Study of Some Important Air Microflora in Sikkim

A Thesis Submitted

To

Sikkim University

In Partial Fulfilment of the Requirement for the

Degree of Doctor of Philosophy

By

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and

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[DECEMBER 2021]

DEDICATED

TO

LATE Dr. HARE KRISHNA TIWARI

DECLARATION

I declare that the present Ph.D thesis entitled "**Study of Some Important Air Microflora in Sikkim**" submitted by me for the award of degree of **Doctor of Philosophy in Microbiology** in Sikkim University under the supervision of **Late. Dr. Hare Krishna Tiwari**, Associate Professor, Department of Microbiology and **Professor. Dr. Jyoti Prakash Tamang**, Head of the Department, Department of Microbiology, School of Life Sciences, Tadong, Sikkim University, is my original research work solely carried out by me in the Department of Microbiology, School of Life Sciences, Sikkim University, Sikkim. No part thereof has been submitted for any degree or diploma in any university/Institution.

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This is to certify that the PhD thesis entitled **"Study of some important air microflora in Sikkim"** submitted to **SIKKIM UNIVERSITY** in partial fulfilment for the requirement of the Doctor of Philosophy in Microbiology, embodies the work carried out by **Ms. Nilu Pradhan** for the award of PhD Degree in Microbiology, Department of Microbiology, School of Life Sciences, Sikkim University, Gangtok, Sikkim. It is a record of bonafide investigation carried out and completed by her under the supervision of **Late Dr. Hare Krishna Tiwari** of Department of Microbiology, Sikkim University, who expired on 27th September 2020. As per the Ordinance of the University, she was assigned to me for completion and submission of her Thesis. She has followed the rules and regulations laid down the University. The results are original and have not been submitted anywhere else for any other degree or diploma.

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"Study of some important air microflora in Sikkim"

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Date: 29th December 2021 **NILU PRADHAN**

CONTENTS

INTRODUCTION

The most essential factor that affects the health and wellbeing of people is the quality of air we breathe. An average air inhaled by a person in a single day has been reported to be approximately $10m^3$ per day (Daccaro et al. 2003). The air includes a large portion of the primary biological aerosols (PBA), which are atmospheric particles of biological origin consisting of microorganisms (bacteria, fungal spores and viruses) (Jaenicke 2005) archaea, algae, plant pollen and excreta of organisms (Despres et al. 2012). Bioaerosols, have gained much attention recently because of their probabilities to cause human, plant, and animal diseases (Abdel-Hafez 1982; Kim et al. 2018), while others are identified as beneficial to human health and the environment **(**Agranovski et al. 2004). The tendency of bioaerosols to get dispersed covering wide geographical distance, resulting in exchange of genetic material between distant bioaerosols can get dispersed over a large area, allowing the exchange of organisms and genetic material between distant environments (Prussin et al. 2015; Xie et al. 2021).

Consequently, bioaerosols are not limited to its distribution in the biogeography and dynamics of ecosystems, but also has a role in dispersal of pathogens and allergens with implications for human, plant and animal health (Despres et al. 2012; Frohlich-Nowoisky et al. 2016). Recent studies have suggested the account of bioaerosols up to 30% of the total aerosol mass in urban and rural air (Frohlich-Nowoisky et al. 2016). The PBA being an important constituent of total aerosol mass; its emission, deposition, residence time, and transformation may influence the local as well as global climate by absorption of radiation and scattering of light (Frohlich-Nowoisky et al. 2016). Furthermore, it has also reported to act as cloud condensation nuclei, affecting the hydrological cycle and climate (Despres et al. 2012; Frohlich-Nowoisky et al. 2016). Recent studies have shown that PBAs represent up to 46% of the total aerosol load with particles sizes between 2 μm and 4 μm (Wu et al. 2019). Moreover, it has been

established that temporal variation of the concentration of microbial air particles is influenced by meteorological parameters, such as relative humidity, solar radiation, and temperature (Moran-Zuloaga et al. 2018). There have been many studies to examine the distribution and concentrations of airborne bacteria and fungi in both indoors and outdoors (Lighthart and Stetzenbach 1994; Prussin et al. 2015). There has been reports that typically, concentration of bacteria and fungi is approximately 10^2 to 10^6 CFU m^{-3} and 10^2 to 10^3 spores m^{-3}, respectively. (Lee et al. 2006; Bowers et al. 2009)

The upper respiratory tract, the nose and nasopharynx of humans is resided by a large number of airborne particles (Zeterberg 1973: Xi and Longest 2008; Thomas 2013). These PBA particles has been found to have negative impact on the health of plants, animals and humans as specific PBA particles are known to act as allergens and/or pathogens (Husman 1996; Pillai et al. 1996; Hirano and Upper 2000; Michalska et al. 2019). Most of them originate from natural sources such as soil, lakes, animals and humans (Lindemann and Upper, 1985; Korzeniewska 2011: Hu et al. 2017). Nonetheless, the air people inhale is loaded richly with air pathogens which is the major components of the atmospheric aerosol (Jaenicke 2005) that comprises of thousands and millions of cells/m^3 of air (Lighthart 2000).

Airborne microorganisms can transmit disease from one region to another and impact agriculture and human health (Fujiyoshi et al. 2017). Microorganisms as bioaerosols may result in the spread of plant diseases and loss of agricultural productivity (Kim et al. 2018). The microbial aerosols from sources both anthropogenic and environmental sources are associated with public health concerns (Winn 1988; Calvo et al. 2013; Querol et al. 2019)**.** Among the microorganisms present in the atmosphere, bacteria are often the highest in number, despite their high death rate due to environmental factors producing stress of various kinds, of the major being dehydration stress (Madrioli et al.

1998). Moreover, agricultural practices, healthcare facilities and industrial units such as sewage treatment plants, animal farming houses, fermentation and food processing units also emit viable microorganisms into the air (Cullinan et al. 2001; Mouli et al. 2005). Airborne bacteria have also been found to play an essential role in the global climate system by absorbing or reflecting incoming sunlight (Mohler et al. 2007), acting as cloud condensation nuclei (Bauer et al. 2003; Mohler et al. 2007) and potentially serving as ice nucleating particles (Schnell and Vali 1972: Constantinidou et al. 1990; Mohler et al. 2007, 2008; Santl-Temkiv et al. 2019).

The study of airborne microorganisms' dates back to Pasteur's work in the mid-19th century (Ariatti and Comtois 1993; Griffin et al. 2017) but the full extent of microbial diversity in the atmosphere still remains poorly understood. Sikkim as tourism hub and also an important strategic point becomes crucial to study air microflora which directly affects the inhabitants in this location. It is very essential to know the distribution pattern of live bioaerosols at different sites in urban as well as rural environment. As such, it is important to assess the possibility of microbial air pollution and associated risks, also current monitoring of viable microbial level must be performed (Rangaswamy et al. 2012)**.** Mold, a wide range of fungal organisms that thrive well under damp and humid conditions indoors and outdoors, and in humans' hence, exposure to it is associated with the aggravation of asthma, allergic rhinitis and occasionally infection (Hesseltine and Shotwell 1973; Mendell 2011) but these conditions caused by such molds require further detailed investigation (Mazur and Kim 2006). Genus of bacteria *Bacillus, Paeudomonas, Arthrobacter, Pseudomonas, Paenibacillus Cryptococcus, Corynebacterium* (Kellogg et al. 2004; Griffin et al. 2006; Zhai et al. 2018) including the common genera of outdoor mold and yeast *Alternaria, Penicillium, Aspergillus, Cladosporium, Fusarium Candida, Botrytis and Helminthosporium* (Griffin et al. 2006; Shams-Ghahfarokhi et al.

2014; Reddy et al. 2015; Black 2020) have been frequently obtained from open space air samples. Exposure to mold varies depending on a range of factors including regional differences, local climate notably humidity and wind, shade, organic debris, landscape maintenance, etc. (Mazur and Kim 2006). Various molds, yeasts, other fungi and many organisms saprophytically degrade organic matter such as fallen leaves, trees and others and are generally known to be beneficial for the ecosystem (Black 2020).

A well-established method of collecting viable airborne particles is the Anderson air sampler (Andersen, 1958), where the air trapped is collected on the petri-plates containing a suitable agar medium (Black 2020). Many methods of measuring different airborne microorganisms have shown successful results, however, the techniques used for measurement of airborne microorganisms has been broadly classified as active and passive method (Haas et al. 2017). Two methods commonly used are impaction sampling (active process) and passive sedimentation (passive process) where the microorganisms settle directly on the agar media (Pasquarella et al. 2000; Sandle and Satyada 2015), For qualitative and quantitative determination of microorganisms in indoor and outdoor environment an active impactor sampling is normally preferred to measure the degree of contamination (Haas et al. 2007). Passive sampling relies on settlement of particles by gravity on the selected medium, it is a method of choice as it is most readily available, cost effective and modest method of bioaerosol sampling (Haas et al. 2007).

Fungal spores are present everywhere in outdoor air and often are the dominant biological component of air (Gregory 1973). Most fungal spores fall in the range 2–10 mm diameter, this allows effortless penetration of the lower airways of the human respiratory tract, from where the dissemination of infection by the pathogens follows (O'Gorman and Fuller 2008). The allergenic and pathogenic potential of a vast range of

fungi is well recognized, for example, spores of *Alternaria, Aspergillus, Penicillium* and *Cladosporium* are responsible for causing many respiratory conditions, from allergic rhinitis to asthma (Kurup et al. 2002). Although, exposure to fungal spores occurs mostly indoors, outdoor air is equally important source of both aeroallergens and pathogens (Curtis et al. 2006). However, major hindrances in the study of potential health consequences of exposure to bioaerosols in the agricultural setting have been a lack of information on exposure and difficulties in disentangling the effects of bioaerosol emissions from those of other emissions (Douglas et al. 2018).

There is paucity of information regarding the abundance of microbial population resident in the air environment also, there is a limited understanding of the quantities and types of bacteria found in the atmosphere due to technical challenges and underappreciated importance (Womack et al. 2010). This scanty information could be due to methods used in the isolation and identification of microorganisms associated with the air environment. However, with recent advances in high-throughput sequencing, the dynamics of bacteria in the atmosphere can be better understood (Bowers et al. 2009), and thus provide a more comprehensive data set for deciphering those bacteria found in the atmosphere and the control of their populations (Griffin 2007; Bowers et al. 2011b). Recent advances of competent air sampling tools, in combination with high-throughput sequencing technologies, have created better opportunities for studying PBA at an unparalleled scale and with much detail (Mayol et al. 2017; Archer et al. 2019; Gusareva et al. 2019). In spite of increasing advancements in research effort and awareness on significance of air microflora on the climate and hydrological cycles, nonetheless, the effect of ongoing global and regional changes in land, properties, and function of bioaerosols remains indefinable (Santl-Temkiv et al. 2020).

Population and the type of microorganisms present in aerosol micro environments particularly in densely populated areas are of relevance for maintenance of public health particularly for children and immunocompromised patients. Having a direct relation with climate change the study of air flora becomes more significant. The aim of the study was to examine aerosolized bacteria and fungi in the environment in Sikkim, a mountainous state of India. Sikkim, as tourism hub and also an important strategic point, becomes crucial to study air microflora which directly affects the inhabitants in this location. The study area consists of both urban and rural area of the State. Therefore, the study was conducted to understand the basic prevalent microbes in the study area in different seasons.

REVIEW OF LITERATURE

Microbes are all-pervasive on the earth (Timmis et al. 2019). Microorganisms existing in the air originate from natural sources such as plants, soil and water (Smets et al. 2016) and it plays important role in maintaining the ecological balance (Fang et al. 2007) and atmospheric processes (Gandolfi et al. 2013). Although the atmospheric air is not favorable environment for the growth of microorganisms due to low levels of moisture, nutrients availability, high solar radiation, and a large dispersal ability (Gandolfi et al. 2013) many species of bacteria and fungi however manage to survive in the atmosphere in large populations (Ruiz-Gil et al. 2020). The abundance of airborne bacteria in the atmosphere ranges from 10^4 to 10^8 cells per m^3, which depends on their habitats, such as agricultural fields, urban areas, forests, and mountains (Bowers et al. 2011a, 2012; Cao et al. 2014; Mhuireach et al. 2019).

The presence of microbes in the atmosphere was discovered by the ingenious experiments of Spallanzani in the middle of the 18^{th} century (Capanna 1999) and at the end of the 19^{th} century (Pasteur 1890). Late back, it was studied that some of the pathogens dispersed in the atmosphere has the ability to cause diseases, such as wound infections (Whyte et al. 1982) allergies and inflammation (Polymenakou 2012). The airborne bacteria to which people are regularly exposed occasionally cause health risks in human however, some bacteria act as an agent responsible for hypersensitivity, infectious, or inflammatory diseases (Tsai and Macher 2005). Endotoxin, a constituent of the outer membrane of Gram-negative bacteria, has been identified as a health hazard in various occupations (Radon 2006; Liebers et al. 2020) and are found to be associated with severity of asthma (Reed and Milton 2001; Freitas et al. 2016). Many microbes have crucial roles to play to the earth's ecology and on human health, for example, gram-positive and gram-negative bacteria in soil, water, and on leaf surfaces; gram-positive

bacteria reside on human skin and scalp likewise gram-negative as intestinal bacteria (Tsai and Macher 2005).

Bioaerosols

Bioaerosols are known as the airborne biological particles or droplets containing biological material (dead or alive), usually 0.5-30 μm (Kim et al. 2018), which are originated biologically from plants and/or animals and can hold living organisms (Georgakopoulos et al. 2009; Bulski 2020). Pollens from anemophilous plants have characteristic diameters of 17 to 58 μm (Stanley and Linskens 2012), fungal spores are typically 1 to 30 μm in diameter (Gregory 1973), bacteria are typically 0.25 to 8 μm in diameter (Thompson 1981), while viruses have diameters usually less than 0.3 μm (Taylor 1988). Bioaerosol material is derived from biological origins, including aerial suspensions of bacteria, viruses, fungi, enzymes, and pollen (Londahl 2014). If carried by a favorable air flow, bioaerosol material may be distributed over large distances with potentially fatal results (Han and Mainelis 2012).

Microorganisms including pathogenic or non-pathogenic dead or alive may exist in bioaerosols (Mandal and Brandl 2011). Bioaerosols consists of microorganisms and their components such as endotoxin, mycotoxins, and allergens. Such organisms are well recognized typical components of both indoor and outdoor air (Kim et al. 2018). Spore-forming bacteria and fungi are able to survive in bioaerosols and stay viable for a long time in the air (Smith et al. 2011). The bacteria may occur as clusters of cells, or may be transported into the air on plant or animal fragments, on soil particles, on pollen, or on spores which have themselves become airborne (Reponen et al. 1997). Viruses may be transported in larger droplets emitted by animals (Donaldson et al. 1983; Jayaweera et al. 2020). Dispersal of microbes (bacteria, fungi, algae and viruses) may occur through

various vectors such as animals migrating from one place to another or hydrological cycle (Gerba 2015), nonetheless, dispersal through wind is considered the most common method (Smith et al. 2011). However, rudimentary environmental factors, such as relative humidity and temperature have been found to considerably influence the degree of their formation and dispersion of microorganisms (Dedesko et al. 2015). Having the small size and light weighted, bioaerosols are known to be easily shifted from one environment to another (Van Leuken et al. 2016). In recent years, exposure to bioaerosols in occupational as well as residential environments has gained much attention considering their probable impacts on human health (Kim et al. 2018). Despite of all the disadvantages, however, it has been studied that under certain conditions, exposure to some microbes is beneficial for health particularly in developing of immune immune system and protection of children from developing medical conditions such allergies and asthma (Severson et al. 2010). Even though the significance of bioaerosols and their effect on human health has been documented, it has still been difficult to describe their specific role in the development or worsening of various symptoms and diseases (Kim et al. 2018).

Fungi and bacteria in ambient air

Molds play key role in biodeterioration of wood and paper (Sterflinger and Pinzari 2012), textiles (Boryo 2013), fuels (Das and Chandran 2011) and leather (Rathore et al. 2013). Molds having a high biodeterioration activity (enzymatic and acidic) (Gutarowska and Czyżowska 2009) belongs mainly to the genera- *Aspergillus, Cladosporium, Chaetomium, Paecilomyces, Penicillium* and *Trichoderma*, besides members of some genera such as *Alternaria, Epidermophyton, Microsporum, Scopulariopsis, Stachybotrys* and *Trichophyton* are also reported to be producing

allergens and mycotoxins which cause health threats (Gutarowska 2014). Allergens, toxins, enzymatic proteins and organic compounds (volatile) causing allergies, toxic effects, infections and irritations could be produced from fungi risking human health (Reinmuth-Selzle et al. 2017). Important associations have been found between meteorological parameters (especially, relative humidity) and bacterial and fungal growth (Niazi et al. 2015). It has been recorded that the outdoor concentrations of fungal and bacterial bio-aerosols increased rapidly during monsoons (Kang et al. 2015).

The microbial distribution in community places is usually dominated by bacteria associated with human and soil (Bowers et al. 2011b; Hewitt et al. 2012; Hoisington et al. 2016). The exposure to the area with frequent visit by people and types of human activities occurring can affect the microbial composition of the place (Hoe et al. 2017) which is considered as one of the prime factors that influence the composition of indoor bioaerosols (Adam et al. 2015). The microbial community in open air spaces is known to be more diverse as their source of origin are many which includes water, plants, soil and various living carriers example; insects (Fujiyoshi et al. 2017). The composition of microorganisms in the air is also influenced by geographical variations such as landscapes, land use, types of crops etc. (Fujiyoshi et al. 2017).

Air microflora and its potential threat

Microorganisms are found richly loaded in the air during the time favorable for development of disease in crop plants caused mainly by fungal spores disseminated through air (Stakman and Christensen 1946; Frohlich et al. 2016) and through anthropogenic activities resulting in release of microbial particles into the atmosphere particularly during harvesting of crops (Lighthart 1997; Morris et al. 2014). Bacterial load have been observed ranging from thousands to 10^8 bacteria per m^3 in an area close

the canopy level (Lighthart 1997; Morris et al. 2004). Among the bacteria detected in the atmosphere, majority of them are gram-positive spore formers of genera *Bacillus* and *Microbacterium*, which are particularly found dominant throughout a dust event (Kellogg and Griffin 2006; Ruiz-Gil et al. 2020). Besides, gram negative bacteria with a cell wall more fragile than gram positive has also been recovered in the air environment (Lighthart 1997).

Among the fungi, spores related to those from *Aspergillaceae, Alternaria, Botrytis Cladosporium* and various Basidomycetes (*Coprinus*, *Ustilago*) have been frequently obtained from the atmosphere (Gregory 1961; Kellogg and Griffin 2006; Morris et al. 2011), but then spores of *Cladosporium* sp. seem to be numerically the most dominant among others (Bensch et al. 2018). Viruses have also been detected from the atmosphere, especially in air over the sea surface (Aller et al. 2005; Yahya et al. 2019) and in clouds (Castello et al. 1995), and virus-like particles have been reported to be associated with transoceanic dust (Griffin et al. 2001). The most abundant species of *Staphylococcus* found on human skin such as *S. epidermidis, S. hominis, S. cohnii, S. caprae* and *S. haemolyticus* has been reported (Robertson et al. 2013). In an outdoor facility such as composting facility predominant bacterial genera recovered are *Bacillus, Staphylococcus Streptococcus, Acinetobacter and Kocuria*, which pose a potential hazard to air quality and human health (Pahari et al. 2016).

It is commonly known that microorganisms present in the air can enter the human body via respiratory route affecting human health causing mostly respiratory and related diseases (Bugajny et al. 2005). Airborne microbes are either be pathogenic or nonpathogenic, however causing sensitivities with exposure for long duration to the contaminated air (Griffin 2007). The mode of entry of the airborne microbes usually occurs through inhalation otherwise indirectly by ingestion of contaminated foods and

water, the microbes bind to dust particles, precipitates and then enter into the human body (Gorbushina and Palinska 1999) eventually leading to disease development (Heldman 1974). The other health problems reported due to exposure to bioaerosols include gastrointestinal discomfort, exhaustion, weakness and headache (Douwes et al. 2003). However, it would be unfair to assume only the health risks from bioaerosols as beneficial health effects may also occur (Douglas et al. 2018). Bioaerosols containing viruses (enteric viruses, including Noro-and rotaviruses, and some respiratory virus such as influenza and coronaviruses), bacteria (staphylococci, legionellae, tuberculous and nontuberculous), bacterial spore formers (*Clostridium difficile* and *Bacillus anthracis*) and fungi (*Aspergillus, Penicillium,* and *Cladosporium sp.* and *Stachybotrys chartarum*) are of concern (Jiayu et al. 2019).

Many species of bacteria as *Streptococcus pyogenes, Mycobacterium tuberculosis* or *Legionella pneumophila* or viruses may cause severe human infections and diseases (Mehdinejad et al. 2011). One of the studies has suggested the exposure to some frequent microorganisms such as *Streptococcus pneumoniae, Streptococcus pyogenes, Mycoplasma pneumoniae, Haemophilus influenzae, Klebsiella pneumoniae, Pseudomonas aeruginosa, and Mycobacterium tuberculosis* have wide ranges of ill effects on health increasing possibility of food pollution, deterioration of cosmetics and medicine, corrosion of metallic materials for infrastructure ultimately leading to economic losses (Smets et al. 2016). The potential hazard caused by bioaerosols is dependent on the specificity and the community structure (Mbareche et al. 2019). In some instances, even though the strains identified as non-opportunistic pathogens, it can serve as a source of antibiotic resistance in the environment posing threat to public health (Brągoszewska and Biedron 2018). However, both significant and burdensome for humans are filamentous molds occupying sometimes 70% of all microorganisms present

in the air (Gutarowsk and Jakubowska 2001). Exposure to bio-aerosols, containing airborne microorganisms and their by-products, can result in respiratory disorders and other adverse health effects such as infections, hypersensitivity pneumonitis and toxic reactions (Gorny et al. 2002; Fracchia et al. 2006).

It has been reported that atopic allergy affects over 25% of the population in the industrialized world (Kurup et al. 2002) and it is estimated that in the year 2020, 50% of total world population could be affected by different forms of allergies (Sweeney et al. 2021). The major allergic diseases induced by fungi are asthma, rhinitis, allergic broncho pulmonary mycoses, and hypersensitivity pneumonia (Kurup et al. 2002; Sarica et al. 2002). Predominantly sensitive to allergens are the elderly, infants, people on chemotherapy, AIDS patients, etc. (Ventura et al. 2017). Many genera of fungi can cause allergic responses, such as *Cladosporium, Alternaria, Penicillium,* and *Aspergillus* (Zukiewicz-Sobczak et a. 2013). *Cladosporium* and *Alternaria* are recurrent and predominant genera present mostly in the outdoor air and found throughout the world, whereas *Penicillium* and *Aspergillus* species are generally isolated from indoor environments (Akerman et al. 2003). Some molds also produce mycotoxins, while some mold species become particularly toxigenic by producing dangerous volatile compounds (Akerman et al. 2003). Subsequently, the prevalence of bioaerosols has been associated with certain human diseases, such as pneumonia, influenza, measles, asthma, allergies, and gastrointestinal illness (Srikanth et al. 2008). Studies of suspected airborne transmission routes of various pathogens have been undertaken with differing degrees of success (Hobday and Dancer 2013). The microbiome of concern are the fungal bioaerosols as they can produce mycotoxins (Kim et al. 2018), which can lead to diverse health effects causing non-infectious diseases (i.e., weakening of immune systems, allergies, irritation, many identifiable diseases, and even death) usually through

ingestion, inhalation and dermal contact (Andretta et al. 2011). Airborne biological particles have widespread impacts not only limited to human health but also climate and microbial biogeography (Smets 2016). One of the examples is plant pathogen *Pseudomonas syringae* and related phylloplane bacteria, which have strong ice nucleation ability at a temperature (33°C to −5°C) than the homogeneous freezing temperature of cloud droplets solely composed of pure water (Morris et al. 2013; Failor et al. 2017).

Influence of Environmental factors on air borne microorganisms

Most studies have found that air temperature is directly corelated to both culturable and total bacteria concentrations (Bovallius et al. 1978; Harrison et al. 2005). Seasonal and daily variation in the amount and kinds of microorganisms in the air has also remarkably varied in many studies (Nunez et al. 2021). High concentrations of air-borne bacterial and fungal spores frequently occur from spring to fall in temperate areas of the world, mainly due to the fact that leaf surfaces are a major source of fungi (Mitakakis et al. 2001; Levetin and Dorsey 2006; Ruiz-Gil et al. 2020) and bacteria (Tong and Lighthart 2000) in the air. On land, aerial parts of plants are considered a principal source of air-borne micro-organisms (Lighthart 1997; Lymperopoulou et al. 2016).

Concerning the chemical composition of the atmosphere, air-borne microbial concentrations have been observed to increase with increase in atmospheric CO_2 concentrations (Klironomos et al. 1997; Pandey et al. 2014). Total fungal spores and other biological materials constituted 6% in air of Shillong by Satpute et al. 1983. It was reported that the low incidence of spores was due to high elevation of the place and also a smaller number of anemophilous plants in the locality (Damialis et al. 2017). The frequency of fungal spores was maximum during September when the humidity was

high and minimum in March during low humidity) (Damialis et al. 2017). The relative humidity and/or the moisture content of the materials determine the degree at which different micro-organisms can grow on both indoor and outdoor materials (Dhanasekaran et al. 2009).

Since airborne bacteria can affect the visibility, climate and quality of life (Beggs 2003; Martiny 2006; Griffin 2007), it is noted as very essential to know the distribution pattern of live bioaerosols at different sites in urban environment. Bacterial species form part of the many types of bioaerosol particles, and they are known to be abundant in the atmosphere (Jaenicke 2005). Bacteria can enter the atmosphere as aerosol particles from all surfaces, including soil, water, and plant surfaces (Jones and Harrison 2004). Once in the air, they are raised upwards by air currents and may remain in the atmosphere for several days before being removed by precipitation or direct deposition onto surfaces (Rai 2016). Hence, meteorological parameters like wind direction, wind speed, temperature, and relative humidity play a key role in determining the suspension, transportation, and deposition of airborne microbes (Burrows et al. 2009)

Sampling of bioaerosol

Air sampling of microorganisms is a popular method of conducting microbial examinations by direct toxicological evaluation (Yassin and Almouqatea 2010). There are several types of microbial air sampler accessible to the researchers, which are broadly divided into four categories; cyclones, impactors, impingers, and filtration (Haig et al. 2016). The types cyclones and impingers allow collection of airborne particles into a liquid medium, while impactors collect particles on to solid agar medium and filtration method traps the particles on fine fibers or on surface of porous filter membrane (Haig et al. 2016). The final concentration or microbial load is expressed in colony forming

units per cubic meter (CFU/m^3) (Yassin and Almouqatea 2010). It is reasonable to expect that environmental parameters and the mechanical stress caused during sampling affects the recovery of each type of bioaerosols. (Morris et al. 2013). Therefore, the tools to be for sampling of bioaerosols should be more subtle that the ones used for studying the general particle composition of air (Haig et al. 2016). Such precise and delicate methods for such specific purposes are not yet well established, hence the general methods developed for air sampling such as such as impactors, impingers, cyclones, filtration, spore traps, precipitators, condensation traps, gravitational samplers or passive method (gravitational settle plate) can be useful with or without modifications (Haig et al. 2016). Generally, these sampling tools are designed for the purpose of steady sample collection in a selected culture medium allowing separation of particles from the air stream (Jensen and Schafer 1998: Morris 2000). Gravitational sampling is known as the passive method (non-volumetric) of sampling to aid the collection of particles by gravitational force usually on a solid agar medium, on coated microscope slide etc. (Wang et al. 2001; Ariya et al. 2013). In bioaerosol samplers, where the samplers are kept undisturbed (inertial) there are many options that one can choose, from impactors, sieves to stacked sieves (Haig et al. 2016). The advantage of these arrangements is that they facilitate collection of bioaerosol particles by size selection as they rely on particle properties to deviate from airflow streamlines by virtue of inertia (Haig et al. 2016). Filtration, electrostatic precipitation, thermal precipitators, and condensation traps are non-inertial samplers that operate without the inertia of particles; as a result, they do not rely on particle size. (Ghosh et al. 2015). Impactors, on the other hand, rely on particle inertia to gather bioaerosol samples. As a result, the bioaerosol-containing air sample is pushed to pass through a collection of curve streamlines, where low-inertia particles are moved to evade capture. (Mainelis 2020). Particles with a high inertia, on the other side,

cannot transit through the streamlines' 90° curve. Alternatively, while being influenced by centrifugal force, they can impact the agar plate. (Xu et al. 2013). This property of the impactor sampler makes it ideal for classifying particles into various size fractions, as larger particles fall out of the air flow, the smaller particles still remain airborne. (Xu et al. 2013). Resistibility of bioaerosol, testing time, and stream rate are considered as the foremost critical variables influencing microbial collection and their survival in bioaerosol samplers (Macher and Willeke 1992; Therkorn and Mainelis 2013). Largely, impactor is more commonly favored over impinger and filter in ambient and/or indoor environments due to its advantage of direct sample collection without post collection sample processing (Li 1999; Kumar et al. 2021b).

Bioaerosol sampling

There are a variety of commercially available bioaerosol samplers and many new sampler concepts are being developed, but the great majority of samplers utilize impaction, filtration, impingement, or electrostatic precipitation, or a combination of these techniques, to capture the particles of interest (Mainelis 2020). Moreover, the many ways for collecting air samples from the environment have been broadly categorized into two methods: passive or active sampling methods, as mentioned below; in active sampling devices, mechanical components are also implicated (Gollakota et al. 2021)

Passive sampling of microbial air samples

Passive sampling is perhaps considered as the most easily available, cost effective the most readily available, economic, and modest method of bioaerosol sampling which relies on settlement of particles by gravity on the selected medium (Cox et al. 2020).

The number of colony-forming units (cfu) inside the surface area of the settling plates exposed for a specified time length (for example, in units of cfu/m2/h) is commonly used to quantify the collected particles. (Haig et al. 2016). This sampling method does not require any mechanical assistance, such as a pump (Haig et al. 2016). This method offers the advantage of collecting data without affecting the environment or ambient air. The settling velocity of a particle, which is determined by particle size and density, is the rate at which the particle drops in undisturbed air (Hinds 1999). Smaller and lighter particles remain airborne for longer period of time than the larger and denser particles, in case of increase in the air speed over the settling velocity the particle has tendency to remain suspended unlimitedly (Canha et al. 2015). In addition, the airflow, even within an enclosed room, will be driven by subtle variations in temperature, the source volume of air for the passively collected sample will remain unknown (Fox et al. 2011). The combination of these factors has allowed passive sampling to be regarded as both quantitatively and qualitatively inaccurate, as a subordinate collection method to active sampling (Ghimire et al. 2019). However, this is an oversimplification to the invariably complex nature of bioaerosol sampling (Haig et al. 2016). Many efforts have been made to standardize the use of settle plates or gravitational method to study the microbial surface contamination in consideration with plate size, position, and duration of exposure (Pasquarella et al. 2000).

Active Sampling of microbial air samples

Collection of the bioaerosol samples is usually carried out on to nutrient medium or a filter or into liquid medium, liquid collection method is normally used as it places less stress on the bioaerosol particles as it is not dried out and are more likely to sustain the viability of collected methods (Sykes 2005).

Impactors

Impactors use the inertia of a particle for collection of air samples (Laszlo 2018). The air sample is fed through a series of nozzles that drive a flow of particle-laden air across a gap and onto an agar culture plate that is parallel to the nozzle exit. Through the sampler, the air flow passes through streamlines, which are curves loosely related to the flow's velocity vectors. The air flows past the plate and through the passage between the plate and the tool walls, bouncing the curves by 90^{o} (Haig et al. 2016). (Haig et al. 2016). The particles having a low inertia will flow through the streamlines and avoid being captured. High inertia particles, on the other hand, will be unable to pass through the streamlines' 90^{o} curve and will collide with the solid medium plate due to centrifugal force. The diameter and density of the particle to be collected, the diameter of the nozzle, and the air velocity of the jet all influence the impactor's collection ability. The impactor should have a sharp cutoff curve that acts like a sieve, allowing the agar plate to capture any particles larger than the cutoff size. Impactors are ideal for particle size classifiers because they can filter particles larger than a certain size from the air flow while allowing smaller particles to remain airborne. Because a single-stage impactor has only one cut-off size and single agar plate, it is called a single-stage impactor. (Haig et al. 2016).

Sampling by filters

In this method of sampling, the bioaerosol particles are collected on to filters which is further transferred on to plates or dissolved into a liquid solution for culturing, or microscopy (Burdsall et al. 2021). Filtration sampling is frequent and regarded successful, however it is less popular for collecting bioaerosol particles due to the loss of bio-efficiency caused by microbial desiccation. (Mainelis 2020).

Other sampling techniques

Electrostatic precipitation and condensation procedures are two less commonly employed approaches. (Hosansky 2021). The bio-aerosol particles are electrically charged on entering the inlet of an electrostatic precipitator, they are separated from the air flow and deposited on to charged plates. Although there is active research on this method, it has been a matter of concern that the electric field destabilizes the viability of microbes, hence much extensive research in this sampling technique is required (Lancereau et al. 2013; Wei et al. 2014). Sampling of bioaerosol through condensation techniques has also been studied, it involves flow of the air sample being processed through a humidifier. Subsequently, there are possibility that the warm, humid air is rapidly cooled with the bioaerosol particles acting as condensation nuclei (Oh et al. 2010). Despite the fact that this technology can be used to amplify microscopic bacteria, the system is difficult to apply, and heat transmission to the microbes may reduce the survivability of collected microbes. (Park et al. 2011)

Selection and bio-efficiency of microbial air samplers

While selecting a microbial air sampler the type and size of microorganisms under investigation, the sampling environment and cost should be considered (Napoli et al. 2012). Other considerations for active samplers include ease of cleaning and disinfection, as well as procedures to prevent exhaust air from contaminating the sampling environment. (Rayisnia 2016). Sampling for airborne pathogens posing a health risk is unlimited, besides healthcare environments, wastewater treatment plants, farms and slaughterhouses, public and residential buildings, compost facilities and the ambient outdoor environment have all been the focus for bioaerosol studies (Persoons et al. 2010; Dungan 2012). The bio efficiency of the sampling device is the most vital

aspect of bioaerosol sampling (Breeding 2003). It is affected by the mechanical stress and desiccation experienced by the pathogen which varies with the type of sampling device, the sampling time, the type of pathogen under study and environmental factors (Wang et al. 2001; Yao and Mainelis 2006). Many studies have been conducted to compare the effectiveness of various samplers on bio-efficiency (Shintani 2004; Han and Mainelis 2012; King and Mc Farland 2012; Li et al. 2018).

The limitations of various sampling devices have long been recognized, therefore the bio-efficiency of the chosen sampler against the target microorganisms is a noteworthy contribution to the field of bioaerosol sampling in itself (Pasquarella et al. 2000; (Ghosh et al. 2015). Through CFU calculation and visual enumeration, various PCR techniques, epifluorescence microscopy, mass spectrometry, metagenomics, matrix-assisted laser desorption/ionization mass spectrometry, and other sources, it provides a good prospect to test the storage, enumeration, and identification procedure. (Lavine et al. 2012; Pankhurst et al. 2012). Quantification of the pathogens obtained by active samplers is generally expressed per cubic meter of air, which is a reason why it is necessary to determine accurately the air flow rate of the device and the sampling time-period. (Pasquarella et al. 2000; Persoons et al. 2010).

Sampling of bioaerosols in open spaces

The sampling period has been studied and found to be influenced by a variety of factors including sampling device, operational limitations, the rate of evaporation of the collecting liquid while using a liquid collection medium, solid medium drying out, and the amount of time one has access to a site. (Haig et al. 2016). However, with all of the above characteristics taken into account, the sampling time intervals used by investigators ranged from 3 minutes to several hours (Uhrbrand 2011). As long as the

bio-efficiency of the sampler does not decline with time, it has been noted that the longer the sampling period, the more air is collected, and hence the higher the chance of capturing airborne pathogens. (Fernstrom and Goldblatt 2013). While the device suggests a brief sampling duration, repeated sampling, usually in triplicates, should be taken into account (Pankhurst 2011). The overall duration of the study could last anything from a single day to several years (Adhikari et al. 2004). Recent advances in real-time online biological particle sensors, such as light-induced fluorescence (LIF), have enabled continuous on-site measurement of bioaerosol counts. (O'Connor 2015; Huffman et al. 2020).

