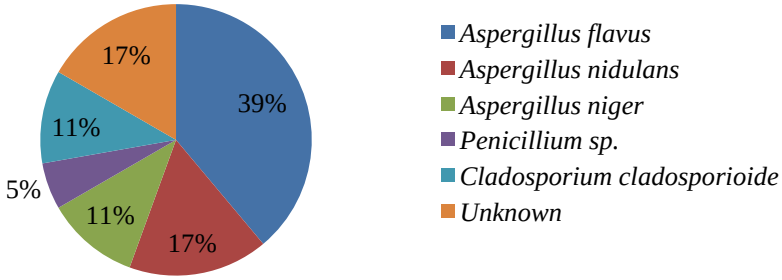
G


5%
15%
30%
10%
20%
20%
Penicillium sp
Aspergillus nidulans
Aspergillus niger
Claddosporium cladosporioides
Aspergillus flavus
Unnknown

H


6%
11%
22%
39%
22%
Aspergillus nidulans
Aspergillus niger
Aspergillus flavus
Claddosporium cladosporioides
Unknown

I


17%
39%
11%
5%
11%
17%
Aspergillus flavus
Aspergillus nidulans
Aspergillus niger
Penicillium sp.
Cladosporium cladosporioide
Unknown

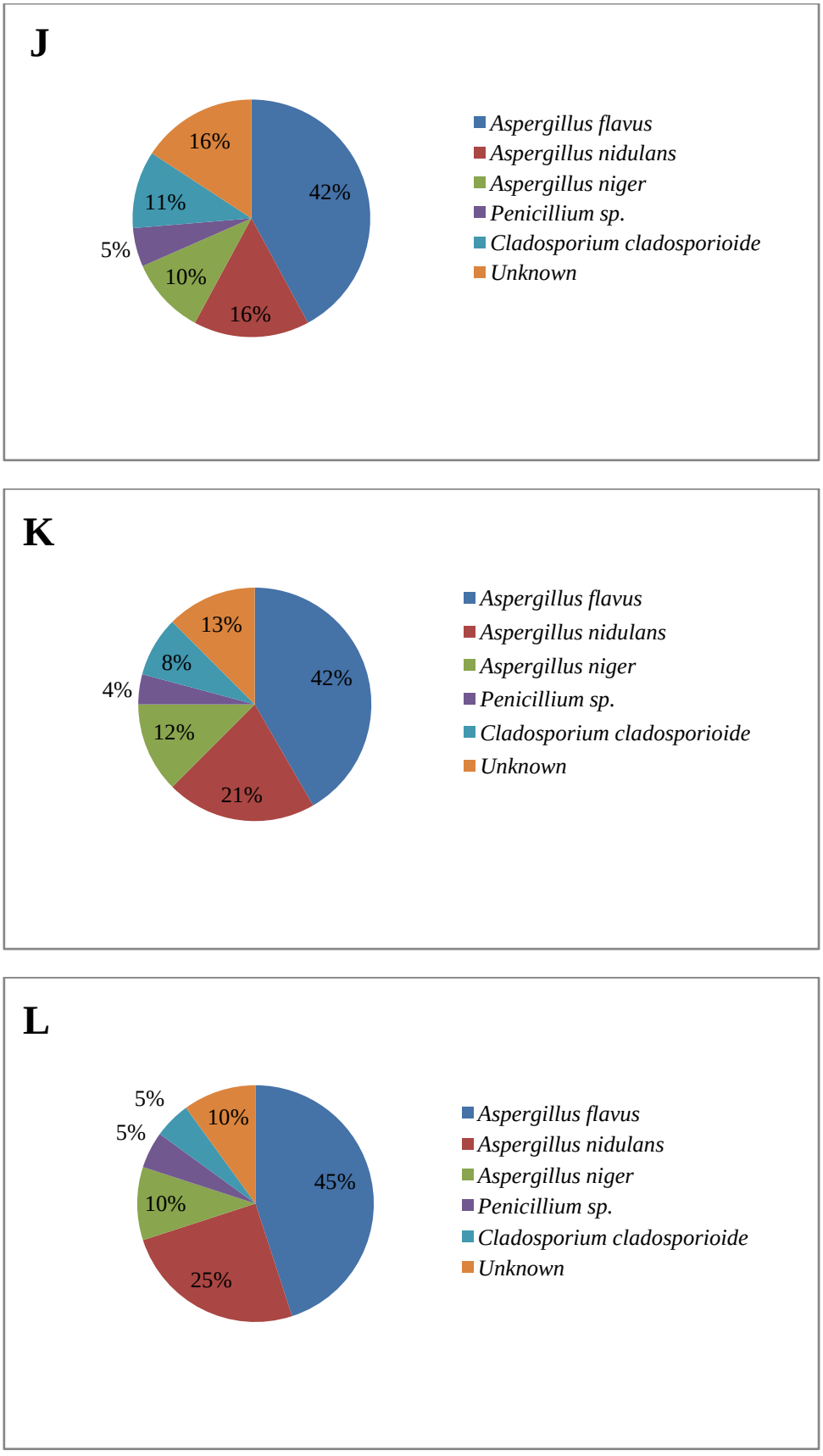


Figure 3.9: Percentage representation of the culturable fungal community structure present in dust samples (A, C, E, G, I and K, respectively) and building materials (B, D, F, H, J and L respectively) collected from sites 1 to 6, respectively.

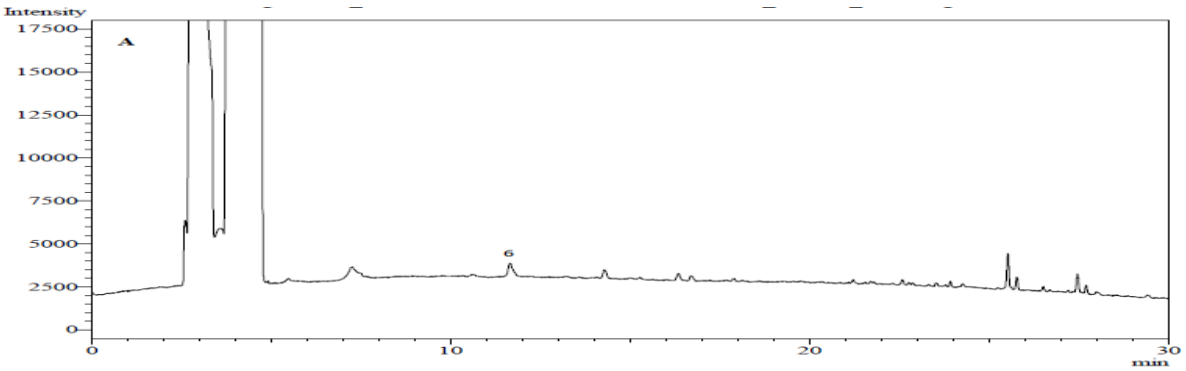
3.3.2. MVOCs detection and quantified after 8th day of passive sampling over isolated fungal species

As seen in table 3.4 and figure 3.10, among all the 6 MVOCs studied upon, only 3, namely 1-hexene, 3-methyl-1-butanol and 1-pentanol could be detected to be released by some of the isolated fungal species from both dust and building material samples from all the four sites after 8th day. *Aspergillus nidulans* and *Curvuleria sp.* were found to produce all the 3 compounds followed by *Penicillium sp.* which produced 2 compounds in comparison to other fungal species. Amongst the 3, 1-pentanol was detected from all the six fungal species and quantified for 4 fungal species namely *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus nidulans* and *Cladosprium sp*. Highest concentration was detected from *Cladosprium sp*. with 0.37 µg/ml, followed by *Aspergillus niger*, *Aspergillus flavus* and *Aspergillus nidulans* with concentrations 0.32 µg/ml, 0.26 µg/ml and 0.24 µg/ml respectively. Similar to 1-pentanol, 1-hexene was released by *Penicillium sp.* whereas both 1-hexene and 3-methyl-1-butanol were released by both *Aspergillus nidulans* and *Curvuleria sp.* however none of them could be quantified due to very low concentrations.

Table 3.4: Detection and quantification of MVOCs from six fungal species after 8th day (µg/ml)

MVOCs	**Fungal Species**					
	A.niger	*A.flavus*	*A.nidulans*	*Cladosporium cladosporioide*	*Curvuleria lunata*	*Penicilium sp.*
1-octen-3-ol	-	-	-	-	-	-
3-octanone	-	-	-	-	-	-
1-hexene	-	-	+ NQ	-	+ NQ	+ NQ
3-methyl-1-butanol	-	-	+ NQ	-	+ NQ	-
Cyclohexanone	-	-	-	-	-	-
1-pentanol	+ 0.32	+ 0.26	+ 0.24	+ 0.37	+ NQ	+ NQ

Note: (+) indicates compound detected; (-) indicates compounds not produced by the species on the specific medium or was present below the detectable level; NQ indicates Not Quantifiable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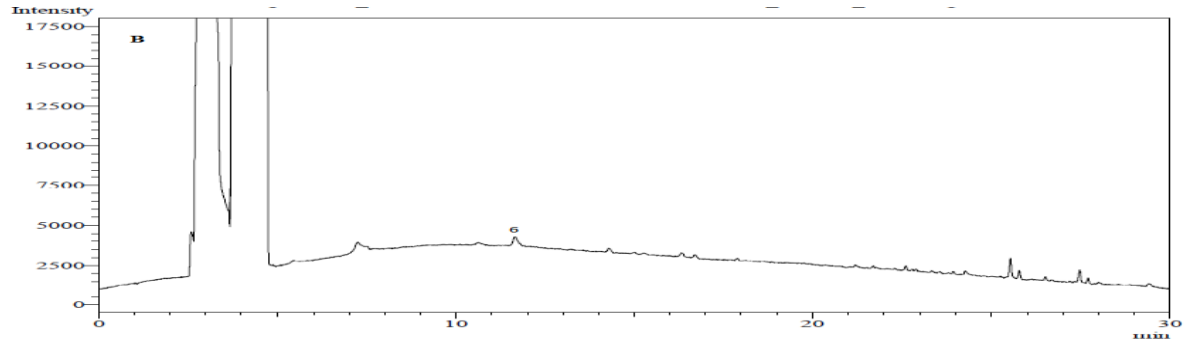
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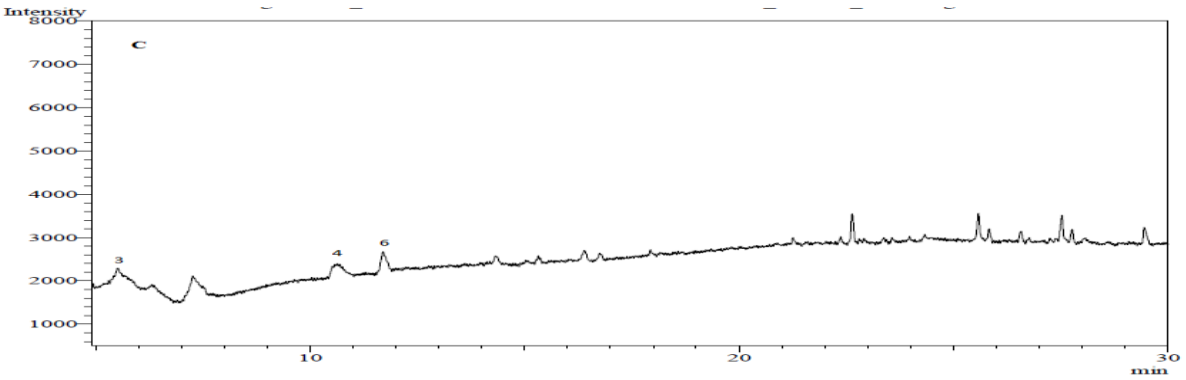
Intensity
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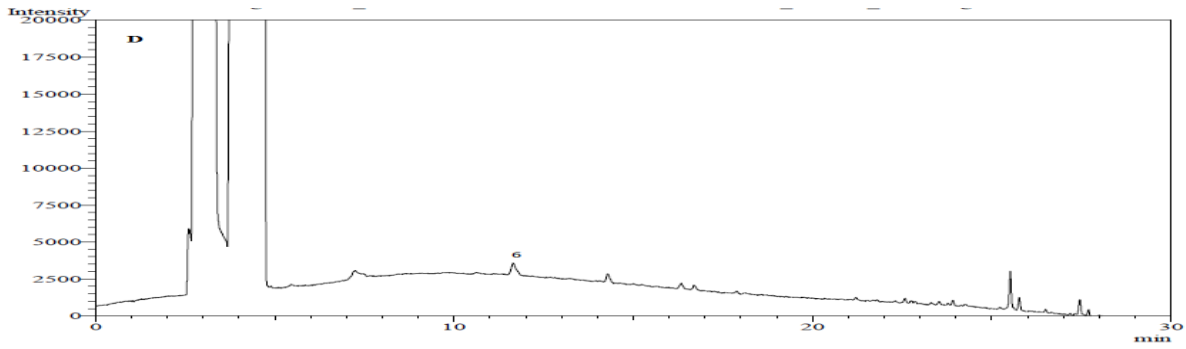
3
4
6
min


Intensity
D
6
min

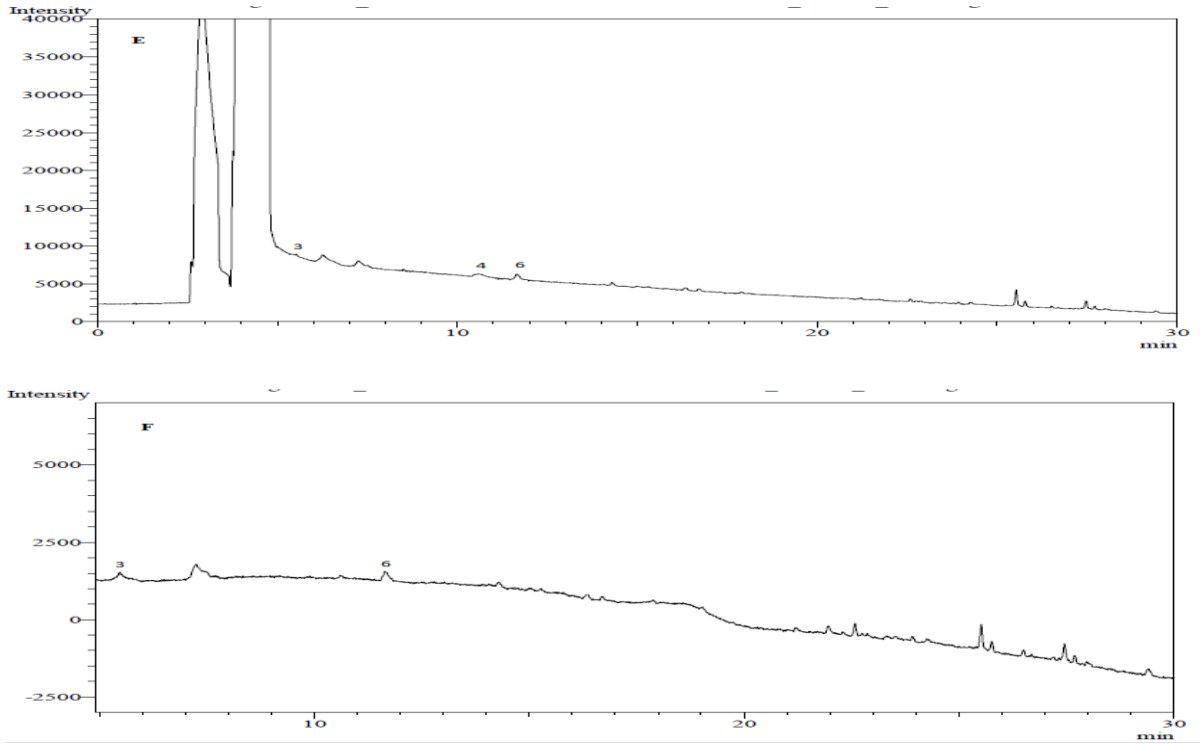


Figure 3.10: MVOCs identified from isolated fungal species from both dust and building materials after 8th day namely; *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus nidulans*, *Cladosporium cladosporioide*, *Curvuleria lunata* and *Penicillium sp*. (A, B, C, D, E and F, respectively)

3.3.3. MVOCs detection and quantified after 16th day of passive sampling over isolated fungal species.

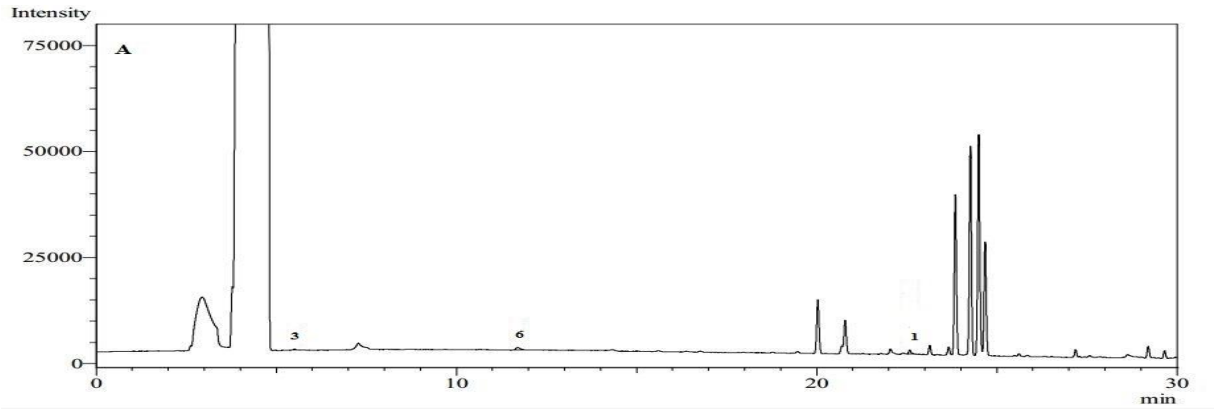
It is clear from table 3.5 and figure 3.11, among all the 6 MVOCs studied upon only 4 namely; 1-octen-3-ol, 1-hexene, 3-methyl-1-butanol and 1-pentanol could be detected to be released by some of the isolated fungal species from both dust and building material samples from all the four sites after 16th day. *Aspergillus nidulans* was found to produce all the 4 compounds. Amongst the 4, 1-octen-3-ol was detected and quantified from all the six fungal species Highest concentration was detected from *Aspergillus niger* and *Penicillium sp*. with 0.09 µg/ml, followed by *Aspergillus nidulans, Curvuleria lunata , Aspergillus flavus* and *Cladosporium cladosporioide.* with concentrations 0.08 µg/ml, 0.06 µg/ml, 0.05 µg/ml and 0.04 µg/ml respectively. Similarly 1-hexene was maximally released by *Aspergillus niger and Curvuleria sp.* with 0.07 µg/ml concentration followed by *Aspergillus flavus* and *Aspergillus nidulans* with 0.06 µg/ml concentration. In case of 1-pentanol, highest