General bioaerosol sampling, at different heights within a room and at several spatial locations, provide a good sampling coverage (Grosskopf and Mousavi 2014). After collecting the samples, they are to be transported and stored in a manner that preserves their efficacy until further cultivation and/or identification. (Robertson et al. 2019). Inferences on bioaerosol concentration and health risk can be formed by combining the information with the genus of the captured microbe, the particle size range, and the health consequences on the human or animal population. (Robertson et al. 2019). Several studies based on bioaerosol-emitting facilities, such as waste water treatment plant (WWTP) and compost facilities has been conducted, where the chances of exposure to harmful microbes is high and is a cause of concern for occupational safety reasons and for risk to health of the population in the surrounding area (Lou et al. 2021). Hence, it is important to establish suitable response relationships to allow health-based exposure limits for bioaerosols (Walser et al. 2015). Such exposure limits would intend to protect the general population from the ill effects of long-term exposure to bioaerosols. There may be many sources of bioaerosols health risk such as staff, patients or their visitors infected with SARS virus, influenza, measles, respiratory syncytial virus, mumps, or

rubella viruses (Ghosh and Srivastava 2015; Bing-Yuan et al. 2018). The wider environment could also be a source of harmful bioaerosols, such as an increased risk of airborne *Aspergillus* during construction activities or the risk of legionella in heating, ventilation, and air conditioning (HVAC) or water systems (Kumar et al. 2021a). At present, international consent on the acceptable exposure limits of bioaerosol concentration is unavailable (Ghosh et al. 2015). A lack of bioaerosol studies targeting viruses and archaea has also been known, further limiting our understanding of the impact of airborne microbes on human health (Blais-Lecours et al. 2015).

Antibiotic susceptibility of airborne bacteria

Methicillin-resistant staphylococci (MRS) have been a matter of concern to human health as these are either pathogenic/toxigenic or are involved in causing many fatal diseases including skin diseases, blood infections, respiratory tissue and soft tissue infections (CDC 2007; Watkins et al. 2012). Antibiotic-resistant bioaerosols, such as Methicillin-resistant Staphylococcus aureus (MRSA), are more commonly reported in houses. (Gandara et al. 2006). Therefore, characterization of antibiotic-resistant bacteria and their mode of transmission during a human gathering is essential (Yadav et al. 2015). Surveillance of antibiotic resistance has been given much attention by the researchers globally as a chief tool to assess health risk by resistant airborne microbes and bacteria from other environmental sources (Naz and Pasha 2019; Akani et al. 2019).

Ambient air quality and its harmful effects

The environmental pollution has become a serious and an emerging topic of debate worldwide. Recently, air pollution is known as one of the major factors in deterioration of environment and human health (Al-Wahaibi and Zeka 2015). Air pollutants leads to

various health hazards which is subjected upon the type, concentration, duration, exposure level, frequency and associated toxicity level (Cooke et al. 2007; Al-Wahaibi and Zeka 2015). Other factors such as age, cultural practices, lifestyle may also influence the exposure to air pollutants (Omidvarborna et al. 2018). As per the report of World health Organisation, in 2019, 99% of the world's population was residing in a place where the WHO air quality guidelines were not met (Shaddick et al. 2020). In the year 2016, ambient air pollution in both cities and rural areas was estimated to cause 4.2 million premature deaths worldwide (Canha et al. 2021). Millions of people are suffering from the preventable chronic respiratory diseases caused by air pollution (GBD 2020). The most important identified air pollutants are carbon dioxide (CO_2), carbon monoxide (CO), particulate matters (PMs), nitrogen oxides, volatile organic compounds (VOCs), polycyclic aromatic hydrocarbons (PAHs), persistent organic pollutants (POPs), ozone (O_3), and sulfur dioxide (SO_2) (Omidvarborna et al. 2018).

Particulate matters are risk factors for cancer, respiratory and cardiovascular systems (Pope and Dockery 2006; Denholm et al. 2016). PMs refer to particles or droplets which are generally classified as coarse (aerodynamic diameter, 2.5–10 μm), fine (<2.5 μm) and ultrafine (<0.1 μm) PM (Fennelly 2020). $PM_{2.5}$ is related to pollution in transportation sector, while PM_{10} is more related to other particulates, such as air born particulates (Han and Naeher 2006). The smaller the particle size, the greater the risk and possibility for damage. (Harrison and Yin 2000). The concentration levels of nitrous oxides (NO_x) are presently under wide investigations, because NO_x emission is known as one of the main vehicle-related air pollutants (Omidvarborna et al. 2015). As a respiratory irritant, it penetrates deep into the lungs, causing respiratory disorders such as bronchospasm, coughing, dyspnea, wheezing and even pulmonary edema with extended exposure and inhalation (Manisalidis et al. 2020). It is estimated that T-

lymphocytes (CD8+ cells and NK cells) are affected at concentrations over 0.2 ppm, while concentrations greater than 2.0 ppm affect T-lymphocytes (CD8+ cells and NK cells). (Hesterberg et al. 2009). Long-term exposure to NO_2 has been shown to deteriorate the sense of smell. (Chen et al. 2007). Other systems that have been targeted include eye, throat, and nasal irritation, as well as diminished visibility. (Chen et al. 2007). Nitrogen dioxide levels above a certain threshold have been shown to impair agricultural output and plant growth efficiency. (Chen et al. 2007)

Sulfur dioxide is a hazardous gas that is emitted mostly as a result of the use of fossil fuels or industrial processes (Perera 2018). Respiratory irritation, bronchitis, mucus production, and bronchospasm, which leads to bronchoconstriction, are the most common health concerns linked to sulphur dioxide emissions in industrialised areas (Manisalidis et al. 2020). In addition, the pollutant causes skin redness, eye irritation (lacrimation and corneal opacity), mucous membrane irritation, and exacerbation of pre-existing cardiovascular diseases (Jung et al. 2018). Sulfur dioxide emissions are also linked to negative environmental impacts such as soil acidification and acid rain (Cape et al. 2003).

MATERIALS AND METHODS

MATERIAL AND METHODS

MEDIA USED

1. Nutrient Agar

Peptone	5 g
Nacl	5 g
Beef Extract	3 g
Agar	15 g
Distilled water	1000 ml

2. Eosin Methylene Blue
3. Mac Conkey Agar
4. Muller Hinton Agar

Muller Hinton Agar	38 g
NaCl	2%
Distilled water	1000 ml

Used to perform antimicrobial susceptibility test for control organisms.

5. Potato Dextrose Agar (MH096, Himedia, Mumbai)
6. Sabouraud Dextrose Agar (MH063, Himedia, Mumbai)
7. Mannitol Salt Agar (MH118, Himedia, Mumbai)
8. SIM Medium (M181, Himedia, Mumbai)
9. Simmons Citrtae Agar (M099, Himedia, Mumbai)
10. Nutrient Broth

Peptone	5 g
Nacl	5 g
Beef Extract	3 g
Distilled water	1000 ml

11. MR-VP Medium (LQ082, HiMedia, Mumbai)

12. Coagulase Plasma

13. Carbohydrate fermentation medium

14. Peptone 10 g

Yeast extract	5 g
Potassium phosphate	2 g
Tri-sodium citrate	2 g
Carbohydrate	0.5 %
Tween 80	1 ml
Sodium acetate	5 g
Magnesium sulphate	0.58 g
Manganese sulphate	0.28 g
Phenol red `	0.004 %
Distilled water	1000 ml

15. Urease reaction test

Peptone	1 g
Dextrose	1 g
Sodium chloride	5 g
Potassium phosphate, monobasic	2 g
Urea	20 g
Phenol red	0.012 g
Agar	15 g

16. Physiological saline (0.85%)

17. Alkaline Peptone water

REAGENTS

18. Methylene blue

19. Mac Farland 0.5 (R092-INO, HiMedia, Mumbai)

20. 3% hydrogen peroxide

21. Kovac's indole reagent (R008, HiMedia, Mumbai)

22. Methyl Red indicator (1007, HiMedia, Mumbai)

23. Barrit Reagent A

α-Napthol	5 g
Absolute alcohol (99.98%	100 ml

24. Barrit Reagent B

Potassium hydroxide	40 g
Distilled water	100 ml

25. Iodine solution (M425, HiMedia, Mumbai)

Iodine	1.0 g
Potassium oxide	2.0 g
Distilled water	300 ml

26. Gram's Crystal Violet (S012, HiMedia, Mumbai)

27. Safranin (S027, HiMedia, Mumbai)

Safranin	2.5 g
95%ethanol	100 ml

28. Ethanol (MB106, HiMedia, Mumbai)

29. Ethidium bromide (RM813, HiMedia, Mumbai)

30. 1XTAE buffer (ML016, HiMedia, Mumbai)

31. Agarose (MB002, HiMedia, Mumbai)

32. Gel loading dye (G1881, Promega, US)

33. Nuclease free water (P1193, Promega, USA)

34. Sodium hydroxide solution (TL002, HiMedia, Mumbai

35. Green Master Mix (M7122, Promega, US)

36. Proteinase K (RM2957, HiMedia, Mumbai)

37. RNase (MB087, HiMedia, Mumbai)

38. Sodium acetate (MB048, HiMedia, Mumbai)

39. DNA Ladder (100bp) (MBT049, HiMedia, Mumbai)

40. DNA Ladder (1kb) (MBT051, HiMedia, Mumbai)

41. Phenol: Chloroform:Isoamyl alcohol 25:24:1 (MB078, HiMedia, Mumbai)

42. PCR primers

43. Mercuric chloride (RM1383, HiMedia, Mumbai)

44. Potassium chloride (PCT0012, HiMedia, Mumbai)

45. EDTA di sodium salt (GRM1195, HiMedia, Mumbai)

46. Sulphamic acid (RM6363, HiMedia, Mumbai)

47. Formaldehyde (MB059, HiMedia, Mumbai)

48. Iodine (GRM1064, HiMedia, Mumbai)

49. Starch (RM3029, HiMedia, Mumbai)

50. Potassium iodate (GRM1086, HiMedia, Mumbai)

51. Sodium thiosulfate pentahydrate (GRM1314, HiMedia, Mumbai)

52. Sodium carbonate (MB253, HiMedia, Mumbai)

53. Sodium hydroxide (PCT1325, HiMedia, Mumbai)

54. Hydrogen Peroxide - 30% (PCT1511, HiMedia, Mumbai)

55. Phosphoric Acid - 85%

56. Sodium nitrite (GRM417, HiMedia, Mumbai)

57. Filter Media

Antibiotic discs (commercial discs from HiMedia, Mumbai).

Ampicillin (10 mcg)	(SD002, HiMedia, Mumbai)
Amikacin (10 mcg)	(SD035, HiMedia, Mumbai)
Amoxycillin (30 mcg)	(SD063, HiMedia, Mumbai)
Clindamycin (2 mcg)	(SD164, HiMedia, Mumbai)
Chloramphenicol (5 mcg)	(SD006, HiMedia, Mumbai)
Erythromycin (15 mcg)	(SD013, HiMedia, Mumbai)
Methicillin (5mcg)	(SD019, HiMedia, Mumbai)
Norfloxacin (10 mcg)	(SD057, HiMedia, Mumbai)
Penicillin G (10 mcg)	(SD028, HiMedia, Mumbai)
Streptomycin (10 mcg)	(SD031, HiMedia, Mumbai)
Tetracycline (30 mcg)	(SD037, HiMedia, Mumbai)
Vancomycin (10 mcg)	(SD045, HiMedia, Mumbai)

Test organisms

Bacteria	**Obtained from**
Gram negative	MTCC, Chandigarh
Escherichia coli	(MTCC 1089)

Gram positive	MTCC, Chandigarh
Staphylococcus aureus	(MTCC 7443)

INSTRUMENTS/EQUIPMENTS

1. Air sampler	
2. High Volume sampler	(APM 460, Envirotech India)
3. Analytical balance	(AX 204, Mettler, Kolkata)
4. Spectrophotometer	(Eppendorf, Germany)
5. Titrator	
6. Desiccator	
7. Biological incubator	(Accumax, CIS-24BL, Kolkata)
8. Water Bath	(RIME-1322, Remi, Mumbai)
9. Water Distillation Unit	(RIME-1322, Remi, Mumbai)
10. Laminar Air Flow	(1386, Thermo Scientific, USA)
11. Autoclave	(Instrumentation India, Kolkata)
12. Centrifuge	(CL21, Thermo Scientific, USA)
13. Gel Doc Imaging System	(Bio Rad, USA)
14. Thermal Cycler	(2720, Applied Biosystems, USA)
15. Freezer (-80°C)	(T S E240A, Thermo fisher, USA)
16. Freezer (-20°C)	(R OFV-170, Remi, Mumbai)
17. Refrigerator	
18. Electrophoresis Unit	(Bio Rad, USA)
19. Turbidometer	
20. BIOLOG System	

21. Compound Microscope (EX1000, Olympus, Japan)

22. Phase Contrast Microscope (CKX41, Olympus, Japan

Field survey

Field survey was conducted from ten different locations (Deorali, Singtam, KSC, Tadong, Changu Lake, Pelling, Lachung, Chungthang, Mangan and Ravangla) in Sikkim using the questionnaire (Annexure I). All age group of participants were surveyed. During the survey various factors such as types of kitchens, current health conditions and history of diseases, if any, were considered.

Annexure I: STUDY OF AIRBORNE DISEASES QUESTIONNAIRE BASED

A. General Information

1. Name
2. Age
3. Gender (M/F)
4. Population Type (Rural/Urban)
5. Name of the place ……
6. District (East/West/North/South)
7. Socio economic status *BPL/Non-BPL*
8. Total number of

B. Kitchen and cooking habits

1. Separate Kitchen (Yes/No)
2. Kitchen ventilation (Yes/No)
3. Cooking fuel (electricity/ fuel/coal/Kerosene/ others)
4. Cross ventilation?

D. Human Diseases

1. Any skin/respiratory infections?.....
2. Type of infection?.....
3. For how long is the infection?.....
4. If under medication at present? .....

C. Economy and Health

1. Working/ Non-working, if Yes (Govt. employee/ Private sector employee/ Farmer/ Self owned business animal rearing/ poultry others).
2. Last medication used (within 48 hrs/ a week ago/ 3 months ago/6 months/ 1 yr/> 1 yr)
3. Any recent visit to hospital? (Yes/No)
4. Reason for Visit……
5. Past history of hospitalisation (Yes/ No).
6. Reason for hospitalisation……..
7. Any recent or past respiratory disorders? What were the signs and symptoms?
8. Any Upper respiratory tract problems *(Throat problems/ fever/ rhinitis/ headache)*
9. Allergy? If yes to which allopathic drugs/ Traditional medicines/ Ayurveda/ Homeopathy *(Never/ occasional/ Regular).*
10. Suspect of TB (Yes/ No)
11. Ever Asthma? Or ever in family?
12. Breathlessness on dust exposure?
13. Smoking habit (Never/occasionally/frequently)
14. If, Yes (Cigarette/ Bidi/ hookah)
15. Others.....

D. Plant health

1. Name of crops mostly grown in this area: …….
2. From where do you get mother plant in case of cardamom/Ginger/Turmeric etc?
3. Did you observe/ (hear about) any plant disease(s)? Name……Location…….When…….
4. Since when the disease has been observed?
5. What is your approach for the disease control?
6. What is economic importance of this crop for your livelihood?…….
7. Do you get any support from any agency like Govt./NGOs?…….
8. Do you use biofertilizer?……

Identification of the sampling sites

The outdoor air samples were collected from ten different locations (Deorali, Singtam, KSC, Tadong, Changu Lake, Pelling, Lachung, Chungthang, Mangan and Ravangla) of four districts in Sikkim (Table A, Fig. A). Prior to the sample collection permission was granted for sample collection from Department of Forest Environent and Wildlife Management, Govrenment of Sikkim. Permission was also granted to simulatneously work collaborately with the State Pollution Control Board, Sikkim under National Ambient Air Monitoring Programme.

Table A: Table showing study sites and its coordinates.

Study sites	District	Coordinates	Elevation (in m)
Deorali	East Sikkim	27°19' 06"N, 88°36' 22"E	1484
Singtam	East Sikkim	27°13' 55"N, 88°29' 53"E	358
Kanchenjunga Shopping Complex (KSC)	East Sikkim	27°19' 37"N, 88°36' 43"E	1590
Tadong	East Sikkim	27°18' 51"N, 88°36' 02"E	1350
Changu	East Sikkim	27°22' 25"N, 88°45' 49"E	3794
Pelling	West Sikkim	27°19' 14"N, 88°14' 59"E	1458
Lachung	North Sikkim	27°41' 54"N, 88°44' 56"E	2746
Chungthang	North Sikkim	27°35' 46"N, 88°41' 56"E	1815
Mangan	North Sikkim	27°29' 46", 88°31' 53"E	1258
Ravangla	South Sikkim	27°18' 33"N, 88°21' 47"E	1999

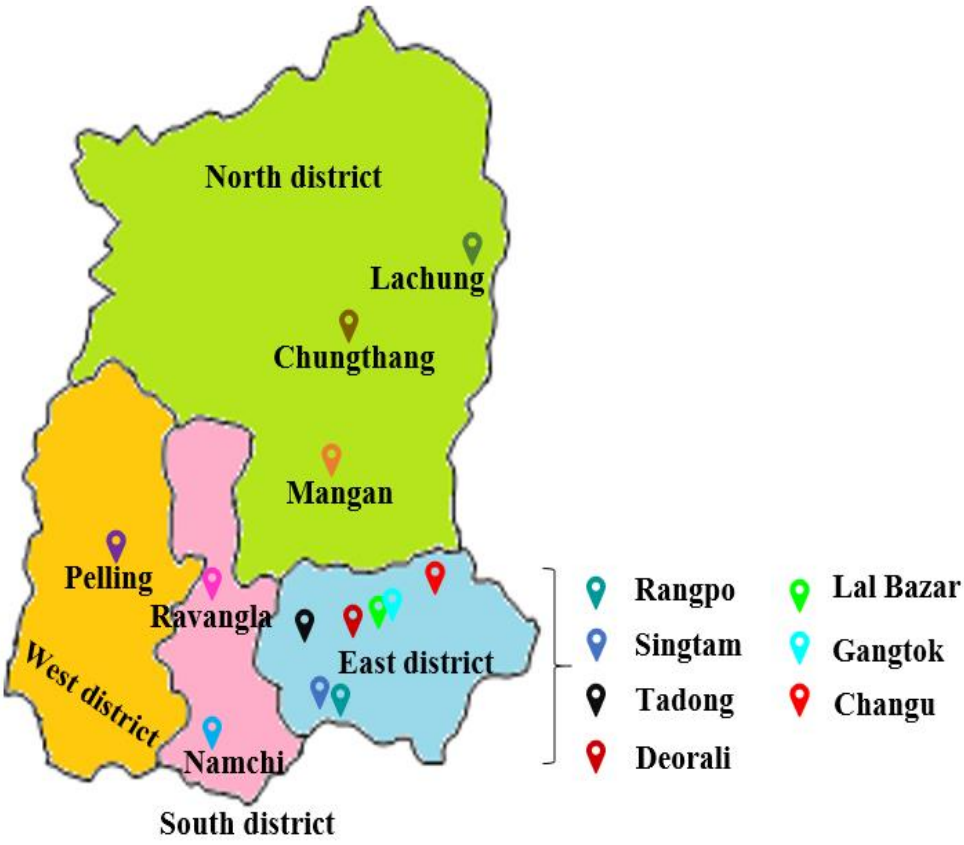


Figure A: Map showing study sites of sample collection in Sikkim

Air Sampling

The air samples for culturable bacteria and fungi during the study period were collected by passive method (Settle plate or Gravitational method) (Stryjakowska et al. 2007) and Active method (Impaction method) (Sveum et al. 1992; Senior et al. 2006) using Basic air sampler having 400 holes cap and volume sampling capacity of 100L/min; focusing mainly on predominant bacteria and fungi. The two seasons dry (December -February) and wet (May-August) were considered. The solid media used for air samplings were Nutrient Agar and Potato Dextrose Agar. Total 188 samples were collected during the studty period. For collection of sample, the air microbes were allowed to settle on to solid culture medium Nutrient Agar and Sabouraud Dextrose Agar (SDA) for bacteria and fungi respectively. The culture medium-containing petri plates were exposed for 30 minutes for the passive approach and 5 minutes for the impaction method, with the plates placed 1.5 meters above the ground to imitate the human breathing zone. (Tong and Lighthart 2000). Temperature and relative humidity were monitored using a digital hygrometer during the sampling process (HTC-1). The impactors were cleaned using cotton swabs dipped in isopropyl alcohol before each session of sampling. (USEPA 2000). The Petri plates were sealed immediately with paraffin after sampling (Huang et al. 2013) and further transported to laboratory on ice boxes.

The exposed nutrient agar plates for bacteria were incubated at 37^{o}C for 24-48 hours (Yassin, and Almouqatea 2010), simultaneously, SDA plates for fungi were incubated at 28^{o}C for 5- 7 days followed by further incubation when required, monitoring the growth of fungus. The plates were exposed in triplicate at each sampling site, with the exception of the impaction method, which had a 15-minute time delay in each sampling.

MICROBIAL ANALYSIS

Microbial load

For both the passive and active sampling methods, the colony forming unit was estimated after proper incubation for the specified period. With the use of correction factors from the sampler manufacturer's conversion tables, specific statistical corrections were produced. The number of each colony was considered for determination of the microbial load. The probable statistically total (Pr) was calculated according to the number of holes (N) and the real count (r).

Pr = N [1/N + 1/ (N-1) + 1/(N-2) + …….. + 1/ (N-r+1)]

The total number of colony-forming units (CFU) was enumerated and expressed in terms of colony forming units (CFU) per unit volume of air using equation as given by Senior (2006).

a) Calculation of CFU/m^{-3} for impaction method

CFU=1000P/RT CFU/$m^{-3.}$

'P' is the number of colonies counted on the sample plate after correction by using the positive hole conversion table provided in the manual by the manufacturer. 'T' is the duration of the plates exposed in minutes (5 min), and 'R' is the rate of air sampling in Liters/min (100 L min-1) as suggested by the manual.

b) Calculation of CFU/m^{-3} for settle plate method using Omelinsky formula:

$N=5a \times 10^4(bt)^{-1}$ (Abdel and Hanan 2012).

N= CFU per cubic meter of air (CFU/m^3)

a= number of colonies per petridish; *b*= surface area of petridish in cm2; *t*= time exposure in minutes.

Microbiological data of the numbers of colonies per cubic meter (CFU/m^3) obtained was transformed into logarithms of the numbers of colony forming unit per cubic meter (log CFU/m^3) of the sample.

Isolation of bacteria and fungi

The bacteria were isolated by re-streaking on the culture medium till isolated colony was obtained and further confirmed through microscopic examination for each isolation. Pure culture of each bacterial isolates was preserved in 15 % (v/v) glycerol at -86 °C and stabs of bacteria in nutrient agar was also preserved at -20 °C for working cultures. These isolates were sub-cultured after every 4 weeks. The pure colonies of fungi were obtained and further transferred into PDA slants and distilled water for preservation (Upendra et al. 2013).

Phenotypic characterization

Cell morphology

A 24 hour-old bacterial culture was taken, smear was made in a grease free slide, allowed to air dry (not heated-fixed), smear was stained for 30 seconds with safranin and rinsed in water, air-dried and was viewed under oil- immersion objective (Harrigan 1998).

Gram's staining

A suspension of a 24 hour-old bacterial culture on slant was prepared, and smear was made in a grease-free slide. The smear was heat-fixed, flooded by crystal violet stain for 1 minute, and washed for 5 seconds with water. The smear was flooded with Gram's iodine solution, allowed to stay for 1 min, and washed again for 5 sec with water. 95% ethanol was poured drop- wise from the top edge of the slide until no more colour came out from the lower edge of the slide. After washing with water, the smear was stained with safranin for 1 minute and washed again with water. The slides were air-dried and observed under oil-immersion objective. The specimen

was examined and compared with both negative and positive control **(**Bartholomew 1962).

Potassium hydroxide (KOH)

Potassium hydroxide **(**KOH) test was performed following the method of Halebian et al. (1981). The test was performed by placing 3% KOH solution on microscope slide followed by addition of generous amount of 24 h culture to the drop of KOH with an inoculating loop. The KOH culture mixture was stirred in circular motion with the loop and occasionally raising the loop. The positive result was indicated with the presence of mucoid or viscous appearance while raising the loop. Absence of mucoid or viscous appearance and no string of mixture indicated negative result. Gram positive bacteria are negative for KOH test while Gram negative bacteria are positive for this test.

Acid from carbohydrates

Bacterial isolates were screened for their ability to ferment seven different carbohydrates using carbohydrate fermentation broth medium. Tubes of 4 ml 1% (w/v) carbohydrate fermentation broth medium containing supplemented with 0.25% phenol red indicator was inoculated with fresh bacterial culture at 37°C for 24 h. Colour change from red to yellow indicated acid production (Hugenholtz and Fuerst 1992).

Catalase production

The production of gas bubbles was observed by adding 0.5 ml of 10 % hydrogen peroxide solution to the cultures as described by Reiner (2010). The production of

gas bubbles indicated the presence of catalase, while absence of gas bubbles indicated absence of catalase.

Coagulase Test

A drop of physiological saline was placed on either end of a clean glass slide. With the help of loop a colony was emulsified in each drop of saline to make thick suspensions. A drop of plasma was added to one of the suspensions and mixed it gently. Clumping of the organisms within 10 seconds indicated positive result, while absence of clot formation indicated negative result (Banaji 2013).

Indole test

This test was conducted to screen the ability of bacteria to degrade the amino acid tryptophan to indole, mediated by tryptophanase enzyme. The isolates were inoculated in to SIM agar and incubated at 37°C for 24 h. After incubation Kovac's reagent an indicator was added to the culture to check the indole production by forming a pink or red color ring at the interface of reagent and broth (Collee et al. 1996).

Methyl red test

This quantitative test was performed to detect the ability of bacterial isolates to perform mixed acid fermentation. The methyl red indicator shows bright red color at pH below 4.4 indicating positive result, yellow color at pH above 6.2 while gives orange color at pH in between. The bacterial culture was inoculated in MR-VP broth containing glucose, peptone and phosphate buffer. The inoculated broth was further incubated at 37°C for

overnight. Few drops of methyl red indicator were added to the medium, the results were observed and noted (Collee et al. 1996).

Voges- Proskauer (VP) test

This test was done to determine acetoin in culture broth. The MR-VP broth was inoculated with bacterial isolates and incubated at 37°C for overnight. After the incubation 5% alpha-naphthol and 40% potassium hydroxide was added to the broth and mixed. Alpha-naphthol and potassium hydroxide react with acetoin to give a cherry red color, indicating positive result. If yellow color occured the test was considered negative, which implies acetoin was not produced in the medium (Collee et al. 1996).

Citrate utilization test

This test was done to detect the ability of bacterial isolates to utilize citrate as the sole carbon source in the absence of glucose or lactose. Simmon's citrate agar, a medium containing sodium citrate as the only source of carbon and bromothymol blue, an indicator was used for the test. Slants of Simmon's citrate were prepared and the bacterial culture was stabbed in the butt and streaked on the slant surface using a straight inoculation wire. The slants were then incubated at 37°C for 24 h and the results were noted. Intense blue color in the slant indicated the ability of isolates to utilize citrate as the carbon source, hence positive result. No change in the color of the media was considered a negative result (Banaji 2013).

Nitrate reduction test

This test was conducted to detect the ability of isolates to produce the enzyme nitrate reductase and hence reduce nitrate to nitrite and nitrogenous gases. Nitrate broth was

inoculated with the test isolates and incubated at 37°C for 24 h. After incubation, six drops of nitrate reagent A followed by six drops of nitrate reagent B was added to the broth. Appearance of red color indicated a positive result. Zinc powder was added to the broth tubes with no color change. Appearance of red color in the broth after the addition of zinc powder indicated a negative result. If color change was not observed even after addition of zinc, the test was considered as positive result (Collee et al. 1996).

Oxidase test

The test was performed to demonstrate the presence of an intracellular enzyme cytochrome oxidase. This enzyme oxidizes the reagent N-N-N tetramethyl para-phenylene diamine hydrochloride (a colourless reagent). Oxidase disc (HiMedia, Mumbai India) was laced in a clean petri plate, with the help of sterile toothpick, a colony was picked and rubbed on to the disc. Appearance of blue or purple colour indicated positive result, while no appearance of colour indicated negative result (Banaji 2013).

Urease test

This test was performed to screen the ability of bacterial isolates to produce urease enzyme. The urease enzyme produced by many bacteria has ability to hydrolyze urea and release ammonia and carbon dioxide. A pure colony of bacterial was streaked onto the slant of Christensen's urea agar and incubated at 37°C for 24 h. Appearance of magenta pink color was taken as positive result which was due to reaction of ammonia in the solution to form ammonium carbonate, leading to increased pH in the medium. While no colour change was taken as negative result (Banaji 2013).

Haemolysis test

Samples were added to the flasks containing sterile Blood Agar Base, added with 5% (v/v) sterile defibrinated blood and mixed thorough to avoid any formation of bubbles, and were dispensed into sterile plates. Plates were incubated at 37°C for 24 h and observed for haemolysis (Buxton 2005)

PHYSIOLOGICAL TESTS

Growth at different NaCl concentration

Salt tolerance of bacterial isolates were tested by inoculating a loop-full of culture in nutrient broth supplemented with 6.5 %, 10.0 % and 18.0 % NaCl, respectively and incubated for 3 days at 30º C in a slanting position to improve aeration (Schillinger and Lücke 1987). Cultures were observed for growth after incubation.

Growth at different temperatures

Nutrient broth was inoculated with 24 hour-old cultures and incubated at 10º C 7 days and 45º C for 3 days, after the incubation period growth observed (Dykes and Carson 1994).

Growth at different pH

Using 1 N HCl or 10% w/v NaOH, the pH of Nutrient broth was adjusted to 3.9 and 9.6. 5ml of filter sterilised broth was poured to each tube and inoculated with 24-hour-old nutrient broth culture. The tubes were incubated at 37º C for 24-72 h and observed for the growth (Dykes and Carson 1994).

Tentative Identification

The bacterial isolates were tentatively identified up to genus level on the basis of phenotypic and biochemical tests using the taxonomical keys of Bergey's manual of bacteriological classification (Holt et al. 1994).

ANTIMICROBIAL SUSCEPTIBILITY TESTING

Preparation of test organisms

The test organism for the study included one genus of gram-negative bacteria (*Escherichia coli*) and one genus of gram-positive bacteria (*Staphylococcus aureus*). The test organisms were maintained at 4^{o}C on a nutrient agar slant. Active cultures for experiments were prepared by transferring loopful of culture in a flask containing nutrient broth, followed by incubation at 37^{o}C for 24 h.

Preparation of bacterial suspension

Two to three isolated colonies from each organism were selected from a fresh agar plate and put into a tube containing 3-4 ml of nutrient broth medium. The broth cultures were incubated at 37°C for 24 hours after adequate mixing. The absorbance of the suspension was measured with a Spectrophotometer to determine turbidity. At 625 nm, the absorbance should be between 0.08 and 0.13 OD (Optical Density), which is similar to McFarland standard 0.5 (CLSI 2014). When the turbidity seemed higher than necessary, sterile saline was added, and when the turbidity was too low, a bacterial colony was added and incubated longer (Wiegand et al. 2008).

Antibiotic susceptibility test by disc diffusion method

The antimicrobial susceptibility was studied by using the method described by

Bauer et.al (1966). Nutrient broth was inoculated with the isolates and incubated at 37^{o}C for 24 hours. Turbidity of bacterial cultures was adjusted to 0.5 McFarland standards, equivalent to cell density of (10^{8} CFU/ml). The bacterial lawn was made on Mueller Hinton Agar (Collee et al. 1996) using sterile cotton swab. The streaking was done on the entire surface of the MHA agar plates three times; the plate was turned 60° between streaking to achieve uniform inoculation. The plates were allowed to dry for 10-15 min. Using sterile forceps, the antibiotics discs were placed on the surface of MHA agar aseptically. The MHA plates were incubated at 37^{o}C for 24-48 h. After incubation the diameter of zone of inhibition was measured using Hi media scale. Results were expressed as per the instructions given by CLSI 2014. The test was done with *Escherichia coli* MTCC 1089 and *Staphylococcus aureus* MTCC 7443 as control strain. The results obtained were compared to these two strains as published by the CLSI (2014).

Phenotypic identification using Omnilog Plus system (BIOLOG)

Phenotypic characterization of selected bacterial isolates was conducted using Omnilog Plus system (BIOLOG), a fully automated high throughput aerobic identification system. The test was conducted as per the user manual provided by the manufacturer. The system evaluates samples against various biochemical tests which includes 94 different carbon compounds including sugars, carboxylic acids, amino acids and peptides. Two additional wells are present which serves as a positive and negative control. For this process, tetrazolium redox dyes are used to show utilisation of the carbon sources or resistance to inhibitory chemicals colorimetrically (Berridge et al. 2005). For the process, the isolate to be identified

was grown on agar medium which was then suspended into a special "gelling" inoculating fluid 3 (IF) at the cell density of 95-98%T. The cell suspension was then inoculated into the GEN III MicroPlate (100 µl per well) with the help of multi-channel pipette. The microplate was incubated for 22-24 h at 33ºC to allow the phenotypic fingerprint to form. All of the wells are initially colourless after being inoculated. Increased respiration in the wells occur during incubation when cells begin to use a carbon source. The tetrazolium redox dye is reduced by increased respiration, resulting in a purple colour. Negative wells, as well as the negative control well (A-1) with no carbon supply, remained colourless. A positive control well (A-10) is also included as a reference for the chemical sensitivity tests in columns 10-12. The end result was a sequence of reactions that turned colourless into purple (named as "metabolic fingerprint" by the manufacturer) that enabled unknown microorganisms to be identified. (Miller and Rhoden 1991). After incubation, the phenotypic fingerprint of purple wells was compared to Biolog's extensive species library (Sandle et al. 2013). The metabolic profiles acquired were matched to a database (the GEN III database) including characteristic profiles of a variety of microorganisms for identification purposes (Stager and Davis 1992).

MOLECULAR IDENTIFICATION OF BACTERIAL ISOLATES

Genomic DNA Extraction

Genomic DNA of the chosen bacterial isolates was extracted using by Cheng and Jiang (2006) standard phenol/chloroform method with minor modifications. One millilitre of overnight grown culture on nutrient agar was centrifuged for 10 min at 8000 rpm. The pellets were washed twice with 1 X PBS (phosphate buffer saline, pH 7) after the supernatant was removed. Pellets were recovered and suspended in 40 µl 1X TE buffer

after centrifugation at 3000 rpm. The pellets were mixed with15 µl of freshly prepared lysozyme and 15 µl of RNAse and incubated in water bath at 37° C for 3 hours. Following the incubation period, 15 µl of 20% SDS (sodium dodecyl sulphate) and 15 µl of Proteinase K were added to the centrifuge and incubated for another 3 hours at 55°C. Equal volume of phenol-chloroform-isoamyl solution (25:24:1) was added to the above prepared mixture and centrifuged at 10,000 rpm for 15 min, the uppermost aqueous layer obtained was transferred to a new vial containing 15 µl of 3M sodium acetate and 400 µl of cold absolute alcohol was added, and kept undisturbed for minimum 1 hour at -20°C. he pellets were obtained after centrifuging the aqueous phase containing the precipitated DNA at 10,000 rpm for 30 minutes. The supernatant was discarded and the pellets were air dried, then suspended in 30 µl of 1 X TE buffer and kept at -20°C until further analysis. To evaluate the purity of the extracted DNA, it was electrophoresed in a 0.8 percent agarose gel and measured with a Nano-Drop ND-1000 spectrophotometer. (Nano drop technologies, Willington, USA) (Kumbhare et al. 2015).

PCR amplification of the 16S rRNA gene

The PCR amplification of 16S rRNA gene from the extracted genomic DNA was done using a thermal cycler (Applied biosystems-2720, USA) (Lane 1991). Universal oligonucleotide primers p27F 5'-AGAGTTTGATC[A,C]TGGCTCAG- 3' and p1492R 5'-GG[C,T] TACCTTGTTACGACTT- 3' was used for the amplification (Park et al. 2010). Each reaction mixture contained 12.5µl GoTaq Green (M7122) master mix, reaction buffer (pH 8.5), 400µM dATP, 400µM dGTP, 400µM dCTP, 400µM dTTP and 3mM $MgCl_2$), each primer was maintained at a concentration of 1µl, 8.5µl nuclease free water and 2µl of template DNA, making a final volume of 25µl. The following were the

PCR conditions: 95°C for 10 minutes (denaturation of DNA), 95°C for 30 seconds (35 denaturation cycles), 65°C for 30 seconds (annealing), 72°C for 1 minute (extension), 72°C for 10 minutes (final extension) (Chagnaud et al. 2001). The PCR products were detected by electrophoresis using 1% agarose gel. The bands were stained with 7µl/100mL ethidium bromide (RM813, HiMedia, Mumbai) and viewed using UV source Gel-Doc 1000 (Bio-Rad, 97-0186-02, US). The size of the amplicon was checked using a standard 100 base pair DNA ladder.

Purification of amplified PCR products

The amplified PCR products were then purified using the standard PEG-NaCl (Poly ethylene glycol-sodium chloride) precipitation method with some modification (Schmitz and Riesner 2006). Approximately, 0.6 volume of PEG-NaCl (20% w/v of PEG, 2.5 M NaCl) was added to the final volume of PCR product and incubated at 37°C for 30 min. The mixture was centrifuged for 30 minutes at 4°C at 12,000 rpm, following which the tubes were emptied. The previous process was carried out twice. After allowing the tubes to air dry overnight 20 µl of nuclease free water was added to them. he resulting mixture was incubated for 30 minutes, well mixed, and loaded in 1 % agarose gel.

Phylogenetic analysis

The quality of the sequence was tested by Sequence Scanner v.1.0 (Applied Biosystems, Foster City, CA, USA). After the sequence quality was screened, the sequences were assembled using Chromas Pro 1.5 (McCarty 1998). he orientation of the assembled sequences was checked using Orientation Checker v.1.0. The DNA sequences of the bacterial isolates were compared to those already in the GenBank NCBI (National Center for Biotechnology Information) database using BLAST (basic local alignment search tool) 2.0 program (Altschul et al. 1990). he sequences were then aligned using clustalW pairwise alignment, and a phylogenetic tree was built using MEGA7.0 (Pennsylvania State University, Vl.7.0.26, USA) software using the neighbour joining method (Gascuel and Steel 2006; Kumar et al. 2016). Diversity indices were calculated using PAST (PAleontological STatistics) (Palaeontological Association-V4.0, Norway) (Hammer et al. 2001). Chao 1 value for species richness was further determined (Chao and Chiu 2016).

Data Availability of 16S rRNA Gene Sequencing

The sequences retrieved from the 16S rRNA gene sequencing were deposited at GenBank-National Centre for Biotechnology Information (NCBI) with nucleotide accession number: MW536490, MW548737, MW536491, MW536492, MW536493, MW536494, MW536495, MW536496, MW709569, MW709570, MW709571, MW709572.

MOLECULAR IDENTIFICATION OF FUNGAL ISOLATES

Genomic DNA Extraction

Genomic DNA of fungi was extracted following the method of Umesha et al. (2016). Mycelial mass from the culture plate was scraped out by sterile blade and ground in sterile mortar pestle using 500 µL of extraction buffer [100-mM Tris–HCl (pH 8.0), 20-mM EDTA (pH 8.0), 1.4M NaCl, 2% CTAB and 0.2% 2 mercaptoethanol]. 1.5 ml clean tube was taken where the mixture was transferred, added 4-µL RNase, vortexed and incubated at 37 °C for 60 min and kept further in a water bath at 55°C for 60 min. 500 µL of phenol: chloroform: isoamyl alcohol (25:24:1) was added to the prepared solution, thoroughly mixed for nearly 5 min, then centrifuged for 10 min at 14,000 rpm. The transparent aqueous phase was obtained and mixed with chloroform: isoamyl alcohol (24:1), centrifugation at 12,000 rpm for 5 min was done, then the aqueous phase was recovered. Added 0.8 volume of cold 7.5 M ammonium acetate and 0.54 volume of ice-cold isopropanol to the above mixture, and thoroughly mixed and kept at -80° for 12 hours for precipitation of DNA. Centrifugation of the solution was carried out at 14,000 rpm for 3 min, precipitated with absolute ethanol for recovery of DNA. The recovered DNA was rinsed twice with 1 ml of 70 % ethanol and further resuspended in 100 µL of 1X TE [200-mM Tris–HCl (pH 8.0), 20-mM EDTA (pH 8.0)] buffer for further use. The DNA was stored at -20ºC. Following the approach outlined by Kumbhare et al. (2015) DNA quality was assessed on an agarose gel and its concentration was measured using a nanodrop spectrometer (2015).

PCR amplification

PCR of the internal transcribed spacer (ITS) region of filamentous molds was amplified using the primer ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC- 3′) (Adekoya et al. 2017). PCR reactions were performed in 25 µL of PCR pre-master mix solution (Promega, USA). The amplification steps were followed: initial denaturation at 94ºC for 5 min followed by 35 cycles

consisting of 94ºC for 1 min, 54 C for 1 min, and 72ºC for 1 min, respectively; and final extension at 72ºC for 10 min in a Thermal Cycler. The PCR products were verified by electrophoresis on 1.0% agarose gel containing 0.7 mg/ml of ethidium bromide and visualized under UV light Gel doc. Approximate size of amplicons was determined using standard molecular markers.

Purification of the PCR amplicons

The amplified PCR products were purified using PEG (polyethylene glycol)-NaCl (sodium chloride) and precipitation solution (20% w/v of PEG, 2.5 M NaCl) with addition of 0.6 volumes of 20% PEG-NaCl to final volume of PCR products (Schmitz and Riesner 2006). The mixture was centrifuged at 12,000 rpm for 30 min, incubated at 37ºC for 30 min, the aqueous solution was discarded and pellet was washed twice with 1 mL ice cold 70% freshly prepared ethanol (70%). The collected pellet was then air dried prior to elution in 20µl of nuclease-free water and finally the purified product was loaded in 1% agarose gel.

ITS Sequencing

The PCR-amplified products had been sequenced in a forward and reverse direction using ITS 1 primer (5'-TCCGTAGGTGAACCTGCGG-3′) and ITS 4 primer (5′-TCCTCCGCTTATTGATATGC-3′) primer, respectively as per the method of Martin and Rygiewic (2005). The PCR reaction was carried out in 50 µL reaction volume containing 2.0 mM $MgCl_2$, 0.2 µM each primer, 0.2 mM dNTP, 0.5 mg [mL]-1 bovine serum albumin (BSA) and 0.04 U [µL]-1 tTaq DNA polymerase on a thermal cycler equipped with a heated lid. The thermal program included initial denaturation, enzyme activation at 95°C (6-10 min) followed by 35 cycles of complete the step [95°C for 1

min, 40°C for 2 min and 72°C for 1 min and one cycle at 72°C for 10 min]. The amplified products were sequenced by an automated DNA Analyzer. These high-quality, double-stranded sequence data were analysed with the help of the BLASTn program and multiple sequence alignment.

Bioinformatics

The qualities of the raw sequences were checked by Sequence Scanner version 1.0 and were edited using software ChromasPro version 1.34. Sequences were compared with sequence entries in the GenBank of NCBI (National Center for Biotechnology Information) (http://www.ncbi.nlm.nih.gov/Blast.cgi) using the Basic Local Alignment Search Tool for nucleotides (BLASTn) on the NCBI website (Pinto et al. 2012). For phylogenetic analysis, the available sequence of similar related organisms was retrieved in FASTA format and aligned using the clustal-W. Sequence alignment and phylogenetic tree were constructed using MEGA7.0 software by Neighbor-Joining methods using 1000- bootstrap replicates (Lutzoni et al. 2004).

Data Availability

The sequences obtained were deposited at GenBank-National Center for Biotechnology Information (NCBI) with nucleotide accession number: MH393340, MH393341, MH393342, MH393343, MG745321, MG745325, MH379771, MH379773, MH379774.

AMBIENT AIR QUALITY MONITORING

Ambient air quality monitoring was done from seven stations (Deorali, Singtam, Rangpo, Ravangla, Chungthang, Namchi and Pelling) in Sikkim. The study was conducted in collaboration with State Pollution Control Board, Sikkim. Interpretation of the result recorded was done as per the national ambient air quality monitoring standards (Annexure II).

Annexure II: NATIONAL AMBIENT AIR QUALITY MONITORING STANDARDS				
Pollutants	**Time Weighted Average**	**Concentration in Ambient Air**		**Methods of Measurement**
		Industrial, Residential, Rural and other Areas	**Ecologically Sensitive Area (Notified by Central Government)**	
Sulphur Dioxide (SO_2), µg/m^3	Annual * 24 Hours **	50 80	20 80	-Improved West and Gaeke Method -Ultraviolet Fluorescence
Nitrogen Dioxide (NO_2), µg/m^3	Annual * 24 Hours **	40 80	30 80	-Jacob & Hochheiser modified (NaOH-$NaAsO_2$) Method -Gas Phase Chemiluminescence
Particulate Matter (Size less than 10µm) or PM_{10}, µg/m^3	Annual * 24 Hours **	60 100	60 100	-Gravimetric -TEOM -Beta attenuation
Particulate Matter (Size less than 2.5µm) or $PM_{2.5}$, µg/m^3	Annual * 24 Hours **	40 60	40 60	-Gravimetric -TEOM -Beta attenuation
Ozone (O_3) µg/m^3	8 Hours * 1 Hour **	100 180	100 180	-UV Photometric - Chemiluminescence -Chemical Method
Lead (Pb) µg/m^3	Annual * 24 Hours **	0.50 1.0	0.50 1.0	-AAS/ICP Method after sampling on EPM 2000 or equivalent filter paper

				-ED-XRF using Teflon filter
Carbon Monoxide (CO), mg/m^3	8 Hours * 1 Hour **	02 04	02 04	-Non dispersive Infrared (NDIR) Spectroscopy
Ammonia (NH_3), µg/m^3	Annual * 24 Hours **	100 400	100 400	- Chemiluminescence -Indophenol blue method
Benzene (C_6H_6), µg/m^3	Annual *	05	05	-Gas Chromatography (GC) based continuous analyzer -Adsorption and desorption followed by GC Analysis
Benzo(a)Pyrene (BaP) Particulate phase only, ng/m^3	Annual *	01	01	-Solvent extraction followed by HPLC/GC analysis
Arsenic (As), ng/m^3	Annual *	06	06	-AAS/ ICP Method after sampling on EPM 2000 or equivalent filter paper
Nickel (Ni), ng/m^3	Annual *	20	20	-AAS/ ICP Method after sampling on EPM 2000 or equivalent filter paper
* Annual Arithmetic mean of minimum 104 measurements in a year at a particular site taken twice a week 24 hourly at uniform intervals. ** 24 hourly or 8 hourly or 1 hourly monitored value as applicable, shall be compiled with 98% of the time in a year 2% of the time, they may exceed the limits but not on two consecutive days of monitoring. **NOTE:** Whenever and wherever monitoring results on two consecutive days of monitoring exceeds the limits specified above for the respective category, it shall be considered adequate reason to institute regular or continuous monitoring and further investigations.				

Determination of Sulfur Dioxide concentration in Air (West and Gaeke, 1956)

Preparation of Reagents

Absorbing Reagent (TCM. 0.04M): In 1000 mL distilled water, 10.86 g Mercuric Chloride, 0.066 g EDTA, and 6.0 g Potassium Chloride were dissolved.

Precaution: The reagents are extremely toxic.Personal protection equipment was worn wherever possible to avoid spills on the skin, and any exposed skin was quickly cleansed with water if an accident occurred.

Sulphamic acid (0.6%): Dissolved 0.6 g sulphamic acid in 100 ml distilled water.

Preparation of Formaldehyde solution (0.2%): 1.25 ml Formaldehyde solution (36-38%) was taken and made the final volume of 250 ml by adding distilled water.

Stock PRA Solution: The 0.5 g of specially purified pararosaniline was dissolved in 100 ml distilled water and kept for 48 h in room temperature before analysis.

Working PRA Solution: 10 ml stock PRA solution and 15 ml concentrated Hydrochloric acid was taken, to make a final volume of 250 ml distilled water was added.

For preparation of calibration curve:

Stock Iodine Solution (0.1N): Dissolved 1.27 g iodine, 4.0 g potassium iodine and 25 ml distilled water by mixing until dissolved completely, made final volume of 100 ml by adding distilled water.

Working Iodine solution (0.01N): 50 ml of stock Iodine solution was taken and made final volume of 500 ml with distilled water. The prepared reagent was stored in amber glass bottle.

Starch Indicator: It was prepared by dissolving 0.40 g soluble starch and 0.002 g mercuric Iodide in distilled water to make a paste. Added 200 ml distilled water in the paste allowing the solution to boil until clear solution was observed.

Primary standard Potassium Iodate solution: Dissolved1.5gm Potassium Iodate (oven dried at 180^{o}C) and made volume of 500 ml with distilled water. The solution was prepared freshly for every test.

Stock Sodium Thiosulphate solution (0.1N): Dissolved 6.25 g sodium thiosulphate and 0.025 g sodium carbonate in 250 ml boiled and cooled distilled water. The solution was allowed to stand for one day before standardization. Hence, this solution was prepared a day prior to the standardization process.

Stock sulphite solution: Dissolved 0.15 g sodium metabisulphite in 250 ml boiled and cooled distilled water.

Standardization of sodium thiosulphate solution:

- 50 ml of potassium iodate solution was taken in 250 ml Iodine flask.
- Added 2.0 g potassium Iodine and 10 ml of (1:10) HCL.
- Stopper was placed on the flask and allowed to react for 5.0 min.
- Titrated with stock sodium thiosulphate solution to a pale-yellow color.
- Added 2-5 ml starch indicator, which gave blue color.
- Continued titration until the disappearance of color.
- Calculated the normality as follows:

$$N = \frac{W \times 10^3 \times 0.1}{V \times 35.67} \quad \text{OR} \quad N = \frac{2.8 \times W}{V}$$

where,

W = Weight of potassium iodate in gm (Primary Standard)

V = Volume of Sodium thiosulphate solution consumed (m1)

35.67 = Equivalent weight of Potassium iodate

N = Normality of sodium thiosulphate

(a) **Working Sodium thiosulphate solution (0.01N):** Calculated the required volume time of stock sodium thiosulphate solution and prepared a required volume of working sodium thiosulphate solution by using the formula:

N1V1 = N2V2

Where,

N1 = Normality stock sodium thiosulphate solution (0.1N)

V1 = ?

N2 = Normality of working sodium thiosulphate solution (0.01N)

V2 = Required volume of working sodium thiosulphate solution

Took the required amount of stock sodium thiosulphate solution and made the final volume with freshly boiled and cooled distilled water.

(b) Working sulphite solution (for SO_2 calibration curve): Took 2.0 ml of stock sulphite solution (C µg SO_2/ml) make up to 100ml with TCM 0.04 M Absorbing Reagent.

Calculated the strength of working sulphite solution:

$\frac{C \, x \, 2}{100}$ = …… µg SO_2/ml

[1 ml of this solution = …….. µg SO_2/ml]

Sampling

Sampling was done for four hours at the flow rate of 1L/min by placing 30 ml of absorbing solution in an impinge. After sampling, the volume of sample was measured and transfer to a sample storage bottle.

Analysis

Any water loss by evaporation during sampling was replaced by adding distilled water up to the calibration mark on the absorber. Mixed thoroughly and pipetted out 10/20 ml of the collected sample into a 25 ml volumetric flask. Added 1 ml 0.6% sulphamic acid and allowed to react for 10 minutes in order to destroy the nitrite resulting from oxides of nitrogen. Added 2 ml of 0.2% formaldehyde solution and 2 ml pararosaniline solution and made up to 25 ml with distilled water. Prepared a blank in the same manner using 10 ml of unexposed absorbing reagent. After a 30 min colour development interval and before 60 min, measured and recorded the absorbance of samples and reagent blank at 560 nm. For the optical reference distilled water was used.

Calibration

The actual strength of the sulphite solution was determined by addition of surplus iodine and back titrating with the standard sodium thiosulfate solution. To back-titrate 50 ml of 0.01N iodine solution was pipetted into each of two 500 ml iodine flasks labelled ‘A’ and ‘B’. To flask A (blank) added 25 ml distilled water and into flask B (sample) measure pipetted 25ml sulphite solution. Stoppered the flasks and allowed to react for 5 minutes. The working sulphite-TCM solution was freshly prepared and iodine solution is added to the flasks. Using the burette containing standardized 0.01 N thiosulfate,

titrated each flask till the colour turned pale yellow. Then added 5 ml starch solution and continued titration until the blue colour disappeared.

Preparation of SO_2 Calibration curve

Measured 0.5 ml, 1.0 ml, 1.5 ml, 2.0 ml, 2.5 ml, 3.0 ml, 3.5 ml and 4.0 ml of working sulphite TCM solution in 25 ml volumetric flask. Added sufficient TCM solution to each flask to bring the volume to approximately 10 ml. Then added the remaining reagents as described in the procedure for analysis. A reagent blank with 10 ml absorbing solution was also prepared. Recorded the absorbance of each standard and reagent blank. A curve was plotted absorbance (Y axis) versus concentration X axis. A line of best fit was drawn and determined he slope**.** The concentration of Sulphur dioxide in $\mu g/m^3$ was determined using the following calculations:

- Total volume of air passed through SO_2 impinger,

  v (liter) = Avg. flow rate (LPM) X Time (min)

  Flow Rate = $\frac{\text{Initial Flow(LPM)+ Final Flow(LPM)}}{2}$

- Concentration ($\mu g\ SO_2/m^3$) = $\frac{C \times A \times 1000 \times D}{V \times B}$

  where,

  C = SO_2 curve value (µg in 25 ml)

  A = Absorbing reagent taken for sampling (ml)

  V = Volume of air passed (liter)

  B = Volume taken for sample analysis (ml)

  D = Dilution factor (D=1 for no dilution, D=2 for 1.1 dilution)

  1000 = Conversion factors liter to m^3

  SO_2 Conc. = $\mu g/m^3$

DETERMINATION OF NITROGEN DIOXIDE CONCENTRATION IN AIR

(Modified Jacobs and Hochheiser 1958)

Preparation of Reagents

Absorbing Reagent: Dissolved 4.0 g sodium hydroxide into 1 g sodium arsenite and made up to 1000 ml with distilled water.

Sulfanilamide Solution: Dissolved 20 g sulfanilamide into 50 ml of 85% phosphoric acid and made up to 1000 ml with distilled water. The solution was stored in refrigerator for further use.

Hydrogen Peroxide Solution: Diluted 0.2 ml of 30% Hydrogen Peroxide solution and made up to 250 ml with distilled water. The solution was stored in refrigerator for further use.

N-(1-Napthyl)-ethyenediamine Di-hydrochloride (NEAD) Solution: Dissolved 0.5 g NEAD and make up to 500 ml with distilled water.

Stock Sodium Nitrite solution (1000 µg NO_2/ml): Dissolved 1.5 g sodium nitrite and made up to 1000 ml with distilled water. The amount of $NaNO_2$ to be used if the assay percent is less than 100% was calculated as follows

$$\mathbf{G= \frac{1.5}{A} X\ 100}$$

where,

G = Amount

1.5 = Gravimetric Conversion factor

A = Assay percent (should be 97 or greater)

Working solution Nitrite standard solution:

Solution A (10.0 µg NO_2/ml): Diluted 5 ml of stock sodium nitrite solution and made up to 500 ml with distilled water.

Solution B (1.0 µg NO_2/ml): Diluted 25 ml Solution A and made up to 250 ml with absorbing solution.

Sampling

Placed 30 ml of absorbing solution in an impinger and sampled for four hour at the flow rate of 0.2 to 1 L/min. After sampling measured the volume of sample and transferred to a sample storage bottle.

Preparation of NO_2 calibration curve: -

Analysis

Pipetted 10 ml of the collected sample into a 50 ml volumetric flask. Added 1 ml of hydrogen peroxide solution, 10 ml of sulphanilamide solution, and 1.4 ml of NEDA made up to 50 ml with distilled water. A blank was prepared in same way using 10 ml of unexposed absorbing reagent. Kept the solution for 10 minutes to react. The Spectrophotometer was set at zero with distilled water, measured the absorbance at 540 nm. Samples with an absorbance greater than 1.0 was re-analysed after diluting the aliquot of the collected samples with an equal quantity of unexposed absorbing reagent.

Randomly selected 5-10% of the samples were re-analysed as part of an internal quality assurance program.

Concentration of nitrogen dioxide in µg/m^3 was calculated using the following calculations:

Total volume of air passed through NO_2 impinger,

v (liter) = Avg. flow rate (LPM) X Time (min)

$$\text{Flow Rate} = \frac{\text{Initial Flow(LPM)} + \text{Final Flow(LPM)}}{2}$$

$$\text{Concentration (µg } NO_2/m^3) = \frac{C \times A \times 1000 \times D}{V \times B \times 0.82}$$

Where,

C= NO_2 curve value (µg in 50ml)

A= Absorbing reagent taken for sampling (ml)

V= Volume of air passed (liter)

B= Volume taken for sample analysis (ml)

D= Dilution factor (D=1 for no dilution, D=2 for 1.1 dilution)

1000= Conversion factors liter to m^3

0.82= Sampling efficiency

NO_2 Concentration= µg/m^3

DETERMINATION OF PARTICULATE MATTER $_{10}$ IN AMBIENT AIR

Sampling

A glass fibre filter of 20.3 x 25.4 cm size was used for sampling. The filter paper was checked for any physical damage. The filter paper was marked with an identification number Prior to the use, the filter paper was conditioned in desiccator for 24 h within

20-30°C and 40-50% relative humidity. The initial weight of the paper was recorded. The filter paper was placed on the sampler allowing the sampler to run for 8 h, and the flow rate was recorded on every hour. Once the sampler was run for 8 h, the filter paper was removed from the sampler. Kept the exposed filter in desiccator 24 h within 20-30°C and 40-50% relative humidity and noted the final weight of the filter paper. Recorded the total time of sampling and average flow rate, this process was repeated two times i.e. three times in 24 h. was marked.

Calculated the concentration of PM 10 using the following formula:

C PM $_{10}$μg/m^3 = (Wf - Wi) x 10^6 / V 10

where,

C PM_{10} = Concentration of PM_{10}, μg/m^3

Wf = Initial weight of filter in g

Wi = Initial weight of filter in g

10^6 = Conversion of g to μg

V = Volume of air sampled, m^3

RESULTS

FIELD SURVEY

The air samples were collected from ten different locations (Deorali, Singtam, KSC, Tadong, Changu Lake, Pelling, Lachung, Chungthang, Mangan and Ravangla) in Sikkim (Table 1). Along with the collection of air samples, the field survey was conducted based on the prepared questionnaire to identify individuals who were prone to illnesses due to airborne microorganisms.

Table 1: Table showing study sites and its coordinates and elevation

Study sites	Coordinates	Elevation (in m)
Deorali	27°19' 06"N, 88°36' 22"E	1484
Singtam	27°13' 55"N, 88°29' 53"E	358
KSC	27°19' 37"N, 88°36' 43"E	1590
Tadong	27°18' 51"N, 88°36' 02"E	1350
Changu	27°22' 25"N, 88°45' 49"E	3794
Pelling	27°19' 14"N, 88°14' 59"E	1458
Lachung	27°41' 54"N, 88°44' 56"E	2746
Chungthang	27°35' 46"N, 88°41' 56"E	1815
Mangan	27°29' 46", 88°31' 53"E	1258
Ravangla	27°18' 33"N, 88°21' 47"E	1999

The field survey was conducted along the area of study sites randomly irrespective of age and gender; however, the individuals were grouped in to age groups which ranged

from age 10-90 years (Table 2). In total, 561 individuals were surveyed, 360 (64.17%) individuals from rural area and 201 (35.82%) from urban area of Sikkim. From the total individuals surveyed, 250 were female and 165 were male participants. The number of participants from each district were 182 (32.44%), 131 (23.35%), 146 (26.02%) and 102 (18.18%) from east, west, north and south district, respectively (Figure 2).

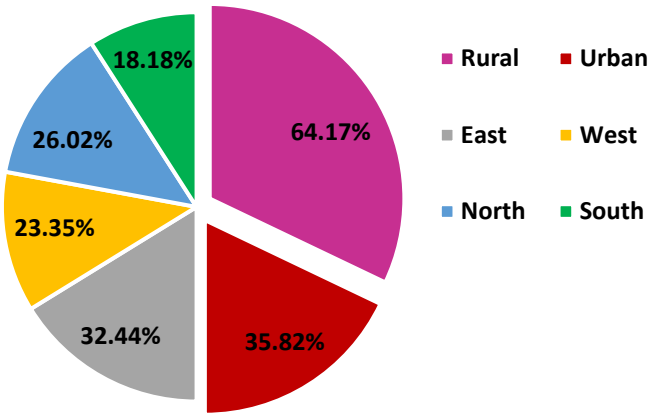


Figure 2: Distribution pattern of participants from different districts in Sikkim.

It was recorded that many individuals had the medical conditions such as severe or mild allergies, skin infections, throat problems and headache. These were the medical conditions which the participants expressed without any hesitation. According to the information given by the participants, 92 (16.39%) complained of headache. Throat problems, skin infections and allergies were reported among 73 (13.01%), 37 (6.59%) and 59 (10%) of the individuals, respectively. Allergies (16.36%) and skin infections (10.53%) were found highest among the age group of 20-30 years. While Skin infections (12%) were prevalent among the age group 80-90 years. Throat infections were common

(17.89 %) among the age group 10-20 years, while headache (28%) was prevalent among age groups 80-90 years. (Table 2; Figure 3).

Table 2: Categories and medical conditions of the surveyed individuals.

Categories		Medical conditions							
		Allergies		Skin infections		Throat problem		Headache	
Age group	No. of participants	No.	%	No.	%	No.	%	No.	%
10-20	95	11	11.58	10	10.53	17	17.89	14	14.73
20-30	110	18	16.36	11	10	12	10.9	21	19.09
30-40	125	10	8	7	5.6	9	7.2	16	12.8
50-60	92	11	11.95	3	3.26	14	15.21	15	16.3
60-70	78	8	10.25	2	2.56	7	8.97	13	16.67
70-80	36	1	2.78	1	2.78	9	25	6	16.67
80-90	25	0	0	3	12	5	20	7	28

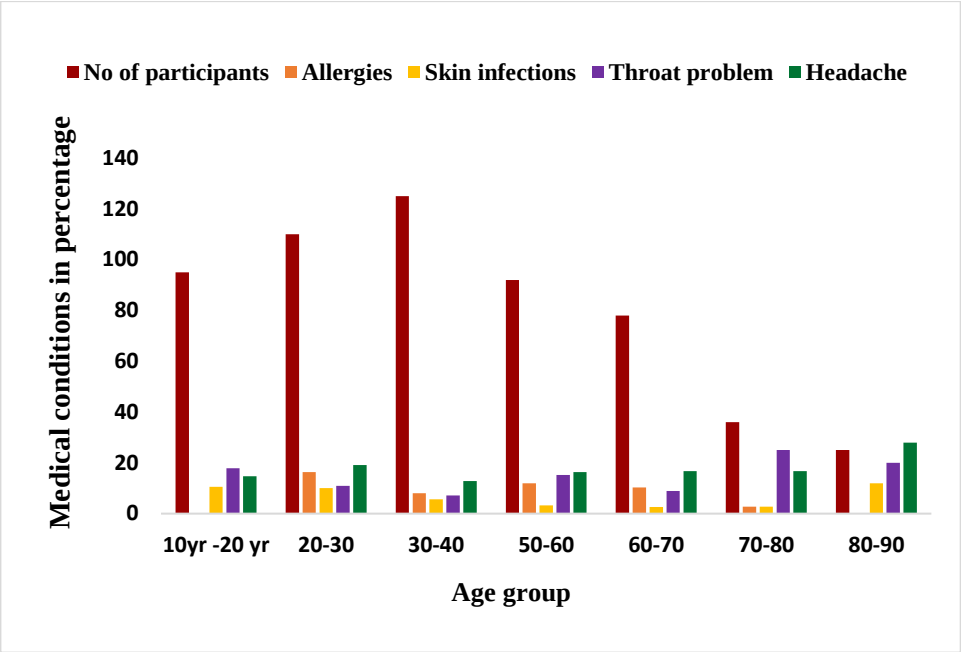


Figure 3: Distribution of medical conditions of the surveyed participants.

MICROBIAL ANALYSIS

The air sampling from various sampling sites was carried out both by passive (Plate sedimentation method) and active method (Impaction method) considering the two seasons, dry and wet. Overall, the bacterial load during the study period ranged from 1.41- 3.78 log CFU/m^3. The lowest bacterial load (1.41 log CFU/m^3) was observed at Changu lake in wet season and the highest bacterial load (3.78 log CFU/m^3) was observed at Mangan in wet season in both the cases the samples were collected by passive method. The fungal load recorded from air samples during the study period ranged from 1.32- 3.71 log CFU/m^3 the lowest fungal count was observed at Lachung and the highest fungal load was observed at Pelling both the counts were observed in dry season.

In Deorali, the highest bacterial count (3.24 log CFU/m^3) was observed during wet season while the lowest bacterial count (1.95 log CFU/m^3) was recorded during both dry and wet season. The fungal load ranged from 1.60-3.17 log CFU/m^3), both the lowest and the highest in in dry season and the mode of sample collection was passive method. In Deorali the highest fungal count (3.29 log CFU/m^3) was recorded in dry season, followed by (3.01 log CFU/m^3) in wet season and (2.95 log CFU/m^3).

In Singtam, the bacterial count ranged from 2.01- 3.74 log CFU/m^3, the least count of (2.01 log CFU/m^3) were observed in both dry and wet season, while the highest (3.74 log CFU/m^3) count was observed in wet season. The fungal load ranged from 1.81-3.59 log CFU/m^3 in dry and wet season respectively. Higher count (3.28 log CFU/m^3) was also observed in dry season.

In Kanchenjunga Shopping complex (KSC), the bacterial load ranged from 1.72-3.55 log CFU/m^3. The lowest bacterial load (1.72 log CFU/m^3) in wet season and the highest (3.55 log CFU/m^3) bacterial load in wet season. The higher range of bacterial load observed was 3.34 log CFU/m^3 and 3.10 log CFU/m^3 in dry season by passive method, 3.16 log CFU/m^3 in dry season by passive method. The fungal load ranged from 1.82-3.54 log CFU/m^3 both the highest and lowest were recorded in dry season.

In Tadong, the bacterial load ranged from 1.99-3.48 log CFU/m^3, the lowest (1.99 log CFU/m^3) in wet and dry season by active and passive method and the highest (3.48 log CFU/m^3) in dry season. Fungal load ranged from 1.89-3.40 log CFU/m^3. The highest as well as the lowest fungal count was observed in dry season.

In Changu, the bacterial load ranged from 1.41-3.12 log CFU/m^3, the lowest in wet season and he highest in wet season by passive sampling. Fungal load ranged from 1.49-

3.12 log CFU/m^3 the lowest (1.49 log CFU/m^3) in dry season and the highest (3.12 log CFU/m^3) in wet season.

In Pelling, the bacterial load ranged from 1.80-3.27 log CFU/m^3, both the lowest and the highest in wet season, the significant of bacteria (3.00, 2.83, 2.82 log CFU/m^3) was also observed in dry season. While the fungal load ranged from 1.88-3.71 log CFU/m^3, both the highest and lowest count was recorded in dry season on different year of sampling.

In Lachung, the bacterial load ranged from 1.39- 3.27 log CFU/m^3 both in the wet season. The significant bacterial count of 2.57 log CFU/m^3, 2.52 log CFU/$m^3$and 2.70 log CFU/m^3 was also recorded in dry season. Lachung, recorded the highest fungal count of 3.68 log CFU/m^3 in wet season and the lowest of 1.32 log CFU/m^3 in dry season.

In Chungthang, the bacterial count ranged from 2.05-3.34 log CFU/m^3 the lowest (2.05 log CFU/m^3) in wet season and the highest (3.34) log CFU/m^3 in dry season. The notable bacterial load was frequently observed in dry season except of microbial load (3.13 log CFU/m^3) in wet season in 2016. Fungal load ranged in this sampling site from 1.81- 3.34 log CFU/m^3 in wet season and dry season respectively.

In Mangan the bacterial load ranged from 2.04- 3.78 log CFU/m^3, the lowest in dry season and the highest in wet season, the fungal load ranged from 1.79-3.38 log CFU/m^3 in wet season. In Ravangla the bacterial count recorded ranged from 1.98-3.38 log CFU/m^3 both in the dry season. The fungal load ranged from 1.72-3.41 log CFU/m^3 in dry season.

Bacterial load (log CFU/m^3) from air samples in dry season by passive sampling method (2015-16)

The microbial load of bacteria with passive method ranged from 3.48 log CFU/m^3 to 2.57 log CFU/m^3. The highest microbial load recorded was 3.48 log CFU/m^3 at Tadong, East Sikkim, an urban area followed by 3.34 log CFU/m ^{3} at Kanchenjunga Shopping Complex, East Sikkim and Chungthang, North Sikkim a rural and urban areas, respectively. The lowest was 2.57 log CFU/m^3 at Lachung, North Sikkim a rural area, 2.71 log CFU/m ^{3} at Changu Lake, East Sikkim a rural area followed by 3.00 log CFU/m ^{3} at Pelling, a rural area. (Table 3, Figure 4).

Bacterial Load (log CFU/m^3) from air samples in dry season by active sampling method (2015-16)

The microbial load of bacteria with passive method ranged from 2.38 log CFU/m^3 to 1.71 log CFU/m ^{3}. The highest bacterial load recorded was 2.38 log CFU/m^3 at Chungthang, North Sikkim followed by 2.30 log CFU/m ^{3} at Deorali, and Tadong, east Sikkim, a rural area. The log CFU/m^3 by the active sampling showed less variation. The lowest bacterial load observed was 1.71 log CFU/m^3 in Changu Lake, followed by Lachung, 2.02 log CFU/m ^{3} (Table 3, Figure 4).

Table 3: The bacterial load (log CFU/m^3) from air samples by passive and active methods of sampling methods in dry season (2015-16).

Location	Altitude (metre)	CFU/m^3 (P)	CFU/m^3 (A)	log CFU/m^3 (P)	log CFU/m^3 (A)
Deorali	1484	1748.5	220	3.24	2.30
Singtam	358	2401	172	3.30	2.23
KSC	1590	2218.5	135	3.34	2.10
Tadong	1350	3040.5	209	3.48	2.30
Changu	3794	521.5	51	2.71	1.713
Pelling	1458	1017.5	129	3.00	2.11
Lachung	2746	378	117	2.57	2.02
Chungthang	1815	2231.5	240	3.34	2.38
Mangan	1258	1996.5	193	3.29	2.28
Ravangla	1999	1644	118	3.20	2.06

Note: P stands for "Passive" and A stands for "Active" method of sampling. The CFU/m^3 and the log CFU/m^3 of bacteria, considered here is the average.

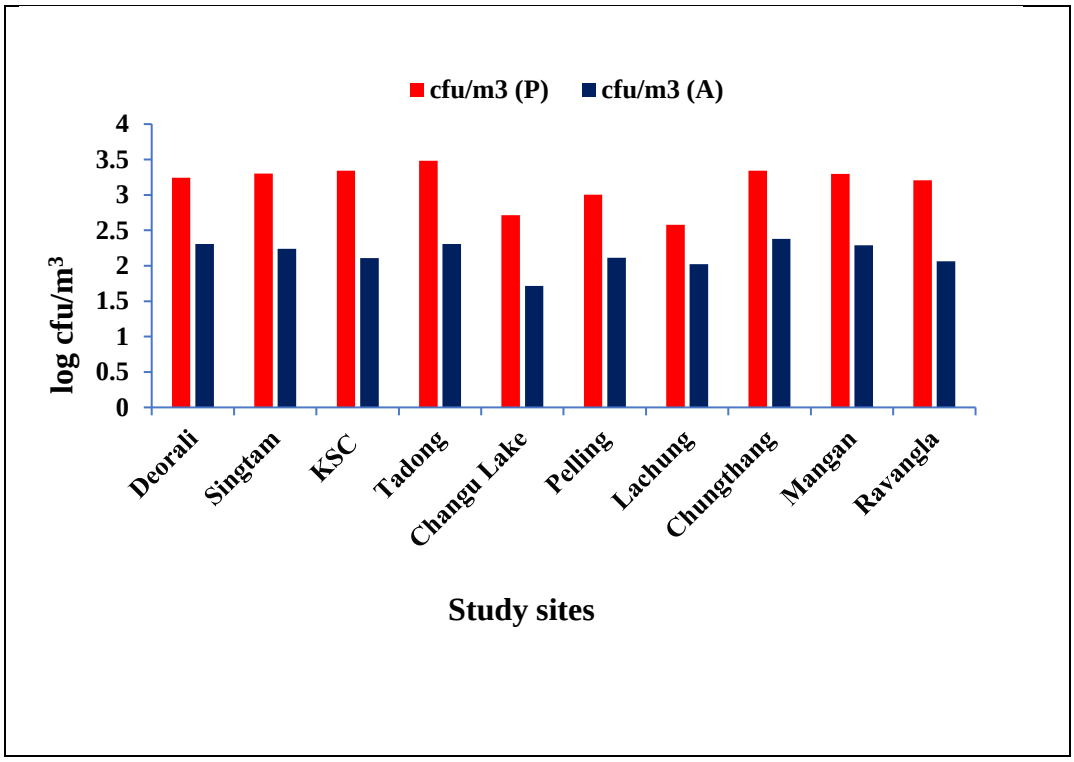


Figure 4: Bacterial Load (log CFU/m^3) from air samples by active and passive in dry season (2015-16).

Bacterial Load (log CFU/m^3) from air samples by passive sampling in wet season (2015-16).