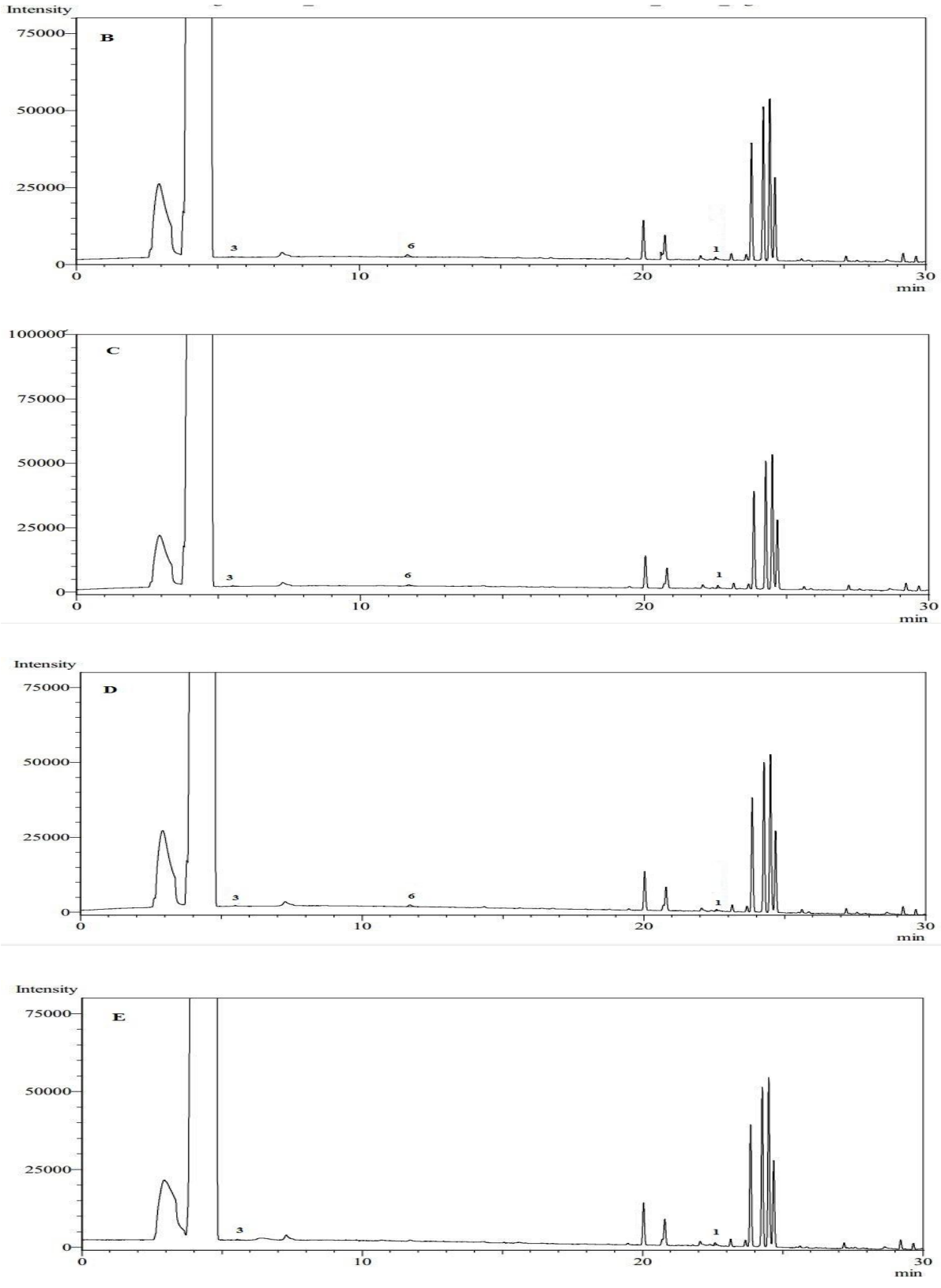
concentration was released by *Aspergillus flavus* and lowest by *Aspergillus nidulans* i.e., 0.30 µg/ml and 0.18 µg/ml respectively. Unlike the other three MVOCs, 3-methyl-1-butanol was released only by *Aspergillus nidulans* and *Curvuleria lunata* however none of them could be quantified due to very low concentrations.

Table 3.5: Detection and quantification of MVOCs from six fungal species after 16th day (µg/ml)

	Fungal Species					
MVOCs	*A.niger*	*A.flavus*	*A.nidulans*	*Cladosporium cladosporioide*	*Curvuleria lunata.*	*Penicilium sp.*
1-octen-3-ol	+ 0.09	+ 0.05	+ 0.08	+ 0.04	+ 0.06	+ 0.09
3-octanone	-	-	-	-	-	-
1-hexene	+ 0.07	+ 0.06	+ 0.06	+ 0.04	+ 0.07	-
3-methyl-1-butanol	-	-	+ NQ	-	+ NQ	-
Cyclohexanone	-	-	-	-	-	-
1-pentanol	+ 0.23	+ 0.30	+ 0.18	+ 0.26	-	+ 0.20

Note: (+) indicates compound detected; (-) indicates compounds not produced by the species on the specific medium or was present below the detectable level; NQ indicates Not Quantifiable




Intensity
B
75000
50000
25000
0
3
6
1
0
10
20
30
min
100000
C
75000
50000
25000
0
3
6
1
0
10
20
30
min
Intensity
D
75000
50000
25000
0
3
6
1
0
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Intensity
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1
0
10
20
30
min

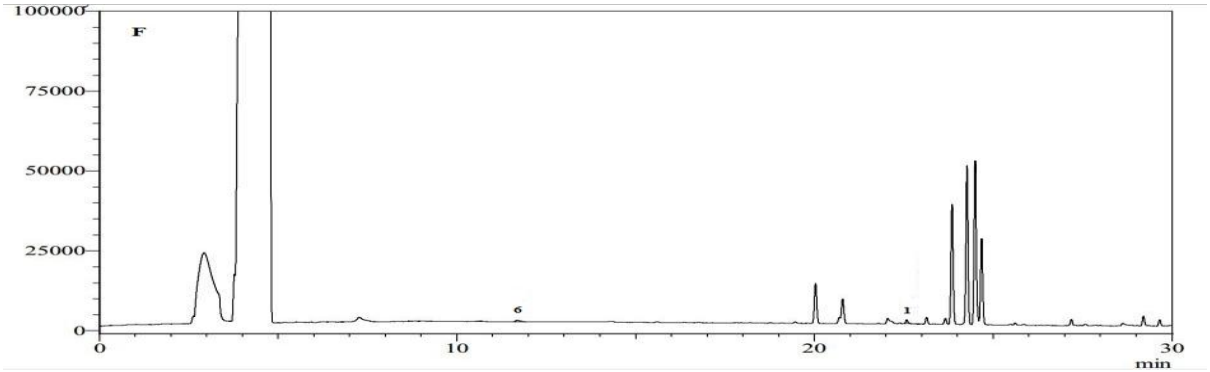
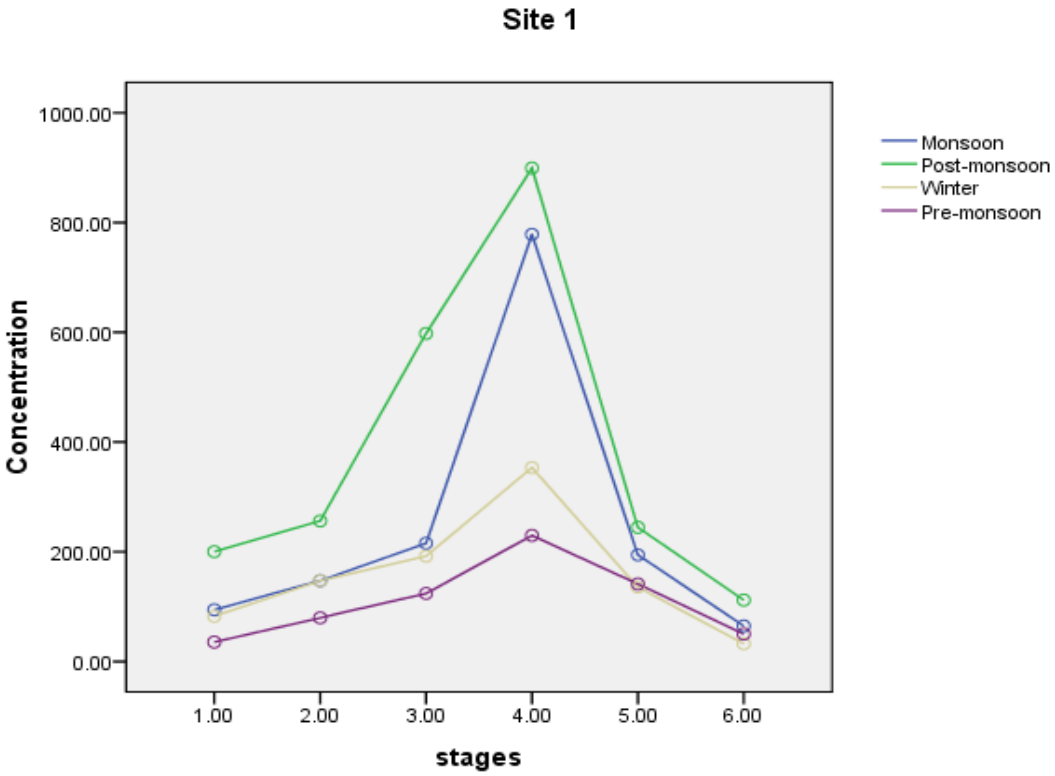


Figure 3.11: MVOCs identified from isolated fungal species from both dust and building materials after 16th day namely *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus nidulans*, *Cladosporium cladosporioide*, *Curvuleria lunata* and *Penicillium sp*. (A, B, C, D, E and F, respectively)

3.4. Characterization and seasonal variation of bioaerosol in different indoor environments

3.4.1. Size segregated fungal bioaerosol in indoor environment

The concentration of fungal bioaerosol found in different stages at each site seems to follow a typical pattern in all the four season. According to figure 3.12 there is an increasing trend in concentration from stage 1 to 4 with a further decrease from stage 4 to 6 at all the six sites. In all the six figures the highest concentration of fungus is found at stage 4 (diameter ranging from 2.1 to 3.3 µm) and lowest concentration at stage 6 (diameter ranging from 0.6 to 1.1 µm).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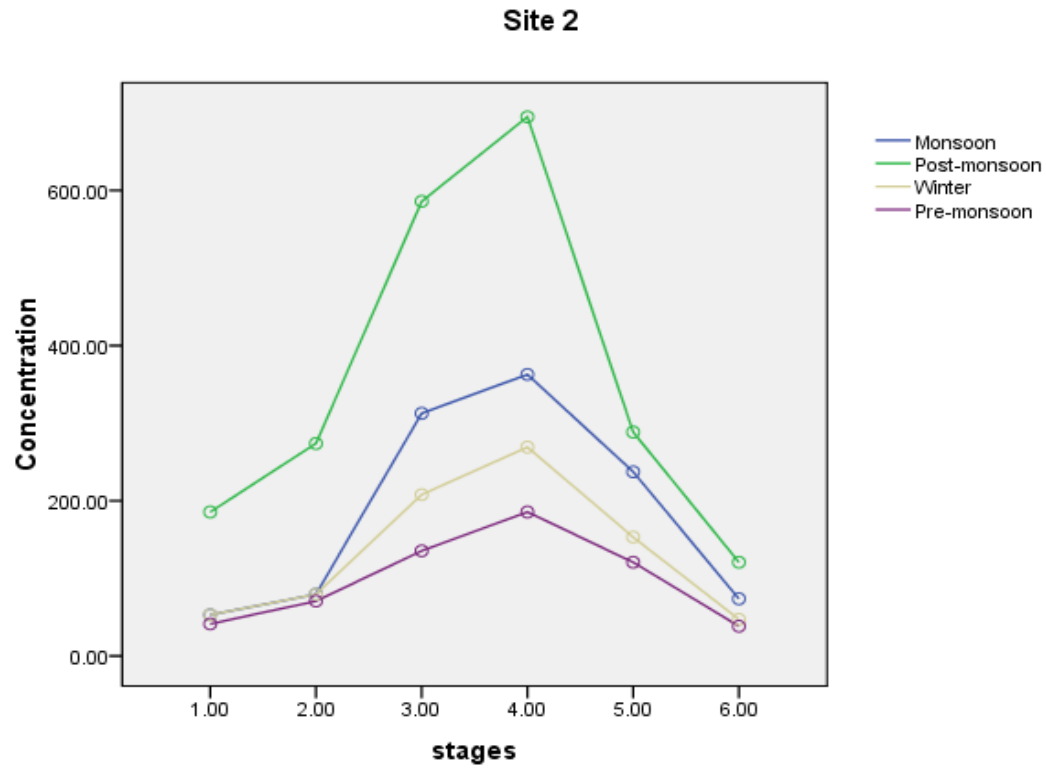
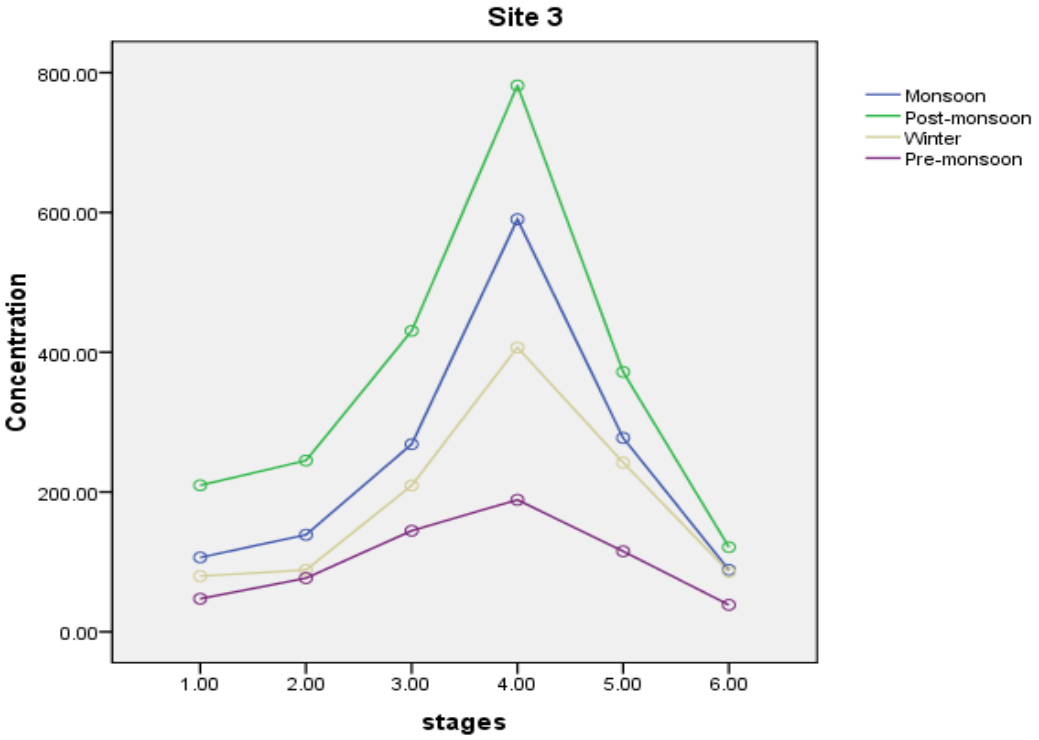
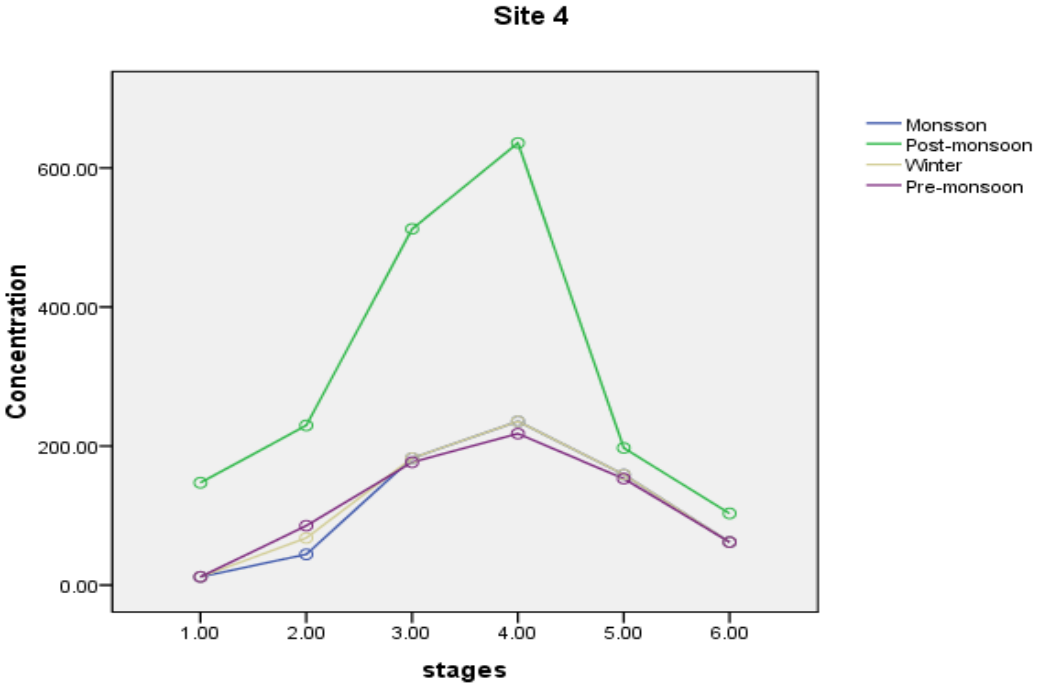
1000.00
800.00
600.00
400.00
200.00
0.00
Concentration
1.00
2.00
3.00
4.00
5.00
6.00
stages
Monsoon
Post-monsoon
Winter
Pre-monsoon


Site 2
600.00
400.00
200.00
0.00
Concentration
1.00
2.00
3.00
4.00
5.00
6.00
stages
Monsoon
Post-monsoon
Winter
Pre-monsoon


Site 3
800.00
600.00
400.00
200.00
0.00
Concentration
1.00
2.00
3.00
4.00
5.00
6.00
stages
Monsoon
Post-monsoon
Winter
Pre-monsoon


Site 4
600.00
400.00
200.00
0.00
Concentration
1.00
2.00
3.00
4.00
5.00
6.00
stages
Monsson
Post-monsoon
Winter
Pre-monsoon

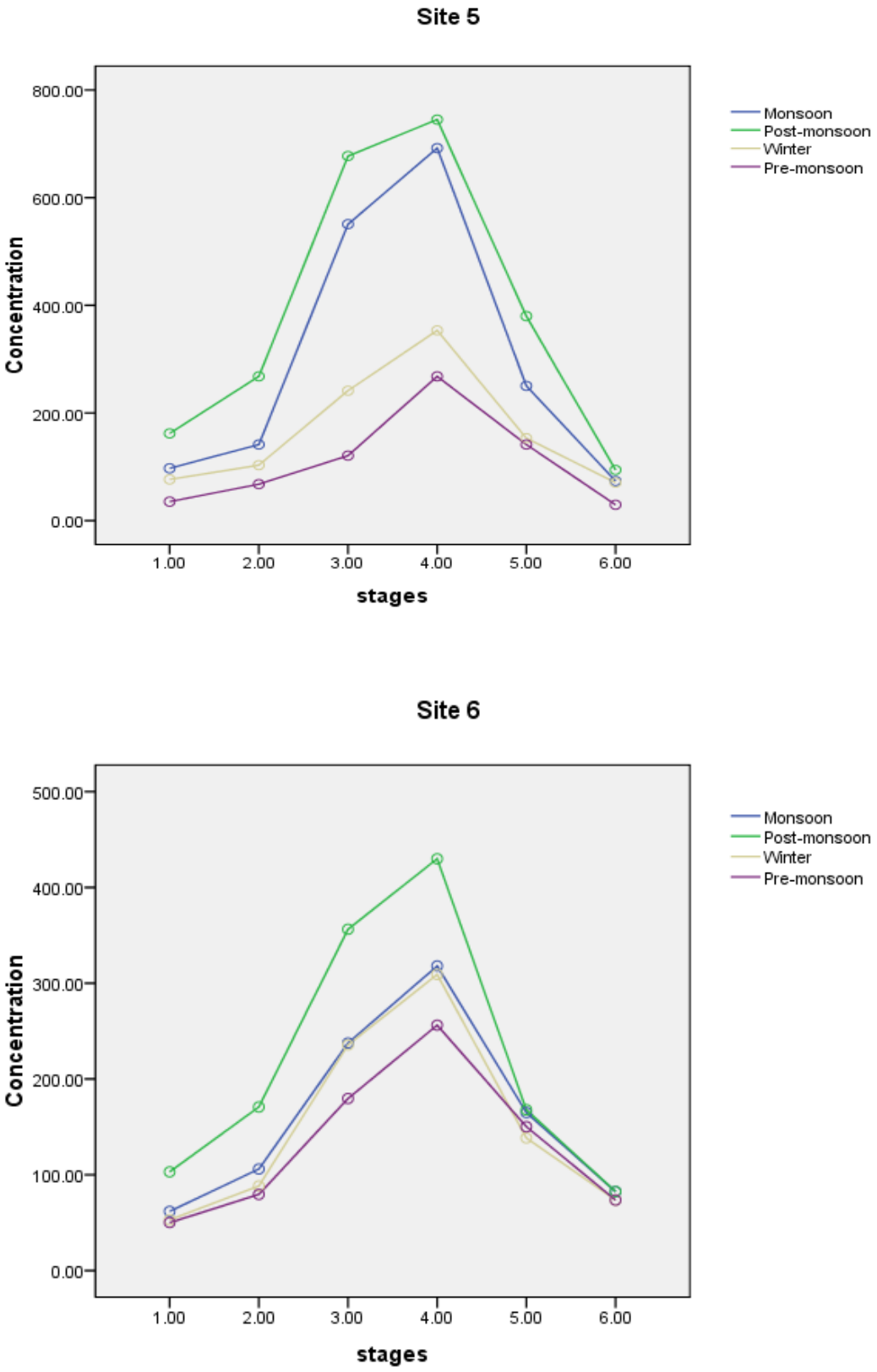


Figure 3.12: Size segregated distribution of fungal bioaerosol in different season at different sites.

3.4.2. Size segregated bacterial bioaerosol in indoor environment

Unlike fungi, bacterial concentration does not follow any typical pattern of size distribution in indoor environment. The concentration of bacterial bioaerosol was different for different sites and seasons.

As seen in figure 3.13 at site 1, concentration of all the sizes of bacterial bioaerosol in post-monsoon season were higher than other three seasons wherein stage 6 (diameter ranging from 0.6 to 1.1 µm) was found to have the highest concentration while the lowest was at stage 3. In monsoon season highest concentration was found at stages 3 (diameter ranging from 3.3 to 4.7 µm) and 5 with the lowest being at stage 4. Similar to post-monsoon season highest concentration of bacteria was found at site 6 and lowest at stage 3 in winter season. Unlike all the other seasons, not much variation was found among all the size segregated bacterial bioaerosol in pre-monsoon season.

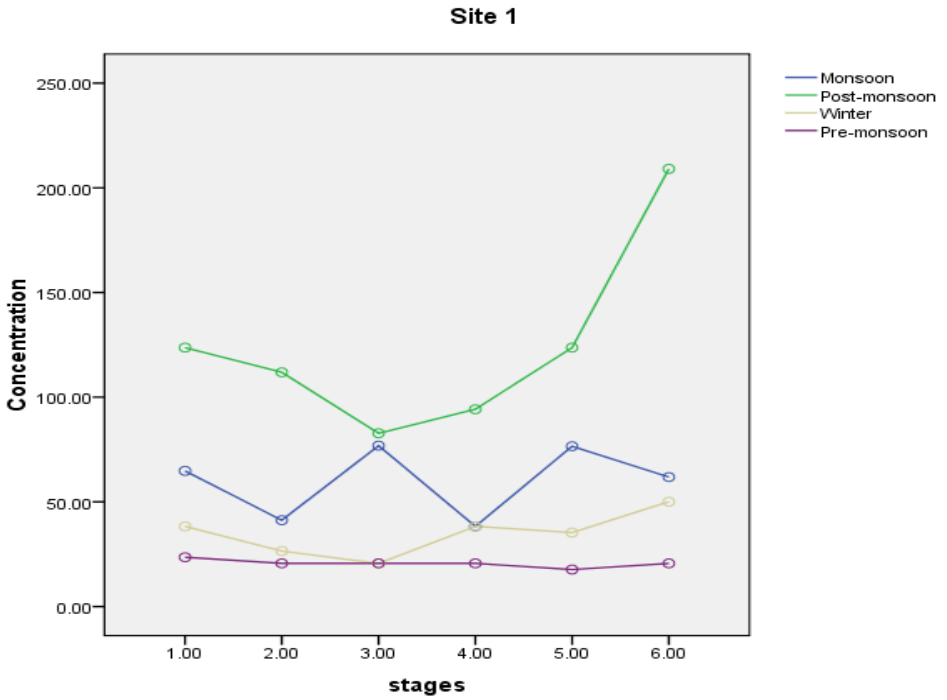
At site 2 in monsoon and post-monsoon season highest bacterial concentration was found at stages 6 and 1 (diameter >7 µm) respectively while the lowest at stage 4 for both. In winter season highest bacterial concentration was found at stage 6 and the lowest at stage 1. Similar to site 1, at site 2 not much variation was found among all the size segregated bacterial bioaerosol in pre-monsoon season.

At site 3 in monsoon season highest bacterial concentration was found at stage 3 while the lowest at stage 6. In post-monsoon season, stage 6 depicted the highest bacterial concentration and stage 4 the lowest. In winter season not much variation was found among all the size segregated bacterial bioaerosol. Similarly in pre-monsoon season though the variations were very less yet comparatively the highest bacterial concentration was found at stage 5 while the lowest at stage 2.

Similar to site 1, at site 4 post-monsoon season too depicts the highest concentration of bacteria for all the size ranges in comparison to other seasons. In the post monsoon season the highest bacterial concentration was found at stage 6 and the lowest at stage 3 that was entirely vice versa in case of monsoon season. In winter and pre-monsoon season, size variation was found to follow a similar pattern with highest bacterial concentration at stage 4 and lowest at stage 1.

At site 5, both monsoon and post-monsoon season was found to follow a similar pattern with highest bacterial concentration at stage 1 and lowest at stage 5. In winter season highest bacterial concentration was found at stage 3 while lowest at stage 2. In pre-monsoon season though the variations were very less yet comparatively the highest bacterial concentration was found at stage 6 while the lowest at stage 4.

At site 6, post-monsoon season depicts the highest concentration for all the sizes except stage 3. Thus in this season the highest bacterial concentration was found at stage 6 while the lowest at stage 3. In monsoon season the highest concentration was found at stage 4 (diameter ranging from 2.1 to 3.3 μm) and the lowest at stage 1. In winters, stages 5 and 1 have similar and highest concentration while stage 3 the lowest concentration. Similar to site 1 and 2 not much variation was found among all the size segregated bacterial bioaerosol in pre-monsoon season at site 6.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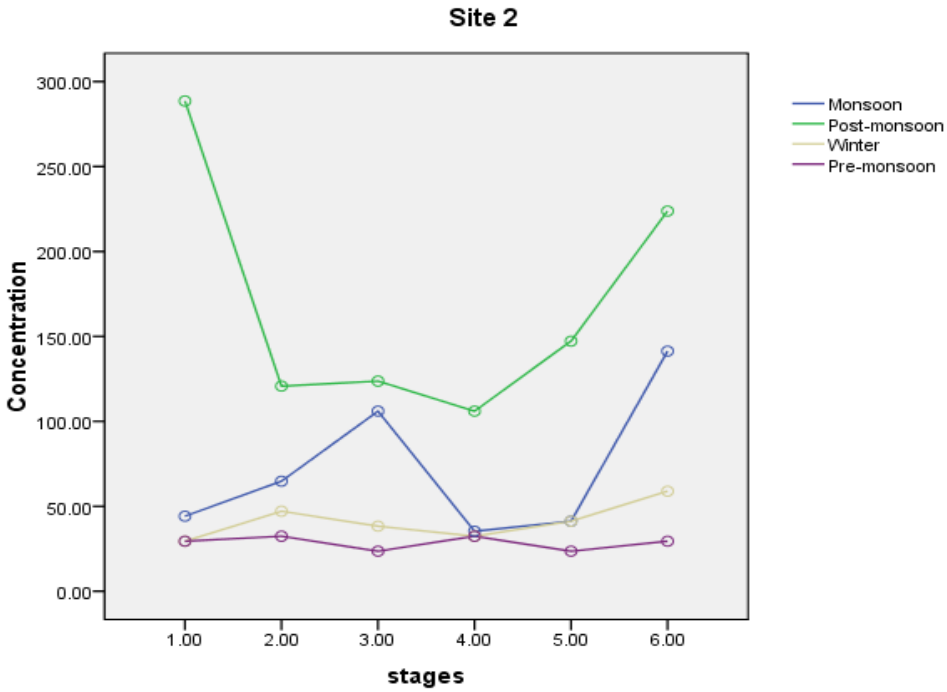
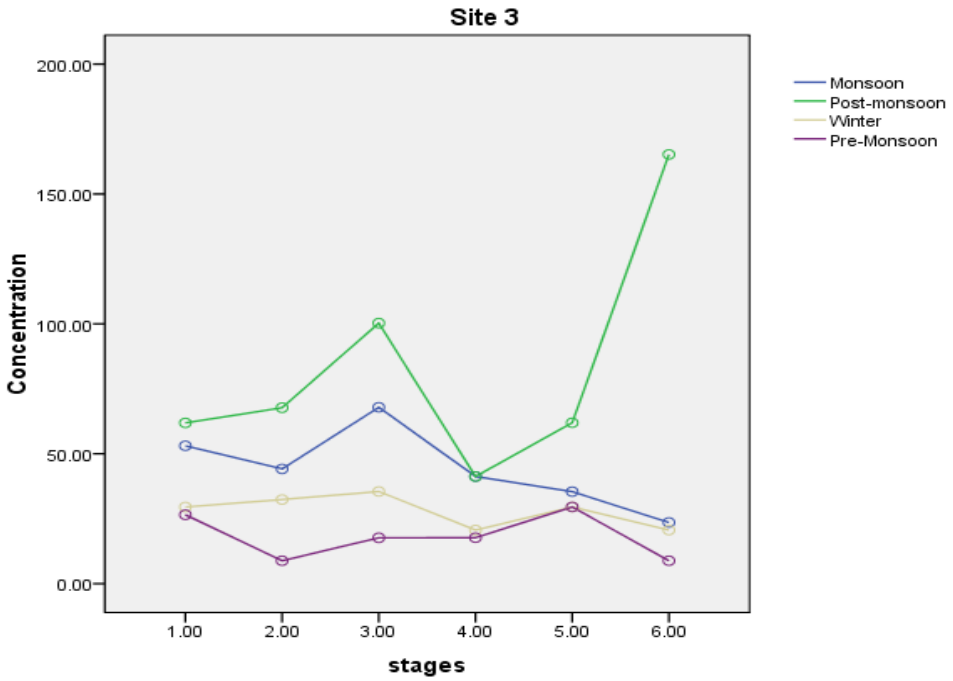
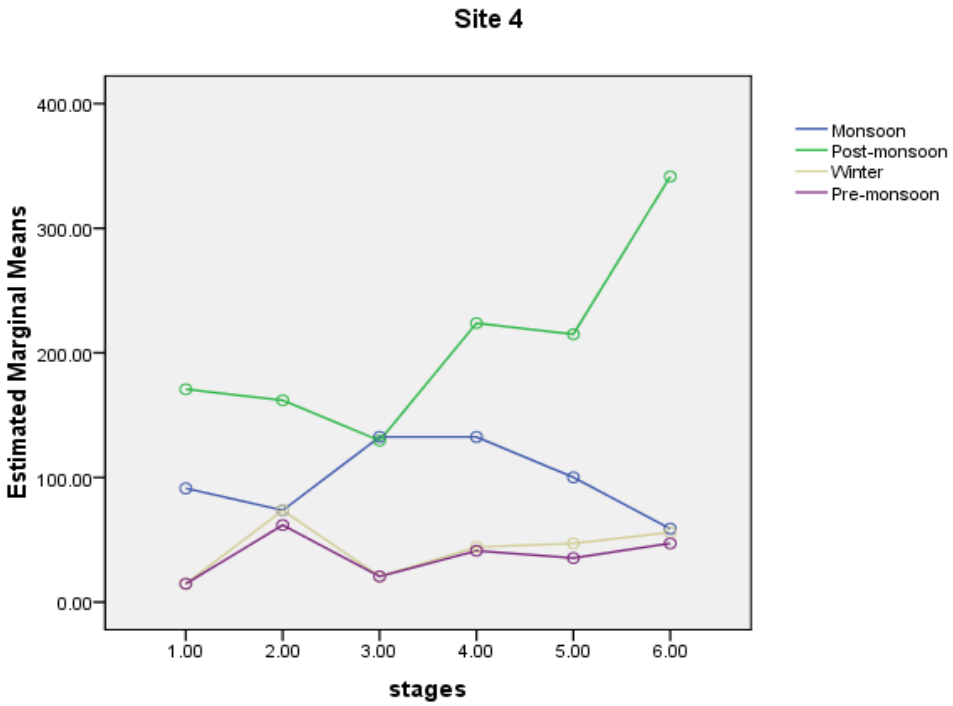
Concentration
250.00
200.00
150.00
100.00
50.00
0.00
1.00
2.00
3.00
4.00
5.00
6.00
stages
Monsoon
Post-monsoon
Winter
Pre-monsoon


Site 2
Concentration
300.00
250.00
200.00
150.00
100.00
50.00
0.00
1.00
2.00
3.00
4.00
5.00
6.00
stages
Monsoon
Post-monsoon
Winter
Pre-monsoon


Site 3
Concentration
200.00
150.00
100.00
50.00
0.00
1.00
2.00
3.00
4.00
5.00
6.00
stages
Monsoon
Post-monsoon
Winter
Pre-Monsoon