The log CFU/m^3 of bacteria with passive sampling in wet season ranged from 3.78 log CFU/m^3 to 3.13 log CFU/m^3. The highest load of 2.38 log CFU/m^3 was recorded at Chungthang, North Sikkim followed by 2.30 log CFU/m^3 at Deorali, and Tadong, east Sikkim a rural area. The CFU by the active sampling showed less variation. The lowest load of 1.71 log CFU/m^3 was observed at Changu Lake, a rural but also a tourist destination during this season, followed by Lachung, 2.02 log CFU/m^3 which is less populated yet a tourist hub during this time of the year. Irrespective of the sampling

location, time and the season, in active sampling it was observed that the bacterial load variation was not remarkable (Table 4, Figure 5).

Bacterial load (log CFU/m^3) from air samples by active sampling in wet season (2015-16)

The bacterial load by active sampling in wet season ranged from 2.66 log CFU/m^3 to 1.39 log CFU/m^3. The highest load of 2.66 log CFU/m^3 was recorded at Chungthang followed by Singtam (2.5 log CFU/m$^{3)}$, Tadong (2.48 log CFU/m ^{3}), Pelling (2.37 log CFU/m$^{3)}$, Deorali (2.36 log CFU/m^3), Pelling (2.35 log CFU/m^3), KSC (2.32 log CFU/m ^{3}) and x Ravangla (2.32 log CFU/m ^{3}), respectively. The lowest bacterial load was observed at Lachung (1.39 log CFU/m ^{3}) followed by Changu Lake (1.54 CFU/m^3). It was observed that the variation in log CFU/m ^{3} in wet season did not vary significantly as well (Table 4, Figure 5).

Table 4: The bacteria load (log of CFU/ m^3) from air samples by passive and active sampling methods in wet season (2015-16).

Location	Altitude (metre)	CFU/m^3 (P)	CFU/m^3 (A)	Log CFU/m^3 (P)	Log CFU/m^3 (A)
Deorali	1484	3236.5	297.5	3.50	2.36
Singtam	358	5755	350	3.74	2.5
KSC	1590	3589	229.5	3.55	2.32
Tadong	1350	1696	352	3.29	2.48
Changu Lake	3794	1370	34	3.12	1.54

Pelling	1458	1905	236.5	3.27	2.37
Lachung	2746	1879	24	3.27	1.39
Chungthang	1815	1422	466	3.13	2.66
Mangan	1258	6277.5	256.5	3.78	2.35
Ravangla	1999	2427	171.5	3.38	2.20

Note: P stands for "Passive" and A stands for "Active" method of sampling. The log CFU/m^3 considered here is the average.

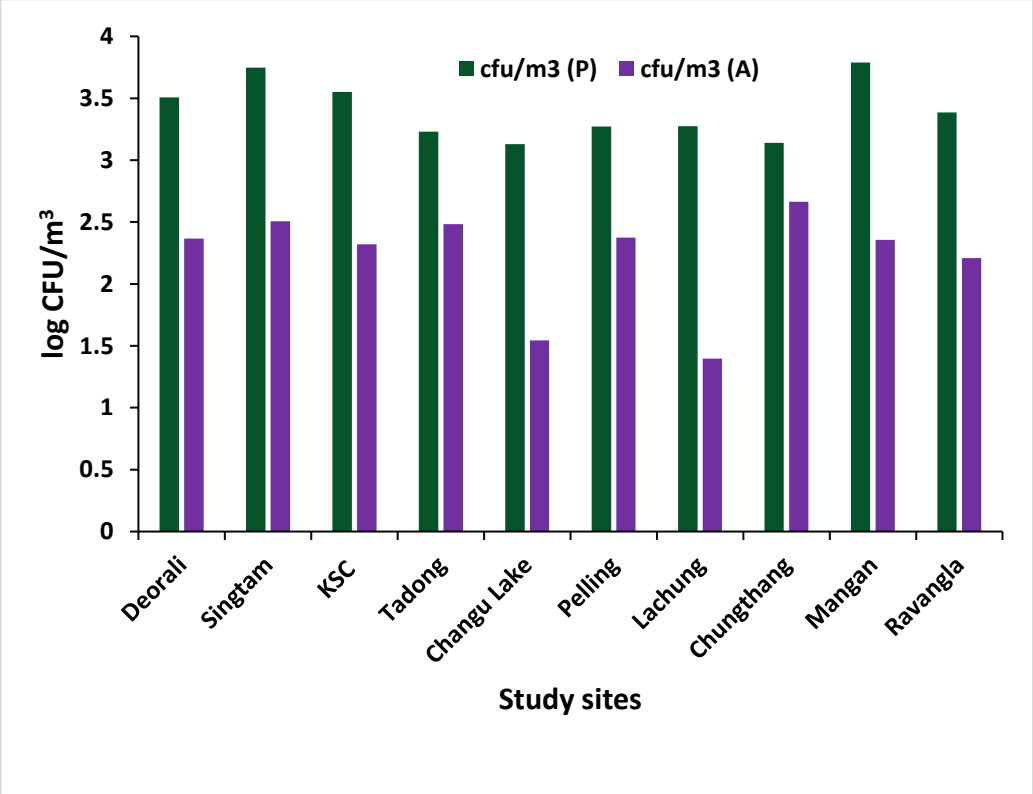


Figure 5: Bacterial load (log CFU/m^3) by active and passive in wet season (2015-16).

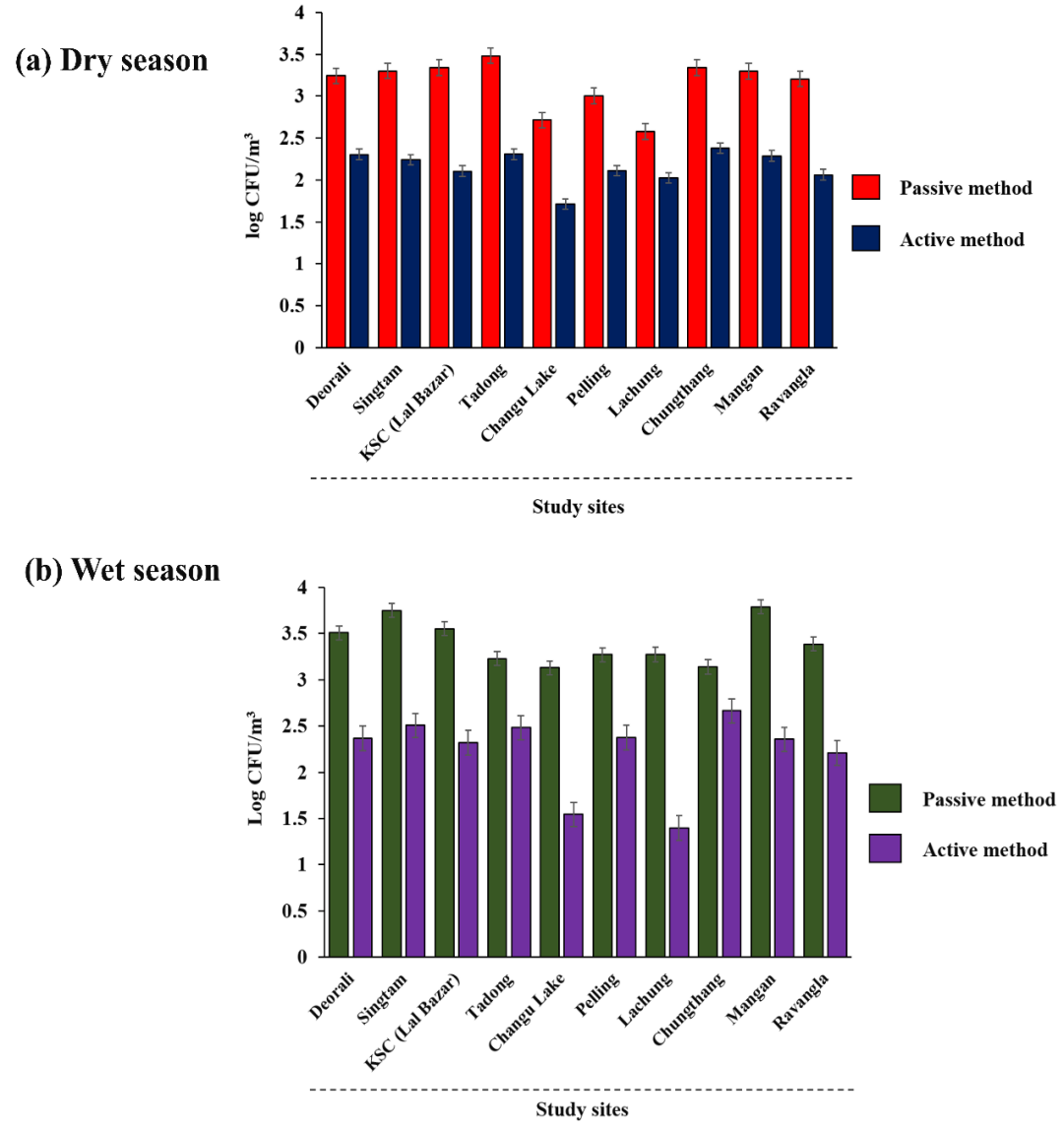


Figure 6: Bacterial Load (log CFU/m^3) in dry season by passive and active sampling method (2015-16).

Bacterial Load (log CFU/m^3) from air samples by passive sampling in dry season (2016-17)

The bacterial load by passive method of sampling in dry season ranged from 3.39 log CFU/m^3 to 2.52 log CFU/m^3. The highest bacterial load of 3.39 log CFU/m^3 was observed at Singtam followed by Tadong (3.15 log CFU/m^3), Chungthang (3.14 log CFU/m^3), KSC (3.10 log CFU/m^3), Ravangla (2.98 log CFU/m^3), Pelling (2.83 log CFU/m^3), Mangan (2.77 log CFU/m^3), Deorali and Changu Lake (2.59 log CFU/m^3) and Lachung (2.5 log CFU/m^3), respectively (Table 5, Figure 7).

Bacterial Load (log CFU/m^3) from air samples by Active sampling in dry season (2016-17)

The CFU of bacteria with active sampling in dry season ranged from 2.96- 1.83 log CFU/m^3. The microbial load by active method in dry season was highest in Singtam (2.96 log CFU/m^3) followed by Mangan (2.64 log CFU/m^3), KSC (2.58 log CFU/m^3), Chungthang (2.56 log CFU/m^3), Ravangla (2.38 log CFU/m^3), Deorali (2.36 log CFU/m^3), Pelling (2.41 log CFU/m^3), Tadong (2.27 log CFU/m^3), Lachung (2.01 log CFU/m^3) and Changu Lake (1.83 log CFU/m^3), respectively (Table 5, Figure 7).

Table 5: The bacterial load (log CFU/m^3) from air samples by passive and active method of sampling in dry season (2016-17).

Location	Altitude (metre)	CFU/m^3 (P)	CFU/m^3 (A)	Log CFU/m^3 (P)	Log CFU/m^3 (A)
Deorali	1484	389.75	230	2.59	2.36
Singtam	358	2483.5	919	3.39	2.96
KSC	1590	1279.75	387	3.10	2.58
Tadong	1350	1436.75	187	3.15	2.27
Changu Lake	3794	389.75	67	2.59	1.83
Pelling	1458	686.25	262	2.83	2.41
Lachung	2746	332.25	126	2.52	2.10
Chungthang	1815	1392	364	3.14	2.56
Mangan	1258	598.25	441	2.77	2.64
Ravangla	1999	973.5	243	2.98	2.38

Note: P stands for "Passive" and A stands for "Active" method of sampling. The log CFU/m^3 considered here is the average.

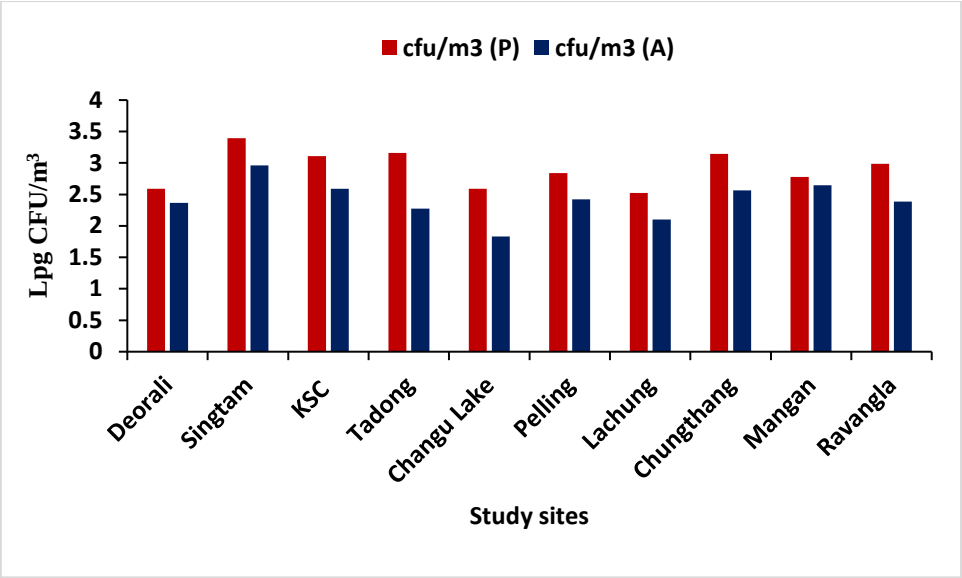


Figure 7. Bacterial load (log CFU/m^3) from air samples by active and passive in dry season (2016-17).

Bacterial load (log CFU/m3) from air samples by passive sampling in wet season (2016-17)

The bacterial load with passive sampling in wet season ranged from 2.35- 1.41 log CFU/m^3. The microbial load by passive method in wet season was highest in Mangan (2.35 log CFU/m^3) followed by Singtam and KSC (2.21 log CFU/m^3), Tadong (2.06 log CFU/m^3), Chungthang (2.05 log CFU/m^3), Deorali (1.99 log CFU/m^3), Ravangla (1.98 log CFU/m^3), Pelling (1.80 log CFU/m^3), Lachung (1.76 log CFU/m^3) and Changu Lake (1.41 log CFU/m^3), respectively (Table 6, Figure 9).

Table 6: The bacterial load (log CFU/m^3) from air samples by passive and in wet season (2016-17)					
Location	**Altitude (metre)**	**CFU/m^3 (P)**	**CFU/m^3 (A)**	**Log CFU/m^3 (P)**	**Log CFU/m^3 (A)**
Deorali	1484	98.75	131	1.99	2.12
Singtam	358	163.25	366.5	2.21	2.56
KSC	1590	162.5	313.25	2.21	2.49
Tadong	1350	114.75	137.75	2.06	2.14
Changu Lake	3794	25.25	60.75	1.42	1.79
Pelling	1458	63.25	193.5	1.81	2.29
Lachung	2746	56.75	54.75	1.76	1.74
Chungthang	1815	113.5	302.25	2.05	2.48
Mangan	1258	226.5	327.5	2.36	2.52
Ravangla	1999	96.5	164.75	1.99	2.22
Note: P stands for "Passive" and A stands for "Active" method of sampling. The log CFU/m^3 considered here is the average.					

Bacterial Load (log CFU/m^3) from air samples by active sampling in wet season (2016-17).

The log CFU/m^3 of bacteria with active sampling in wet season ranged from 2.56- 1.74 log CFU/m^3. The bacterial load by active method in wet season was highest in Singtam (2.56 log CFU/m^3) followed by Mangan (2.51 log CFU/m^3), Chungthang (2.48 log CFU/m^3), KSC (2.49 log CFU/m^3), Pelling (2.28 log CFU/m^3), Ravangla (2.21 log CFU/m^3), Tadong (2.14 log CFU/m^3), Deorali (2.12 log CFU/m^3), Changu Lake (1.79 log CFU/m^3 and Lachung (1.74 log CFU/m^3), respectively (Table 6, Figure 9).

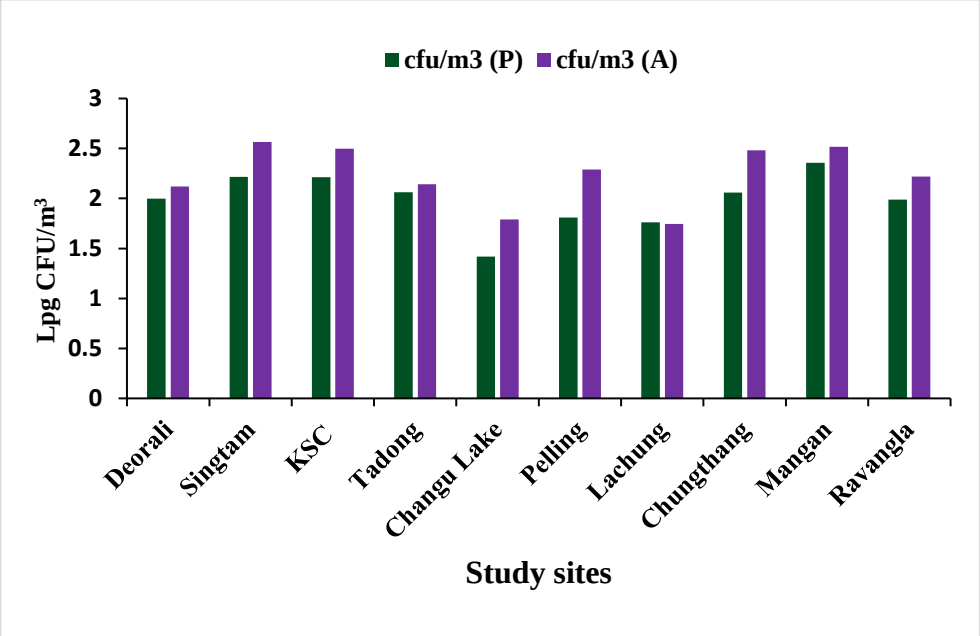


Figure 9: Bacterial load (log CFU/m^3) from air samples by active and passive in wet season (2016-17).

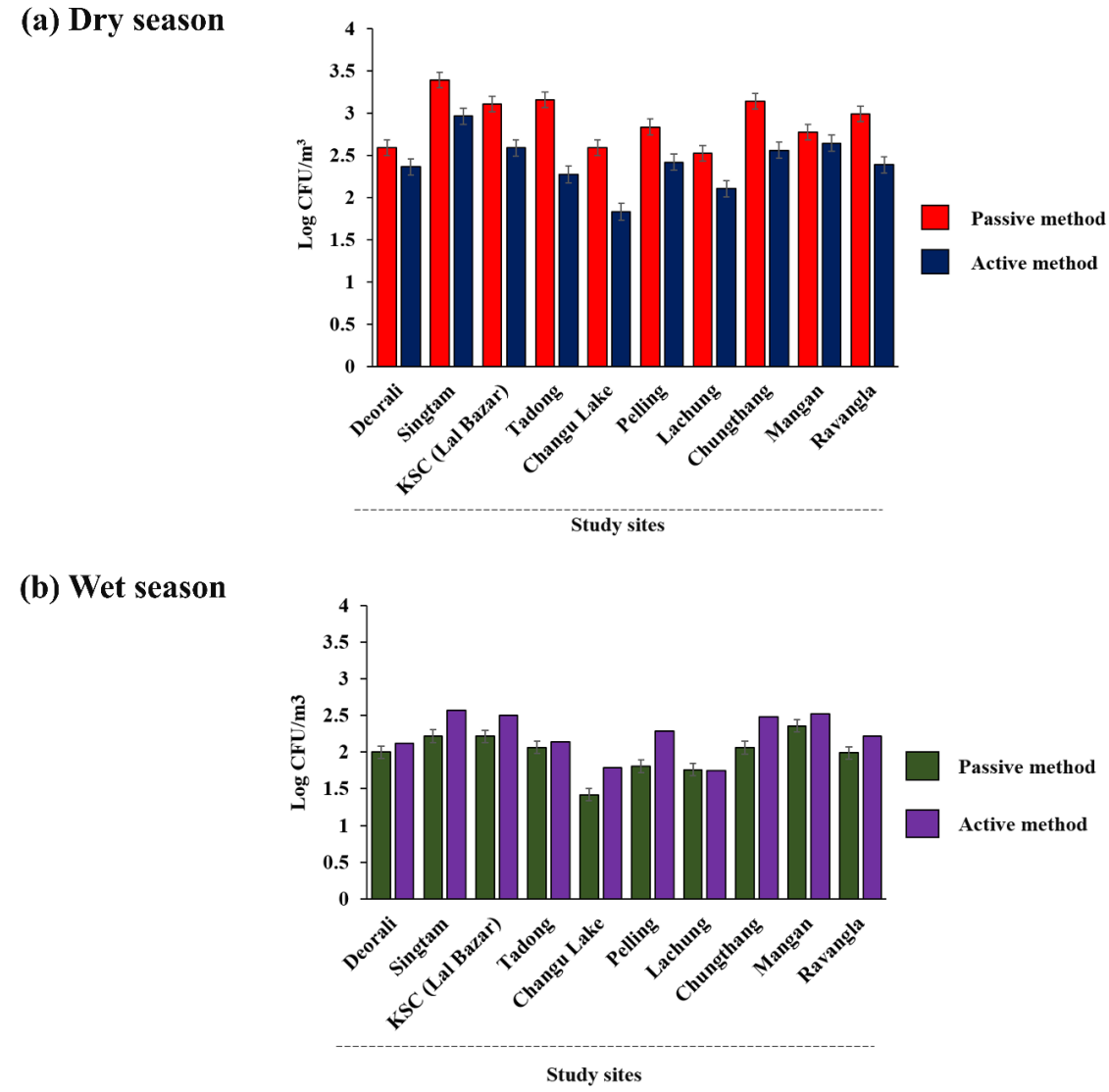


Figure 10: Bacterial load (log CFU/m^3) from air samples by active and passive in dry and wet season (2016-17).

Bacterial load (log CFU/m^3) from air samples by passive sampling in dry season (2017-18).

The log CFU/m^3 of bacteria with passive sampling in dry season ranged from 3.41- 2.70 log CFU/m^3. The bacterial load by passive method in dry season was recorded highest in Tadong (3.41 log CFU/m^3) followed by Singtam (3.27 log CFU/m^3) followed by

Mangan (3.19 log CFU/m^3), Ravangla (3.17 log CFU/m^3), KSC (3.16 log CFU/m^3), Changu Lake (3.81 log CFU/m^3), Chungthang and Deorali (2.98 log CFU/m^3), Pelling (2.82 log CFU/m^3) and Lachung (2.70 log CFU/m^3), respectively (Table 7. Figure 10).

Bacterial load (log CFU/m^3) from air samples by active sampling in dry season (2017-18).

The bacterial load by active sampling in dry season ranged from 2.18- 1.52 log CFU/m^3. The highest bacterial load was observed in Chungthang (log 2.18 CFU/m^3) and the lowest in Changu lake (log 1.52 CFU/m^3). However, the bacterial load in the sample collected by active sampling, in this season was found not exceeding beyond log 2.18 log CFU/m^3 (Table 7, Figure 10).

Table 7: The Bacterial load (log CFU/m^3) from air samples collected by passive and active sampling method in dry season (2017-18).

Location	Altitude (metre)	CFU/m^3 (P)	CFU/m^3 (A)	Log CFU/m^3 (P)	Log CFU/m^3 (A)
Deorali	1484	973	89.25	2.99	1.95
Singtam	358	1899.5	103.5	3.28	2.02
KSC	1590	1451	72	3.16	1.86
Tadong	1350	2620.5	97	3.42	1.99
Changu Lake	3794	714.75	32.25	2.85	1.52

Pelling	1458	668.75	82.25	2.82	1.92
Lachung	2746	506.25	71	2.705222	1.86
Chungthang	1815	954.25	151	2.98	2.18
Mangan	1258	1576.25	110.5	3.19	2.04
Ravangla	1999	1493.75	133.5	3.174569	2.12
Note: P stands for "Passive" and A stands for "Active" method of sampling. The log CFU/m^3 considered here is the average.					

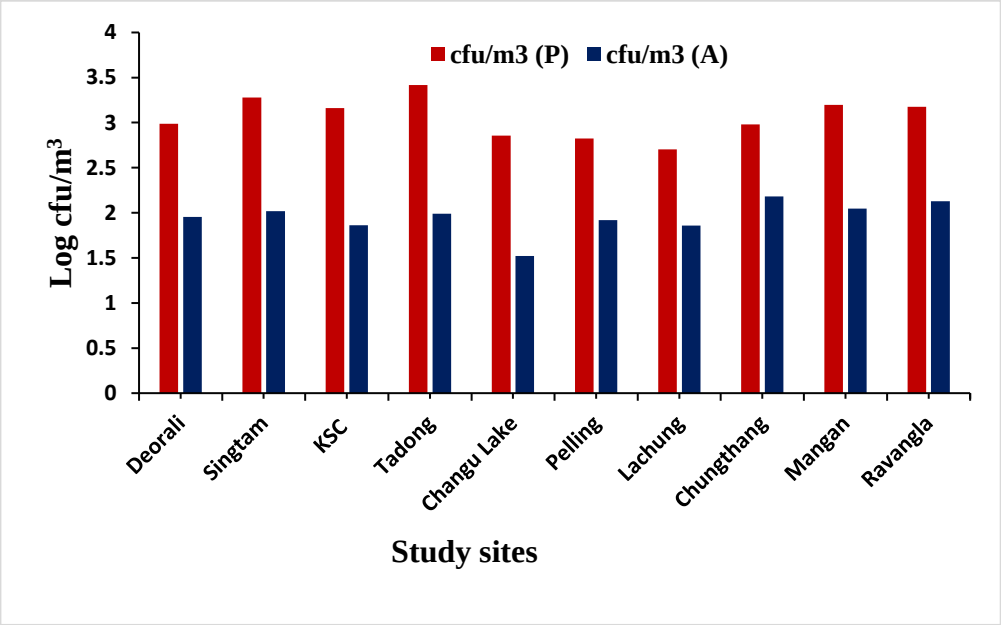


Figure 10: Bacterial load (log CFU/m^3) from air samples by active and passive in dry season (2017-18).

Bacterial load (log CFU/m^3) from air samples by passive sampling in wet season (2017-18).

The microbial load in this season by passive sampling ranged from 2.18-1.85 log CFU/m^3. The highest load was at Chungthang and the lowest at Lachung, both the rural areas and lightly populated areas. Other than Chungthang (2.18 log CFU/m^3), Ravangla (2.12 log CFU/m^3), Singtam (2.01 log CFU/m^3) and Tadong (1.99 log CFU/m^3) showed a higher microbial load (Table 10, Figure 11).

Bacterial load (log CFU/m^3) from air samples by active sampling in wet season (2017-18)

The microbial load in this season by active sampling ranged from 2.37-1.68 log CFU/m^3. The highest microbial load recorded at Chungthang (2.37 log CFU/m^3) and the lowest at Changu lake (1.76 log CFU/m^3) followed by KSC (1.72 log CFU/m^3). The variation in the microbial load was not remarkable for sampling locations, Deorali, Singtam, Tadong, Pelling, Lachung, Chungthang, Mangan and Ravangla which ranged from 2.37-2.09 log CFU/m^3 (Table 8, Figure 12).

Table 8: Bacterial load (log CFU/m^3) of air samples collected by passive and active method in wet season (2017-18)

Location	Altitude (metre)	CFU/m^3 (P)	CFU/m^3 (A)	Log CFU/m^3 (P)	Log CFU/m^3 (A)
Deorali	1484	89.25	123.5	1.95	2.09
Singtam	358	103.5	159.75	2.01	2.20
KSC	1590	72	52.25	1.86	1.72
Tadong	1350	97	133.5	1.99	2.12
Changu Lake	3794	32.25	47	1.52	1.68
Pelling	1458	82.25	129.5	1.92	2.11
Lachung	2746	71	122	1.85	2.08
Chungthang	1815	151	234.5	2.18	2.37
Mangan	1258	110.5	164.75	2.04	2.219
Ravangla	1999	133.5	183.25	2.12	2.26

Note: P stands for "Passive" and A stands for "Active" method of sampling. The CFU considered here is the average.

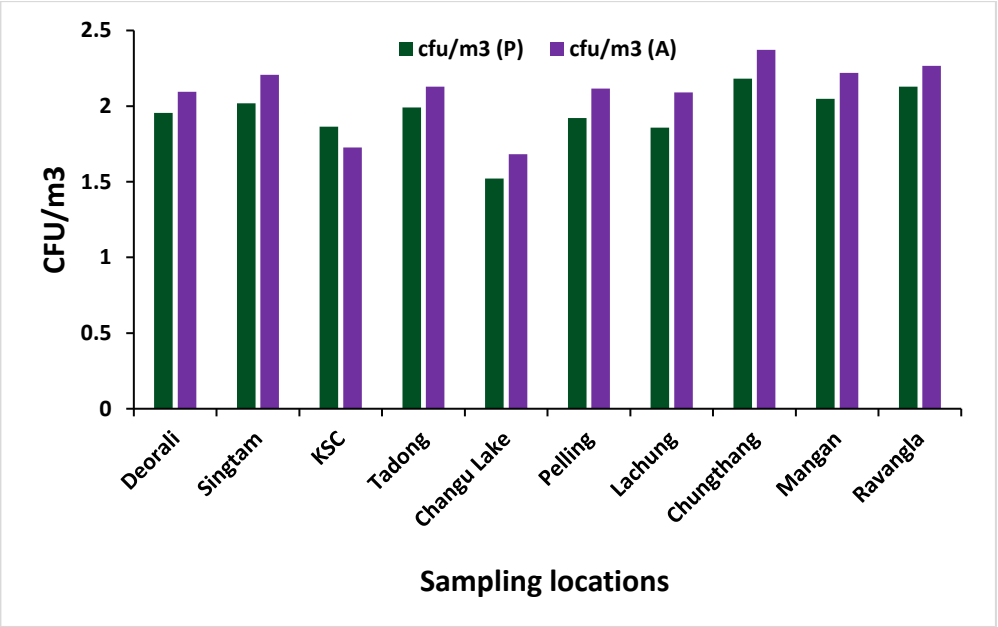


Figure 12: Bacterial Load (log CFU/m^3) of air samples collected by passive and active method in wet season (2017-18)

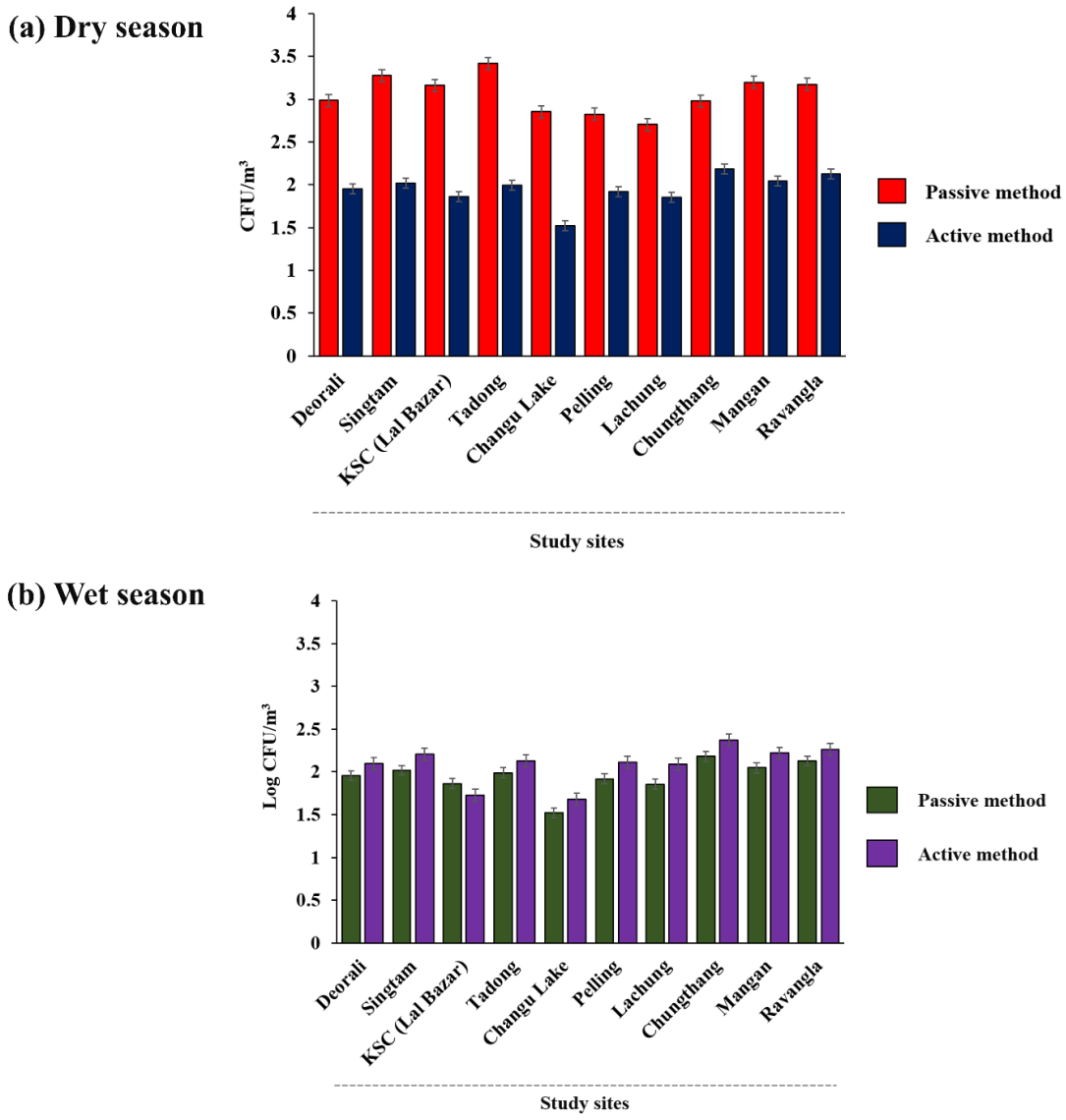


Figure 13: Bacterial Load (log CFU/m^3) from air samples with active and passive sampling in dry and wet season (2017-18).

Fungal Load (log CFU/m^3) from air samples in dry season by passive sampling method (2015-16).

The fungal load in dry season by passive method of sampling ranged from 3.54-2.16 log CFU/m^3. The highest fungal load was recorded at KSC (3.54 log CFU/m^3) and very close to it were recorded at Tadong (3.40 log CFU/m^3), Pelling (3.39 log CFU/m^3), Singtam (3.21 log CFU/m^3) and Deorali (3.17 log CFU/m^3). Chungthang and Ravangla recorded a fungal load of 2.96 log CFU/m^3 and 2.97 log CFU/m^3.

Fungal Load (log CFU/m^3) from air samples in dry season by active sampling method (2015-16)

The fungal load in dry season by active method of sampling ranged from 3.20-1.93 log CFU/m^3. The significantly higher fungal load was recorded at Singtam (3.20 log CFU/m^3) and Ravangla (3.14 log CFU/m^3) followed by KSC (2.96 log CFU/m^3), Tadong (2.99 log CFU/m^3) and Deorali (2.95 log CFU/m^3) and Mangan (2.90 log CFU/m^3) while the lowest fungal load observed at Lachung (1.93 log CFU/m^3) (Table 9, Figure 14)

Table 9: Fungal load (log CFU/m^3) from air samples collected by passive and active method in dry season (2015-16)

Location	Altitude (metre)	CFU/m^3 (P)	CFU/m^3 (A)	Log CFU/m^3 (P)	Log CFU/m^3 (A)
Deorali	1484	1500.5	910.5	3.17	2.95
Singtam	358	1644	1584	3.21	3.20
KSC	1590	3471.5	912	3.54	2.96
Tadong	1350	2531.5	981	3.40	2.99
Changu Lake	3794	182	473.5	2.26	2.67
Pelling	1458	2466.5	917.5	3.39	2.96
Lachung	2746	195	86	2.29	1.93
Chungthang	1815	926	301.5	2.96	2.48
Mangan	1258	146	796	2.16	2.90
Ravangla	1999	952	1392.5	2.97	3.14

Note: P stands for "Passive" and A stands for "Active" method of sampling. The CFU considered here is the average of multiple sampling.

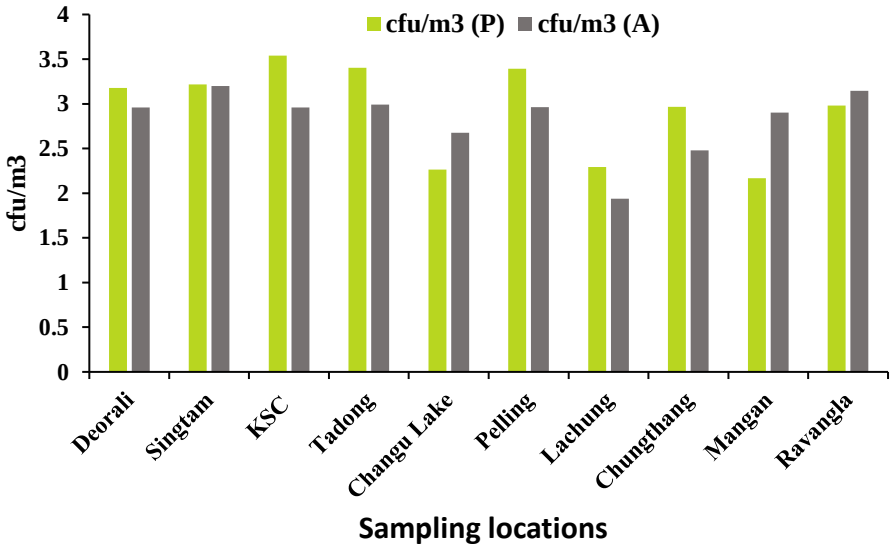


Figure 14: Fungal loads (log CFU/m^3) from air samples by passive and active method in dry season (2015-16).