Site 4
Estimated Marginal Means
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300.00
200.00
100.00
0.00
1.00
2.00
3.00
4.00
5.00
6.00
stages
Monsoon
Post-monsoon
Winter
Pre-monsoon

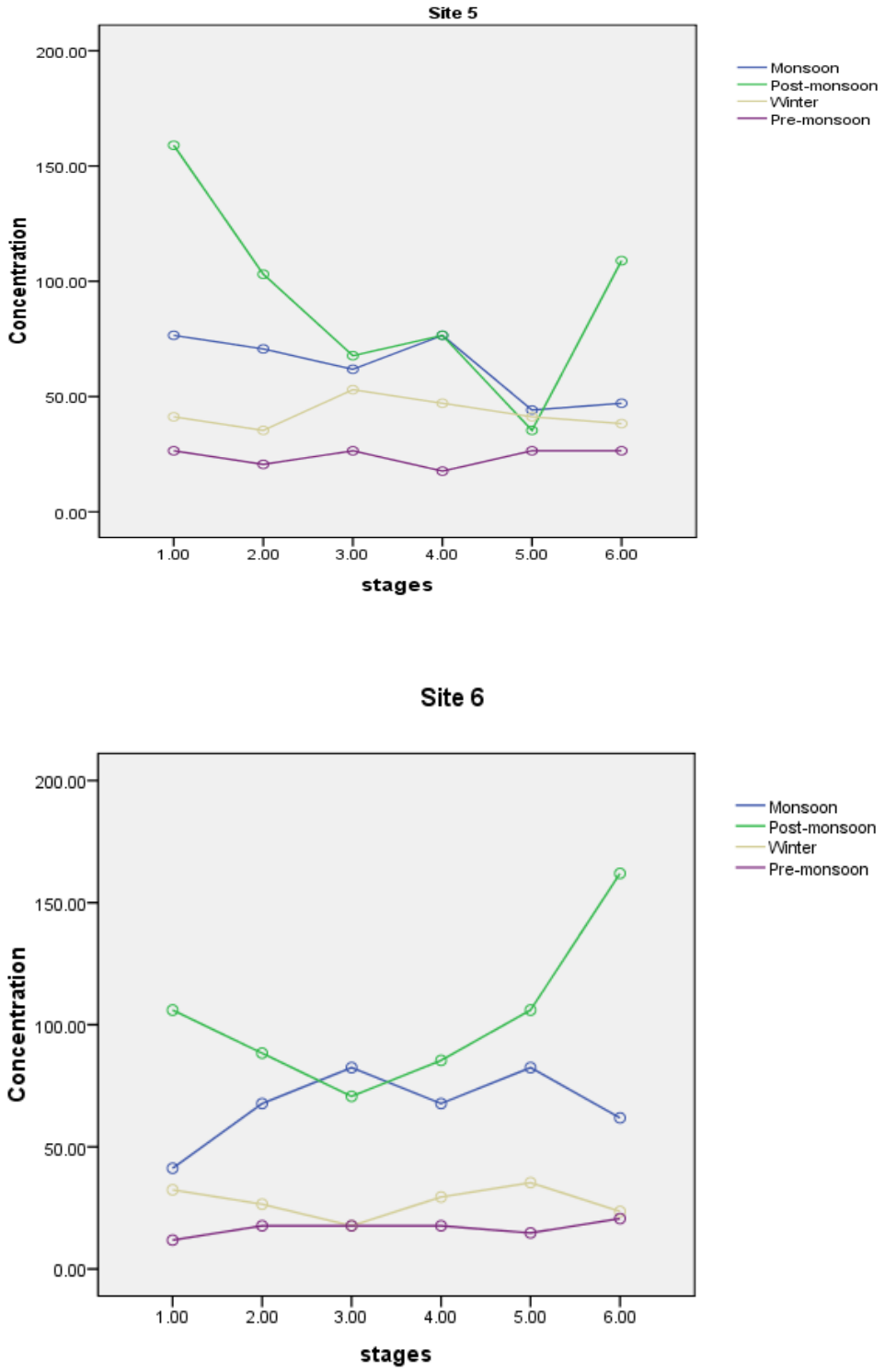

Figure 3.13: Size segregated distribution of bacterial bioaerosol in different season at different sites.

3.4.3. Seasonal variation of fungal bioaerosol concentration at different sites.

Table 3.6 to 3.9 shows fungal bioaerosol concentration in various indoor environments in four seasons of India. In monsoon season, fungal bioaerosol concentration was found within a range of 653-1925 $CFUm^{-3}$. Minimum mean concentration was found at site 2 (111 $CFUm^{-3}$) and maximum mean concentration at site 6 (980 $CFUm^{-3}$).

In post-monsoon season, fungal bioaerosol concentration was found within a range of 812-3462 $CFUm^{-3}$. Minimum mean concentration was found at site 6 (133 $CFUm^{-3}$) and maximum mean concentration at site 1 (230 $CFUm^{-3}$).

In the winter season, range of fungal bioaerosol concentration was 547-1784 $CFUm^{-3}$. Minimum mean concentration was found at site 3 (111 $CFUm^{-3}$) and maximum mean concentration at site 5 (965 $CFUm^{-3}$).

In pre-monsoon or summer season, range of fungal bioaerosol concentration was 318-1007 $CFUm^{-3}$. Minimum and maximum mean concentration was found to be 577 $CFUm^{-3}$ and 789 $CFUm^{-3}$ at sites 5 and 6 respectively.

Table 3.10 and figure 3.14 depict the seasonal variation of fungal bioaerosols concentration at six different indoor sites. According to figure 3.14 highest fungal concentrations was found in post-monsoon season while lowest concentration in pre-monsoon season at all the sites.

Table 3.11 depicts the mean fungal outdoor concentration at each site in different season. Figure 3.15 to 3.18 represents the seasonal variation of fungal bioaerosol concentration both indoor and outdoor at all the six sites. According to the figures outdoor fungal bioaerosol concentration was found to be higher than indoor concentration at all sites in all three seasons except monsoon season. Thus I/O ratio (indoor/outdoor) is less < 1 for all three seasons namely post-monsoon, winter and pre-monsoon while > 1 for monsoon season at all the six sites.

Table 3.6: Fungal bioaerosol concentration range in $CFUm^{-3}$ at different sites in monsoon season

Sites	N	Mean	SD	Standard Error	Minimum	Maximum	Temperature Range (ºC)	Relative Humidity Range (%)
Site 1	6	1.4947E3	303.32144	1.23830E2	1008.83	1819.79	27.5-35.2	58.2-72.1
Site 2	6	7.3910E2	71.91196	29.35794	653.71	830.39	25.2-34.8	54.3-69.8
Site 3	6	1.4693E3	117.29964	47.88738	1307.42	1625.00	25.3-35.2	56.2-79.3
Site 4	6	1.1190E3	123.75902	50.52441	1024.73	1360.42	26.2-36.2	55.6-72.3
Site 5	6	1.7344E3	119.52485	48.79582	1572.44	1925.80	26.1-35.9	59.9-74.8
Site 6	6	9.8057E2	168.26245	68.69286	812.72	1183.75	28.1-33.7	58.7-73.5

Table 3.7: Fungal bioaerosol concentration range in $CFUm^{-3}$ at different sites in post-monsoon season

Sites	N	Mean	SD	Standard error	Minimum	Maximum	Temperature Range (ºC)	Relative Humidity Range (%)
Site 1	6	2.3098E3	531.26673	2.16889E2	1678.45	3162.54	28.6-30.1	56.1-58.7
Site 2	6	1.8257E3	661.98249	2.70253E2	1130.74	2879.86	26.8-32.1	53.2-54.2
Site 3	6	2.1595E3	692.25602	2.82612E2	1307.42	3250.88	26.8-30.1	54.8-60.3
Site 4	6	2.1808E3	586.21223	2.39320E2	1583.04	3268.55	27.2-29.4	59.6-61.1
Site 5	6	2.1790E3	672.74054	2.74645E2	1572.44	3462.90	29.0-31.2	54.8-59.2
Site 6	6	1.3310E3	421.46852	1.72064E2	812.72	1819.79	26.2-31.8	54.4-58.2

Table 3.8: Fungal bioaerosol concentration range in $CFUm^{-3}$ at different sites in winter season

Sites	N	Mean	Standard Deviation	Standard Error	Minimum	Maximum	Temperature Range (ºC)	Relative Humidity Range (%)
Site 1	6	9.4287E2	70.85371	28.92591	812.72	1008.83	14.1-24.2	58.1-65.2
Site 2	6	8.0978E2	206.46951	84.29082	583.04	1077.74	16.1-23.3	57.3-66.3
Site 3	6	1.1117E3	340.41219	1.38973E2	742.05	1554.77	15.0-25.0	54.0-59.1
Site 4	6	7.3910E2	71.91196	29.35794	653.71	830.39	14.0-26.1	50.9-62.1
Site 5	6	9.6584E2	437.03072	1.78417E2	547.70	1784.45	15.0-23.0	52.2-66.1
Site 6	6	8.9812E2	169.98511	69.39613	689.05	1183.75	16.1-22.1	58.2-72.1

Table 3.9: Fungal bioaerosol concentration range in $CFUm^{-3}$ at different sites in pre-monsoon season

Sites	N	Mean	SD	Standard error	Minimum	Maximum	Temperature Range (ºC)	Relative Humidity Range (%)
Site 1	6	6.8640E2	184.70715	75.40638	459.36	865.72	34.3-36.1	32.4-36.2
Site 2	6	5.9187E2	115.99044	47.35290	441.70	759.72	35.6-36.1	30.7-35.0
Site 3	6	6.1068E2	140.18544	57.23047	406.36	795.05	27.1-32.1	39.9-41.2
Site 4	6	7.2144E2	87.57027	35.75041	618.37	830.39	26.7-32.5	40.3-42.1
Site 5	6	5.7715E2	189.85056	77.50617	388.69	830.39	35.7-37.2	30.9-40.3
Site 6	6	7.8916E2	252.26481	1.02987E2	318.02	1007.07	33.9-37.2	31.9-42.1

Table 3.10: Average fungal bioaerosol indoor concentration (in $CFUm^{-3}$) in different season

	Site 1		Site 2		Site 3		Site 4		Site 5		Site 6	
Seasons	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
Monsoon	1494.7	303.3	739.1	71.9	1469.3	117.2	1118.9	123.7	1734.4	119.5	980.6	168.2
Post-monsoon	2309.7	531.3	1825.7	661.9	2159.5	692.2	2180.8	586.2	2179	672.7	1330.9	421.4
Winter	942.8	70.8	809.8	206.5	1111.7	340.4	739.1	71.9	965.8	437	898.1	169.9
Pre-monsoon	686.4	184.7	591.9	115.9	610.7	140.2	721.4	87.6	577.2	189.8	789.1	252.3

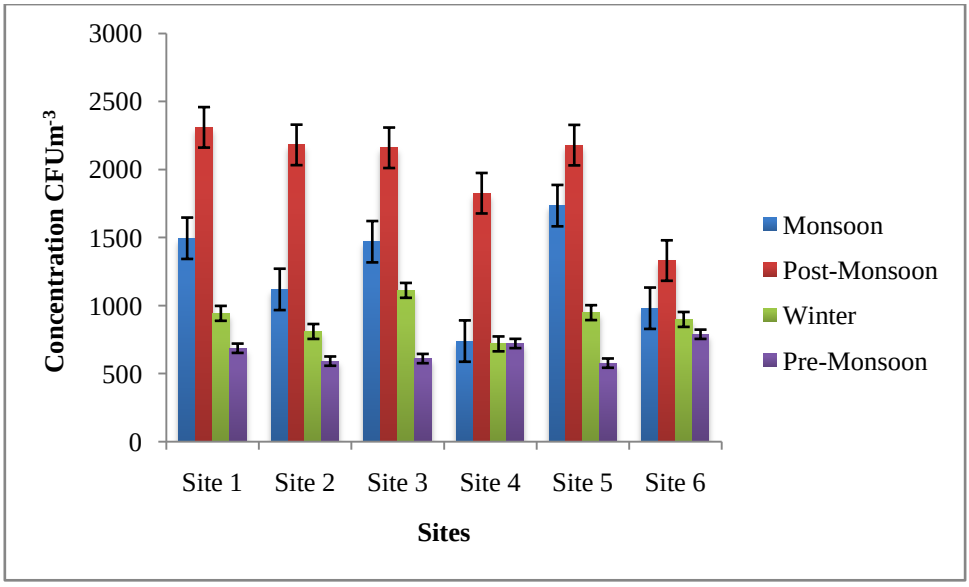

Figure 3.14: Seasonal variation of fungal bioaerosol concentration at different sites

Table 3.11: Average fungal bioaerosol outdoor concentration (in $CFUm^{-3}$) in different seasons

Seasons	Sites	Mean Concentration	Temperature Range (^0C)	Relative Humidity Range (%)
Monsoon	Site 1	980.01	30.0-32.3	74.2-79.1
	Site 2	948.20	29.8-31.8	67.9-79.0
	Site 3	1118.05	30.2-33.8	66.3-80.1
	Site 4	739.80	30.1-32.4	69.1-77.8
	Site 5	1010.20	29.3-33.0	66.8-74.3
	Site 6	745.50	29.8-32.8	65.2-79.8
Post-monsoon	Site 1	3018.30	28.8-30.1	58.5-59.1
	Site 2	2209.05	27.8-31.8	50.2-54.3
	Site 3	2810.06	29.1-32.1	52.8-56.9
	Site 4	2630.50	26.8-31.8	54.2-59.6
	Site 5	3368.23	27.4-32.1	55.8-58.9
	Site 6	2089.56	28.1-33.1	54.6-60.1
Winter	Site 1	1118.58	15.6-21.2	59.6-62.3
	Site 2	1469.24	14.9-20.3	57.2-63.2
	Site 3	1494.23	15.1-24.2	56.3-64.4
	Site 4	1090.85	16.2-23.1	54.5-61.1
	Site 5	990.25	15.7-22.8	56.8-60.7
	Site 6	948.96	15.1-20.7	55.1-61.7
Pre-monsoon	Site 1	865.01	34.3-41.2	20.3-27.3
	Site 2	844.05	35.6-42.3	19.4-26.1
	Site 3	662.04	35.7-40.9	18.9-25.6
	Site 4	774.65	37.1-43.4	20.3-27.2
	Site 5	768.54	38.1-41.5	20.5-26.8
	Site 6	681.85	37.1-39.7	21.3-28.9

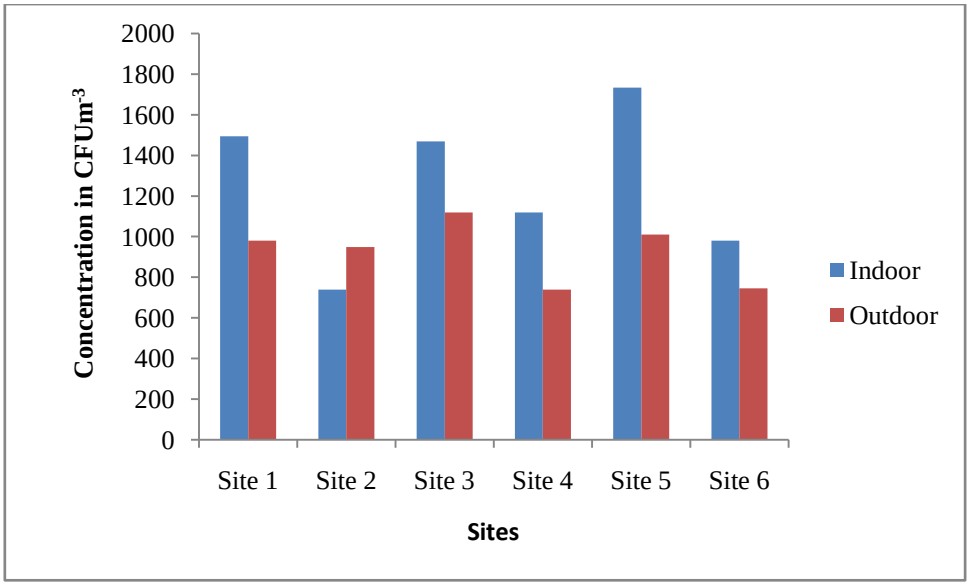

Figure 3.15: Fungal bioaerosol indoor and outdoor concentration in monsoon season

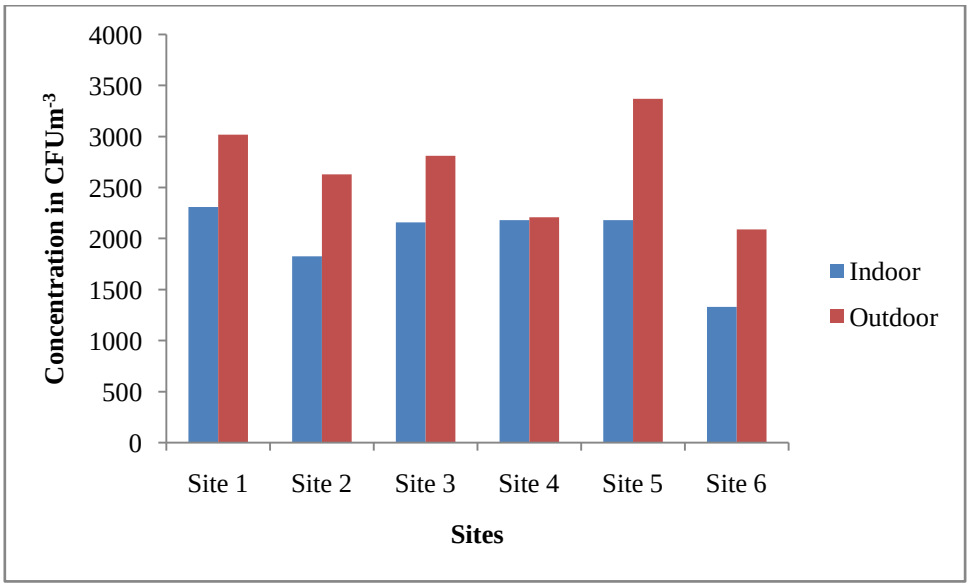

Figure 3.16: Fungal bioaerosol indoor and outdoor concentration in post-monsoon season