Fungal Load (log CFU/m^3) from air samples in wet season by passive sampling method (2015-16).

The fungal load in wet season by passive sampling ranged from 3.13- 1.66 log CFU/m^3 which was recorded for Ravangla and Changu Lake, respectively. The significantly higher fungal load was observed at Mangan (3.04 log CFU/m^3), Singtam (3.02 log CFU/m^3), Pelling (2.99 log CFU/m^3) and Tadong (2.90 log CFU/m^3). Another significantly low fungal load was observed at Lachung (1.89 log CFU/m^3) and Chungthang (1.81 log CFU/m^3), respectively (Table 10, Figure 15).

Fungal Load (log CFU/m^3) from air samples in wet season by active sampling method (2015-16).

The fungus load in wet season ranged from 2.72- 2.18 log CFU/m^3. The highest at Ravangla (2.72 log CFU/m^3) and Tadong (2.62 log CFU/m^3). The minimum fungal load was observed at Chungthang (2.18 log CFU/m^3) (Table 10, Figure 15).

Table 10: The fungal load from air samples in wet season by passive and active sampling in wet season (2015-16).

Location	Altitude (metre)	CFU/m^3 (P)	CFU/m^3 (A)	Log CFU/m^3 (P)	Log CFU/m^3 (A)
Deorali	1484	353	239.75	2.54	2.38
Singtam	358	1056.25	299.5	3.02	2.47
KSC	1590	571.25	275.75	2.75	2.44
Tadong	1350	802	419.75	2.90	2.62
Changu	3794	45	212.75	1.66	2.32
Pelling	1458	835.5	446.25	2.92	2.65
Lachung	2746	77.5	188	1.89	2.27
Chungthang	1815	64	151	1.81	2.18
Mangan	1258	1120.5	370.75	3.04	2.57
Ravangla	1999	1363	532.5	3.13	2.72

Note: P stands for "Passive" and A stands for "Active" method of sampling. The log CFU/m^3 considered here is the average.

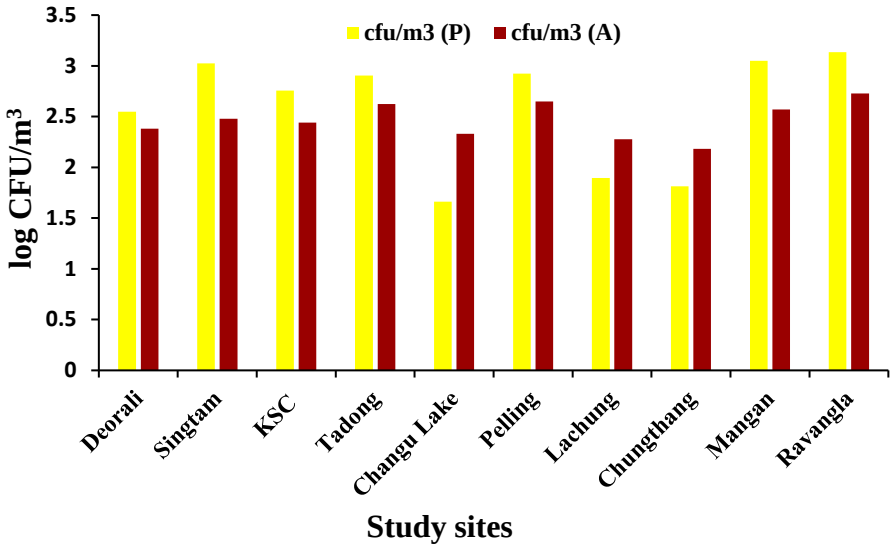


Figure 15: The fungal load (log CFU/m^3) from air samples in wet season by passive and active sampling (2015-16).

Fungal Load (log CFU/m^3) from air samples in dry season by passive sampling method (2016-17).

The fungal load in 2016-17 ranged 1.89-1.32 log CFU/m^3. The fungal load in this season of 2016-17 remained comparatively low, all the sampling locations had fungal load below 1.89 log CFU/m^3. The fungal load was observed at Chungthang and Tadong (1.89 log CFU/m^3) and the lowest fungal load was observed at Lachung (1.32 log CFU/m^3). However, in the remaining sampling locations the variation in the fungal load was not significant (Table 13, Figure 16)

Fungal Load (log CFU/m^3) from air samples in dry season by active sampling method (2016-17).

The fungal load ranged from 2.46-1.66 log CFU/m^3, the highest at Singtam (2.46 log CFU/m^3) and the least at Changu Lake (1.66 log CFU/m^3). After Singtam (2.46 log CFU/m^3), KSC (2.44 log CFU/m^3), Chungthang (2.40 log CFU/m^3), Deorali (2.39 log CFU/m^3), Pelling (2.37 log CFU/m^3) and Ravangla (2.34 log CFU/m^3), respectively had a significantly high fungal load.

Table 11: The fungal load (log CFU/m^3) from air samples in dry season by passive and active sampling (2016-17).

Location	Altitude (metre)	CFU/m^3 (P)	CFU/m^3 (A)	Log CFU/m^3 (P)	Log CFU/m^3 (A)
Deorali	1484	39	247.15	1.60	2.39
Singtam	358	64.5	293.75	1.81	2.46
KSC	1590	66.25	275.75	1.82	2.44
Tadong	1350	77.25	187.5	1.89	2.27
Changu Lake	3794	30.5	45.75	1.49	1.66
Pelling	1458	76	235.25	1.88	2.37
Lachung	2746	20.25	193.75	1.32	2.28
Chungthang	1815	77.25	254.25	1.89	2.40

Mangan	1258	61.75	257	1.79	2.41
Ravangla	1999	51.5	218.25	1.72	2.34
Note: P stands for "Passive" and A stands for "Active" method of sampling. The log CFU/m^3 considered here is the average.					

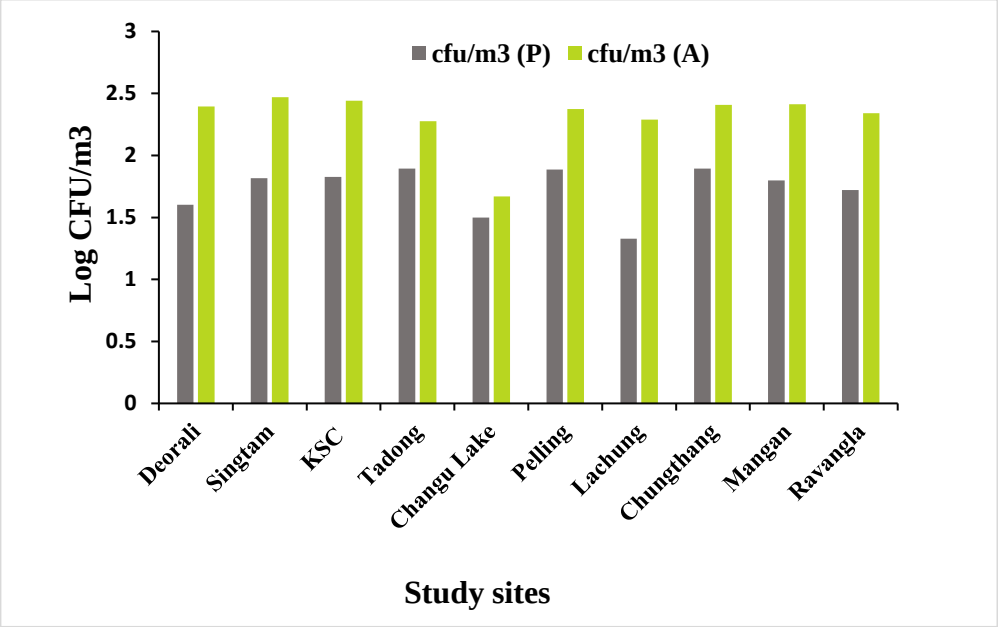


Figure 16. The fungal load (log CFU/m^3) from air samples in dry season by passive and active sampling (2016-17)

Fungal Load (log CFU/m^3) from air samples in wet season by passive sampling method (2016-17).

The fungal load in this year in wet season the ranged from 3.59- 2.79 log CFU/m^3. The highest 3.59 log CFU/m^3 was recorded at Singtam and the least 2.79 log CFU/m^3 was recorded at Changu Lake. Other significantly low fungal load was observed at Lachung (2.84 log CFU/m^3). The fungal load ranged from 2.57- 2.30 log CFU/m^3, in this method

of sampling for this season of this year the fungal load did not show significant variation. However, the highest was recorded at Mangan (2.57 log CFU/m^3) and the lowest at Changu Lake (2.30 log CFU/m^3). Ravangla (2.55 log CFU/m^3) had the next highest fungal load, while Singtam (2.51 log CFU/m^3) and Chungthang (2.51 log CFU/m^3) had the equal fungal load observed (Table 12, Figure 17).

Table 12: Fungal load (CFU/m^3) from air samples in wet season by active sampling method (2016-17).

Location	Altitude (metre)	CFU/m^3 (P)	CFU/m^3 (A)	Log CFU/m^3 (P)	Log CFU/m^3 (A)
Deorali	1484	1043.5	326.25	3.01	2.51
Singtam	358	3901.5	330	3.59	2.51
KSC	1590	1800.5	253.25	3.25	2.40
Tadong	1350	1552.5	272	3.19	2.43
Changu Lake	3794	616.5	201.75	2.79	2.30
Pelling	1458	1265.5	233.75	3.10	2.37
Lachung	2746	702	212.5	2.84	2.32
Chungthang	1815	1517	327.5	3.18	2.51
Mangan	1258	1970	376.5	3.29	2.57
Ravangla	1999	2055.42	360	3.31	2.55

Note: P stands for "Passive" and A stands for "Active" method of sampling. The log CFU/m^3 considered here is the average.

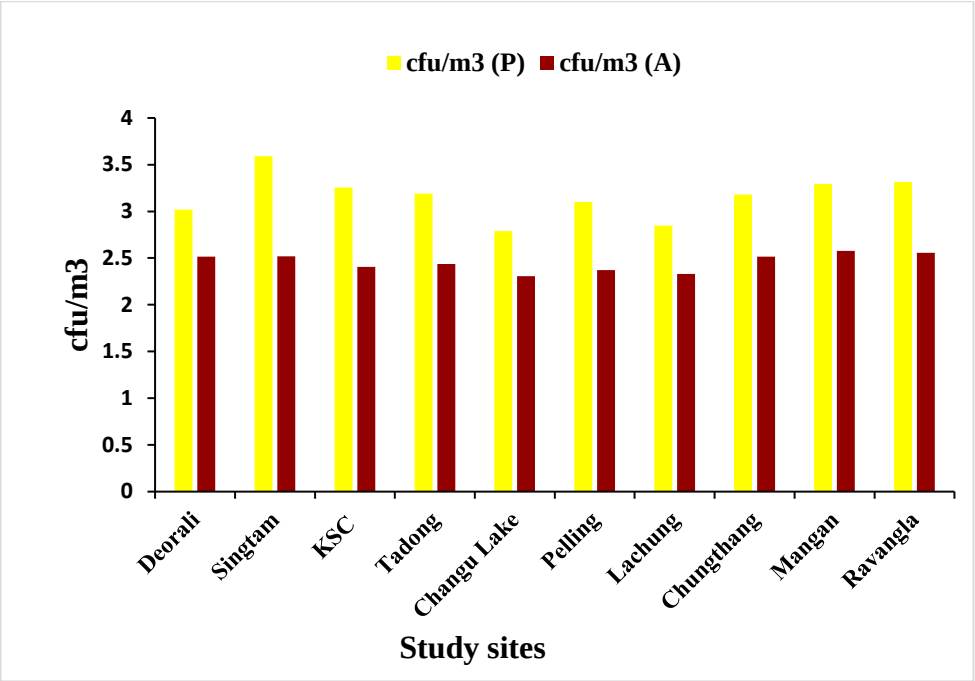


Figure. 17: The fungal load (log CFU/m^3) from air samples in wet season by passive and active sampling (2016-17).

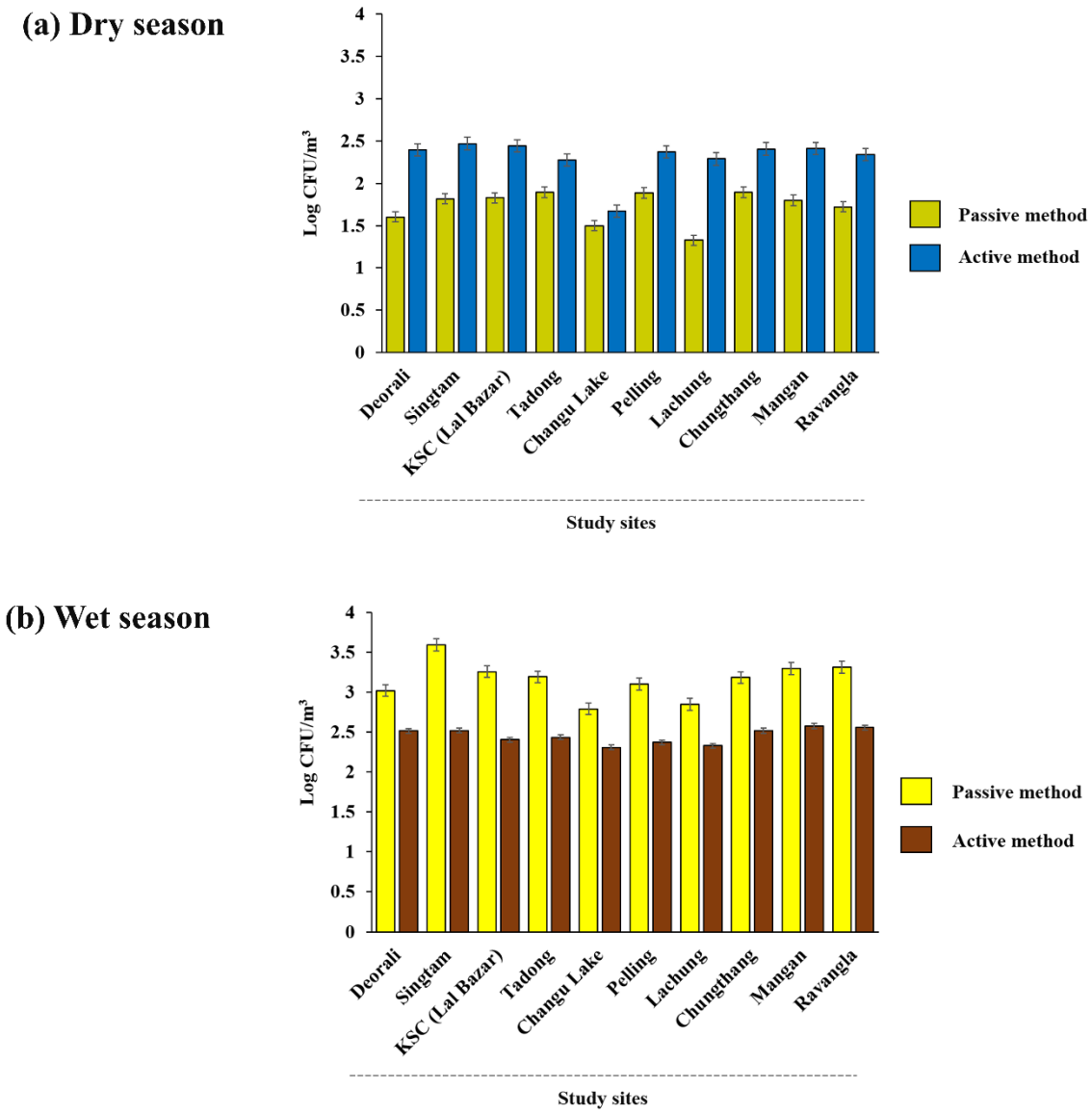


Figure 18: Fungal Load (log CFU/m^3) from air samples in wet season by passive and active method.

Fungal Load (log CFU/m^3) from air samples in dry season by passive sampling method (2017-18).

The fungal load in this year in dry season by passive sampling ranged from 2.99-3.71 log CFU/m^3. The lowest load was recorded at Changu lake and the highest at Pelling.

The other sampling locations that showed significantly high fungal load were Ravangla (3.41 log CFU/m^3), Mangan, (3.38 log CFU/m^3) Tadong (3.36 log CFU/m^3), Chungthang (3.34 log CFU/m^3), Deorali (3.29 log CFU/m^3), and Singtam (3.28 log CFU/m^3), respectively (Table 13, Figure 19).

Fungal Load (log CFU/m^3) from air samples in dry season by active sampling method (2017-18).

The fungal load ranged from 2.18-2.94 log CFU/m^3, at Lachung and Ravangla, respectively. The fungal load of the sampling locations at ascending order are Lachung (2.18 log CFU/m^3), Changu Lake (2.48 log CFU/m^3), Pelling (2.44 log CFU/m^3), Mangan (2.49 log CFU/m^3), Chungthang (2.59 log CFU/m^3), Tadong (2.65 log CFU/m^3), Singtam (2.73 log CFU/m^3). Deorali and KSC had similar fungal load of 2.90 log CFU/m^3 and the highest at Ravangla with fungal load of 2.94 log CFU/m^3, respectively (Table 13, Figure 19).

Table 13: The fungal load (log CFU/m^3) from air samples in dry season by passive and active sampling (2017-18)

Location	Altitude (metre)	CFU/m^3 (P)	CFU/m^3 (A)	Log CFU/m^3 (P)	Log CFU/m^3 (A)
Deorali	1484	1951	808	3.29	2.90
Singtam	358	1937.5	537.5	3.28	2.73
KSC	1590	1355.5	804.75	3.13	2.90
Tadong	1350	2342	447.25	3.36	2.65
Changu Lake	3794	978.5	301	2.99	2.48

Pelling	1458	5194	276.25	3.71	2.44
Lachung	2746	1135	151.75	3.05	2.18
Chungthang	1815	2205	393	3.34	2.59
Mangan	1258	2433.5	314.5	3.38	2.49
Ravangla	1999	2597	878.5	3.41	2.94
Note: P stands for "Passive" and A stands for "Active" method of sampling. The CFU considered here is the average of multiple samples					

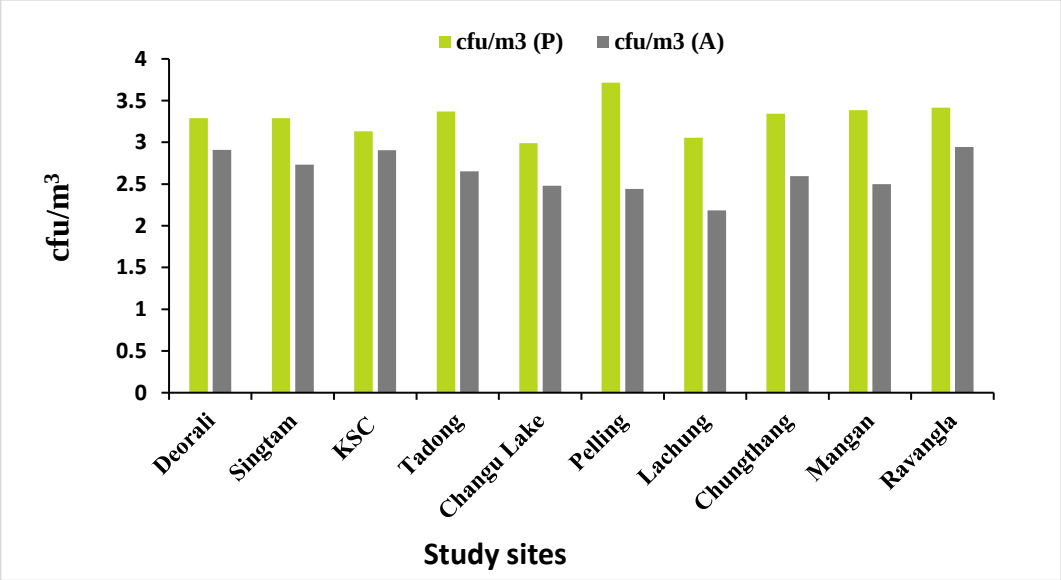


Figure. 19: The fungal load (log CFU/m^3) from air samples in dry season by passive and Active sampling (2017-18).

Fungal Load (log CFU/m^3) from air samples in wet season by passive sampling method (2017-18).

The fungal load ranged from 2.85-3.68 log CFU/m^3, the lowest at Deorali and the highest at Lachung. Majority of the locations had a fungal load above 3.12 log CFU/m^3, two of

the locations Singtam and Changu Lake had the same fungal load of 3.12 log CFU/m^3. The other locations having fungal load higher than 3.12 log CFU/m^3 are Pelling (3.18 log CFU/m^3), Chungthang (3.21 log CFU/m^3), Mangan (3.31 log CFU/m^3), Ravangla (3.35 log CFU/m^3), and Lachung (3.68 log CFU/m^3), respectively (Table 16. Figure 20).

Table 14: The fungal load (log CFU/m^3) from air samples in wet season by passive and active sampling (2017-18).

Location	Altitude (metre)	CFU/m^3 (P)	CFU/m^3 (A)	Log CFU/m^3 (P)	Log CFU/m^3 (A)
Deorali	1484	710.5	180	2.85	2.25
Singtam	358	1327	480	3.12	2.68
KSC	1590	750	541.5	2.8	2.73
Tadong	1350	874	408	2.94	2.61
Changu Lake	3794	1331	336	3.12	2.52
Pelling	1458	1513.5	124	3.18	2.09
Lachung	2746	4789.5	134	3.68	2.13
Chungthang	1815	1650.5	352	3.21	2.54
Mangan	1258	2042	356.5	3.31	2.55
Ravangla	2128	2250.5	430.5	3.35	2.63

Note: P stands for "Passive" and A stands for "Active" method of sampling. The CFU considered here is the average of multiple sampling.

Fungal Load (log CFU/m^3) from air samples in wet season by active sampling method (2017-18).

The fungal load in this season by active sampling ranged from 2.09-2.73 log CFU/m^3. KSC recorded the highest fungal load of 2.73 log CFU/m^3 followed by Singtam (2.68 log CFU/m^3), Ravangla 2.63 (log CFU/m^3), Tadong (2.61 log CFU/m^3), Mangan (2.55 log CFU/m^3), Chungthang (2.54 log CFU/m^3), Changu Lake (2.52 log CFU/m^3), Deorali (2.25 log CFU/m^3) and Lachung (2.13 log CFU/m^3), respectively (Table 16. Figure 20).

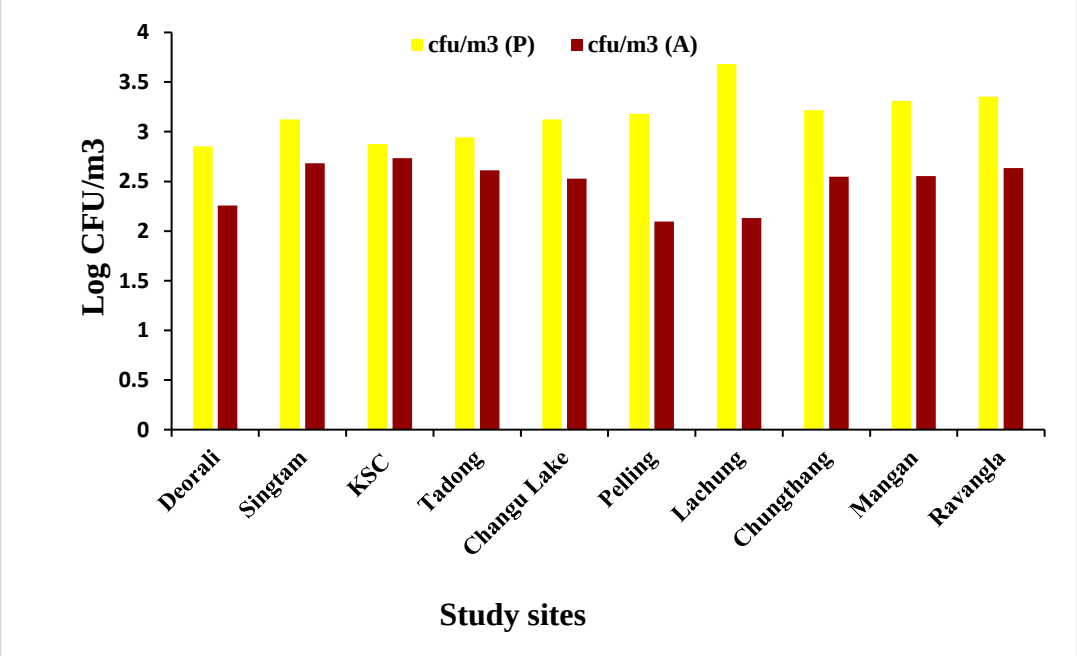


Figure 20: The fungal load (log CFU/m^3) from air samples in wet season by passive and active sampling (2017-18)

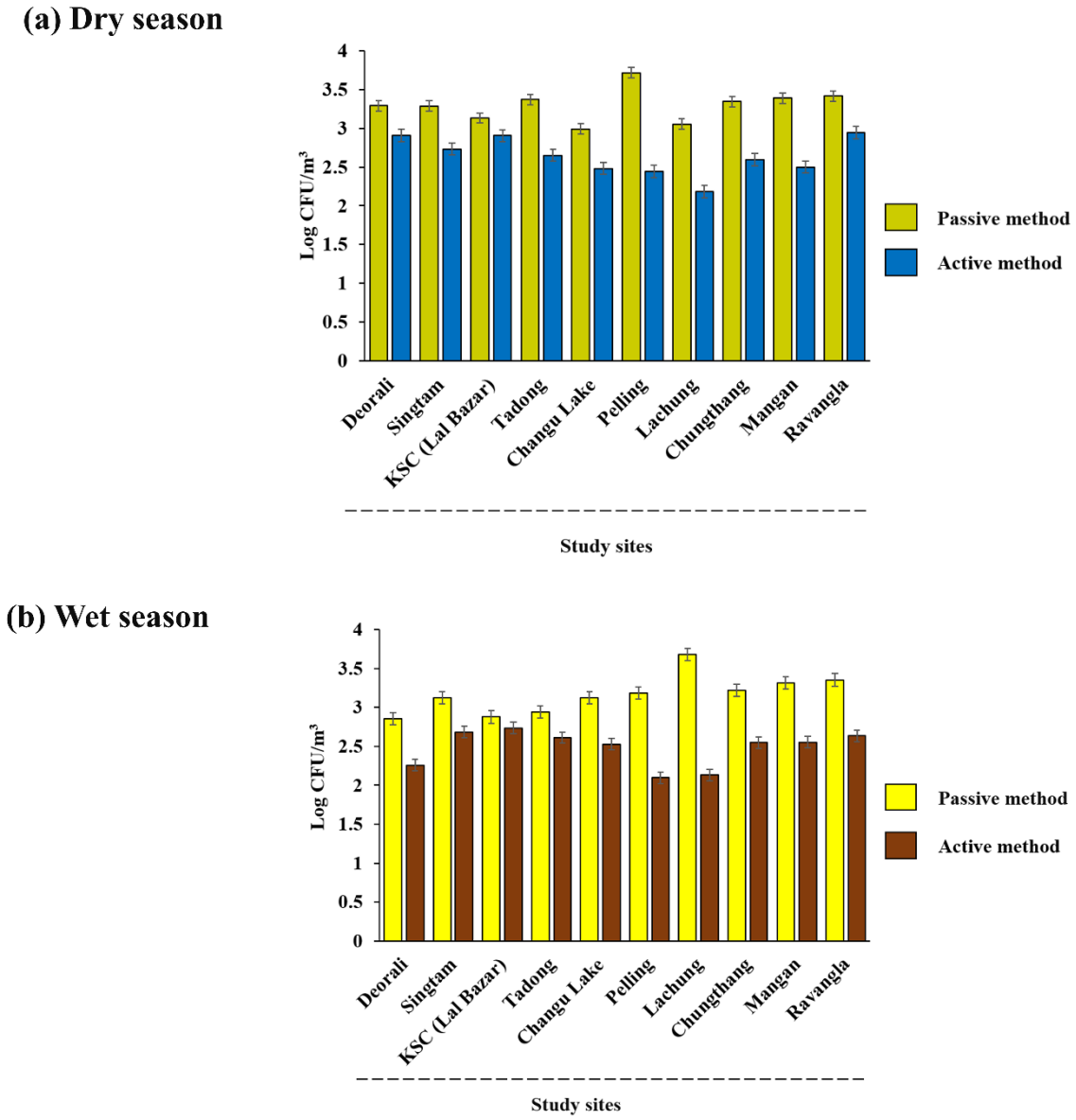


Figure 21: The fungal load (log CFU/m^3) from air samples in wet season by passive and active method (2017-18).

PHENOTYPIC CHARACTERIZATION

Phenotypic characterization of bacterial isolates

A total of 570 bacteria isolates were isolated from 188 air samples of four districts of Sikkim. Of which 226 survived subculturing and retained its morphological and cultural characteristics, which was considered for further study.

Morphological characterization

The morphological characterization of the bacterial isolates was based on cultural characteristics, cell morphology, Gram's reaction and KOH (potassium hydroxide test) and catalase test (Table 15). Out of 226 bacterial isolates 68 were Gram positive rod (30.88%), 91 (40.26%) Gram positive cocci and 67 (29.64%) Gram negative rod, it was further confirmed by the KOH test. Majority of Gram-positive rods and cocci showed positive test for catalase except few rods. Many isolates were circular, large, elevated, large or small pale yellowish elevated, milky white colonies, shiny off white, irregular, fuzzy colonies. Some were neon colored, reddish, orange, transparent and pink colonies. The cell morphology observed for gram positive rods were endospores on terminal, some were clustered, in long and short chains some singles. Gram negative rods observed were slender, tiny rods most in single and in chains. Gram positive cocci observed were large in clusters, chains, tetrads, diplococci. Some of the colonies were brownish, yellowish wrinkled and dry (Table 15).

Table 15: Morphological characteristics of bacteria isolated from air samples					
Isolates	**Colony morphology**	**Cell morphology**	**Gram reaction**	**String test**	**Catalase test**
AS:De:B3	Irregular, creamy colonies with swarming growth.	Rod	+	+	+
AS:De:15d	Dark creamy colored colonies, pointed in center.	Rod	+	+	+
ASDe:17d	Creamy, irregular edged, medium sized flat colonies	Rod	+	+	+
ASDe:16:v	Large, creamy colonies, with irregular edges.	Rod	+	+	+
ASDe:18:f	Yellowish, small colonies with swarming growth.	Rod	+	+	+
AS:De:16:4	Colonies with brown pigmentation, irregular colonies	Rod	-	-	-
ASDe:16:i	Yellowish, large colonies with smooth surface, pointed in center.	Rod	+	+	+
ASDe:6	Small, yellowish, sharp circular colonies.	Cocci	+	+	+
ASDe:17 b	Yellowish, small, circular colonies.	Cocci	+	+	+
ASDe :21	Yellowish, large circular colonies with sharp edges.	Cocci	+	+	+
ASDe18s	Small, circular, sharp circular colonies.	Cocci	+	+	+
AS:De:17:5	Smooth, convex, small, translucent, creamy colonies	Rod	-	-	-

ASDe:P1	Yellowish, medium sized, circular colonies.	Cocci	+	+	+
AS:De:17:14	Red pigmented, medium sized, round colonies.	Rod	-	-	+
ASDe:18i	Circular, off white, small colonies.	Cocci	+	+	+
ASDe:18a	Light yellow, circular, small colonies	Cocci	+	+	+
ASDe:18c	Circular, pale, large, elevated colonies.	Cocci	+	+	+
AS: Deu	Small circular, brownish, transparent colonies.	Rod	+	+	+
AS:DEB4	Brown, medium sized colonies, with fine edges.	Rod	+	+	+
ASDE:B5	Small, circular, transparent colonies.	Rod	+	+	+
AASDe17:d	Light yellow colonies with green pigmentation, slightly irregular.	Rod	+	+	+
ASDe:6	Light yellow, circular, small colonies.	Rod	+	+	+
ASDE:16s	Transparent, tiny, shiny colonies.	Rod	+	+	+
AS:De16h	Yellow colonies, with convex surface and irregular edges.	Cocci	+	+	+
AS:De:18: 5 (M)	dark yellow, dry, elevated circular colonies	Cocci	+	+	+
AS:Si 1g	Irregular, creamy colonies with fuzzy growth.	Rod	+	+	+
ASSi:18:a	Dark creamy slimy colonies, elevated in center.	Rod	+	+	+

ASSi:16d	Thick colonies, yellowish, circular	Rod	+	+	+
ASSi:16:g	Large, creamy colonies, with irregular edges.	Rod	+	+	+
ASSi:17:a	Yellowish, small colonies with swarming growth.	Rod	+	+	+
ASSi:17r	Shiny, tiny, circular colonies.	Rod	+	+	-
AS:Si:16:8 (M)	pink and yellow mixed, elevated colonies	Cocci	+	+	+
ASSi:2n	Yellowish, large colonies with smooth surface, pointed in center	Cocci	+	+	+
AS:Si:18:6 (P)	Colonies with brown pigmentation, irregular colonies	Rod	-	-	+
Assing:1	light yellow, circular, thick mucoid	Cocci	+	+	+
ASSi:17k	light yellow, circular, medium sized mucoid colonies.	Cocci	+	+	+
Assing:12	Mucoid, yellow, circular average colonies.	Cocci	+	+	+
Assing:18	Small, yellow colonies with sharp circular edges.	Cocci	+	+	+
ASSg: 14n	Small, elevated, circular yellowish colonies.	Cocci	+	+	+
ASSg: 14k	Small, circular, shiny off-white colonies	Cocci	+	+	+
AS:KSC:14m	Shiny, small, circular convex colonies.	Cocci	+	+	+
AS:Sg3	Milky white, small, circular colonies	Cocci	+	+	+

AS:Sg6	White, medium sized, circular colonies.	Cocci	+	+	+
ASSi:19r	Yellowish, small, circular, shiny colonies.	Cocci	+	+	+
ASSi:T4	Small, yellow, circular colonies.	Cocci	+	+	+
ASSi:2n	small, yellow, circular	Cocci	+	+	+
ASSi16a	Light yellow colonies with mild pigmentation.	Rod	+	+	+
AS:Si:17:g (P)	Colonies with brown pigmentation, irregular colonies	Rod	-	-	+
AS:KSC9	Large, umbonate, yellowish colonies.	Rod	+	+	+
AS:Ksc7d	Off white, filamentous colonies.	Rod	+	+	+
ASKC: 16:i	Flat, circular, large colonies.	Rod	+	+	+
AS:Ksc5	Smooth, clear, round colonies.	Cocci	+	+	+
AS:KSC7	Yellowish white, circular, regular sized colonies	Cocci	+	+	+
ASKsc:12	Mucoid, round, small colonies	Cocci	+	+	+
AS:KSC: 18:9 (M)	small, yellow, circular colonies	Cocci	+	+	+
ASKsc:29	White, small, round, shiny colonies.	Cocci	+	+	+

ASKSc:14a	Small, yellow, round colonies.	Cocci	+	+	+
AS:KSC:17:f (S)	Red pigmented, medium sized, round colonies.	Rod	-	-	+
AS:KSC18	Rigid, dry, irregular colonies, that was fixed compactly with the growth medium	Cocci	+	+	+
ASKSC:1d	Dry powdery, yellow, irregular colonies.	Cocci	+	+	+
ASKSC:16d	Dry, irregular shaped, yellow colonies.	Cocci	+	+	+
AS:KSC:17:21 (C)	Smooth, convex, small, translucent, creamy colonies	Rod	+	+	+
ASKSC:17i	Shiny, off white, circular colonies.	Rod	-	-	-
ASKSC:18l	Off white, tiny, round, elevated, colonies.	Rod	-	-	-
ASTd :3d	Bright yellow, medium sized, circular colonies.	Rod	+	+	+
ASTd:2b	Small, shiny, bright yellow, round colonies.	Rod	+	+	+
AS:Td (a)	Large, slightly dry, fuzzy colonies.	Rod	+	+	+
ASTd: Pi	Off white, thin colonies with spreading edges.	Rod	+	+	+
ASTD: 3it	Opaque, waxy, white colonies.	Rod	+	+	+
AS:Td3	Colonies with rough white edges, protruding yellow color in center.	Rod	+	+	+