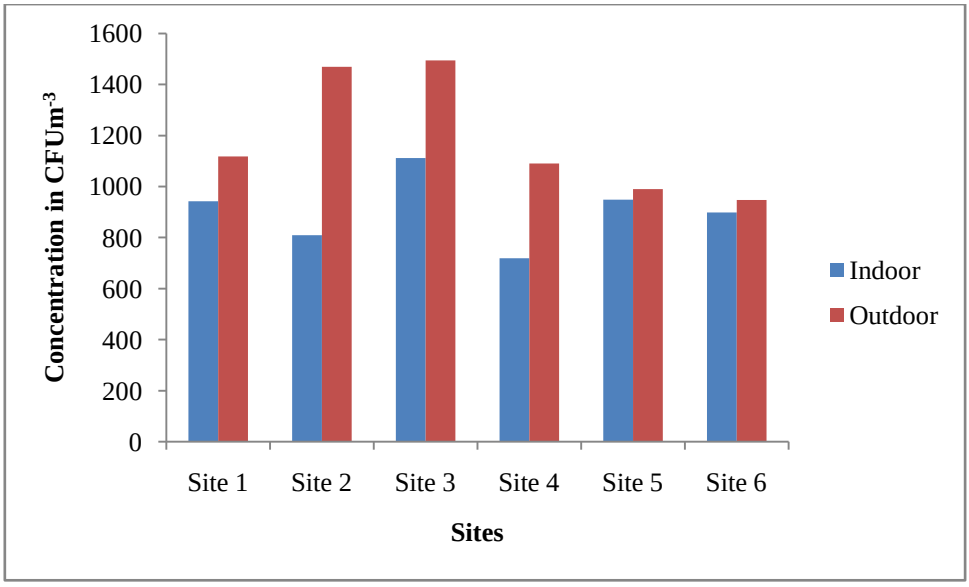

Figure 3.17: Fungal bioaerosol indoor and outdoor concentration in winter season

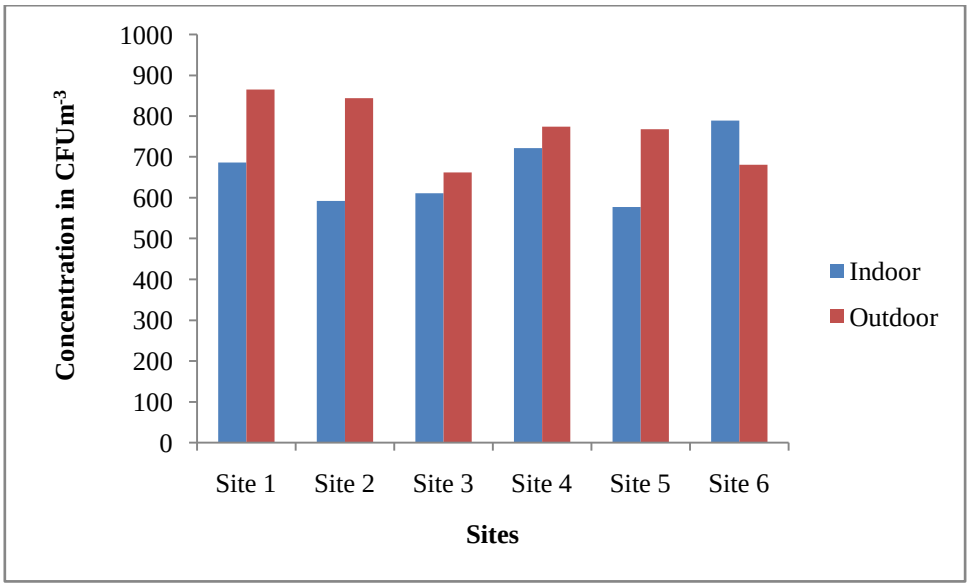

Figure 3.18: Fungal bioaerosol indoor and outdoor concentration in pre-monsoon season

3.4.4. Seasonal variation of bacterial bioaerosol concentration at different sites

Tables 3.12 to 3.15 show bacterial bioaerosol concentration at various indoor environments at four seasons in India. In monsoon season, bacterial bioaerosol concentration was found within a range of 141.34-742.05 $CFUm^{-3}$. Minimum mean concentration was found at site 3 (265 $CFUm^{-3}$) and maximum mean concentration at site 2 (432 $CFUm^{-3}$).

In post-monsoon season, bacterial bioaerosol concentration was found in a range of 318.02-1519.43 $CFUm^{-3}$. Minimum mean concentration was found at site 3 (498 $CFUm^{-3}$) and maximum mean concentration at site 4 (1242 $CFUm^{-3}$).

In the winter season, bacterial bioaerosol concentration ranged 141.34-388.69 $CFUm^{-3}$. Minimum mean concentration at site 6 (164 $CFUm^{-3}$) and maximum mean concentration was at site 4 and 5 (256 $CFUm^{-3}$).

In pre-monsoon or summer season, range of bacterial bioaerosol concentration was 35.34-265.02 $CFUm^{-3}$. Minimum mean concentration was at site 6 (100 $CFUm^{-3}$) and maximum mean concentration was at site 4 (220 $CFUm^{-3}$).

Table 3.16 and figure 3.19 depict the seasonal variation of bacterial bioaerosols concentration at six different indoor sites. According to figure 3.19, highest bacterial concentrations were found in post-monsoon season, while lowest concentration in pre-monsoon season at all the sites.

Table 3.17 depicts the mean bacterial outdoor concentration at each site in different season. From figures 3.20 to 3.23, represent the seasonal variation of bacterial bioaerosol concentration both the indoor and outdoor at all the six sites. According to the figures, outdoor bacterial bioaerosol concentration was found to be higher than indoor concentration at all sites in all three seasons except monsoon. Thus, I/O ratio (indoor/outdoor) is < 1 for all three seasons namely; post-monsoon, winter and pre-monsoon while, it is > 1 for monsoon season at all the proposed six sites.

Table 3.12: Bacterial bioaerosol concentration range in $CFUm^{-3}$ at different sites in monsoon season

Sites	N	Mean	SD	Standard Error	Minimum	Maximum	Temperature Range (ºC)	RH Range (%)
Site 1	6	3.5954E2	98.45762	40.19516	247.35	531.80	27.5-35.2	58.2-72.1
Site 2	6	4.3286E2	97.89325	39.96475	265.02	530.04	26.2-36.2	55.6-72.3
Site 3	6	2.6536E2	53.04399	21.65512	196.43	335.69	25.3-35.2	56.2-79.3
Site 4	6	5.8893E2	140.30835	57.28064	388.69	742.05	25.2-34.8	54.3-69.8
Site 5	6	3.7691E2	243.70527	99.49226	141.34	689.05	26.1-35.9	59.9-74.8
Site 6	6	4.0342E2	122.61874	50.05889	229.68	512.37	28.1-33.7	58.7-73.5

Table 3.13: Bacterial bioaerosol concentration range in $CFUm^{-3}$ at different sites in post-monsoon seasoon

Sites	N	Mean	SD	Standard error	Minimum	Maximum	Temperature Range (ºC)	RH Range (%)
Site 1	6	7.4529E2	43.57073	17.78768	689.05	795.05	28.6-30.1	56.1-58.7
Site 2	6	1.0100E3	155.40340	63.44317	830.39	1201.41	27.2-29.4	59.6-61.1
Site 3	6	4.9831E2	125.83934	51.37370	375.00	653.71	26.8-30.1	54.8-60.3
Site 4	6	1.2426E3	178.66958	72.94155	1042.40	1519.43	26.8-32.1	53.2-54.2
Site 5	6	5.5065E2	164.00354	66.95416	318.02	777.39	29.0-31.2	54.8-59.2
Site 6	6	6.1837E2	59.12792	24.13887	530.04	689.05	26.2-31.8	54.4-58.2

Table 3.14: Bacterial bioaerosol concentration range in $CFUm^{-3}$ at different sites in winter season

Sites	N	Mean	Standard Deviation	Standard Error	Minimum	Maximum	Temperature Range (ºC)	RH Range (%)
Site 1	6	2.0907E2	40.92945	16.70938	141.34	247.35	14.1-24.2	58.1-65.2
Site 2	6	2.4735E2	54.74181	22.34825	176.68	335.69	16.1-23.3	57.3-66.3
Site 3	6	1.6819E2	33.39657	13.63409	123.67	212.01	15.0-25.0	54.0-59.1
Site 4	6	2.5618E2	38.30297	15.63712	212.01	318.02	14.0-26.1	50.9-62.1
Site 5	6	2.5618E2	104.67359	42.73281	141.34	388.69	15.0-23.0	52.2-66.1
Site 6	6	1.6490E2	84.11576	34.34012	88.34	300.35	16.1-22.1	58.2-72.1

Table 3.15: Bacterial bioaerosol concentration range in $CFUm^{-3}$ at different sites in pre-monsoon season

Sites	N	Mean	SD	Standard error	Minimum	Maximum	Temperature Range (ºC)	RH Range (%)
Site 1	6	1.2367E2	24.98611	10.20053	88.34	159.01	34.3-36.1	32.4-36.2
Site 2	6	2.2085E2	33.05351	13.49404	159.01	247.35	26.7-32.5	40.3-42.1
Site 3	6	1.0911E2	35.95806	14.67982	53.00	159.01	27.1-32.1	39.9-41.2
Site 4	6	1.7079E2	76.33381	31.16315	70.67	265.02	35.6-36.1	30.7-35.0
Site 5	6	1.4429E2	28.30534	11.55560	106.01	176.68	35.7-37.2	30.9-40.3
Site 6	6	1.0012E2	39.76901	16.23563	35.34	141.34	33.9-37.2	31.9-42.1

Table 3.16: Average bacterial bioaerosol concentration (in $CFUm^{-3}$) in different season

	Site 1		Site 2		Site 3		Site 4		Site 5		Site 6	
Seasons	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
Monsoon	359.5	98.5	432.9	97.9	265.4	53.05	588.9	140.3	376.9	243.7	403.4	122.6
Post- monsoon	745.3	43.6	1010.0	155.4	498.3	125.8	1242.6	178.7	550.7	164.0	618.4	59.1
Winter	209.1	40.9	247.4	54.7	168.2	33.4	256.2	38.3	256.2	104.7	164.9	84.1
Pre-monsoon	123.7	24.9	220.8	33.1	109.1	35.9	170.8	76.3	144.3	28.3	100.1	39.8

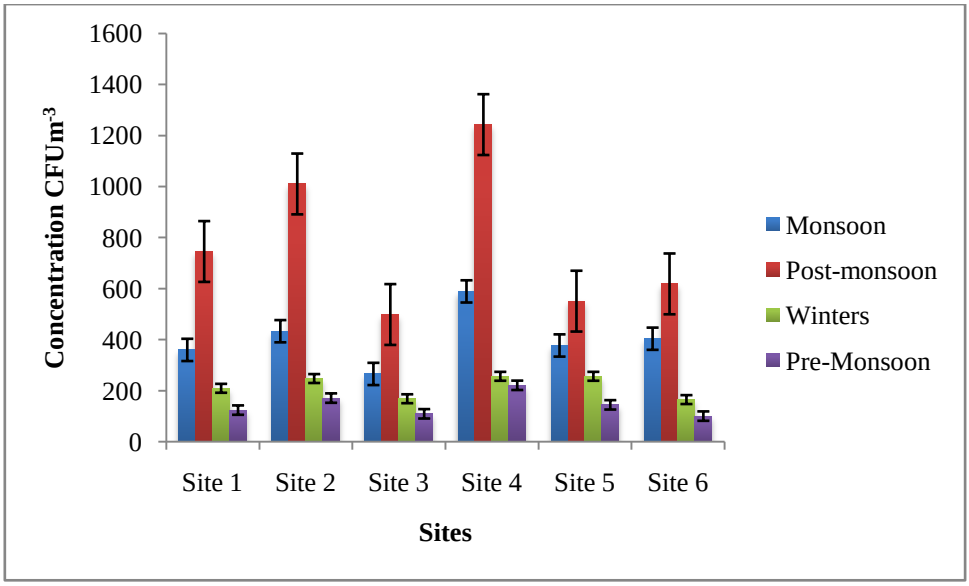

Figure 3.19: Seasonal variation of bacterial bioaerosol concentration at different sites

Table 3.17: Average bacterial bioaerosol outdoor concentration (in $CFUm^{-3}$) in different seasons

Seasons	Sites	Mean	Temperature Range (^0C)	RH Range (%)
Monsoon	Site 1	371.10	30.0-32.3	74.2-79.1
	Site 2	401.23	29.8-31.8	67.9-79.0
	Site 3	354.07	30.2-33.8	66.3-80.1
	Site 4	523.3	30.1-32.4	69.1-77.8
	Site 5	356.2	29.3-33.0	66.8-74.3
	Site 6	389.65	29.8-32.8	65.2-79.8
Post-monsoon	Site 1	640.85	28.8-30.1	58.5-59.1
	Site 2	784.15	27.8-31.8	50.2-54.3
	Site 3	510.01	29.1-32.1	52.8-56.9
	Site 4	1045.30	26.8-31.8	54.2-59.6
	Site 5	621.45	27.4-32.1	55.8-58.9
	Site 6	550.41	28.1-33.1	54.6-60.1
Winter	Site 1	543.12	15.6-21.2	59.6-62.3
	Site 2	426.52	14.9-20.3	57.2-63.2
	Site 3	450.54	15.1-24.2	56.3-64.4
	Site 4	387.74	16.2-23.1	54.5-61.1
	Site 5	389.45	15.7-22.8	56.8-60.7
	Site 6	268.25	15.1-20.7	55.1-61.7
Pre-monsoon	Site 1	200.35	34.3-41.2	20.3-27.3
	Site 2	230.05	35.6-42.3	19.4-26.1
	Site 3	150.03	35.7-40.9	18.9-25.6
	Site 4	321.85	37.1-43.4	20.3-27.2
	Site 5	254.95	38.1-41.5	20.5-26.8
	Site 6	185.96	37.1-39.7	21.3-28.9

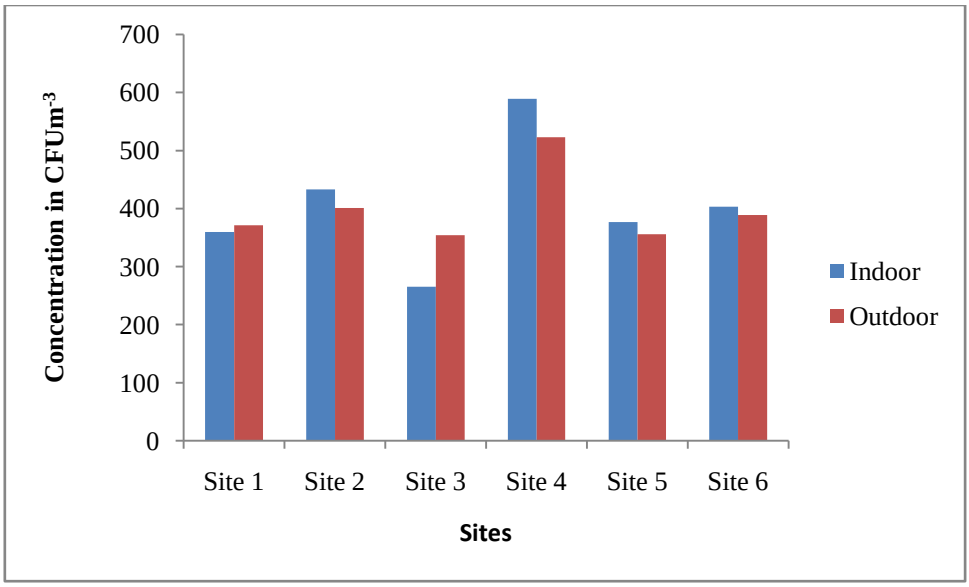

Figure 3.20: Bacterial bioaerosol indoor and outdoor concentration in monsoon season

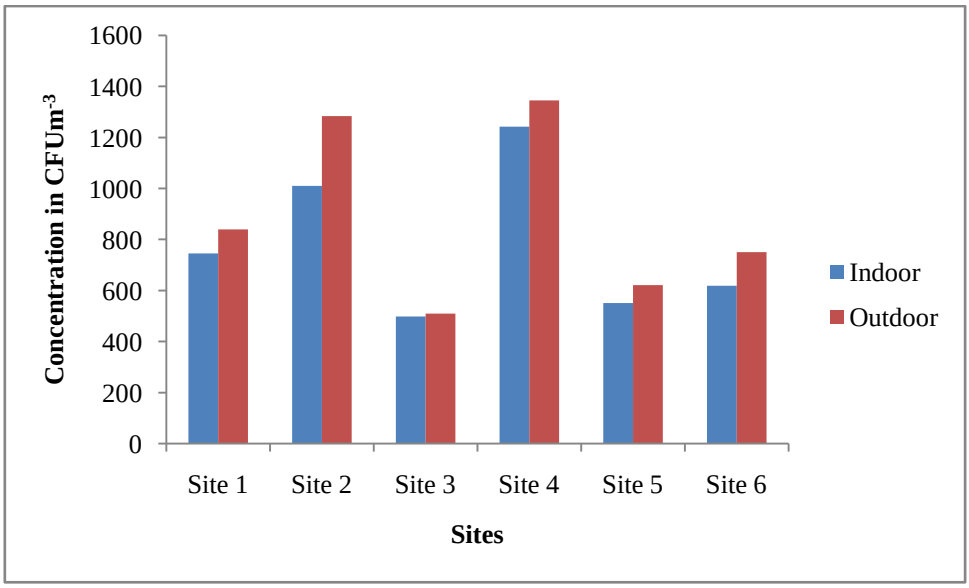

Figure 3.21: Bacterial bioaerosol indoor and outdoor concentration in post-monsoon season