AS Td: Pi	Bright yellow, small, shiny, circular colonies	Rod	+	+	+
AS Td: 1g	Spindle shaped, dry, off-white colonies.	Rod	+	+	+
AS:Td: 17:2	pink and yellow mixed, elevated	Cocci	+	+	+
As:Lu5	Light orange, tiny, circular colonies.	Rod	+	+	+
AS Td: 1g	Entire, convex, smooth colonies.	Cocci	+	+	+
AS: Td13	Yellow, mucoid, round colonies	Cocci	+	+	+
AS:TD9	Light pink colonies, with entire edges.	Cocci	+	+	+
ASTd:3e	Saffron colored colonies, circular and elevated.	Cocci	+	+	+
AS:Td 3a	Bright yellow, dry colonies, circular colonies.	Cocci	+	+	+
AS:Td:18a	Yellow, dry, elevated, round colonies.	Cocci	+	+	+
AS:Td:18d	Yellow, round elevated, medium sized colonies.	Cocci	+	+	+
AS:Td:18e	Dry, elevated, circular colonies.	Cocci	+	+	+
P1Td.b1	Small, shiny, circular, yellow colonies.	Rod	-	-	-
ASTd:12u	Tiny, white, circular colonies.	Rod	-	-	-
ASTd:1e	Shiny, off white, circular colonies.	Rod	-	-	-
AS:Td:17:18	Colonies with brown pigmentation, irregular colonies	Rod	-	-	-
ASTd:16e	off white, circular, small, shiny colonies.	Rod	+	+	+

ASTd:18e	Shiny, white, small colonies.	Rod	+	+	+
ASCL:18:1	Thick, opaque, large, waxy colonies	Rod	+	+	+
AS:Ch:18:C	Greyish smooth colonies, round.	Rod	-	-	+
ASCL:17:h	Filamentous growth, dry, white colonies.	Rod	+	+	+
ASCL: 16:a	Off white, rhizoid colonies.	Rod	+	+	+
ASCL:15:3i	Spindle shaped, medium sized colonies	Rod	+	+	+
ASCL:18:23	Translucent colonies, medium sized, growth compact with the growth medium	Rod	+	+	+
AS:Cl:17:6 (S)	Red pigmented, round, medium sized colonies	Cocci	+	+	+
ASCL:18:14	Dry, white, fuzzy colonies.	Rod	+	+	+
ASCl:2a	Large, flat, waxy colonies.	Rod	+	+	+
ASCl:17a	Large, circular, milky white colonies.	Rod	+	+	+
AS: Cl 18g	White, small, circular, mucoid colonies.	Cocci	+	+	+
AS:BM1	Bright yellow, small, shiny, circular colonies.	Cocci	+	+	+
ASCL:2g	Yellowish, large, round colonies.	Cocci	+	+	+
AS: Cl: 17: 11	pink and yellow mixed, elevated colonies	Cocci	+	+	-
ASCL:16f	Light pink, round, smooth colonies.	Cocci	+	+	+

AS:Ct1	Light yellow, circular, convex colonies.	Cocci	+	+	+
ASCL:17k	Off white, small, round colonies.	Cocci	+	+	+
AS:Pe:16:D3	Red, irregular edged colonies.	Cocci	+	+	+
AS:Pe2	fuzzy white colonies with, rough edges, protruding sharply in center	Cocci	+	+	+
ASPe:7	Wavy edges, white, raised colonies.	Rod	+	+	+
AS:Pe:18:11	Smooth, convex, small, translucent, creamy colonies	Rod	-	-	-
AS:Pe:18:9	Creamy, small, shiny colonies	Rod	-	-	+
ASPe:16	Thick, mucoid, waxy colonies.	Rod	+	+	+
ASPe:15:b	White, umbonate, large colonies.	Rod	+	+	+
ASPe:18:s	Thick, large, irregular edged colonies.	Rod	+	+	+
ASPe:1a	Medium sized, smooth, flat colonies.	Rod	+	+	+
ASPe:16g	Milky white, circular, small, round colonies.	Cocci	+	+	+
ASPe:16u	Off white, round, medium sized colonies.	Cocci	+	+	+
ASPe:1	Smooth, white, circular, convex colonies.	Cocci	+	+	+
ASPe:5	Dry, yellowish colonies with irregular edges.	Cocci	+	+	+
ASPe:Da	Smooth, white, medium sized colonies.	Cocci	+	+	+

AS: Pe: 17:1	Dry, dark yellow, dry colonies	Cocci	+	+	+
AS:Pe:18:W5	Swarming growth, with light pigmentation.	Rod	-	-	+
ASPe:18	Smooth, round, white colonies.	Cocci	+	+	+
ASPe:18c	Milky white, circular, clear edged colonies.	Cocci	+	+	+
ASPe:16:6	Shiny, off white, small colonies.	Cocci	+	+	+
AS:Pe:17:N (S)	Red pigmented, medium sized, round colonies.	Rod	-	-	+
ASPe:1a	White, small, round colonies.	Rod	+	+	+
ASPe:16g	Yellow, small, shiny, circular colonies.	Rod	+	+	+
ASPe:17(2)	White, small, shiny, circular colonies.	Rod	+	+	+
ASPe:18g)	Transparent, small, round colonies.	Rod	+	+	+
ASPe:18:9	Yellow, small shiny, circular colonies.	Rod	+	+	+
ASLa:1h	White, raised undulate colonies.	Rod	+	+	+
ASLa:4i	Thick, mucoid, waxy colonies.	Rod	+	+	+
ASLa:8	White, large colonies, raised in center.	Rod	+	+	+
AS:La:18:I (En)	Irregular, large undulate colonies	Rod	+	+	+

ASLa:21f	Thick, large, irregular edged colonies.	Rod	+	+	-
ASLa:22a	Medium sized, smooth, white colonies.	Rod	+	+	-
ASLa:21	Wavy edged, white, raised colonies.	Rod	+	+	+
AS: La: 16:1 (C)	Smooth, convex, small, translucent, creamy colonies	Rod	-	-	-
ASLa:21g	Off white, large, waxy colonies.	Rod	+	+	+
ASLa:21k	White, umbonate, large colonies.	Rod	+	+	+
ASLa:2	Yellow, large lobate colonies.	Cocci	+	+	+
ASLa:5	White, flat, large colonies.	Cocci	+	+	+
ASLa:17b	Raised, wavy, white colonies.	Cocci	+	+	+
ASLa:17h	White, small, circular, shiny colonies.	Cocci	+	+	+
ASLa:17c	Small, circular, smooth, white colonies.	Cocci	+	+	+
AS: La: 16:a (M)	Dark yellow, smooth colonies.	Cocci	+	+	+
ASLa:16h	Small, circular, shiny, yellow colonies.	Cocci	+	+	+
ASLa:17(10)	Off white, medium sized circular colonies.	Cocci	+	+	+
ASCh13b	Translucent, colonies compact in media, dry.	Rod	+	+	+

ASCh:11c	White, circular, large, raised colonies.	Rod	+	+	+
AS: Ch2	Slightly yellow colonies with fuzzy growth.	Rod	+	+	+
ASCh:5i	White, dry, fuzzy colonies	Rod	+	+	+
ASCh:5b	Translucent, medium sized, flat colonies.	Rod	+	+	+
AS:Ch:1r	Thick mucoid, spindle shaped colonies.	Rod	+	+	+
ASCh:17d	Mucoid, small, circular, smooth colonies.	Cocci	+	+	+
AS:Ch 17	Mucoid, small, smooth colonies.	Cocci	+	+	+
ASCh:A1	Light yellow, circular, thick, waxy colonies.	Cocci	+	+	+
ASCh:14	Thick, mucoid, off-white colonies.	Cocci	+	+	+
AS:CH: 17:13	Opaque, circular, yellow colonies.	Cocci	+	+	+
AS:Ch 28	small, yellow, circular colonies	Rod	-	-	-
AS: Td: 19	Light yellow, round, smooth, colonies.	Cocci	+	+	+
AS: Ch15	Bright yellow, dry, elevated, circular colonies.	Cocci	+	+	+
AS:Ch(a)	Dry, yellow, circular colonies.	Cocci	+	+	+
ASCh:P2	Smooth, elevated, small, circular colonies.	Cocci	+	+	+
AS:CH:17:16	Red, irregular edged colonies.	Cocci	+	+	+
AS:Ch: 18:14	Medium, flat, circular, glossy colonies	Rod	-	-	+

AS:CH: 17:5	Creamy, small, shiny colonies	Rod	-	-	+
ASCH:c	Small shiny, circular, off-white colonies.	Rod	-	-	-
ASCh:1d	Small, white, round colonies.	Rod	-	-	-
ASCh:16i	Small, yellow, shiny, circular colonies.	Rod	+	+	+
ASCh:17m	Small, yellow, smooth, circular colonies.	Rod	+	+	+
AS:Ch:18:3	Irregular, large undulate colonies	Rod	+	+	+
ASCh:17o	Small, white, irregular edged colonies.	Rod	+	+	+
AS:CH:18:10 (S)	Red pigmented, medium sized, round colonies.	Rod	-	-	+
AS:Mn11	Large, white colonies with irregular edges.	Rod	+	+	+
AS: Mn26	Opaque off white small shiny, circular colonies	Rod	+	+	+
AS Mn: 1d	Large, white, umbonate colonies.	Rod	+	+	+
AS:Mn:1c	Large, off-white colonies with irregular margin	Rod	+	+	+
ASMB:1d	Yellow, small shiny, circular, convex colonies.	Cocci	+	+	+
AS:Mn:16:5 (M)	Red, irregular edged colonies.	Cocci	+	+	+
ASMB:B24	Medium sized, shiny, circular colonies.	Cocci	+	+	+

ASMn:12	Small, yellow, shiny, circular colonies.	Cocci	+	+	+
AS:Mn3	bright yellow, small shiny, circular	Cocci	+	+	+
AS: Mn: 17:4 (M)	small, yellow, circular colonies	Cocci	+	+	+
AMn:18	Medium sized, clear, smooth, round colonies.	Cocci	+	+	+
ASMn:2a	Small, circular, smooth, off-white colonies.	Rod	-	-	-
ASMn:16j	Brownish, tiny, round colonies.	Rod	-	-	-
ASMn:17m	Large, mucoid, filamentous colonies.	Rod	+	+	+
AS: Ra1d	large, flat yellowish colonies, with irregular edges.	Rod	+	+	+
ASRa:6d	Large, dry, irregular shaped colonies.	Rod	+	+	+
AS Ra: 18:g	Large, circular, shiny, flat colonies.	Rod	+	+	+
AS Ra: 16 b	Large, mucoid colonies with wavy margin.	Rod	+	+	+
ASRa:17c	White, flat, large colonies.	Rod	+	+	+
AS:Ra:18:5	Irregular, large undulate colonies	Rod	+	+	+
ASRa17:e	Yellow, large, wavy colonies.	Cocci	+	+	+
AS Ra: 2d	White, medium sized, round colonies.	Cocci	+	+	+
ASRa:16a	Off white, medium sized, smooth colonies.	Cocci	+	+	+

ASRa:18e	Small, yellow, circular colonies.	Cocci	+	+	+
AS: RA 18	small, off white, circular colonies.	Cocci	+	+	+
AS: RA 18z	Medium sized, yellow, smooth colonies.	Cocci	+	+	+
AS:RA:18m	Clear, smooth colonies, convex.	Cocci	+	+	+
ASRa:18 a	Yellow, circular, medium sized colonies.	Cocci	+	+	+
ASRa:1b	Thick, tiny, circular, white colonies.	Rod	+	+	+
ASRa:16e	Thick large yellow, umbonate colonies.	Rod	+	+	+
ASRa:17(13)	Thick, flat surface, elevated in center.	Rod	+	+	+
AS: Ra: 16:7 (P)	Swarming growth, with light pigmentation.	Rod	-	-	+
ASRa17d	Thin, small, off-white colonies.	Rod	-	-	-
AS:Ra:18c (M)	Pink, medium sized, round colonies	Cocci	+	+	+
AS:Ra:17:9 (E)	Greyish smooth colonies, round.	Rod	-	-	+
Note: +, positive; -, negative.					

Table 16: Biochemical tests of Gram-positive rods isolates from air samples.

Representative Isolates	Growth in/at different						Sugar fermentation							Biochemical tests						Tentative identification
	NaCl conc.		Temp.		pH															
	6%	10%	10°C	45°C	3.9	9.6	Dextrose	Fructose	Mannose	Mannitol	Raffinose	Sucrose	Glucose	Starch	VP	Citrate	Nitrate	Oxidase	Urease	
As:Lu5	+(11)	+(6) -(5)	+(11)	+ (4) -(7)	+(11)	+(4) -(7)	+(7) -(4)	+(11)	+(7) -(4)	+(4) -(7)	-(11)	+(7) -(4)	+(11)	-(11)	+(4) - (7)	+(4) -(7)	+(4) -(7)	+(8) -(3)	-(11)	*Bacillus* sp.
As:Mn26	+(10)	+(8)-(2)	+(10)	+(6)-(4)	+(10)	+(4)-(6)	+(7)-(3)	+(10)	+(7)-(3)	+(3)-(7)	-(10)	+(8)-(2)	+(10)	+(7)-(3)	+(2)-(8)	+(4)-(6)	+(4)-(6)	+(3)-(7)	-(10)	*Bacillus* sp.
A:Ksc5	+(7)	+(4)-(3)	+(7)	+(4)-(3)	+(7)	+(3)-(4)	+(4)-(3)	+(7)	+(4)-(3)	+(3)-(4)	-(7)	+(4)-(3)	+(7)	-(7)	+(5)-(2)	+(5)-(2)	+(3)-(4)	+(2)-(5)	-(7)	*Bacillus* sp.
AS:Ra1d	+(7)	+(3)-(4)	+(7)	+ (4)-(3)	+(7)	+(2)-(5)	+(2)-(5)	+(7)	+(5)-(2)	+(2)-5()	-(7)	+(2)-(5)	+(7)	+(1)-(6)	+(6)-(1)	+(4)-(3)	+(2)-(5)	+(2)-(5)	+(1)-(6)	*Bacillus* sp.

-, negative; +positive; the number in the parenthesis () indicates, number of isolates.

AS-De16(h)	AS:Mn11	AS:Td3	AS:Lu2	AS:Ch2	AS:Pe2
+(3)	+(6)	+(5)	+(6)	+(7)	+(6)
+(3)	+(4)-(2)	+(3)-(2)	+(1)-(5)	+(4)-(3)	+(4)-(2)
+(3)	+(6)	+(5)	+(6)	+(7)	+(6)
+(3)	+(4)-(2)	+(4)-(1)	+(4)-(2)	+(5)-(2)	+(4)-(2)
+(3)	+(6)	+(5)	+(6)	+(7)	+(6)
-(3)	+(3)-(3)	+(2)-(3)	+(3)-(3)	+(2)-(5)	+(4)-(2)
+(3)	+(2)-(4)	+(2)-(3)	+(3)-(3)	+(3)-(4)	+(2)-(4)
+(3)	+(6)	+(5)	+(6)	+(7)	+(6)
+(3)	+(2)-(4)	+(2)-(3)	+(3)-(3)	+(3)-(4)	+(2)-(4)
+(3)	+(4)-(2)	+(3)-(2)	+(3)-(3)	+(4)-(3)	+(4)-(2)
-(3)	-(6)	-(5)	-(6)	-(7)	-(6)
-(3)	+(2)-(4)	+(2)-(3)	+(3)-(3)	+(3)-(4)	+(2)-(4)
-(3)	+(6)	+(5)	+(6)	+(7)	+(6)
-(3)	-(6)	-(5)	-(6)	-(7)	+(1)-(5)
+(3)	+(3)-(3)	+(2)-(3)	+(3)-(3)	+(4)-(3)	+(5)-(1)
-(3)	+(3)-(3)	+(2)-(3)	+(3)-(3)	+(5)-(2)	+(5)-(1)
-(3)	+(3)-(3)	+(2)-(3)	+(3)-(3)	+(2)-(5)	+(4)-(2)
+(3)	+(2) W+(3)	+(3)-(2)	+(3)-(3)	+(2)-(5)	+(2)-(4)
+(3)	-(6)	-(5)	+(1)-(5)	-(7)	-(6)
unidentified	*Bacillus* sp.	*Bacillus* sp.	*Bacillus* sp.	*Bacillus* sp.	*Bacillus* sp.

Table 17: Biochemical tests of Gram-positive cocci from air samples.

Representative Isolates	Growth in/at different						Sugar fermentation							Biochemical tests									Tentative identification
	NaCl conc.		Temp.		pH																		
	6%	10%	10°C	45°C	3.9	9.6	Dextrose	Fructose	Mannose	Mannitol	Raffinose	Sucrose	Glucose	Coagulase	Colour change in MSA	Starch	VP	Citrate	Nitrate	Oxidase	Urease	Hemolysis	
AS:Ksc:17(a)	+(16)	+(16)	+(16)	+(16)	-(16)	-(16)	-(16)	+(16)	+(16)	+(4)	-(16)	+13)	+(16)	-(16)	+(9)	-(16)	+8)	-(16)	+(11)	(16)	+(9)	-(16)	*Staphylococcus* sp.
A:Cnt:1	+(22)	+(22)	+(22)	+(16)	-(22)	-(22)	-(22)	+(22)	+(22)	+(2)	-(22)	+(20	+(22)	-(22)	+(12)	-(22)	+(7)	-(22)	+(13)	-(22)	-(22)	-(22)	*Staphylococcus sp.*
AS:La8	+(21)	+(21)	+(21)	+(16)	-(21)	-(21)	-(21)	+(19)	+(21)	+(12)	+(2) -(19)	+(2-(19)	+(10)	-(21)	+(14)	-(21)	-(21)	-(21)	-(21)	-(21)	-(21)	-(21)	unidentified

P3Tdm	+(20)	+(20)	+(20)	+(16)	-(20)	-(20)	-(20)	+(20)	+(20)	+(15)	-(20)	+(4)	+(20)	-(20)	-(8)	-(20)	+(12)	-(20)	-(20)	-(20)	+(6)	-(20)	unidentified
AS:Ra:18c	+(12)	+(12)	+(12)	+(12)	+(12)	-(12)	-(12)	+(12)	+(12)	-(12)	+(12)	+(12)	-(12)	-(12)	-(12)	-(12)	-(12)	-(12)	-(12)	-(12)	-(12)	-(12)	*Micrococcus sp.*

-, negative; +positive; the number in the parenthesis () indicates, number of isolates.

Table 18: Biochemical tests of Gram-negative bacteria isolates from air samples.

Representative isolates	AS:KSC18	AS:De5	AS:Mb7	AS:Ch:19
Dextrose	-(10)	-(10)	-(12)	-(9)
Fructose	-(10)	-(10)	-(12)	-(9)
Mannose	-(10)	-(10)	-(12)	-(9)
Mannitol	+(10)	+(10)	+(12)	+(9)
Raffinose	-(10)	-(10)	-(12)	-(9)
Sucrose	-(10)	-(10)	-(12)	-(9)
Glucose	-(10)	-(10)	-(12)	-(9)
Indole	-(10)	-(10)	-(12)	-(9)
MR	-(10)	-(10)	-(12)	-(9)
VP	-(10)	-(10)	-(12)	-(9)
Citrate	+(8)-(2)	+(8)-(2)	+(10)-(2)	+(9)
Nitrate	+(10)	+(10)	+(12)	+(9)
Oxidase	+(10)	+(10)	+(12)	+(9)
Urease	-(10)	-(10)	-(12)	-(9)
Tentative identification	*Pseudomonas* sp.	*Pseudomonas* sp.	*Pseudomonas* sp.	unidentified

-, negative; +positive; the number in the parenthesis () indicates, number of isolates.

AS:Ra:18:5	AS: La: 16:1	AS:CH:18:10	AS:De:16:4	AS:Ch:18:14	ASRa17d
-(3)	-(4)	-(4)	-(6)	-(5)	-(4)
-(3)	-(4)	+(4)	-(6)	-(5)	-(4)
+(3)	+(4)	+(4)	-(6)	-(5)	-(4)
+(3)	+(4)	+(4)	-(6)	+(5)	+(4)
+(3)	-(4)	-(4)	-(6)	-(5)	-(4)
-(3)	+(4)	+(4)	-(6)	+(2)-(3)	-(4)
+(3)	-(4)	+(4)	+(6)	+(5)	-(4)
-(3)	-(4)	-(4)	-(6)	+(5)	-(4)
-(3)	+(4)	-(4)	+(6)	+(5)	-(4)
+(3)	-(4)	+(4)	-(6)	-(5)	-(4)
+(3)	+(4)	+(4)	+(6)	-(5)	+(4)
+(3)	+(4)	+(2)-(2)	+(6)	+(5)	+(4)
-(3)	-(4)	-(4)	-(6)	-(5)	+(4)
-(3)	+(2) -(2)	+(4)	+(6)	-(5)	-(4)
Enterobacter	*Citrobacter*	*Serratia*	*Proteus* sp.	*Escherichia coli*	Unidentified

Table 19: Representative strains of bacteria from air samples.	
Isolate code	**Representative isolate**
ASLa:4i, ASCh:11c, AS:De:B3, ASTd:3d, ASCh:5i, ASMn:3t, ASDe:17d, ASCL:15:3i ASRa:18:g, ASKC: 16:I, ASRa: 2d	AS:Lu5
ASLa:21f, ASRa:1g, ASCh:13o, ASPe:3f, AS:Si:1g, ASLa:21, ASCL:18:1, ASCL:18:14, ASRa:17c, AS:Ch:1r	As:Mn26
ASTd:2b, ASKC17a, ASPe:7, ASDe:15:d, ASCL:17:h, ASRa17:e,ASSi:16d	A:Ksc5
ASLa:22a, ASMn:1A, ASMn:1d, ASRa:6d, ASCL: 16:a, ASCL: 16:g, AS: Ra1d	AS:Ra1d
AS: Ch2, AS Td: Pi , AS:Ksc9, AS:Ksc7d, ASDe:18:f, ASCL:18:23, ASPe:18:s	AS:Ch2
AS Td: Pi , AS:KSC9, ASLa:21k, ASDe:16:v, ASCL:18:23, ASSi:16:g	AS:Lu2
ASCh13b, AS:Pe2, ASMn:1d, AS: Tdq, ASSi:18:a, ASPe:16	AS:Pe2
AS Td: 1g, ASDe:16:v, ASPe:17, AS:Mn:1c, ASSi:17:a	AS:Td3
ASLa:8, AS:Td (a), ASTD: 3it, AS:Mn:1c, AS Td: 1g, ASDe:16:i	AS:Mn11
ASDe:6, ASSi:2n, ASPe:1a, ASDe:1, Assing:1, ASCL:16f, ASPe:16g, ASPe:16u, ASDe:17 b, ASSi:17k, AS:Ksc5, ASCl:2a ASCl:17a, ASPe:1, ASLa:2, ASLa:5	AS:Ksc:17(a)
ASLa:17b, ASLa:17h, ASCh:17d, ASLa:17c, AS:Ch 17, ASCh:A1, ASMB:1d, ASRa:17e, ASDe :21, ASDe:10 ASDe18s ASDe:P1 ASDe:18i ASDe:18a, ASDe:18c, Asde:18 AS:KSC7 Assing:12 Assing:18 ASSg: 14n AsSg: 14k	A:Cnt:1
AS:Sg3 AS:Sg6, ASSi:19r, ASSi:T4, ASKsc:12, ASKsc:29, ASKSc:14a, AsKSc:18d, ASKsc18b, AS: Td13, AS:TD9, P1Tdb, ASTd:3e, AS:Td 3a, AS:Td:18a, AS:Td:18d,	AS:La8

AS:Td:18e, AS: Cl 18g, AS:Ct1, AS:BM1, ASCl:18f, ASCl:p1	AS:Ct:1
ASCL:p5, ASPe:5, ASPe:Da, ASPe:18, ASPe:18c, ASLa:5a, ASLa:10, ASLa:18b, ASCh:14, ASCh:14(B), AS:Ch 28, AS: Ch15, AS:Ch(a), ASCh:P2, ASMB:B24, ASMn:12, AS:Mn3, AMn:18, ASRa:16a, ASRa:18e	P3Tdm
ASSi:2n, ASSi16a, AASDe17:d, ASKSC:1d, ASKSC:16d, ASKSC:17i, ASKSC:18l, ASTd:1e, ASTd:16e, ASTd:18e	AS:Ksc:18
ASCL:2g, ASCL:16f, ASCL:17k, ASLa:2c, ASLa:16h, ASLa:17(10), ASPe:1a, ASPe:16g, ASPe:17(2), ASPe:18g), ASCh:1d,	ASCh:16i
ASCh:17m, ASCh:17o, ASMn:2a, ASMn:16j, ASMn:17m, ASRa:1b, ASPe:18:9, ASLa17:7, ASDe:6	AS:Ch: 18:14
ASDE:16s, ASRa:16e, ASRa:17(13),	ASRa17d
AS:Si:18:6 , AS:Si:17:g , AS:Td:17:18 , AS:Pe:18:W5 , AS: Ra: 16:7	AS:De:16:4
AS:De:18: 5 , AS:Si:16:8 , AS:KSC: 18:9 , AS:Td: 17:2 , AS: Cl: 17: 11 , AS:Pe:16:D3 , AS: Pe: 17:1 , AS: La: 16:a , AS:CH: 17:13, AS:Mn:16:5 , AS:Ra:18c.	AS:CH:17:16
AS:Ch:18:C, AS:Pe:18:9, AS:Ch: 18:14 , AS:CH: 17:5, AS:Ra:17:9	AS:Ra:17:9
AS:De:17:5 , AS:KSC:17:21, AS:Pe:18:11	AS: La: 16:1
AS:De:17:14 , AS:KSC:17:f , AS:Pe:17:N	AS:CH:18:10
AS:La:18:I , AS:Ch:18:3	AS:Ra:18:5

Phenotypic characterization of bacteria isolates using Omnilog Plus system

Phenotypic characterization of selected bacterial isolates was conducted using Omnilog Plus system (BIOLOG), a fully automated high throughput aerobic identification system.

Ten bacteria isolates were subjected to the test however, results of only the four isolates could be obtained. Hence the remaining six bacteria isolates remained unidentified (Table 20).

Table 20: Phenotypic identification of bacterial isolates using Omnilog Plus system.

Isolates	**Protocol category**	**Plate used**	**Fluid**	**Identification**
ASCh1	A	Gen III	A	*Staphylococcus haemolyticus*
P3Tdm	A	Gen III	A	not identified
CH2	A	Gen III	A	*Lysynibacillus sphaerius*
ASPe2	A	Gen III	A	*Corynebacterium sphaerius*
ASLu5	A	Gen III	A	*Bacillus flexus*

Note: For aerobic microorganisms protocol A is followed.

RESULT FOR ANTIBIOTIC SUSCEPTIBILITY TESTING

The strains of a Gram positive and Gram negative was taken as control strains. *Escherichia coli* MTCC 1089, *Staphylococcus aureus* MTCC 7443 were considered for this test. The control was used for every set of tests performed. The test strains were inoculated on Muller-Hinton agar and was incubated at 37° C for 18- 24 h. The zone of inhibition was observed, and the results compared with that recommended by the Clinical and Laboratory Standards Institute, CLSI (2014) with the reference strains *E. coli* ATCC 25922 and *S. aureus* ATCC 25923 (Table 21). The test showed positive results; hence it was evident that the tests were performed well and the antibiotics could be used for further tests. Tests performed with the control strains shown in (Table 21). Results were compared with the standard charts of CLSI (2014). As our results were similar to those of CLSI, 2014 we were convinced that the test was properly performed.

Table 21: Zone interpretation chart

Antibiotics	**Symbol**	**Disc content (mcg)**	***E. coli* MTCC 1089**	***S. aureus* MTCC 7443**
Ampicillin	AMP	10	16	32
Amikacin	AK	10	24	30
Amoxicillin	AMX	30	0	28
Chloramphenicol	C	30	26	26
Clindamycin	CD	2	>10	28
Erythromycin	E	15	21	26
Methicillin	MT	5	0	20
Norfloxacin	Nx	10	29	28
Streptomycin	S	10	0	21
Penicillin-G	PEN-G	10	0	25
Tetracycline	TET	30	25	30
Vancomycin	VAN	10	<10	17

Note: The zone size was matched with the reference strains *E. coli* ATCC 25922 and *S. aureus* ATCC 25923 recommended by CLSI, (2014).

Result for AST pattern of bacteria isolates

The isolates which showed maximum number of occurrences, showed variation in the morphological, physiological and biochemical tests were considered for antimicrobial susceptibility testing (AST). Hence, the species of *Bacillus*, *Staphylococcus* and *Pseudomonas* were tested for antibiotic susceptibility. Thus, the isolates were tested against 12 antibiotics; ampicillin, amikacin, amoxycillin, chloramphenicol, clindamycin, erythromycin, methicillin, norfloxacin, penicillin G, streptomycin, tetracycline and vancomycin.

AST results for *Bacillus* sp. from air samples by disc diffusion method.

In *Bacillus* sp. the observed zone of diameter for susceptible was 14-36mm, for intermediate 10-20mm and for resistance 0- 28mm (Table 22). 100 % of the isolates were susceptible to Streptomycin, followed by amoxycillin (98.7%), erythromycin (97.4%), norfloxacin (97.4%). Isolates showed equal susceptibility to Vancomycin and chloramphenicol (87.01%). The susceptibility to amikacin, clindamycin, methicillin, Pen-G were 83.21%, 56.21%, 50.64% and 54.54%, respectively. Maximum resistance showed by the isolates was against Penicillin-G (45.45%) followed by methicillin (25.97%) (Table 23, Figure 22). The isolates showing maximum intermediate zone of inhibition was methicillin 23.37%. The isolates did not show any resistance against erythromycin, Streptomycin and tetracycline (Table 23, Figure 22).

Table 22: Zone size in *Bacillus* sp. using disc diffusion method												
Strain code	**Antibiotics**											
	(AMP $_{10}$)	**(AK$_{10}$)**	**(AMX30)**	**(C$_{30}$)**	**(CD2)**	**(E$_{15}$)**	**MT$_5$)**	**(NX$_{10}$)**	**(PEN-G)**	**(S$_{10}$)**	**(TET$_{30}$)**	**(VAN$_{10}$)**
ASLa:1h	31-S	22-S	28-S	20-S	25-S	24-S	<10-R	19-S	21-R	30-S	34-S	27-S
ASLa:4i	36-S	21-S	29-S	21-S	23-S	31-S	14-S	24-S	31-S	27-S	18-S	29-S
ASLa:8	25-R	20-S	33-S	15-I	26-S	27-S	15-S	21-S	24-R	27-S	32-S	22-S
ASLa:21f	21-R	>10-R	30-S	27-S	30-S	30-S	10-I	22-S	29-S	28-S	23-S	27-S
ASLa:22a	36-S	32-S	36-S	28-S	21-S	36-S	<10-R	23-S	30-S	36-S	24-S	18-S
ASSi:17r	35-S	21-S	31-S	26-S	20-I	34-S	14-S	28-S	25-R	31-S	30-S	19-S
ASCh13b	33-S	21-S	32-S	31-S	19-I	30-S	18-S	24-S	34-S	20-S	25-S	16-S
ASCh:11c	29-S	32-S	36-S	33-S	0-R	24-S	0-R	21-S	34-S	33-S	26-S	27-S

AS:De:B3	35-S	22-S	26-S	25-S	21-S	25-S	18-S	18-S	30-S	19-S	25-S	16-S
ASMn:1A	32-S	22-S	25-S	13-I	21-S	27-S	16-S	20-S	0-R	27-S	19-S	17-S
ASPe2	33-S	19-S	33-S	14-I	28-S	23-S	<10-R	28-S	34-S	35-S	21-S	15-S
CH2	31-S	21-S	33-S	26-S	33-S	24-S	10-I	26-S	29-S	31-S	20-S	18-S
AS: Ra1d	30-S	27-S	28-S	22-S	25-S	21-I	14-S	22-S	30-S	20-S	35-S	17-S
ASRa:1g	33-S	21-S	32-S	25-S	27-S	31-S	19-S	19-S	14-R	28-S	24-S	20-S
ASTd :3d	>36-S	9-R	21-S	20-S	25-S	26-S	19-S	25-S	32-S	33-S	30-S	17-S
ASTd: Pi	26-R	18-S	21-S	20-S	26-S	25-S	<10-R	22-S	28-R	28-S	16-S	29-S
ASMn: 1d	26-R	29-S	34-S	16-S	28-S	24-S	15-S	21-S	22-R	25-S	31-S	19-S
AS:Mn:1c	36-S	10-R	22-S	22-S	30-S	27-S	10-I	18-S	20-R	31-S	15-S	22-S
ASTd: 1g	33-S	16-R	32-S	21-S	0-R	28-S	0-R	25-S	22-R	28-S	17-S	17-S

AS:KSc7	35-S	21-S	21-S	20-S	18-I	24-S	<10-R	26-S	21-R	30-S	34-S	18-S
ASCh:5i	31-S	18-S	30-S	18-S	21-S	23-S	15-S	21-S	31-S	27-S	18-S	19-S
AS:Ksc9	30-S	22-S	25-S	25-S	20-I	27-S	19-S	19-S	24-R	27-S	19-S	17-S
ASLa:21	29-S	21-S	28-S	23-S	18-I	30-S	16-S	20-S	29-S	28-S	23-S	26-S
AS:Td3	36-S	25-S	36-S	15-I	28-S	36-S	<10-R	23-S	31-S	34-S	24-S	17-S
AS:Lu2	35-S	25-S	31-S	14-I	27-S	34-S	11-I	28-S	28-S	31-S	31-S	17-S
AS:MN11	26-R	21-S	31-S	20-S	19-I	30-S	18-S	17-S	34-S	20-S	23-S	17-S
ASCh:13o	32-S	20-S	30-S	26-S	22-S	24-S	0-R	19-S	34-S	33-S	26-S	18-S
AS: Mn26	35-S	22-S	28-S	25-S	20-I	25-S	18-S	22-S	30-S	19-S	18-S	17-S
ASKC17(a)	33-S	18-S	21-S	18-S	18-I	23-S	<10-R	24-S	0-R	17-S	32-S	21-S
AS:De 16(h)	35-S	26-S	26-S	10-R	17-I	27-S	13-I	10-R	30-S	20-S	22-S	18-S

ASRa:6d	33-S	20-S	21-S	21-S	24-S	29-S	0-R	19-S	33-S	27-S	24-S	17-S
ASMn :3t	>36-S	10-R	30-S	20-S	26-S	28-S	14-S	22-S	31-S	36-S	31-S	18-S
ASlu:5	21-R	24-S	28-S	25-S	28-S	21-I	<10-R	21-S	25-R	18 -S	25-S	18-S
AS:Td q	31-S	22-S	27-S	22-S	32-S	28-S	14-S	21-S	24-R	33-S	32-S	19-S
ASPe: 3f	32-S	22-S	29-S	18-S	20-I	29-S	13-I	25-S	17-R	23-S	31-S	22-S
ASDe: P1	26-R	18-S	30-S	21-S	26-S	25-S	<10-R	22-S	31-S	28-S	16-S	15-R
ASRa: 2d	26-R	29-S	31-S	16-I	28-S	24-S	0-R	19-S	22-R	25-S	31-S	19-S
AS:Ch:1r	25-R	30-S	32-S	22-S	29-S	27-S	10-I	18-S	20-R	31-S	26-S	18-S
AS:Si 1g	32-S	26-S	19-R	22-S	26-S	32-S	15-S	24-S	31-S	28-S	16-S	25-S
ASCh:5b	28-R	21-S	31-S	19-S	27-S	25-S	18-S	23-S	25-R	22-S	24-S	>10-R

AS:Ksc7d	30-S	19-S	23-S	27-S	28-S	23-S	18-S	18-S	21-R	25-S	19-S	20-S
ASLa:21g	35-S	23-S	31-S	16-I	23-S	36-S	16-S	26-S	28-R	31-S	23-S	17-S
ASDe:15:d	29-S	18-S	21-S	22-S	21-S	21-S	15-S	17-S	20-R	29-S	31-S	21-S
ASDe:17d	30-S	17-S	20-S	21-S	24-S	26-S	16-S	20-S	30-S	16-S	16-S	18-S
ASDe:16:v	31-S	21-S	31-S	25-S	28-S	31-S	>10-R	22-S	29-S	26-S	18-S	22-S
ASDe:18:f	33-S	22-S	25-S	32-S	31-S	33-S	12-I	23-S	28-R	22-S	21-S	21-S
ASDe:16:i	34-S	19-S	32-S	28-S	26-S	30-S	16-S	25-S	30-S	25-S	20-S	18-S
ASSi:18:a	36-S	23-S	36-S	24-S	22-S	23-S	15-S	27-S	25-R	29-S	29-S	17-S
ASSi:16d	34-S	21-S	21-S	26-S	21-S	29-S	14-S	21-S	29-S	18-S	26-S	17-S
ASSi:16:g	29-S	20-S	22-S	22-S	27-S	27-S	11-I	20-S	31-S	19-S	21-S	28-S
ASSi:17:a	30-S	18-S	20-S	21-S	24-S	24-S	10-I	17-S	30-S	23-S	22-S	21-S

ASCL:18:1	28-R	17-S	21-S	21-S	27-S	23-S	18-S	19-S	27-R	24-S	15-S	19-S
ASCL:17:h	32-S	21-S	22-S	19-S	29-S	30-S	14-S	23-S	34-S	19 -S	20-S	17-S
ASCL: 16:a	27-R	23-S	25-S	27-S	32-S	27-S	15-S	21-S	30-S	21-S	17-S	18-S
ASCL:15:3i	31-S	24-S	26-S	26-S	22-S	22-S	11-I	24-S	31-S	24-S	18-S	17-S
ASCL:18:23	34-S	21-S	23-S	33-S	28-S	24-S	<10-R	18-S	24-R	25-S	17-S	18-S
ASCL:18:14	25-R	20-S	25-S	22-S	26-S	28-S	12-I	24-S	32-S	22-S	16-S	17-S
ASPe:17	31-S	17-S	21-S	19-S	20-R	21-S	14-S	22-S	29-S	29-S	21-S	18-S
ASSi:16:g	29-S	20-S	22-S	22-S	27-S	27-S	11-I	20-S	31-S	19-S	21-S	28-S
ASSi:17:a	30-S	18-S	20-S	21-S	24-S	24-S	10-I	17-S	30-S	23-S	22-S	21-S
ASCL:18:1	28-R	17-S	21-S	21-S	27-S	23-S	18-S	19-S	27-R	24-S	15-S	19-S
ASCL:17:h	32-S	21-S	22-S	19-S	29-S	30-S	14-S	23-S	34-S	19 -S	20-S	17-S

ASCL: 16:a	27-R	23-S	25-S	27-S	32-S	27-S	15-S	21-S	30-S	21-S	17-S	18-S
ASCL:15:3i	31-S	24-S	26-S	26-S	22-S	22-S	11-I	24-S	31-S	24-S	18-S	17-S
ASCL:18:23	34-S	21-S	23-S	33-S	28-S	24-S	<10-R	18-S	24-R	25-S	17-S	18-S
ASCL:18:14	25-R	20-S	25-S	22-S	26-S	28-S	12-I	24-S	32-S	22-S	16-S	17-S
ASSi:16:g	29-S	20-S	22-S	22-S	27-S	27-S	11-I	20-S	31-S	19-S	21-S	28-S
ASSi:17:a	30-S	18-S	20-S	21-S	24-S	24-S	10-I	17-S	30-S	23-S	22-S	21-S
ASCL:18:1	28-R	17-S	21-S	21-S	27-S	23-S	18-S	19-S	27-R	24-S	15-S	19-S
ASCL:17:h	32-S	21-S	22-S	19-S	29-S	30-S	14-S	23-S	34-S	19 -S	20-S	17-S
ASCL: 16:a	27-R	23-S	25-S	27-S	32-S	27-S	15-S	21-S	30-S	21-S	17-S	18-S
ASCL:15:3i	31-S	24-S	26-S	26-S	22-S	22-S	11-I	24-S	31-S	24-S	18-S	17-S

ASCL:18:23	34-S	21-S	23-S	33-S	28-S	24-S	<10-R	18-S	24-R	25-S	17-S	18-S
ASCL:18:14	25-R	20-S	25-S	22-S	26-S	28-S	12-I	24-S	32-S	22-S	16-S	17-S
ASPe:16	36-S	16-R	35-S	26-S	30-S	22-S	16-S	17-S	24-R	27-S	18-S	19-S
ASPe:15:b	29-S	18-S	27-S	29-S	24-S	26-S	10-I	25-S	14-R	31-S	22-S	20-S
ASRa: 18:g	32-S	19-S	31-S	15-I	28-S	24-S	<10-R	21-S	29-S	25-S	16-S	15-R
ASRa: 16 b	23-R	15-R	21-S	24-S	23-S	21-I	14-S	22-S	30-S	23-S	17-S	19-S
ASRa:17c	32-S	21-S	25-S	21-S	29-S	25-S	<10-R	19-S	29-S	16-S	15-S	21-S
ASRa17:e	29-S	25-S	22-S	27-S	22-S	27-S	10-I	24-S	21-R	22-S	16-S	16-R
ASKC: 16:i	36-S	28-S	26-S	25-S	19-I	32-S	17-S	25-S	27-R	21-S	30-S	27-S
ASPe:18:s	28-S	19-S	22-S	26-S	24-S	23-S	15-S	23-S	30-S	22-S	17-S	24-S

Note: Zone diameter in mm, results were expressed as per CLSI 2014. AMP, ampicillin; AK, amikacin; AMX, amoxycillin; C, chloramphenicol; CD, clindamycin; E, erythromycin; MET, methicillin; NX, norfloxacin; P, penicillin G; S, streptomycin; VA, vancomycin; TET, tetracycline. S, sensitive; I, intermediate, R, resistance.