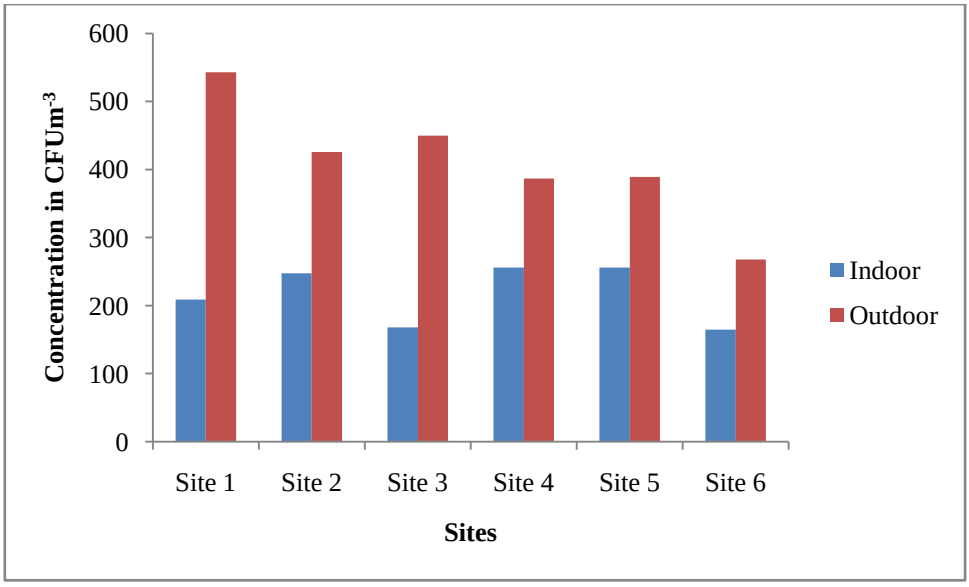

Figure 3.22: Bacterial bioaerosol indoor and outdoor concentration in winter season

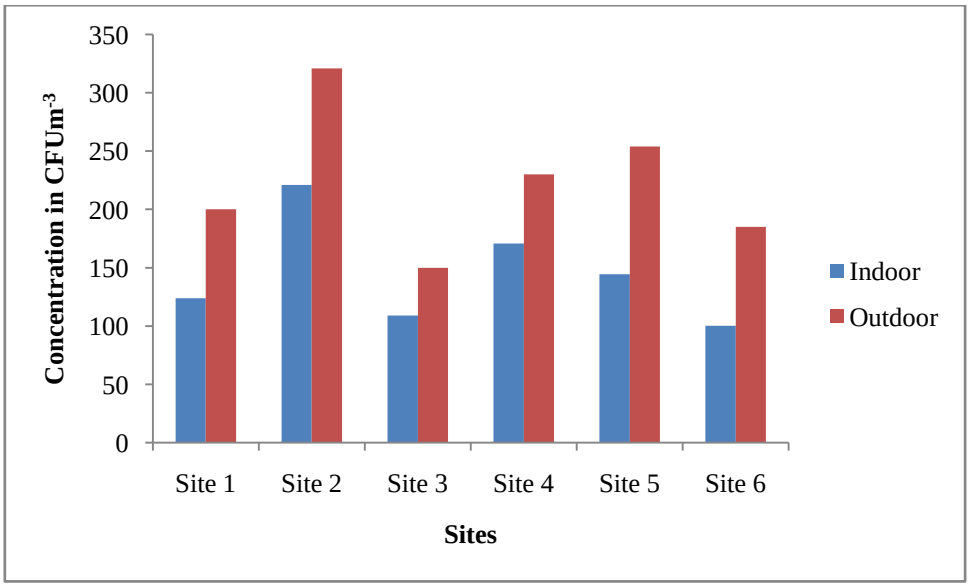

Figure 3.23: Bacterial bioaerosol indoor and outdoor concentration in pre-monsoon season

3.4.5. Identification of fungal bioaerosol

Eight fungal bioaerosols were identified at all the sites during four different seasons as shown in table 3.18. Among the seven genera, three fungal genera namely; *Aspergillus*, *Cladosporium* and *Penicillium* were found to be present at all the six sites at all the four season.

Tables 3.19 to 3.22 represent the percentage distribution of the fungal species at different sites in different season. According to table 3.19, in monsoon season, *Aspergillus* is the dominant species followed by *Alternaria* at sites 1, 2, 3, 4 and 6 while; *Alternaria*, *Curvuleria* and *Penicillium* were maximally found fungal genera at site 5.

According to table 3.20, in post monsoon season, *Cladosporium* is the dominant species followed by *Curvuleria* at sites 1, 2, 3 and 4. At sites 5 and 6, *Curvuleria* is maximally found among all the fungal species.

As seen in tables 3.21 and 3.22, *Cladosporium* is the dominant species followed by *Penicillium* and *Aspergillus* in the winter season at all the six sites while, *Aspergillus* is the found maximally of the fungal genera in pre-monsoon season at all the six sites, respectively. *Curvuleria* and *Drechslera* were absent in both the seasons at both the sites.

Table 3.18: Fungal genus identified at different sites

	Site 1				Site 2				Site 3				Site 4				Site 5				Site 6			
Seasons→	M	PoM	W	PrM	M	PoM	W	PrM	M	PoM	W	PrM	M	PoM	W	PrM	M	PoM	W	PrM	M	PoM	W	PrM
Fungi↓																								
Aspergillus Sp. ≠	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alternaria ×	+	+	-	-	+	+	-	-	+	+	-	-	+	+	+	-	+	+	+	-	+	+	+	-
Curvularia ≠	+	+	-	-	+	+	-	-	+	+	-	-	-	+	-	-	+	+	-	-	+	+	-	-
Cladosporium ×	+	+	+	+	+	+	+	-	+	+	+	-	+	+	+	-	-	+	+	-	-	+	+	-
Penicillium ×	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Drechslera ≠	+	-	-	-	+	-	-	-	+	-	-	-	+	-	-	-	-	-	-	-	+	-	-	-
Fusarium ×	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	-	+	-	-	-	+	-
Rhizopus ×	-	-	-	+	-	-	-	-	-	-	-	+	-	-	-	+	-	-	-	+	-	-	-	+
Unknown	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Note. M: Monsoon; PoM: Post-monsoon; W: Winter; PrM: Pre-monsoon; ≠ Allergic; × Harmless; × Immunotoxic

Table 3.19: Distribution of fungal sp. (in percentage) at six sites in monsoon season

	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6
Aspergillus Sp.	37	20	46	30	16	23
Alternaria	19	20	20	20	17	12
Curvularia	13	7	7	-	17	6
Cladosporium	6	20	13	10	-	-
Penicillium	13	13	-	20	17	18
Drechslera	6	7	7	10		6
Unknown	6	13	7	10	33	23
Total	100	100	100	100	100	100

Table 3.20: Distribution of fungal sp. (in percentage) at six sites in post-monsoon season

	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6
Aspergillus Sp.	-	3	8	5	10	5
Alternaria	3	7	4	5	7	5
Curvularia	24	23	20	31	42	43
Cladosporium	61	48	40	46	32	24
Penicillium	6	3	8	5	3	14
Fusarium	3	3	12	5	-	-
Unknown	3	13	8	3	6	9
Total	100	100	100	100	100	100

Table 3.21: Distribution of fungal sp. (in percentage) at six sites in winter season

	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6
Aspergillus Sp.	17	20	8	9	24	23
Alternaria	-	-	-	5	5	6
Curvularia	-	-	-			
Cladosporium	61	50	59	57	47	55
Penicillium	11	25	17	14	14	10
Fusarium	11	5	8	10	5	3
Unknown	2	-	8	5	5	3
Total	100	100	100	100	100	100

Table 3.22: Distribution of fungal sp. (in percentage) at six sites in pre-monsoon season

	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6
Aspergillus Sp.	65	77	69	74	68	67
Alternaria	-	-	8	-	-	-
Curvularia	-	-	-	-	-	-
Cladosporium	10	-	-	-	-	-
Penicillium	15	15	15	13	18	20
Rhizopus	5	-	8	9	9	6
Unknown	5	8	0	4	5	7
Total	100	100	100	100	100	100

3.5. Statistical Analysis

3.5.1. Analysis of variance (ANOVA)

3.5.1.1. Analysis of variance test for different seasons for MVOCs

Analysis of variance test was performed for six identified microbial volatile organic compounds. In this analysis, MVOCs concentration was considered as a dependent variable and four seasons as factor, which defines the group that is to be compared.

a. Analysis of variance for 1-octen-3-ol in different seasons

Concentration of 1-octen-3-ol was considered as dependent variable and four seasons were considered as factor. Table 3.23 shows, F value with significance level ($p<0.05$) ($p=0.002$), revealing that mean concentration of 1-octen-3-ol vary significantly across all the four seasons. In this case, the null hypothesis thus gets rejected. For 1-octen-3-ol, all the four seasons are not equal; at least one season is different from three others. Sheffee and Bonferroni test for multiple comparisons of seasons was also done for 1-octen-3-ol concentration at different seasons as depicted by table 3.24. According to Sheffee and Bonferroni test significant difference exists between seasons 1 and 3 ($p<0.05$) and seasons 3 and 4 ($p<0.05$) while, no significant difference exist between seasons 1 and 4.

Table 3.23: ANOVA analysis for 1-octen-3-ol

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	4.452	2	2.226	12.204	0.002
Within Groups	2.006	11	0.182		
Total	6.458	13			

Table3.24: Multiple comparisons of 1-octen-3-ol in different seasons

	Sheffee test				Bonferroni test			
Seasons	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**
1								
2								
3	0.004				0.003			
4	0.641		0.007		1.00		0.006	

Note: 1: monsoon season; 2: post-monsoon season; 3: winter season; 4: pre-monsoon season

b. Analysis of variance for 3-octanone in different seasons

ANOVA for 3-octanone was could not be performed due to insufficient data.

c. Analysis of variance for 1-hexene in different seasons

In the case of 1-hexene, null hypothesis is accepted ($p=0.11$), revealing that mean concentration of 1-hexene does not vary significantly across all the three seasons as shown by table 3.25. Sheffee and Bonferroni test was performed which shows that no significant difference exist between seasons 1, 2 and 3 ($p>0.05$) as depicted by table 3.26. Season 4 could not be compared in the test due to insufficient data.

Table 3.25: ANOVA analysis for 1-hexene

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	94.454	2	47.227	2.391	0.111
Within Groups	513.584	26	19.753		
Total	608.037	28			

Table 3.26: Multiple comparisons of 1-hexene in different seasons

	Sheffee test				Bonferroni test			
Seasons	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**
1								
2	0.312				0.392			
3	0.777	0.113			1.00	0.116		
4								

Note: 1: monsoon season; 2: post-monsoon season; 3: winter season; 4: pre-monsoon season.

d. Analysis of variance for 3-methyl-1-butanol in different seasons

ANOVA for 3-methyl-1-butanol has not performed due to insufficient data.

e. Analysis of variance for cyclohexanone in different seasons

Similar to 1-hexene, in case of cyclohexanone no null hypothesis is accepted revealing that mean concentration of cyclohexanone does not vary significantly across the all three seasons with $p>0.05$ as shown by table 3.27. Sheffee and Bonferroni test shows no significant difference between seasons 2 and 3, 1 and 2 as well as seasons 1 and 3 with $p>0.05$ as depicted in table 3.28.

Table 3.27: ANOVA analysis for cyclohexanone

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	6.748	2	3.374	1.446	0.252
Within Groups	70.021	30	2.334		
Total	76.769	32			

Table 3.28: Multiple comparison of cyclohexanone in different seasons

	Scheffe test				Bonferroni test			
Seasons	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**
1								
2	0.382				0.509			
3	0.342	0.995			0.439	1.00		
4								

Note: 1: monsoon season; 2: post-monsoon season; 3: winter season; 4:pre-monsoon season

f. Analysis of variance for 1-pentanol in different seasons

ANOVA analysis for 1-pentanol, reject the null hypothesis (p=0.029), which shows significant differences between four seasons as shown in table 3.29. However, according to Sheffee and Bonferroni test no significant difference could be seen to exist between all the seasons as shown in table 3.30.

Table 3.29: ANOVA analysis for 1-pentanol

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	224.051	3	74.684	3.296	0.029
Within Groups	1042.239	46	22.657		
Total	1266.290	49			

Table 3.30: Multiple comparison of 1-pentanol in different seasons

	Sheffee test				Bonferroni test			
Seasons	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**
1								
2	0.107				0.087			
3	0.688	0.525			1.00	0.835		
4	0.086	1.00	0.464		0.066	1.00	0.680	

Note: 1: monsoon season; 2: post-monsoon season; 3: winter season; 4: pre-monsoon season

3.5.1.2. Analysis of variance of MVOCs concentration between moldy and non-moldy indoor environments

In order to evaluate whether any significant difference exists in various MVOCs concentration, ANOVA was carried out between visibly moldy and non-moldy indoor sites. The results show in table 3.31, reveals that all the six MVOCs which are usually considered to be typical for fungal growth, were found to exist comparatively in higher levels in both the indoor sites with and without visible mold growth. For all the six compounds, no statistical difference could be found in the MVOCs concentration between moldy and non-moldy indoor sites ($p > 0.05$). Among the six MVOCs, three of them namely; 1-octen-3-ol, 3-octanone and 3-methyl-1-butanol were rarely detected in both the moldy and non-moldy indoor sites while; 1-pentanol, cyclohexanone and 1-hexene were found to exist significantly of similar higher frequencies in both moldy and non-moldy indoor sites. The frequencies were 96%, 54% and 65% in visibily moldy sites while 88%, 54% and 62% in non-moldy sites of 1-pentanol, 1-hexene and cyclohexanone respectively.

Table 3.31. Geometric mean and percentile of the proposed MVOCs in moldy and non moldy indoor environments (where, p > 0.005).

Compounds	Visible mold	Percentile					GM	p-value
		5th	10th	50th	90th	95th		
1-Octen-3-ol	Yes	<	<	<	1.171	1.8493	1.47	>0.05
	No	<	<	<	0.567	0.625	0.60	
3-Octanone	Yes	<	<	<	0.3382	0.422	0.38	>0.05
	No	<	<	<	0.422	0.523	0.47	
1-Hexene	Yes	<	<	0.339	8.03	10.29	9.09	>0.05
	No	<	<	0.18	9.75	11.263	10.48	
3-Methyl-1-butanol	Yes	<	<	<	0.1884	1.219	0.48	>0.05
	No	<	<	<	<	0.1813	0.18	
Cyclohexanone	Yes	<	<	0.163	1.846	4.16	2.77	>0.05
	No	<	<	0.29	2.55	3.74	3.09	
1-Pentanol	Yes	<	0.247	17.98	9.6	12.192	10.82	>0.05
	No	<	<	2.68	5.3	10.039	7.29	

Note: GM: Geometric Mean; <: denotes below detection limit 0.04 μgm^{-3}

3.5.1.3.Analysis of variance for fungal bioaerosol

a. Analysis of variance test of fungal concentration at different sites

To conduct ANOVA test in SPSS, fungal concentration was considered as dependent variable and sites were considered as the fixed factor. The factor is the variable, which defines the groups that is to be compared. ANOVA was done to get statistical difference between different indoor environments (sites). Table 3.32 shows, F value with significance level (p>0.05) (p=0.167), shows that no significant difference exits between the mean fungal concentration at all the six sites.

The result of Sheffee and Bonferroni tests for multiple comparisons of different sites for fungal bioaerosol concentration is shown in table 3.33 and 3.34. All the significant values fall in the range of 0.618 to 1.00 for Sheffee test and 0.813 to 1.00 for Bonferroni test also depicting that no significant difference exits between the mean fungal concentration at all the six sites.

Table 3.32: ANOVA analysis of fungal bioaerosols at different sites

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	3255862.698	5	651172.540	1.590	0.167
Within Groups	5.653E7	138	409660.467		
Total	5.979E7	143			

Table 3.33: Multiple comparisons of fungal bioaerosol concentrations at six sites by Sheffee test

Sites	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6
Site 1						
Site 2	0.963					
Site 3	1.00	0.978				
Site 4	0.673	0.987	0.733			
Site 5	1.00	0.971	1.00	0.705		
Site 6	0.585	.969	0.647	1.00	0.618	

Table 3.34: Multiple comparisons of fungal bioaerosol concentrations at six sites by Bonferroni test

Sites	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6
Site 1						
Site 2	1.00					
Site 3	1.00	1.00				
Site 4	1.00	1.00	1.00			
Site 5	1.00	1.00	1.00	1.00		
Site 6	0.813	1.00	1.00	1.00	0.929	

b. Analysis of variance test forfungal concentration in different seasons

To carry out this test, fungal concentration was chosen as dependent variable and seasons were selected as the fixed factor. In this test, season is the variable that defines the groups that is to be compared. Table 3.35 shows, F value with significance level for each sites. In this test, all the F values were with significantly level ($p<0.05$), revealing that mean fungal concentration vary significantly across all the different seasons at all sites. In this case, the null hypothesis thus gets rejected.

Scheffe and Bonferroni tests in comparison of all the four seasons (1-monsoon; 2-post monsoon; 3-winter and 4-pre monsoon) were done for fungal concentration at six different indoor environments as shown in table 3.36 to 3.41. According to the test, at site 1, significant difference existed between all the seasons with ($p<0.05$), except between the seasons 3 and 4. At site 2, significant difference existed between seasons 1 and 2, 1 and 4, 2 and 3 as well as 2 and 4 with ($p<0.05$). At site 3, significant difference existed between seasons 1 and 4, 2 and 3 as well as 2 and 4 with ($p<0.05$). Similar to site 2, at site 4 significant difference existed between seasons 1 and 2, 2 and 3 as well as 2 and 4 with ($p<0.05$). At sites 5 and 6, greater number of significant differences was observed between all the seasons. At site 5, significant differences existed between seasons 1 and 3, 1 and 4, 2 and 3 and between 2 and 4 while, a site 6, significant differences existed between seasons 1 and 2, 1 and 3, 1 and 4, 2 and 3 as well as between 3 and 4 with ($p<0.05$).

Table 3.35: ANOVA analysis of fungal bioaerosol concentrations in four different seasons

		Sum of Squares	df	Mean Square	F	Sig.
Site 1	Between Groups	9287675.146	3	3095891.715	29.956	0.000
	Within Groups	2066926.066	20	103346.303		
	Total	1.135E7	23			
Site 2	Between Groups	8929205.322	3	2976401.774	28.685	0.000
	Within Groups	2075222.565	20	103761.128		
	Total	1.100E7	23			
Site 3	Between Groups	7633928.690	3	2544642.897	16.195	0.000
	Within Groups	3142550.103	20	157127.505		
	Total	1.078E7	23			
Site 4	Between Groups	5371875.351	3	1790625.117	15.699	0.000
	Within Groups	2281160.126	20	114058.006		
	Total	7653035.476	23			
Site 4	Between Groups	9474810.838	3	3158270.279	18.206	0.000
	Within Groups	3469525.570	20	173476.279		
	Total	1.294E7	23			
Site 6	Between Groups	988535.670	3	329511.890	4.416	0.015
	Within Groups	1492402.202	20	74620.110		
	Total	2480937.873	23			

Table 3.36: Multiple comparison of fungal bioaerosol concentration in different seasons at Site 1

	Scheffe test				Bonferroni test			
Seasons	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**
1								
2	0.003				0.002			
3	0.058	0.000			0.045	0.00		
4	0.003	0.000	0.60		0.002	0.00	1.00	

Note: 1: monsoon season; 2: post-monsoon season; 3: winter season; 4: pre-monsoon season

Table 3.37: Multiple comparison of fungal bioaerosol concentration in different seasons at Site 2

	Scheffe test				Bonferroni test			
Seasons	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**
1								
2	0.000				0.00			
3	0.449	0.000			0.672	0.00		
4	0.075	0.000	0.715		0.062	0.00	1.00	

Note: 1: monsoon season; 2: post-monsoon season; 3: winter season; 4: pre-monsoon season

Table 3.38: Multiple comparison of fungal bioaerosol concentration in different seasons at Site 3

	Scheffe test				Bonferroni test			
Seasons	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**
1								
2	0.053				0.041			
3	0.501	0.002			0.803	0.001		
4	0.012	0.000	0.221		0.008	0.00	0.244	

Note: 1: monsoon season; 2: post-monsoon season; 3: winter season; 4: pre-monsoon season.

Table 3.39: Multiple comparison of fungal bioaerosol concentration in different seasons at Site 4

	Sheffee test				Bonferroni test			
Seasons	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**
1								
2	0.000				0.00			
3	1.00	0.000			1.00	0.00		
4	1.00	0.000	1.00		1.00	0.00	1.00	

Note: 1: monsoon season; 2: post-monsoon season; 3: winter season; 4: pre-monsoon season.

Table 3.40: Multiple comparison of fungal bioaerosol concentration in different seasons at Site 5

	Scheffe test				Bonferroni test			
Seasons	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**
1								
2	0.357				0.486			
3	0.038	0.001			0.027	0.00		
4	0.001	0.000	0.473		0.001	0.00	0.73	

Note: 1: monsoon season; 2: post-monsoon season; 3: winter season; 4: pre-monsoon season.

Table 3.41: Multiple comparison of fungal bioaerosol concentration in different seasons at site 6

	Scheffe test				Bonferroni test			
Seasons	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**
1								
2	0.2211				0.228			
3	0.964	0.088			1.00	0.075		
4	0.693	0.023	0.923		1.00	0.016	1.00	

Note: 1: monsoon season; 2: post-monsoon season; 3: winter season; 4: pre-monsoon season.

3.5.1.4. Analysis of variance for bacterial bioaerosol

a. Analysis of variance test for bacterial concentration at different sites

Like fungus, ANOVA test was also performed for bacterial concentrations. Concentration of bacteria was considered as dependent variable and sites were considered as a fixed factor as above the fungal concentration. Table 3.42 shows, F-value with significant level ($p<0.05$, $p=0.005$), which indicates that significant difference observed between the mean bacterial concentration at all the sites, thereby rejecting the null hypothesis. Further, two tests, namely; Sheffee and Bonferroni tests were carried out as shown in table 3.43 and 3.44 in order to find

the specific sites with significant differences in mean bacterial concentration. In the matrix table of both the tests, significant differences was found to be existed between sites 3 and 4 with $p<0.05$ ($p = 0.024$ and $p = 0.005$).

Table 3.42: ANOVA analysis for bacterial concentration at different sites

	Sum of Squares	**df**	**Mean Square**	**F**	**Sig.**
Between Groups	1480424.160	5	296084.832	3.561	0.005
Within Groups	1.147E7	138	83138.413		
Total	1.295E7	143			

Table 3.43: Matrix for multiple comparisons of bacterial concentrations at six sites by Scheffe test

Sites	**Site 1**	**Site 2**	**Site 3**	**Site 4**	**Site 5**	**Site 6**
Site 1						
Site 2	0.898					
Site 3	0.921	0.306				
Site 4	0.305	0.924	**0.024**			
Site 5	1.000	0.803	0.974	0.206		
Site 6	0.999	0.704	0.990	0.137	1.000	

Table 3.44: Matrix table for multiple comparisons of bacterial concentration at six sites by Bonferroni test

Sites	**Site 1**	**Site 2**	**Site 3**	**Site 4**	**Site 5**	**Site 6**
Site 1						
Site 2	1.000					
Site 3	1.000	0.225				
Site 4	0.224	1.000	0.005			
Site 5	1.000	1.000	1.000	0.115		
Site 6	1.000	1.000	1.000	0.061	1.000	

b. Analysis of variance test forbacterial concentration in different seasons

To conduct this test, bacterial concentration was regarded as dependent variable and seasons were considered as the fixed factor accordingly. In this test also, season is the variable that defines the groups that we want to compare. Table 3.45 shows, F value with significance level at each sites. In this test, all the F values were with significant level ($p<0.05$), revealing that mean bacterial concentration vary significantly across the different seasons at all the sites. Thus, similar to fungal concentration, bacterial concentrations also vary significantly across the different seasons at all sites. In this case, the null hypothesis thus gets rejected.

Sheffee and Bonferroni tests in comparison to all the four seasons (1-monsoon; 2-post monsoon; 3-winter and 4-pre monsoon) were also done for mean bacterial concentration at six different indoor environments as shown in table 3.46 to 3.51. According to the tests at sites 1, 2, 4 and 6 significant difference exist between all the seasons with ($p<0.05$), except between seasons 3 and 4. At site 3, significant difference existed between seasons 1 and 2, 1 and 4, 2 and 3 as well as 2 and 4 while, at site 5 significant difference existed between seasons 2 and 3 as well as 2 and 4 with ($p<0.05$).

Table 3.45: ANOVA analysis of bacterial bioaerosol at different sites in different seasons

		Sum of Squares	df	Mean Square	F	Sig.
Site 1	Between Groups	1362453.541	3	454151.180	130.768	0.000
	Within Groups	69459.185	20	3472.959		
	Total	1431912.726	23			
Site 2	Between Groups	2592012.221	3	864004.074	81.209	0.000
	Within Groups	212784.111	20	10639.206		
	Total	2804796.332	23			
Site 3	Between Groups	528092.602	3	176030.867	33.438	0.000
	Within Groups	105287.590	20	5264.379		
	Total	633380.191	23			
Site 4	Between Groups	4037903.666	3	1345967.889	99.390	0.000
	Within Groups	270844.519	20	13542.226		
	Total	4308748.185	23			
Site 5	Between Groups	544849.584	3	181616.528	7.409	0.002
	Within Groups	490235.863	20	24511.793		
	Total	1035085.447	23			
Site 6	Between Groups	1010269.304	3	336756.435	49.544	0.000
	Within Groups	135942.514	20	6797.126		
	Total	1146211.818	23			

Table 3.46: Multiple comparison of bacterial bioaerosol concentration in different seasons at site 1

	Sheffee test				Bonferroni test			
Seasons	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**
1								
2	0.000				0.000			
3	0.003	0.000			0.002	0.000		
4	0.000	0.000	0.132		0.000	0.000	0.125	

Note: 1: monsoon season; 2: post-monsoon season; 3: winter season; 4: pre-monsoon season.

Table 3.47: Multiple comparison of bacterial bioaerosol concentration in different seasons at site 2

	Sheffee test				Bonferroni test			
Seasons	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**
1								
2	0.000				0.000			
3	0.044	0.000			0.033	0.00		
4	0.003	0.000	0.653		0.002	0.00	1.000	

Note: 1: monsoon season; 2: post-monsoon season; 3: winter season; 4: pre-monsoon season.

Table 3.48: Multiple comparison of bacterial bioaerosol concentration in different seasons at site 3

	Sheffee test				Bonferroni test			
Seasons	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**
1								
2	0.000				0.000			
3	0.181	0.000			0.186	0.00		
4	0.013	0.000	0.584		0.008	0.00	1.000	

Note: 1: monsoon season; 2: post-monsoon season; 3: winter season; 4: pre-monsoon

Table 3.49: Multiple comparison of bacterial bioaerosol concentration in different seasons at site 4

	Sheffee test				Bonferroni test			
Seasons	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**
1								
2	0.000				0.000			
3	0.001	0.000			0.000	0.00		
4	0.000	0.000	0.963		0.000	0.00	1.00	

Note: 1: monsoon season; 2: post-monsoon season; 3: winter season; 4: pre-monsoon season

Table 3.50: Multiple comparison of bacterial bioaerosol concentration in different seasons at site 5

	Sheffee test				Bonferroni test			
Seasons	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**
1								
2	0.324				0.414			
3	0.626	0.033			1.000	0.024		
4	0.119	0.003	0.679		0.109	0.001	1.00	

Note: 1: monsoon season; 2: post-monsoon season; 3: winter season; 4: pre-monsoon season

Table 3.51: Multiple comparison of bacterial bioaerosol concentration in different seasons at site 6

	Sheffee test				Bonferroni test			
Seasons	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**
1								
2	0.002				0.001			
3	0.001	0.000			0.000	0.000		
4	0.000	0.000	0.612		0.000	0.000	1.00	

Note: 1: monsoon season; 2: post-monsoon season; 3: winter season; 4: pre-monsoon season

3.5.2. Regression and multiple regression analyses

3.5.2.1. Linear regression and multiple regression for MVOCs

Linear regression and multiple regression analyses were carried out between environmental parameters (temperature and RH) and MVOCs (1-pentanol, 1-hexenen and cyclohexanone). Here, R represents coefficient of correlation. In case of 1-hexene, from the close study of the results shown in table 3.52, it was evident that both RH and temperature had a weak correlation with 1-hexene alone as well as in combination. Although the correlation of temperature (R= 0.362) was found to be almost double in comparison to that of RH (R= 0.190), which also increased when combined (R= 0.390), yet no significance was found for all the three model sets ($p>0.05$).

In case of cyclohexanone too, both RH and temperature had a weak correlation with alone as well as in combination with cyclohexanone. Although the correlation of temperature (R= 0.115) was found to be almost double in comparison to that of RH (R= 0.056) which also increased when combined (R= 0.120), yet no significance was found for all the three model sets ($p>0.05$).

Almost similar result was obtained in case of 1-pentanol wherein both RH and temperature also had a weak correlation alone as well as in combination with 1-pentanol. Even for 1-pentanol, although the correlation of temperature (R= 0.170) was found to be almost double in comparison to that of RH (R= 0.04), which also increased when combined (R= 0.191), yet no significance was found for all the three model sets ($p>0.05$).

Table 3.52: Statistical results of linear regression for MVOCs concentration

	Regression		
Parameters	**1-Hexene**	**Cyclohexanone**	**1-Pentanol**
	R (p)	**R (p)**	**R (p)**
RH	0.190 (0.323)	0.056 (0.752)	0.040 (0.742)
Temperature	0.362 (0.054)	0.115 (0.517)	0.170 (0.460)
RH and Temperature	0.390 (0.117)	0.120 (0.80)	0.191 (0.754)

3.5.2.2. Linear and multiple regression for bioaerosol concentration

Linear regression and multiple regression analyses were carried out between environmental parameters (temperature and RH) and bioaerosol concentration (fungus and bacteria). Here, R represents coefficient of correlation.

In case of fungal concentration, from the close study of the results shown in table 3.53, it was evident that RH had a weak correlation ($R= 0.176$, $p> 0.05$), while temperature had a moderate correlation which is more than twice when compared to RH ($R= 0.537$, $p< 0.05$) fungal concentration alone. Though not much, yet, a substantial improvement could be seen on combination of both the regressors ($R= 0.550$, $p> 0.05$). Maximum incremental impact of 0.361 could be seen of temperature on correlation coefficient (R). However, apart from RH, the remaining two linear regressions studies were found to be significant ($p< 0.05$).

Similar to fungus, in case of bacterial concentration too, RH had a weak correlation ($R= 0.180$, $p> 0.05$), while temperature had a moderate correlation which is more than twice when compared to RH ($R= 0.687$, $p< 0.05$) fungal concentration alone. Though not much, yet, a substantial improvement could be seen on combination of both the regressors ($R= 0.689$, $p < 0.05$). Maximum incremental impact of 0.507 could be seen of temperature on correlation coefficient (R). However, apart from RH, the remaining two linear regression studies were found to be significant ($p< 0.05$).

Table 3.53: Statistical results of linear regression for fungal and bacterial concentration

	Regression	
Parameters	**Fungus**	**Bacteria**
	R (p)	**R (p)**
RH	0.176 (0.276)	0.180 (0.226)
Temperature	0.537 (0.000)	0.687 (0.000)
RH and temperature	0.550 (0.001)	0.689 (0.000)

DISCUSSION

The six MVOCs selected to be studied upon were based on their potential health risks as described earlier as well as whether or not these can be used as marker for the infected buildings under study, as in the case of alcohols such as 1-octen-3-ol and 3-methyl-1-butanol (Miller et al., 1988; Matysik et al., 2008) and ketones such as 3-octanone and heptan-2-one (Elke et al., 1999; Matysik et al., 2008). However, among other ketones, cyclohexanone although found in considerable quantity in indoor environment was more in relevance to carpets and paints other than microbial sources, thereby not been suggested to correlate with indoor molds (Matysik et al., 2009).

Among the six MVOCs, 1-pentanol, cyclohexanone and 1-hexene were maximally found while 1-octen-3-ol, 3-methyl-1-butanol and 3-octanone were comparatively very less in the indoor air samples revealing the fact that majority of the sites may have proper sources for these MVOC productions. According to the seasonal study, all the six MVOCs were found to have different mean concentration in different season. Most of the MVOCs were maximally found in monsoon and winter season while minimally found in post-monsoon and pre-monsoon season at all the sites (figure 3.1 to 3.6). Although, variation in mean MVOCs concentration in different season existed visually as seen in figures 16-21, yet the result varied between different MVOCs statistically. Among the six MVOCs, significant difference existed between the mean concentration in different season for 1-octen-3-ol ($p = 0.002$) and 1-pentanol ($p = 0.029$) only, while, in case of 1-hexene and cyclohexanone no significant difference was found to be existed. Statistical analysis for the rest of the two MVOC namely; 3-octanone and 3-methyl-1-butanol could not be carried out due to insufficient data. Thus, absence of sufficient data can be attributed to be the cause of such results obtained on the application of statistics.

Moreover, no statistical difference could be found in the MVOCs concentration between visibly moldy and non-moldy indoor sites ($p> 0.05$). In fact existence of almost similar higher frequencies of 3 MVOCs such as 1-pentanol, 1-hexene and cyclohexanone in both moldy and non-moldy sites emphasises on the fact that, although, visibly not present yet hidden fungus in building materials and accumulated surface dust can be the reason of the presence of MVOCs in non-moldy sites. Passive samplings over the inoculated microbial cultures as well as isolated fungal species present in dust and building materials collected from all the six sites were found to be the major sources of these MVOCs.

Optimum temperature and RH ranges of 25.3 - 36.2^{0}C and 54.3 - 79.3% found in monsoon season could be attributed to be the reason of microbial proliferation in dust and building materials present in indoor environments, which itself acts as a source of MVOCs. Thus, greater the microbial growth more is the MVOC released in the indoor environment. In winter season, most of the indoor environments were kept minimally ventilated with closed doors and windows. Thus, although the temperature range (14.0 – 26.1^{0}C) was not optimum for microbial growth as for in monsoon season, still absence of ventilation resulted in the entrapment of both microbial species as well as their released MVOCs within the indoor environments greater than in post-monsoon or pre-monsoon season. Statistically too, no linearity was found between temperature and RH with MVOCs concentration, and no significant relationship could be established between the environmental parameters with MVOCs concentration ($p>0.05$) emphasising the fact, that within the indoor environments, other factors especially ventilation rate played the major role for the variation in the MVOCs concentration. In previous study air exchange rate (AER) was also found to be negatively correlated with indoor MVOCs concentration (Schleibinger et al., 2008) supporting the fact that ventilation is an important deciding factor.