Table 23: AST pattern of *Bacillus* sp. from air samples by disc diffusion method.						
Antibiotics	**Susceptible**		**Intermediate**		**Resistant**	
	No	%	No	%	No	%
AMP (10µg)	61	79.22	0	0	16	20.77
AK(10µg)	68	83.31	0	0	9	11.68
AMX (30µg)	76	98.7	0	0	1	1.29
C(30µg)	67	87.01	9	11.6%	1	1.29
CD(2µg)	60	56.21	13	11.68	4	5.19
E(15µg)	73	94.8	4	5.19	0	0
MT(5µg)	39	50.64	18	23.37	20	25.97
Nx(10µg)	75	97.4	1	1.29	1	1.29
S(10µg)	77	100	0	0	0	0
PEN-G(10µg)	42	54.54	0	0	35	45.45
TET(30µg)	77	100	0	0	0	0
VAN (10µg)	67	87.01	0	0	10	12.98

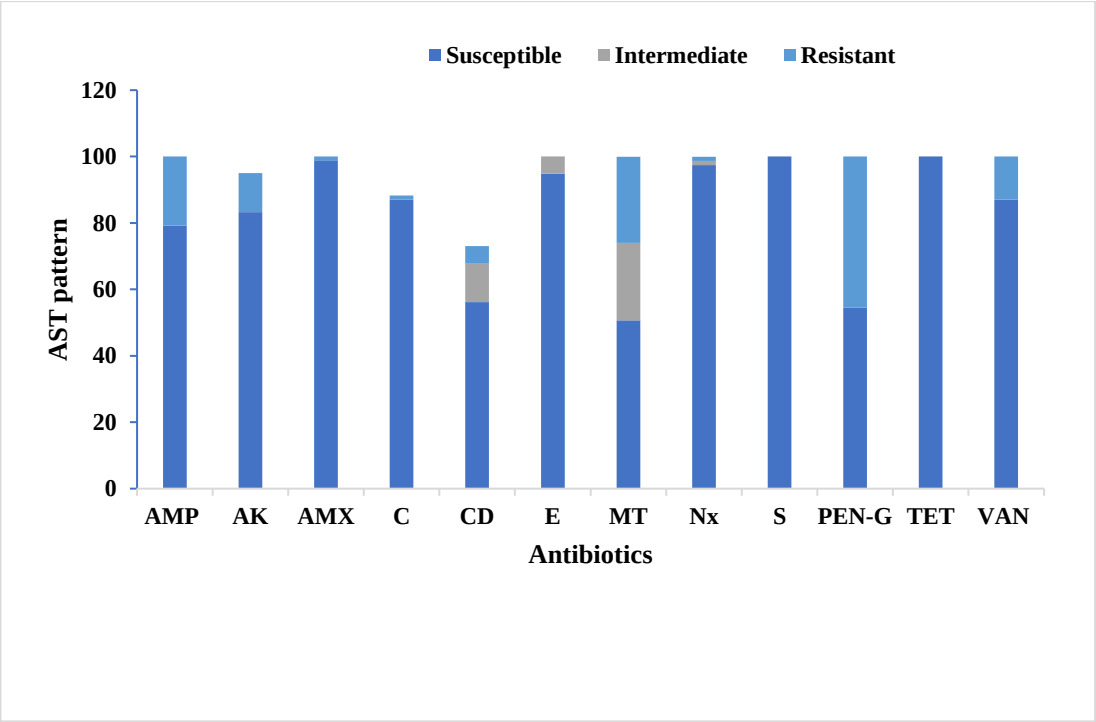


Figure 22: AST pattern of *Bacillus* sp. from air samples.

AST results for *Staphylococcus* sp. from air samples by disc diffusion method.

Staphylococcus sp. showed 100 % susceptibility to chloramphenicol and tetracycline. The isolates were least susceptible to PEN-G (39.75%). The isolates showed higher susceptibility against the antibiotic's amikacin (96.38%), amoxycillin (97.59%), Norfloxacin (86.74%), erythromycin (81.92%, vancomycin (80.72%) and clindamycin (75.9%). Maximum resistance was observed against Pen-G, while no resistance was observed for clindamycin, methicillin, tetracycline. Intermediate zone was observed for amikacin (1%), amoxycillin (1%), clindamycin (11%), erythromycin (12%), norfloxacin (8%) and vancomycin (5%) (Table 24, Figure 23).

Table 24: Zone of inhibition of *Staphylococcus* sp. from air samples by disc diffusion method.											
Strain code	**Antibiotics**										
	(AMP$_{10}$)	**(AK$_{10}$)**	**(AMX30)**	**(C$_{30}$)**	**(CD$_{2}$)**	**(E$_{15}$)**	**MT$_{5}$)**	**(NX$_{10}$)**	**(PEN-G)**	**(TET$_{30}$)**	**(VAN$_{10}$)**
ASDe:6	30-S	20-S	32-S	19-S	15-I	28-S	29-S	33-S	29-S	20-S	16-I
ASSi:2n	20-R	17-S	31-S	20-S	10-R	25-S	31-S	23-S	18-R	25-S	17-S
ASPe:1a	22-R	21-S	32-S	20-S	21-S	20-I	25-S	17-S	19-R	21-S	18-S
ASDe:1	20-R	23-S	34-S	19-S	18-I	23-S	24-S	21-S	21-R	24-S	15--I
Assing:1	36-S	14-R	27-S	31-S	27-S	26-S	15-S	17-S	20-R	18-S	23-S
ASCL:16f	35-S	17-S	31-S	24-S	24-S	34-S	17-S	28-S	21-R	17-S	21-S
ASPe:16g	18-R	18-S	20-S	31-S	12-R	25-S	20-S	24-S	32-S	20-S	17-S
ASPe:16u	15-R	21-S	22-S	19-S	31-S	29-S	26-S	15-I	29-S	22-S	18-S
ASDe:17 b	36-S	20-S	36-S	21-S	30-S	36-S	15-S	23-S	31-S	15-S	21-S

ASSi:17k	33-S	19-S	33-S	35-S	27-S	23-S	29-S	28-S	33-S	31-S	25-S
AS:Ksc5	31-S	20-S	24-S	24-S	27-S	24-S	29-S	20-S	28-R	18-S	23-S
ASCl:2a	20-R	19-S	19-S	20-S	10-R	40-S	18-S	21-S	29-S	29-S	22-S
ASCl:17a	22-R	23-S	18-S	17-S	18-I	35-S	20-S	23-S	24-R	21 -S	20-S
ASPe:1	35-S	24-S	21-S	18-S	28-S	24-S	16-S	31-S	31-S	31-S	28-S
ASLa:2	25-R	17-S	23-S	20-S	32-S	33-S	33-S	28-S	36-S	30-S	27-S
ASLa:5	21-R	21-S	34-S	20-S	11-R	40-S	32-S	26-S	27-R	29-S	22-S
ASLa:17b	20-R	23-S	21-S	19-S	15-R	36-S	28-S	>36-S	31-S	32-S	19-S
ASLa:17h	21-R	28-S	20-S	17-S	24-S	28-S	24-S	17-S	28-R	25-S	20 -S
ASCh:17d	33-S	21-S	17-S	20-S	21-S	26-S	23-S	19-S	32-S	18-S	21-S
ASLa:17c	35-S	34-S	19-S	19-S	32-S	24-S	15-S	21-S	29-S	21-S	18-S

AS:Ch 17	21-R	21-S	20-S	20-S	24-S	23-S	18-S	23-S	27-R	26-S	19-S
ASCh:A1	20-R	23-S	21-S	19-S	14-R	15-I	20-S	18-S	24-R	32-S	28-S
ASMB:1d	>36-S	>36-S	36-S	32-S	36-S	>36-S	15-S	20-S	24-R	19-S	21-S
ASRa:17e	19-R	29-S	32-S	24-S	18-I	25-S	16-S	23-S	32-S	29 -S	21-S
ASDe :21	37-S	31-S	21-S	30-S	35-S	33-S	19-S	25-S	28-S	36-S	22-S
ASDe:10	>36-S	32-S	22-S	31-S	>36-S	>36-S	22-S	14-I	29-S	>36-S	18-S
ASDe18s	31-S	>36-S	32-S	37-S	>36-S	>36-S	21-S	26-S	34-S	34-S	19-S
ASDe:P1	22-R	32-S	31-S	32-S	16-I	28-S	18-S	18-S	21-R	28-S	20-S
ASDe:18i	34-S	>36-S	24-S	36-S	27-S	13-R	24-S	13-I	27-R	36-S	28-S
ASDe:18a	12-R	20-S	21-S	27-S	19-I	12-R	23-S	28-S	28-R	29-S	27-S

ASDe:18c	35-S	38-S	20-S	34-S	17-I	24-S	19-S	22-S	12-R	36-S	19-S
Assing:12	34-S	26-S	24-S	37-S	31-S	26-S	17-S	21-S	21-R	33-S	18-S
Assing:18	31-S	27-S	21-S	28-S	32-S	32-S	21-S	36-S	25-R	30-S	17-S
ASSg: 14n	22-R	28-S	32-S	18-S	20-I	16-I	24-S	16-I	28-R	28-S	13-R
AsSg: 14k	31-S	32-S	22-S	27-S	18-I	29-S	18-S	25-S	27-R	30-S	15-S
AS:Sg3	13-R	24-S	18-S	26-S	27-S	<10-R	21-S	17-S	23-R	27-S	19-S
AS:Sg6	18-R	14-R	17-S	17-S	12-R	22-I	20-S	20-S	24-R	20-S	22-S
ASSi:19r	>36-S	35-S	21-S	31-S	34-S	>36-S	23-S	12-R	31-S	>36-S	23-S
ASSi:T4	31-S	33-S	22-S	26-S	32-S	15-I	19-S	<11-R	24-R	30-S	14-R
ASKsc:12	33-S	25-S	32-S	29-S	27-S	31-S	16-S	0-R	22--R	27-S	23-S
ASKsc:29	31-S	29-S	34-S	18-S	24-S	23-S	19-S	21-S	28-R	20-S	15-I

ASKSc:14a	21-R	30-S	21-S	20-S	27-S	25-S	20-S	17-S	19-R	27-S	11-R
AsKSc:18d	36-S	31-S	17-S	17-S	18-I	19-I	24-S	20-S	18-R	28-S	17-S
ASKsc18b	22-R	36-S	17-S	28-S	20-I	24-S	22-S	26-S	24-R	24-S	17-S
AS: Td13	25-R	30-S	18-S	31-S	24-S	26-S	15-S	31-S	20-R	18-S	12-R
AS:TD9	35-S	>36-S	31-S	28-S	15-R	32-S	24-S	27-S	31-S	32-S	20-S
P1Tdb	18-R	20-S	21-S	21-S	20-I	26-S	18-S	23-S	24-R	25-S	17-S
ASTd:3e	15-R	38-S	22-S	19-S	21-S	19-I	22-S	16-I	26-R	29-S	19-S
AS:Td 3a	36-S	28-S	26-S	18-S	24-S	33-S	19-S	22-S	29-S	28-S	21-S
AS:Td:18a	33-S	32-S	31-S	30-S	20-I	23-S	26-S	26-S	22-R	33-S	18-S
AS:Td:18d	19-R	32-S	32-S	19-S	26-S	26-S	28-S	21-S	32-S	34-S	17-S
AS:Td:18e	21-R	26-S	>36-S	17-S	32-S	18-I	24-S	18-S	33-S	20-S	27-S

AS: Cl 18g	26-R	31-S	21-S	18-S	34-S	22-I	19-S	30-S	29-S	28-S	11-R
AS:Ct1	29-S	>36-S	22-S	20-S	36-S	26-S	15-S	23-S	34-S	22-S	21-S
AS:BM1	26-R	20-S	17-S	30-S	32-S	33-S	31-S	27-S	36-S	30-S	17-S
ASCl:18f	21-R	38-S	17-S	22-S	10-R	>36-S	32-S	18-S	29-S	29-S	18-S
ASCl:p1	>40-S	28-S	21-S	31-S	36-S	36-S	21-S	15-I	29-S	32-S	19-S
ASCL:p5	31-S	32-S	24-S	36-S	36-S	32-S	24-S	26-S	31-S	34-S	27-S
ASPe:5	32-S	32-S	18-S	18-S	21-S	26-S	18-S	19-S	27-R	36-S	21-S
ASPe:Da	34-S	28-S	19-S	28-S	27-S	23-S	26-S	17-S	30-S	19-S	20-S
ASPe:18	12-R	20-S	18-S	27-S	20-S	24-S	27-S	29-S	28-R	29-S	13-R
AS:Td:18e	21-R	26-S	>36-S	17-S	32-S	18-I	24-S	18-S	33-S	20-S	27-S
AS: Cl 18g	26-R	31-S	21-S	18-S	34-S	22-I	19-S	30-S	29-S	28-S	11-R

AS:Ct1	29-S	>36-S	22-S	20-S	36-S	26-S	15-S	23-S	34-S	22-S	21-S
AS:BM1	26-R	20-S	17-S	30-S	32-S	33-S	31-S	27-S	36-S	30-S	17-S
ASCl:18f	21-R	38-S	17-S	22-S	10-R	>36-S	32-S	18-S	29-S	29-S	18-S
ASCl:p1	>40-S	28-S	21-S	31-S	36-S	36-S	21-S	15-I	29-S	32-S	19-S
ASCL:p5	31-S	32-S	24-S	36-S	36-S	32-S	24-S	26-S	31-S	34-S	27-S
ASPe:5	32-S	32-S	18-S	18-S	21-S	26-S	18-S	19-S	27-R	36-S	21-S
ASPe:Da	34-S	28-S	19-S	28-S	27-S	23-S	26-S	17-S	30-S	19-S	20-S
ASPe:18	12-R	20-S	18-S	27-S	20-S	24-S	27-S	29-S	28-R	29-S	13-R
ASPe:18c	29-S	24-S	21-S	17-S	17-I	25-S	22-S	31-S	31-S	28-S	18 -S
ASLa:5a	32-S	31-S	24-S	21-S	32-S	16-I	16-S	19-S	24-R	34-S	28-S
ASLa:10	>40-S	16-I	25-S	32-S	36-S	>36-S	15-S	22-S	25-R	32-S	11-R

ASLa:18b	31-S	32-S	21-S	23-S	32-S	30-S	20-S	27-S	34-S	30-S	17-S
ASCh:14	37-S	31-S	20-S	30-S	35-S	31-S	16-S	25-S	28-R	36-S	11-R
ASCh:14(B)	40-S	32-S	22-S	36-S	32-S	32-S	14-S	14-I	30-S	36-S	23-S
AS:Ch 28	31-S	28-S	21-S	32-S	34-S	28-S	18-S	24-S	32-S	>36-S	18-S
AS: Ch15	33-S	32-S	24-S	28-S	21-S	23-S	19-S	19-S	27-R	28-S	16-I
AS:Ch(a)	34-S	22-S	25-S	21-S	27-S	24-S	21-S	17-S	18-R	32-S	28-S
ASCh:P2	12-R	20-S	21-S	27-S	28-S	26-S	27-S	24-S	20-R	28-S	21-S
ASMB:B24	35-S	>36-S	26-S	34-S	24-S	34-S	21-S	23-S	12-R	22-S	12-R
ASRa:18 a	>36-S	35-S	28-S	27-S	30-S	36-S	15-S	21-S	34-S	>36-S	17-S
AS:Mn3	34-S	26-S	17-S	36-S	32-S	36-S	17-S	25-S	22-R	31-S	18-S
ASMn:18	28-R	23-S	19-S	21-S	19-I	27-S	18-S	36-S	26-R	24-S	21-S

ASRa:16a	31-S	32-S	18-S	18-S	21-S	26-S	21-S	23-S	29-S	29-S	15-I
ASRa:18e	31-S	30-S	16-S	26-S	28-S	29-S	20-S	26-S	26-R	24-S	12-R
AS: RA 18	35-S	31-S	14-I	32-S	30-S	25-S	25-S	17-S	24-R	31-S	11-R
AS: RA 18z	13-R	24-S	13-R	26-S	27-S	30-S	22-S	>10-R	28-R	34-S	19-S
AS:RA:18m	>36-S	34-S	18-S	26-S	15-R	22-I	16-S	18-S	24-R	20-S	20-S

Note: Zone diameter in mm, results were expressed as per CLSI 2014. AMP, ampicillin; AK, amikacin; AMX, amoxycillin; C, chloramphenicol; CD, clindamycin; E, erythromycin; MET, methicillin; NX, norfloxacin; P, penicillin G; VA, vancomycin; TET, tetracycline. S, sensitive; I, intermediate, R, resistance.

Table 25: AST pattern of *Staphylococcus* sp. from air samples by disc diffusion method.

Antibiotics	Susceptible		Intermediate		Resistant	
	No	%	No	%	No	%
AMP (10µg)	35	42.16	0	0	48	57.83
AK(10µg)	80	96.38	1	1.2	2	2.4
AMX (30µg)	81	97.59	1	1.12	1	1.2
C(30µg)	83	100	0	0	0	0
CD(2µg)	63	75.9	11	13.25	9	10.84
E(15µg)	68	81.92	12	14.45	3	3.61
MT(5µg)	83	100	0	0	0	0
Nx(10µg)	72	86.74	8	9.63	3	3.61
PEN-G(10µg)	33	39.75	0	0	50	60.24
TET(30µg)	83	100	0	0	0	0
VAN (10µg)	67	80.72	5	6.02	11	13.25

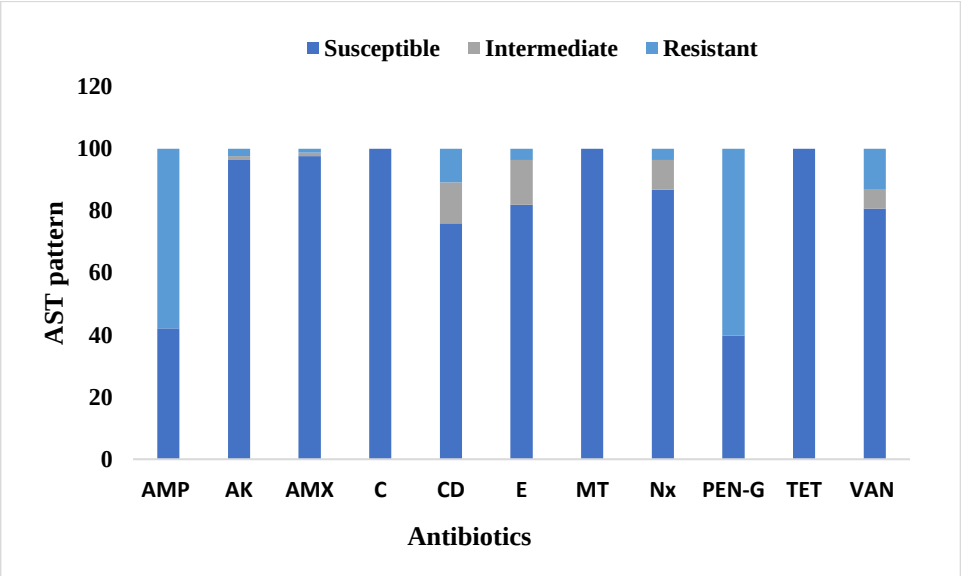


Figure 23: AST pattern of *Staphylococcus* sp. from air samples.

AST results for *Pseudomonas* sp. from air samples by disc diffusion method

In *Pseudomonas* sp. 100% susceptibility was shown against norfloxacin. Against amoxycillin and chloramphenicol the susceptibility was similar i.e., 90.9% and 84.9 % the isolates showed susceptibility to ampicillin and erythromycin. Amikacin, streptomycin showed susceptibility of 84.9% and 81.81% respectively. Resistance was not observed against norfloxacin, while the highest percentage of resistance was shown by erythromycin 15.9% (Table 26, Figure 24).

Table 26: Zone of inhibition of *Pseudomonas* sp. from air samples by disc diffusion method.

Isolate	(AMP$_{10}$)	(AK$_{10}$)	(AMX30)	(C$_{30}$)	(E$_{15}$)	(NX$_{10}$)	(S$_{10}$)	(TET$_{30}$)
AS:P3td	20-S	29-S	19-S	18-S	22-R	31-S	15-S	27-S
P1Td.b1	<10-R	11-R	18-S	12-R	21-R	17-S	17-S	13-I
AS: Deu	17-S	21-S	24-S	17-S	23-S	19-S	11-R	24-S
ASP3:Tdj	19-S	24-S	18-S	18-S	20-R	20-S	16-S	28-S
ASCH:c	22-S	<10-R	13-R	36-S	>40-S	21-S	23-S	36-S
AS:DEB4	23-s	22-S	20-S	31-S	>40-S	24-S	28-S	15-S
ASDE:B5	21-S	17-S	24-S	28-S	21-R	23-S	13-I	18-S
ASTd:12u	18-S	20-S	18-S	24-S	29-S	31-S	20-S	12-I
ASPe:16:6	23-S	23-S	22-S	19-S	31-S	31-S	17-S	31-S
ASPe:18:9	35-S	18-S	35-S	21-S	36-S	24-S	36-S	11-R
ASLa17:7	20-S	17-S	19-S	26-S	28-S	28-S	28-S	26-S
ASDe:6	18-S	17-S	18-S	20-S	23-S	21-S	16-S	15-S
ASDE:16s	20 -S	19-S	19-S	19-S	24-S	18-S	15-S	12-I

ASSi:2n	23-S	24-S	26-S	21-S	22-R	21-S	11-R	25-S
ASSi16a	21-S	25-S	18-S	14-I	24-S	22-S	16-S	21-S
AASDe17:d	13-R	14-R	12-R	18-S	36-S	21-S	14-R	26-S
ASKSC:1d	26-S	28-S	21-S	19-S	29-S	26-S	26-S	27-S
ASKSC:16d	19-S	18-S	26-S	17-S	24-S	24-S	24-S	23-S
ASKSC:17i	20-S	21-S	19-S	18-S	29-S	30-S	21-S	16-S
ASKSC:18l	12-R	15-I	23-S	26-S	30-S	28-S	17-S	28-S
ASTd:1e	17-S	19-S	31-S	21-S	>36-S	24-S	16-S	25-S
ASTd:16e	14-I	13-R	19-S	24-S	29-S	28-S	18-S	23-S
ASTd:18e	25-S	26-S	21-S	28-S	24-S	18-S	17-S	24-S
ASCL:2g	22-S	23-S	19-S	19-S	20-R	20-S	16-S	26-S
ASCL:16f	20-S	21-S	21-S	20-S	23-S	24-S	18-S	30-S
ASCL:17k	19-S	20-S	23-S	18-S	24-S	23-S	10-R	34-S
ASLa:2c	19-S	18-S	14-I	19-S	38-S	21-S	11-R	28-S
ASLa:16h	16-I	17-S	20-S	11-R	25-S	20-S	19-S	14-I
ASLa:17(10)	18-S	17-S	24-S	18-S	31-S	18-S	21-S	31-S
ASPe:1a	19-S	16-I	18-S	18-S	26-S	30-S	29-S	21-S

ASPe:16g	20-S	22-S	22-S	26-S	31-S	29-S	15-S	26-S
ASPe:17(2)	18-S	17-S	30-S	21-S	34-S	21-S	21-S	24-S
ASPe:18g)	20-S	17-S	20-S	24-S	28-S	26-S	15-S	25-S
ASCh:1d	21-S	19-S	21-S	19-S	31-S	23-S	16-S	26-S
ASCh:16i	17-S	18-S	18-R	28-S	34-S	18-S	10-R	28-S
ASCh:17m	20-S	21-S	22-S	29-S	36-S	17-S	15-S	21-S
ASCh:17o	25-S	24-S	18-S	20-S	32-S	27-S	20-S	26-S
ASMn:2a	19-S	23-S	23-S	28-S	32-S	25-S	24-S	28-S
ASMn:16j	24-S	21-S	20-S	17-R	30-S	21-S	14-I	32-S
ASMn:17m	21-S	18-S	21-S	18-S	24-R	18-S	15-S	25-S
ASRa:1b	20-S	19-S	28-S	18-S	31-S	17-S	16-S	18-S
ASRa:16e	19-S	21-S	22-S	30-S	28-S	19-S	30-S	19-S
ASRa:17(13)	19-S	17-S	19-S	28-S	36-S	22-S	22-S	28-S
ASRa17d	16-I	14-R	18-S	20-S	30-S	21-S	16-S	32-S

Note: Zone diameter in mm, results were expressed as per CLSI 2014. AMP, ampicillin; AK, amikacin; AMX, amoxycillin; C, chloramphenicol; E, erythromycin; NX, norfloxacin; S, streptomycin; TET, tetracycline. S, sensitive; I, intermediate, R, resistance.

Table 27: AST pattern of *Pseudomonas* sp. from air samples by disc diffusion method.

Antibiotics	Susceptible		Intermediate		Resistant	
	No	%	No	%	No	%
AMP (10µg)	38	86.36	3	6.81	3	6.81
AK(10µg)	37	84.09	2	4.54	5	11.36
AMX (30µg)	40	90.9	1	2.27	3	6.81
C(30µg)	40	90.9	1	2.27	3	6.81
E(15µg)	37	84.09	0	0	7	15.9
Nx (10µg)	44	100	0	0	0	0
S(10µg)	36	81.81	2	4.54	6	13.63
TET(30µg)	39	88.63	4	9.09	1	2.27

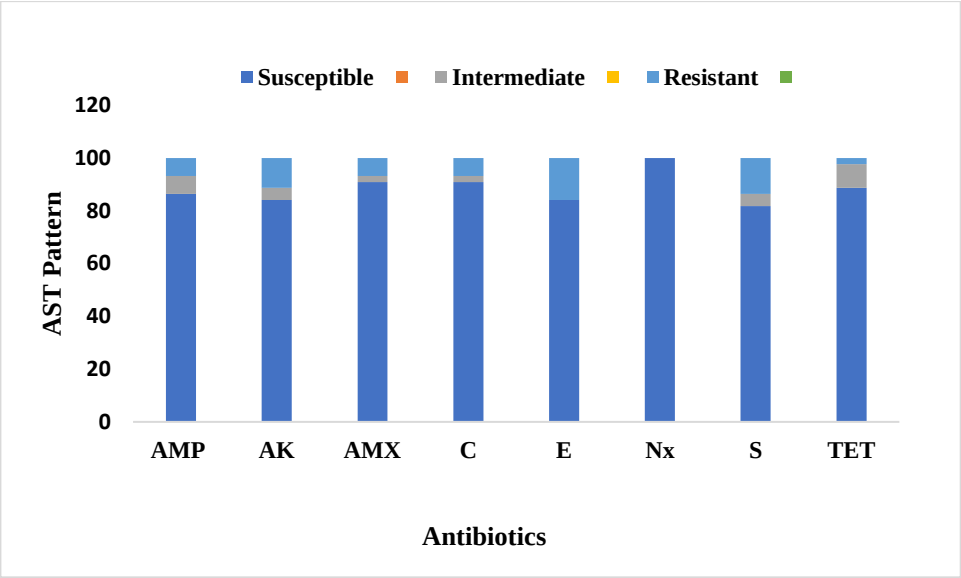


Figure 24: AST pattern of *Pseudomonas* sp. from air samples

Molecular characterisation of bacterial isolates from air samples.

A total of 11 representative strains viz. As:Lu5, As:Mn26, A:Ksc5, AS:Ra1d, AS:Pe2, AS:Ch2, AS:KSC7, AS:KSC18, AS-De16(h), AS:Lu2, AS:Td3 and AS:Mn11, isolated from air samples, were identified by 16S rRNA gene sequencing method (Table 28).

Table 28: Identification of bacterial isolates based on 16S rRNA genes sequencing

Isolates	**Percentage**	**NCBI/EzTaxon Hit**	**Accession number**
As:Lu5	100	*Bacillus safensis* subsp. *safensis*	MW536490
As:Mn26	98.87	*Bacillus safensis* subsp. *safensis*	MW548737
A:Ksc5	100	*Bacillus safensis* subsp. *safensis*	MW536491
AS:Ra1d	100	*Bacillus aryabhattai*	MW536492
AS:Pe2	99.73	*Bacillus altitudinis*	MW536493
AS:Ch2	100	*Bacillus altitudinis*	MW536494
AS:KSC7	100	*Staphylococcus sciuri*	MW536495
AS:KSC18	99.79	*Pseudomonas stutzeri*	MW536496
AS-De16(h)	100	*Cellulosimicrobium cellulans*	MW709569
AS:Lu2	98.96	*Bacillus aryabhattai*	MW709570
AS:Td3	99.6	*Bacillus altitudinis*	MW709571
AS:Mn11	99.64	*Bacillus altitudinis*	MW709572

Molecular Phylogeny of bacterial isolates

Molecular phylogenetic analysis of bacterial isolates from air samples of 11 representative isolates based on 16S rRNA region sequencing were constructed using MEGA 7 software (Kumar et al. 2016). The evolutionary history was inferred using the Neighbour-Joining method (Saitou N. and Nei M. 1987) with *Methanococcus voltae* M59290 as the out group (Figure 25)

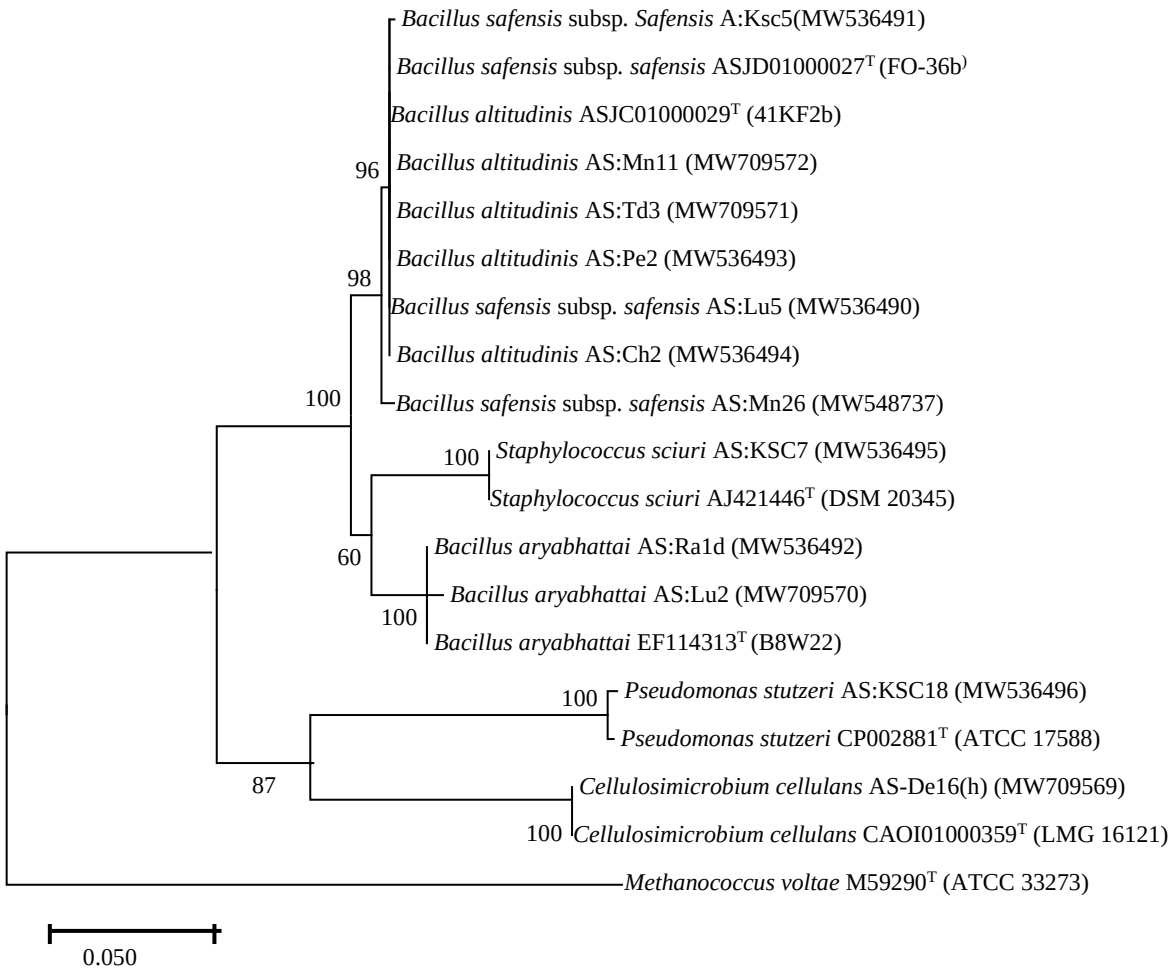


Figure 25: Molecular phylogenetic analysis of 11 bacterial isolates isolated from air samples based on 16S rRNA region sequencing. Neighbouring-joining phylogenetic tree constructed using by MEGA 7 with *Methanococcus voltae* M59290 as the out group.