Although 1-octen-3-ol and 3-octanone have been found to correlate with *Aspergillus sp.* by certain studies (Elke et al., 1999) however, our data after the 8th day passive sampling differed from this statement as both the compounds were neither detected in any of the dust or building material samples collected from all the six sites nor in the headspace of the three *Aspergillus sp*. pure cultures. According to Gao et al., (2002) *Aspergillus flavus* and *Aspergillus niger* released considerable quantity of 1-octen-3-ol and 3-methyl-1-butanol when grown both on media and gypsum board, a laboratory resemblance of natural building material. However, our data do not resemble the previous study as none of the two compounds were released by the above mentioned *Aspergillus* species. Use of different cultural media namely malt extract extract (MEA) in the previous study and PDA in the present study can be pointed to be the main reason in accordance to other studies wherein use of different media or substratum have been found to play a major role in the production difference of MVOCs by the same fungal species. This is because the produced MVOC pattern not only depend on fungal species but also upon the pH, chemical composition, water content and temperature of the substrate be synthetic media in laboratory or building material in problematic indoors (Sunesson et al., 1995; Borjesson et al., 1990; Rose et al., 2000; Pasanen et al., 1991 a.b; Pasasen et al., 1997). In fact, various studies revealed that factors

that might be favourable for the production of one VOC may be totally unfavourable for another one (Korpi, 2001). MVOC emission shows a highly dynamic behaviour during the life span of the fungal cultures wherein, volatile compound emission rate acts as the function of the corresponding culture's cultivation time (Matysik et al., 2008). Presumably, some ketones as well as few alcohols such as 1-octen-3-ol are usually formed at a later stage of growth (Wessen and Schoeps, 1996; Wady et al., 2003). In the present study, a preliminary MVOC analysis was carried out on the 8th day of fungal cultivation while, both 1-octen-3-ol and 3-methyl-1-butanol were found to be released in a quantifiable concentration by *Aspergillus sp*. on the 16th day of cultivation as shown by Gao et al. (2002). However, as shown in table 21, MVOCs analysis carried out on the 16th day of fungal cultivation reveals release of 1-octen-3-ol by all the six fungal species which is in resemblance with the above mentioned studies, e.g , Gao et al. (2002); Wady et al. (2003) and Matysik et al. (2008) as well as justifies the presence of 1-octen-3-ol in the indoor air at various sites.

Most of the previous studies have suggested 3-methyl-1-butanol to be a most reliable indicator of damp and infected buildings in relation to mould formation only (Elke et al., 1999). But, in this study apart from being released from fungus, 3-methyl-1-butanol has been released from bacterial community present in both dust and building material samples. Among the six sites it was maximally released in two sites namely site 1 (9.82 µg/ml and 2.29 µg/ml of dust and building material sample) and 4 (4.93 µg/ml and 2.73 µg/ml in dust and building material sample). Site 1 is a comparatively old built library with infrequent maintenance of the building walls with moderate ventilation, while site 4 is a newly built private hostel but with very low ventilation and absence of fresh air passage resulting in high humidity indoor and damped walls. In a previous study, 1-pentanol was found to be released from fungal cultures grown on gypsum board only and not on malt extract agar (MEA) media while 1-hexene was not detected when grown both on gypsum board and MEA (Claeson et al., 2002). However, in this study, 1-pentanol and 1-hexene were second most highly detected and quantified after 3-methyl -1-butanol in both dust and building materials of almost all the sites, as well as 1-pentanol was released from all the isolated fungal species whereas, 1-hexene from three of the fungal species grown on PDA media. This might be due to the use of totally different fungal species composition in both the previous study (*Aspergillus versicolor, Fusarium culmorum, Ulocladium botrytis* and *Wallemia sebi*) and present study (*Aspergillus flavus, Aspergillus niger, Aspergillus nidulans, Cladosporium cladosporioides,*

Curvuleria lunata, *Penicillium sp.)* as well as the media over which they were grown namely; MEA and PDA respectively (Willkins et al., 2000).

Although, all the three MVOCs namely 1-hexene, 3-methyl-1-butanol and 1-pentanol were detected in dust and building material samples in terms of overall bacterial community as shown in table 3.3, yet none of the bacterial samples could be isolated and detected due to time constraint. However, according to a study carried out by Claeson, (2006), *Streptomyces albidoflavus* was found releasing both 1-pentanol and 3-methyl-1-butanol when it was grown on different substratum namely gypsum board and pinewood respectively.

In case of cyclohexanone, although found to be released by overall fungal and bacterial community present in dust and building materials of site 2 and 5, still it was not detected from any of the isolated identified fungal species. Thus, apart from unknown fungal species as shown in figure 28 and bacterial community present in dust and building materials, other indoor sources such as carpets and paints could be attributed to be the sources of cyclohexanone in the indoor environments as shown by previous study (Matysik et al., 2009). Since 3-octanone was neither released by overall fungal and bacterial community present in dust and building materials nor by any isolated identified fungal species; in this study, unknown fungal species found at different sites as shown in figure 28 can be attributed to be the sources of its presence in the indoor environments in accordance to previous study carried out by Menetrez and Foarde (2002).

Apart from the above mentioned factors, variations of the MVOC analysis may also occur due to the chemical composition of the adsorption tube as MVOCs produced as secondary metabolites have different polarity, volatility and are also present in air at very low concentration levels thereby demanding highly sensitive adsorption material. In this study, as already mentioned before, both passive and active samplings of MVOCs was carried out by charcoal tubes ORBO™ 90 Carboxen® 564, which to the best to our knowledge has been used in adsorbing volatiles released from dairy products (Rankin, 2001) but, not used in the analysis of MVOCs in any context till date thereby making it difficult to compare its adsorption capability with other adsorbents that have been mostly used such as Tenax, Ambersorb XE-340, Carbotrap, GK 26-16 charcoal tubes, Anasorb 747 etc. (Chan et al., 1990; Heavner et al., 1992; Elke et al., 1999; Wessen and Schoeps, 1996). According to a comparative study carried out among various adsorbents such a Tenax TA, Tenax GR, Carbotrap C, Carbotrap B, Anasorb 727 and Anasorb 747, wherein the properties of each of

the adsorbents in context to the amount contained, breakthrough as well as standard deviation during both sampling and analysis (Sunesson et al., 1995). No comparative analysis in regard of literature about the adsorbent properties of ORBO™ 90 Carboxen® 564 is available till date. Hence, the extant of breakthrough and standard deviation during both sampling and analysis is still under question.

Size segregated study of bioaerosol was carried out at each site. Fungal bioaerosol was found to follow a typical pattern in all the four seasons, whereas at every site highest concentration of fungus was found at stage 4 (diameter ranging from 2.1 to 3.3 µm) and lowest concentration at stage 6 (diameter ranging from 0.6 to 1.1 µm). This typical pattern of concentration depicts that majority of the fungal species (their hyphal fragments and spores) have similar diameter as that of stage 4, which is in synonym to the secondary bronchi of the lungs in the human body. This reveals that majority of the immunotoxic and allergic fungi found at this stage are mostly prone to affect the secondary bronchi in human lungs when inhaled.

Unlike fungi, bacterial bioaerosol concentration was found to be different at different sites and season thereby following no typical pattern throughout. In monsoon season, highest bacterial concentration was found at stage 3 (in range to trachea and primary bronchi); stage 6 (in range to alveoli); stage 1 (in range to nasal passage) and stage 4 (in range to secondary bronchi) at various sites. This indicates that in monsoon season, majority of the bacteria found at these stages are mostly prone to affect the trachea, primary bronchi, secondary bronchi as well as alveoli in human lungs when inhaled. Unlike monsoon season, in post-monsoon season highest bacterial concentration was found only at two stages namely; stage 1 (in range to nasal passage) and stage 6 (in range to alveoli) at various sites. This reveals that, in post-monsoon season, majority of the bacteria found at these stages are prone to affect the nasal passage and alveoli in human lungs when inhaled. In winter season, highest bacterial concentration was found at stage 1 (in range to nasal passage); stage 3 (in range to trachea and primary bronchi); stage 4 (in range to secondary bronchi); stage 5 (in range to trachea and terminal bronchi) and stage 6 (in range to alveoli); and at various sites. This reveals that in winter season majority of the bacteria found at these stages are mostly prone to affect the nasal passage, trachea, primary, secondary and terminal bronchi as well as alveoli in human lungs when inhaled. Unlike all other seasons; at sites 1, 2 and 6 not much variation was found among all the size segregated bacterial bioaerosol in pre-monsoon season thereby revealing that at these three sites all the parts of the human lungs are prone to be affected at more or

less in the same rate. However, at the remaining three sites namely; sites 3, 4 and 5 highest bacterial concentration was found at stage 5 (in range to trachea and primary bronchi); stage 4 (in range to secondary bronchi) and stage 6 (in range to alveoli) emphasising the fact that all these mentioned parts of human lungs are prone to be affected on inhalation.

According to the seasonal study both the fungal and bacterial bioaerosol were found to have different mean concentration in different seasons. This difference was also statistically proven as significant difference ($p < 0.05$) existed in the mean fungal and bacterial bioaerosol concentration among different season at each site. A typical pattern could be observed for both fungal and bacterial bioaerosol, wherein highest concentration was in post-monsoon season and lowest in pre-monsoon season at all the sites. Although, the temperature range in both monsoon and post-monsoon season were optimum for microbial growth over various substratum such as accumulated surface dusts, carpet dust, discarded vegetables in kitchen bins etc., in indoor sites yet, higher range of relative humidity (ranging up to 79%) observed in monsoon season could be one of the major reasons of restricted aerosolisation of microbes in monsoon than in post post-monsoon in accordance to studies carried out by De Goffao et al.(2009) and Li et al. (2011). Wherein high relative humidity was found to inhibit airborne microbial growth rate and correlated with low bioaerosol concentration. Statistically too, very weak correlation ($R = 0.176$ and 0.18) and no significance ($p> 0.05$) was observed between RH along with fungal and bacterial bioaerosol respectively denoting the fact that RH alone did not play an important role in the bioaerosol concentration emission. The combined effect of RH and temperature was found to be significant ($p< 0.05$) with both fungal and bacterial bioaerosol concentration emphasising the fact that meteorological parameters in totality play an important role in bioaerosol emission.

Moreover, post-monsoon season is a season of festivals such as Dushehra and Diwali in India (these are the two major festivals in India), which results in high level of activities within residential sites in terms of cleaning and dusting as well as aggregation of people for celebration. Higher occupancy level along with high mechanical movements can be attributed to other reasons of higher bioaerosol concentration (Ghosh et al., 2013) in post-monsoon season in comparison to other seasons.

Study of seasonal variation in mean concentration of indoor and outdoor (for both fungal and bacterial bioaerosol) depicts a typical pattern, wherein apart from monsoon season, in all other three season outdoor bioaerosol concentration was higher than indoor concentration at

all the six sites. In monsoon season, although, temperature range was nearly optimum for microbial growth over their respective substratum yet, relatively high range of relative humidity (ranging up to 80%) that was found to restrict aerosolization of microbes (De Goffao et al., 2009 and Li et al., 2011) as well as rain washout may the major reasons of lower outdoor concentration in comparison to other seasons. Presence of suitable sources such as dense green vegetation, huge municipal dumping site and agricultural field in the vicinity of sites 1, 2 and 3 respectively could be the reason of finding higher bioaerosol concentration outdoor in comparison to indoor. I/O ratio with value < 1 depicts that apart from monsoon, in all the remaining three seasons, outdoor infiltration can be one of the major sources of indoor bioaerosol (Ghosh et al., 2013).

According to certain studies carried out in countries such as India, UK and USA, large number of indoor airborne fungal spores were found to be closely associated with periodicals and books, especially disoriented and discoloured paper and wooden materials (Gutcho, 1974) because microbial proliferation is related to presence of cellulosic matter (Deguchi and Yoshizawa, 1996; Jain, 2000). Thus presence of large amount of cellulosic matter in the form of periodicals, thesis and books can be one of the major sources of high fungal bioaerosol along with outdoor infiltration at site 1 (central library) in comparison to other sites. Apart from outdoor infiltration, surface dust can be attributed to be one of the major indoor sources of fungal bioaerosol especially *Aspergillus*, *Cladosporium*, *Curvuleria* and *Penicillium* as all these mentioned fungal species were found both airborne as well as in the surface dust at almost all the six sites. Thus, aerosolisation of surface dust can also be a reason of above mentioned fungal indoor species due to various mechanical movements such as dusting, cleaning, walking over carpets etc. In case of bacterial bioaerosol, apart from outdoor infiltration and aerosolisation from surface dust, occupancy, although not studied upon in details, can also be attributed to be a major source (Ghosh et al., 2013) as human activities such as sneezing and coughing have been found to release microbes and microbial fragments through nose into the air (Tekonda, 1987; Campbell, 1991).

Presence of *Aspergillus*, *Alternaria* and *Curvuleria* in abundance represents an allergic environment especially in monsoon and post-monsoon season at majority of the sites. Mycotoxins, such as aflatoxins and ochratoxin known to be produced by *Aspergillus* are potentially mutagenic and carcinogenic to humans and have the capability to cause acute hepatic necrosis which ultimately results into carcinoma of liver (Brera et al., 2002; Klich, 2007). *Alternaria* and *Curvuleria* though known plant pathogens yet are capable of acting as

allergens in humans causing hypersensitivity reactions that ultimately lead to asthma. Although *Cladosporium* is mostly known to be harmless, yet in certain studies, respiratory infections have been found to be caused by this fungal genus (Beaumont, 1988). Thus, presence of *Cladosporium* dominantly in post-monsoon and winter season at most of the sites might result into respiratory problems among the inhabitants.

CONCLUSION

The broad conclusions of the present study are as follows:

- Among the six MVOCs, 1-pentanol, cyclohexanone and 1-hexene were maximally found, while 1-octen-3-ol, 3-methyl-1-butanol and 3-octanone were comparatively very less in the indoor air samples revealing the fact that majority of the sites might have had proper sources for these MVOC productions.

- No significant difference was found between moldy and non-moldy indoor environment in terms of MVOCs concentration concluding the fact that, although not visible, yet, hidden fungus in building materials and accumulated surface dust could be the reason of the presence of MVOCs in non-moldy sites.

- Passive samplings over the inoculated microbial cultures as well as isolated fungal species present in dust and building materials collected from all the six sites were found to be the major sources of these MVOCs.

- According to the seasonal study, most of the MVOCs were maximally found in monsoon and winter seasons, while minimally found in post-monsoon and pre-monsoon seasons at all the sites.

- Statistically significant difference was found between the mean concentration in different season for 1-octen-3-ol and 1-pentanol only while; in case of 1-hexene and cyclohexanone no significant difference was found.

- Passive sampling of MVOCs over specific species of fungus revealed that emission from selected fungal species were highly dynamic during the life span of the fungal cultures, wherein, volatile compound emission rate acts as the function of the corresponding culture's cultivation time.

- Use of different media or substratum was found to play a major role in the production difference of MVOCs by the same fungal species because the produced MVOC pattern not only depend on fungal species but also upon the pH value, chemical composition, water content and temperature of the substrate be synthetic media in laboratory or building material in problematic indoors.

- Most of the previous studies suggested that 3-methyl-1-butanol to be a most reliable indicator of damp and infected buildings in relation to mould formation only. But, in

this study, apart from being released from fungus, 3-methyl-1-butanol was released by bacterial community as well in both dust and building material samples.

- 1-pentanol and 1-hexene were second most highly detected and quantified MVOCs after 3-methyl -1-butanol in both dust and building materials of almost all the sites, as well as 1-pentanol was released from all the isolated fungal species.

- No significant relationship could be established between the environmental parameters with MVOCs concentration ($p>0.05$) emphasising the fact, that within the indoor environments, other factors especially ventilation rate played the major role for the variation in the MVOCs concentration.

- The typical pattern of concentration of fungus depicted that majority of the fungal species (their hyphal fragments and spores) were similar diameter as that of stage 4 which is in synonym to the secondary bronchi of the lungs in the human body.

- Statistically no significant difference was found between the mean fungal concentrations ($p> 0.05$) at all the six sites.
- Statistically significant difference was found among the mean fungal concentration across all the different seasons at most of the sites ($p< 0.05$) with very few exceptions.

- Unlike fungi, bacterial bioaerosol concentration was found to be different at different sites and season thereby following no typical pattern.

- In monsoon season, majority of the bacteria found corresponds to trachea, primary bronchi, secondary bronchi as well as alveoli in human lungs when inhaled.

- In post-monsoon season, majority of the bacteria found corresponds to nasal passage and alveoli in human lungs when inhaled.

- In winter season, majority of the bacteria found corresponds to nasal passage, trachea, primary, secondary and terminal bronchi as well as alveoli in human lungs when inhaled.

- Unlike all the other seasons, not much variation was found among all the size segregated bacterial bioaerosol in pre-monsoon season for most of the sites.
- Statistically no significant difference ($p> 0.05$) was found between the mean bacterial concentrations among most of the sites with few exceptions.

- Statistically significant difference was found among the mean fungal concentration across all the different seasons at most of the sites ($p < 0.05$) with few exceptions.
- Relative humidity (RH) alone did not play an important role in the bioaerosol concentration emission. The combined effect of RH and temperature was found to be significant ($p < 0.05$) with both fungal and bacterial bioaerosol concentration emphasising the fact that meteorological parameters in totality play an important role in bioaerosol emission.
- I/O ratio was <1 for all seasons except the monsoon, revealing the fact that for all the other seasons, outdoor infiltration might be one of the sources of indoor bioaerosol.
- Around six fungal species were identified in dust and building material samples namely; *A flavus, A niger, A nidulans, Cladosporium cladosporioides, Curvuleria lunata and Penicillium sp.*
- Apart from outdoor infiltration, surface dust could be attributed to be one of the major indoor sources of fungal bioaerosol especially; *Aspergillus, Cladosporium, Curvuleria* and *Penicillium* as all these mentioned fungal species were found both airborne as well as in the surface dust at almost all the six sites.

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