Molecular characterisation of fungal isolates

A total of 9 fungal representative strains viz. NFGtk2, NfDe3, NFDe4, NFtd5, SU-1b, SU1d, SF11, SF13, SF14, isolated from air samples, were identified using ITS region (Table 29, Figure 26).

Table 29: Identification of bacterial isolates based on ITS

Isolates	**Fungal species**	**Accession number**
NFGtk2	*Penicillium brevicompactum*	MH393340
NfDe3	*Fusarium proliferatum*	MH393341
NFDe4	*Fusarium solani*	MH393342
NFtd5	*Fusarium oxysporum*	MH393343
SU-1b	*Trichoderma reesei*	MG745321
SU1d	*Colletotrichum graminicola*	MG745325
SF11	*Colletotrichum graminicola*	MH379771
SF13	*Phaeosphaeria avenaria f. sp. avenaria*	MH379773
SF14	*Metarhizium granulomatis*	MH379774

Molecular Phylogeny of fungal isolates

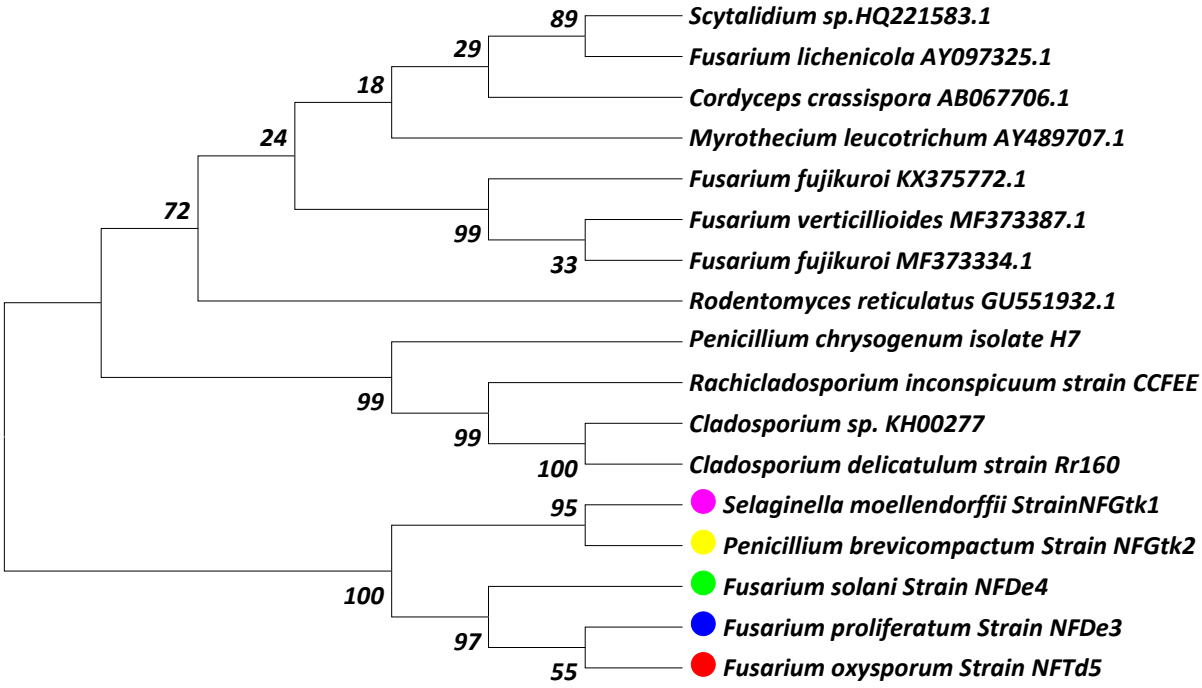


Figure: 26. Phylogenetic tree based upon the Neighbour-joining of 28S rRNA sequences.

AMBIENT AIR MONITORING

Ambient air monitoring of seven stations in Sikkim was conducted using a high-volume air sampler. The pollutants determined were PM10 (Particulate Matter, size less than 10µm) SO_2 (Sulphur dioxide) and NO_2 (Nitrogen dioxide). The National Ambient Air quality standards (2009) is given in (Annexure I).

The annual average of the tested parameters in 2016 at seven stations in Sikkim.

The values of PM 10 ranged from 3.01- 59.33 µg/m^3, the lowest was recorded at Mangan and the highest at Rangpo (Figure 27). However, the value was below the standard given by National Ambient Air quality standards (NAAQS) which is 60 µg/m^3 annual average.

SO_2 value ranged from 1.01- 19.95 µg/m^3 the lowest at Namchi during July and the highest at Mangan during August. Which was far below the annual average standard (50 µg/m^3) given by NAAQS. Significantly high amount of NO_2 was detected in Singtam (31.71 µg/m^3) in November and BDL (Below detection level) in the same month in Ravangla (Figure. 27).

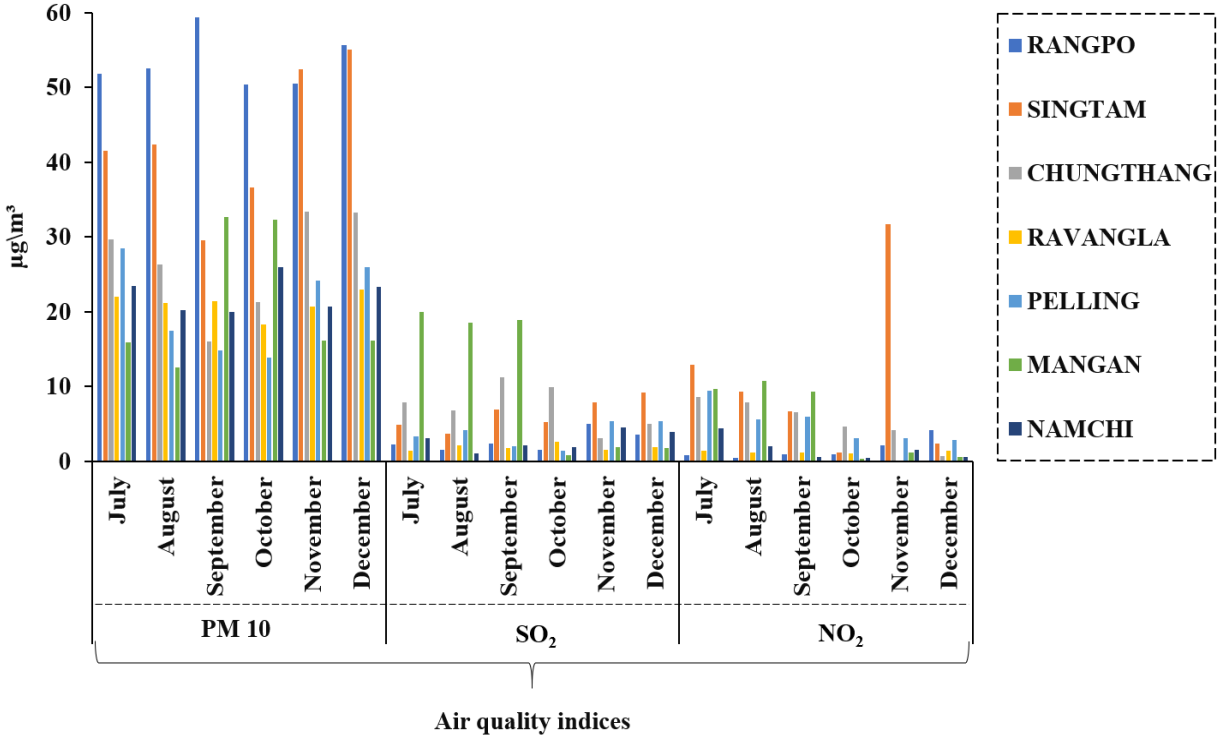


Figure 27: Annual Air quality indices of seven stations in Sikkim in 2016

The annual average of the tested parameters in 2017 at seven stations in Sikkim

The significantly high amount of PM10 was detected in Rangpo in December (84.19 µg/m^3), October (75.85 µg/m^3), September (53.4 µg/m^3) and august (48.5 µg/m^3). In the month of November and December the pollutant level rose above the given standards. The pollutant was high in Singtam reaching up to 67.86 µg/m^3 in December, SO_2 was recorded highest at Mangan (9.25 µg/m^3) in the month of July. The highest NO_2 recorded was 9.06 µg/m^3 at Rangpo and Singtam, which was the highest recorded value in the year. (Figure. 28).

The annual average of the tested air pollutants in 2018 at seven stations in Sikkim

PM10 was recorded highest at Rangpo **(**80.55 µg/m^3) in the month of December which was above the standard limit and the least at Ravangla (11.33 µg/m^3) µg/m^3 in October. SO_2 and NO_2 was very low, the highest SO_2 recorded (9.92 µg/m^3) at Rangpo in July and NO_2 (28.09 µg/m^3) at Singtam in November (Figure 29).

The annual average of the tested air pollutants in 2019 at seven stations in Sikkim

PM10 value was the high in Rangpo in the month of December (57.63 µg/m^3), November (54.19 µg/m^3), September (47.39 µg/m^3) and October (47.3 µg/m^3). And the lowest PM10 value was recorded at Chungthang (8.43 µg/m^3) in December. The pollutant was also high in Singtam in the month of November (52.44 µg/m^3) and in Deorali (53.74 µg/m^3) in the month of December. The highest SO_2 value was recorded in Rangpo (18.2 µg/m^3) in November. the highest NO_2 was recorded in Deorali (10.86 µg/m^3) in December (Figure 30).

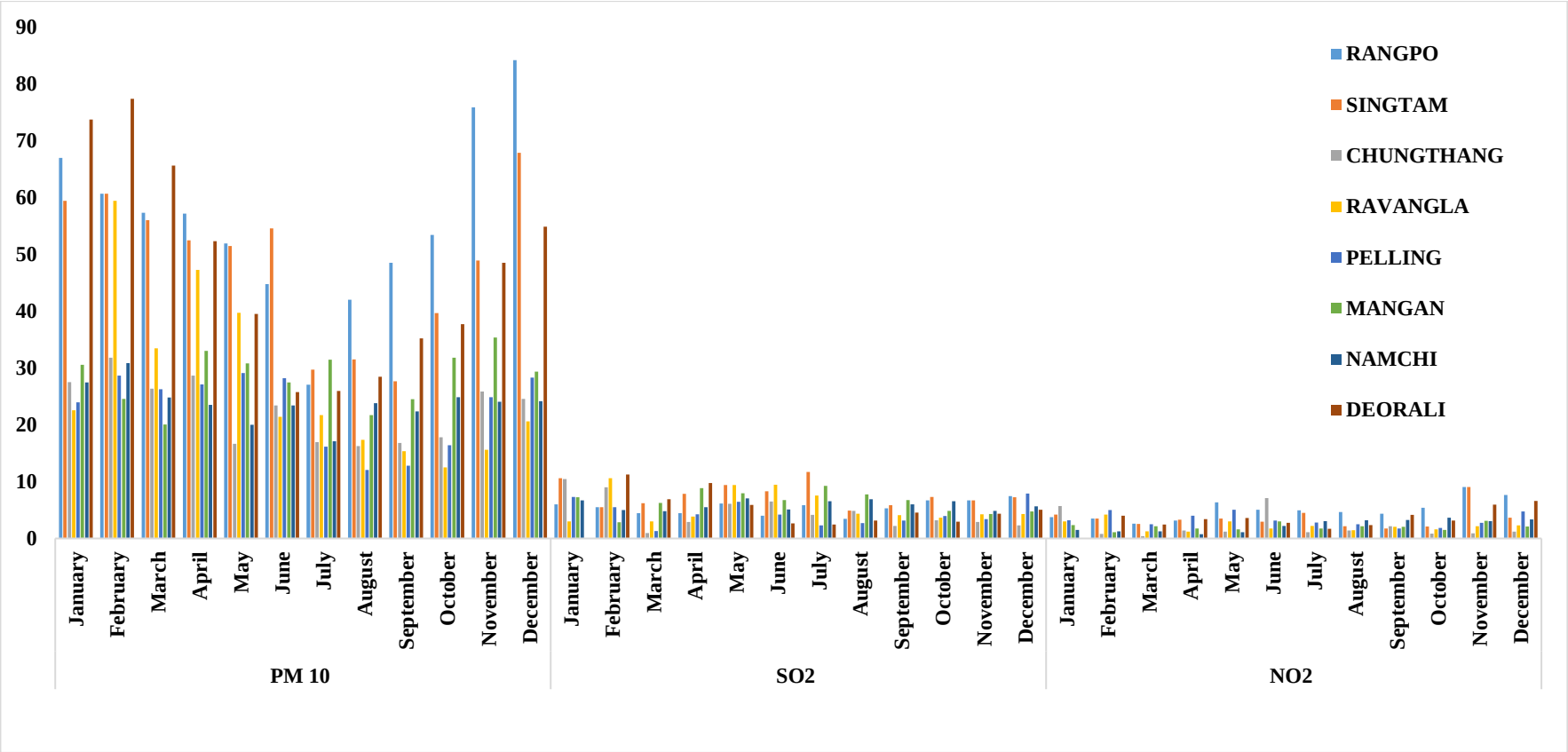


Figure 28: Annual Air quality indices of seven stations in Sikkim in 2017.

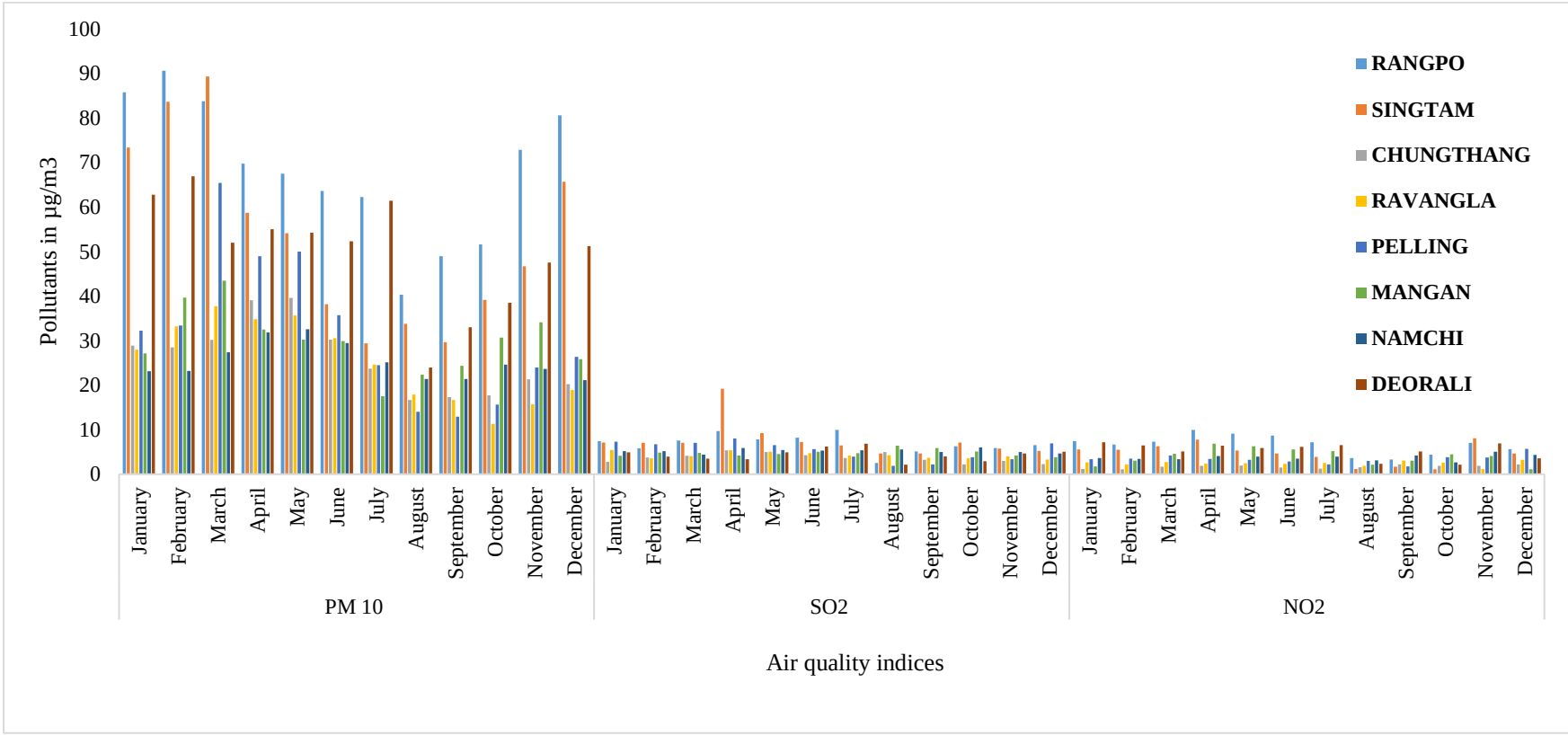


Figure 29: Annual Air quality indices of seven stations in Sikkim in 2018.

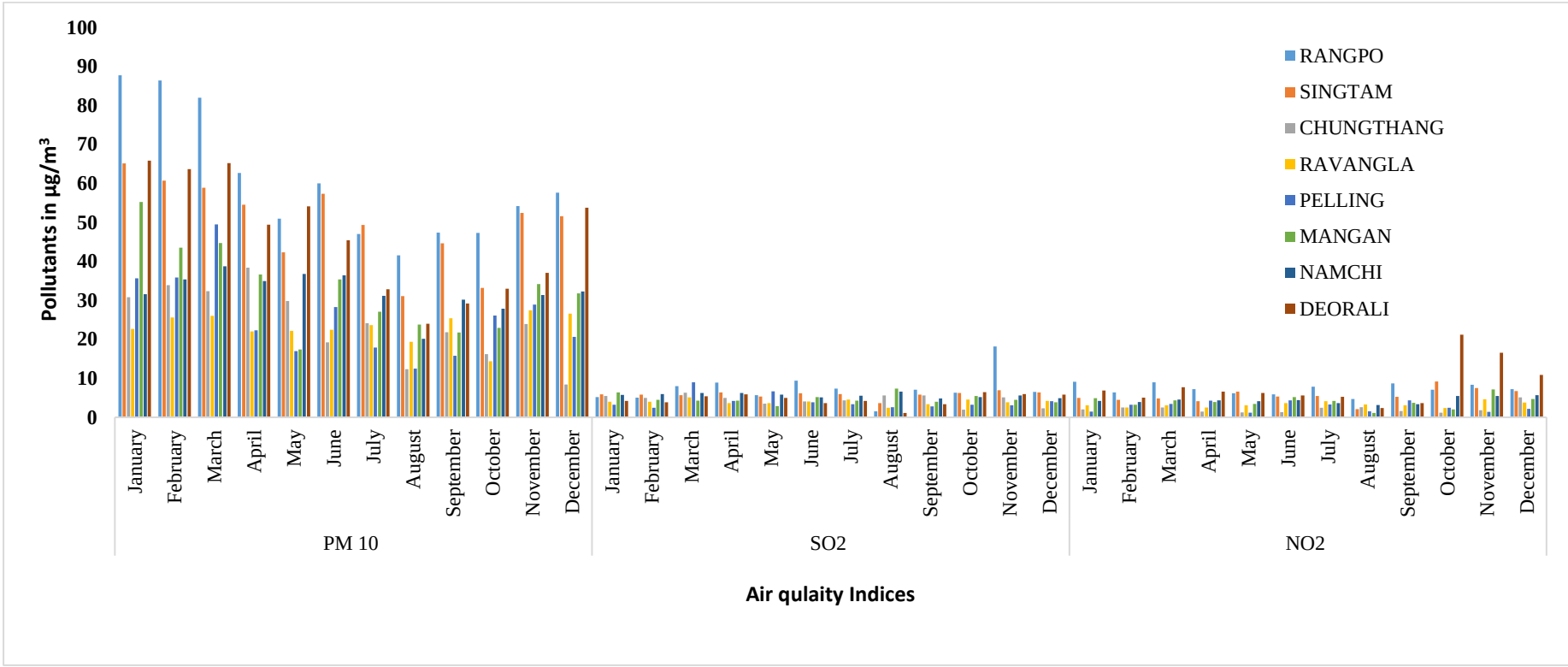


Figure 30. Annual Air quality indices of seven stations in Sikkim in 2019.

DISCUSSION

The main objective of this study was to identify the prevalent microorganisms (bacteria and fungi) in the outdoor environment in various sampling sites of Sikkim in India. Field based surveillance was also incorporated in this study with the help of structured questionnaire. It is commonly known that bacteria (Fujiyoshi et al. 2017), fungi (Nageen et al. 2021) including viruses (Leung 2021) present in the air can affect human health, causing mainly respiratory and related diseases transmitted via respiratory route (Bugajny et al. 2005; Tellier et al. 2019). It was hypothesized that the microflora of Sikkim should be affected by the types of plantations, vegetation and the visitors as the State has been a tourist hub and a place of pilgrimage. Because of the rise in population density, transportation, industries and tourists' influx the occurrence or environmental contamination of resident air microflora from such sources was also expected. We were also hoping that once the baseline data of composition of air is prepared, we will be able to detect any unusual microbes in the air as this study area is strategically important. The climate in Sikkim ranges from sub-tropical in the south to tundra in the north (Kumar et al. 2020). The climate in most of the inhabited regions remain temperate with temperature very seldom exceeding 28°C in summer. The average temperature for most part of the Sikkim is 18°C. Sikkim is situated in an ecological hotspot of the lower Himalayas, one of only three among the ecoregions of India (O'Neill et al. 2017).

The air samples from our study have been collected from various locations that included rural, urban and also tourist hubs viz: Deorali, Singtam, Kanchenjunga Shopping Complex, Tadong, Changu Lake, Pelling, Lachung, Chungthang, Mangan Ravangla and Rangpo. The State observes five seasons, winter, summer, spring, autumn, and monsoon season. However, we broadly categorized our sampling season as dry (November-March) and wet

season (May-October) as samples in places such as Changu Lake, Mangan, Chungthang, Lachung and Pelling in a designated period would not be possible due to the landslides, snowfall and other natural disturbances; particularly in mid of rainy season and extreme winter season. Samples were collected two times for the study period in a place in a year (Agarwal et al. 2016) with some modifications. The preferred method for collection of air sample was passive method (Plate Sedimentation Method or the Gravitational method) (Stryjakowska et al. 2007) and active method (Impaction method) (Sveum et al. 1992) for study of both bacteria and fungi. IUL basic air sampler with air flow of 100L/min was used for active method of sampling as this instrument was already available in the institution and was easy for transportation and could be run on chargeable battery (7.2V) with a run duration of 8 hours once fully charged.

Field survey

The field surveillance was conducted along with sample collection with the prior consent of the participants. The study population cooperated during the survey however, many were reluctant to answer many of the questions during the survey, most importantly many were unaware about their medical history. The study population also found difficulty expressing their medical conditions. No significant data on the diseases due to air borne microorganisms were recorded. Some respiratory diseases, which include asthma, chronic obstructive pulmonary disease (COPD), pulmonary fibrosis, pneumonia, tuberculosis and lung cancer have been reported in Sikkim (Barua et al. 2013; Chettri et al. 2018).

Respiratory diseases are mainly caused by smoking tobacco, or by breathing in second-hand tobacco smoke, radon, asbestos, or other forms of air pollution (Shevade et al. 2015).

Microbiological Analysis

Many media were selected and recommended for culturing the fungi the exceptions were Malt Extract Agar (MEA), Sabouraud Dextrose Agar (SDA), Rose Bengal Chloramphenicol Agar (Nageen et al. 2021). However, we choose using PDA (Potato Dextrose Agar), as we were expecting plant borne pathogens. Similarly, many media were recommended for bacterial isolation from air samples (Weissfeld et al. 2013). The approach of this study was to target both bacteria and fungi in air-microflora. However, in our study, the load of bacteria in average was found to be much higher than fungi. Similar observation was also reported in both indoor and outdoor environment (Jalili et al. 2021). The phenotypic identification of the microbes was time consuming and challenging. We found that the conventional culture-dependent methods for identification of organisms for environmental studies was indeed challenging and laborious. With as many tests we could do for phenotypic identification, the prevalent bacterial species were *Bacillus* sp., *Staphylococcus* sp., *Micrococcus* sp., *Pseudomonas* sp. and *E. coli* sp. One of the aspects of the challenges is that many cultures after sub-culturing tend to lose its original colony pigment and survive sub-culturing and often re-examination of cell morphology varied slightly. The advantage of the culturable dependent methods is that it assumes that the organisms will grow and produce typical characteristics within a definite period (Giuliano et al. 2019). However, microorganisms that are not culturable under the given growth

conditions in the laboratory could remain undetected that could possibly induce adverse health effects (Yoo et al. 2016). These losses due to stress of aerosolization and sampling may result in loss of cultivability, which are difficult to evaluate and may vary within and among species (Yoo et al. 2016). It has been stated that majority of cells believed to be viable might not form colonies on agar plates (Peccia and Hernandez 2006). Hence, cell debris, dead microorganisms which may possess toxic and/or allergenic properties, goes undetectable (Griffin 2007) Therefore, rapid, accurate techniques for monitoring airborne microorganisms are absolutely needed to overcome the constraints of traditional culture-based methods (Hubad and Lapanje 2013).

It is observed that Chungthang despite of being a rural area had a microbial load at par with Tadong, an urban area possibly due to its popularity as a tourist destination. Irrespective of the sampling location, time and the season, in active sampling it was observed that the log CFU/m^3 variation was not remarkable. Gram-positive cocci (40.26%) followed by Gram-positive rods (30.88%) and Gram-negative rods (29.64%) were found the prevalent bacterial genera from air samples in all selected sampling locations, which is similar to the study conducted by Hassan et al. (2021), where the prevalent bacteria genera were Gram-positive cocci (41.1%) and Gram-positive rods (35.3%). In our study, bacterial genera detected were *Bacillus*, *Staphylococcus*, *Pseudomonas*, *Micrococcus*, *Corynebacterium*, *Proteus*, *Serratia* and *E. coli.* Some of these bacteria and fungi have shown to be the prevalent and common bacterial and fungal species in air (Burge and Hoyer 1990).

Further the genotypic identification helped in confirmation of identity of Gram-positive rods viz. *Bacillus safensis* subsp. *safensis*, *Bacillus aryabhattai*, *Bacillus altitudinis* and *Cellulosimicrobium cellulans;* Gram-positive coccus *Staphylococcus sciuri* and Gram-

negative rod *Pseudomonas stutzeri. Bacillus safensis* subsp. *safensis,* is a soil-borne bacterium (Lateef et al. 2015), also isolated from spacecraft and assembly-facility surfaces, is spore-forming rod bacterium, aerobic chemoheterotroph (Satomi et al. 2006), and also silver-resistant bacterium (Ahmed et al. 2020). It also exhibits a broad-spectrum antibacterial activity against multi-drug resistant isolates (Iqbal et al. 2021). *Bacillus safensis* is non-pathogenic and inhibits the growth of *Listeria monocytogenes* (Hascoet et al. 2021) and fungal pathogens *Cryptococcus neoformans* and *Candida albicans* (Mayer and Kronstad 2017). *Bacillus aryabhattai* is a Gram-positive plant growth promoting bacterium, isolated from the rhizosphere of tea (Bhattacharyya et al. 2017). It is used for bioremediation (Elarabi et al. 2020), and also as potent biological control agent against *M. javanica* and a promising substitute of chemical nematicides (Antil et al. 2021). *Bacillus altitudinis* is isolated from air samples, collected from high altitude, hence the name (Shivaji 2006), and also from other environments (Rozanov et al. 2018). It can trigger the plant growth (Zhang et al. 2021). This bacterium produces RNase, which has antitumor and antiviral properties (Shah et al. 2015), and can suppress the fungal pathogen (Sunar et al. 2015). *Cellulosimicrobium cellulans* is a Gram-positive bacterium present in soil and sewage that rarely causes human infection (Zhang et al. 2020a). *Staphylococcus sciuri* is a human pathogenic bacterium, responsible for endocarditis, peritonitis, septic shock, urinary tract infection, pelvic inflammatory disease and wound infections (Zeman et al. 2017). Microorganisms present in air may cause respiratory diseases (Moelling and Broecker 2020; Yang et al. 2020). *Pseudomonas stutzeri* is a ubiquitous bacterium that has been reported as a causative agent of some infections, particularly in immunocompromised patients but has rarely been reported as a cause of infective endocarditis (Alwazzeh et al. 2020). However, it also shows the denitrifying ability in the environment (Silva et al. 2020).

Staphylococcus haemolyticus, Micrococcus luteus, Micrococcus aloeverae, Bacillus pumilus, Lysinibacillus macroides, Bacillus cereus and *Bacillus paralicheniformis* were reported from indoor and outdoor air samples (Hassan et al. 2021). In our study, based on Biolog automated system, we identified from the air samples the following bacteria: *Staphylococcus haemolyticus, Lysynibacillus sphaerius, Corynebacterium sphaerius* and *Bacillus flexus*. *Staphylococcus haemolyticus* has been reported as opportunistic pathogen and are found as commonly on human skin (Robertson et al. 2013). *Bacillus* sp. is spore-forming soil bacteria and the most persistent microorganism in the atmosphere (Shaffer and Lighthart 1994). Prevalence of *Bacillus* sp. on different outdoor locations has been reported on previous studies (Pickering et al. 2018). *Bacillus* sp. are responsible for causing negative health effects (Pickering et al. 2018; Yoo et al. 2018). In this study *Staphylococcus* sp., *Bacillus* sp. and *Pseudomonas* sp. are the most prevalent bacterial species isolated. *Staphylococcus* sp. has been known to be carried easily carried in the nasopharynx, throat, skin, cuts, boils, nails contributing to the microflora in air (Madsen et al. 2018).

In nature the airborne fungal spores are the most common organisms (Soeria-Atmadja et al. 2010). The spores can be dispersed through the atmospheric air and they can also survive as parasites of planta and animals; fungi are pervasive airborne allergens and causal agent of numerous human diseases (Menezes et al. 2004). Species of *Aspergillus, Penicillium* and *Alternaria* are routinely isolated from the air and numerous other substrates (Barnett and *Hunter 1972).* **Overall Fusarium sp. and Penicillium sp. were the most prevalent groups of fungal genera.** *The* genotypically identified fungal genera was *Fusarium proliferatum, Fusarium solani, Fusarium oxysporum, Penicillium brevicompactum, Colletotrichum graminicola, Phaeosphaeria avenaria* f. sp. *avenaria* and *Metarhizium granulomatis*. *Fusarium* spp, have been isolated as air-borne fungi (Zhang et al. 2006; Moretti et al.

2018). *Fusarium proliferatum* is a plant pathogenic fungus Chang et al. 2015), *Fusarium solani* is also plant pathogenic fungus (Sisic et al. 2018). Whereas, *Fusarium oxysporum* (Moretti et al. 2018) is used as bio-control of plant diseases (de Lamo and Takken 2020). *Penicillium* is also known as the most common airborne spores that promote allergic reactions, its exposure to the damp environment and in the homes are a major threat causing asthma (Levetin et al. 2016) and allergies in vulnerable (Flannigan et al. 1991; Makut et al. 2014). *Penicillium brevicompactum* has been reported from air samples (Sen and Asan 2009; Madsen et al. 2016), which produces mycophenolic acid (MPA), a fungal metabolite which is important bioactive substance with immunosuppressive, antiviral, antitumor, antibacterial, antifungal, and various activities (Min et al. 2019). *Colletotrichum graminicola* is a pathogenic fungus causing disease of wheat and maize (Cuevas-Fernández et al. 2019) and is also reported from human patients (Yegneswaran et al. 2010). *Phaeosphaeria avenaria* f. sp. *Avenaria* is a plant pathogen (Bathgate and Loughman 2001). *Metarhizium granulomatis* is a pathogenic fungus causing mycosis (Schmidt et al. 2017). *Fusarium* sp. and *Penicillium* sp. were the most prevalent fungal isolates, while in a similar other study *Aspergillus* sp. and *Penicillium* sp. were found the most predominant species isolated from outdoor air (Maktkovic et al. 2007). Ekhaise et al. (2008) reported *Aspergillus* sp. as the most common genus of fungi in the indoor air environment.

Species of *Aspergillus* and *Penicillium* species are present in outdoor and indoor air (Knutsen et al. 2012). In our study, species of *Fusarium, and Penicillium* were also present in air samples of Sikkim as the most dominant fungal genera. Seasonal variations of the presence of fungal genera did not show significant variation in air samples of Sikkim, where seasonal variations effect the composition of air-born microflora (Kumar et al. 2021c).

There has been reports suggestive of *Alternaria*, *Colletotrichum, Aspergillus* and *Penicillium* being the most common fungi that cause asthma (Zubairi et al. 2014; Forkel et al. 2021). It has been reported that fungal spores are dispersed easily in air and are found abundantly in the outdoor air (Oneto et al. 2020), some of the species of these genera are known to be the source of allergic diseases in sensitive or immunocompromised people (Levetin et al. 2016; Skjoth et al. 2016). It is necessary to also monitor the indoor air, since indoor and outdoor air the composition of outdoor air has suggested to be the major source of fungal contamination in the indoor air (Madureira et al. 2015). Fungal species such as *Aspergillus* sp. *Fusarium* sp. and *Cladosporium* sp. have been found to be involved deterioration of paper under suitable environment (Allsopp et al. 2004).

Microbial contamination in air samples is assessed by two methods viz. active and passive sampling methods (Napoli et al. 2012). We have considered both the active and passive sampling method for semi-quantitative study. It has been observed that in majority of the microbial air sampling the microbial count was high by passive sampling, which was also reported earlier from air samples of other regions (Sampson et al. 2020). Correlation between the active and the passive methods while studying the indoor and outdoor air under normal conditions was reported (Canha et al. 2015). We did not find a large variation in the sampling method except the knowledge of volume of air sampled we get on active method. We found that the passive method was equally reproducible and convenient to perform (Zhang et al. 2020b). However, the duration of exposure of the sample plates may exceed up to 5 hours, in active the sampling duration usually remains short or the colonies of the microorganisms trapped remained impossible (Haig et al. 2016). Active measuring method is reliable over passive measuring method, at lower levels of contamination (Petti et al.

2003; Napoli et al. 2012). For measuring the higher concentrations of microorganisms, the passive method was usually used (Shanmugaraj and Rao 2020), which more affordable the active method (Petti et al. 2003). In a study conducted by Napoli et al. (2012) it has been agreed that the two methods correlate parallelly with the air quality. Some researches contradict with this view and has found that the active method of sampling yielded smaller concentrations of microorganisms than the passive method, with respect to bacteria as well as fungi (Montacutelli et al. 2000; Asefa et al. 2009; Verhoeff 1990). Some researchers are of a view that the passive method is more appropriate for monitoring concentrations of airborne microorganisms in drug manufacturing units (Whyte 1996; Haas et al. 2017). The comparatively high concentrations of fungi in the air pose are a little threat to healthy individuals (Abrego et al. 2020), but would pose a serious health risk to immunosuppressed individuals (Flannigan and Miller 1994; Eades and Armstrong-James 2019).

Exposure to airborne pathogens is a common trait of all human life (Fernstrom and Goldblatt 2013; Tellier et al. 2019). With the advent of advance research methods for studying airborne pathogens, it has been evident that microorganisms (e.g., viruses, bacteria, and fungal spores) from an infectious source that may disperse by air currents and ultimately be inhaled, ingested, or come into contact with individuals (Gonzalez-Martin 2019; Ji et al. 2021). Small percentage of infectious individuals may be responsible for disseminating the majority of infectious particles due to airborne pathogens in air (Fernstrom and Goldblatt 2013. Most of the bacteria and fungi isolated from air samples of different regions and places of Sikkim are non-pathogenic and non-infectious, which indicate the possibly of quality of air or less microbial contaminated air. Though there are reports of cases of respiratory diseases in Sikkim, but most of them are due to smoking and other other factors

(Barua et al. 2013). Air-borne infectious diseases have also been reported in Sikkim (Pandey et al. 2021), however, their sources and identify have been been reported yet. *Mycobacterium tuberculosis*, a causal organism for tuberculosis is an air-borne bacterium (Cambau and Drancourt 2014), which has been detected in environment (Martinez et al. 2019), however in our study, by applied methods, we could not detect *Mycobacterium tuberculosis*. This may be the first report on microbial composition of air samples or aerosol of Sikkim. The sources of these microorganisms are probably from plant, soils and other environments (Ruiz-Gil et al. 2020), which are less toxic and contagious, comparable to air samples of other regions of India (Madamarandawala et al. 2019). Several abiotic and abiotic factors that may influence the composition of airborne transmission of disease (Pica and Bouvier 2012; Cavicchioli et al. 2019; Ruiz-Gil et al. 2020).

Antimicrobial Susceptibility testing

Antimicrobial Susceptibility testing **(**AST) of the culturable air borne bacteria was studied using the antibiotics viz. ampicillin, amikacin, amoxycillin, chloramphenicol, clindamycin, erythromycin, methicillin, norfloxacin, penicillin G, streptomycin, vancomycin and tetracycline as per the CLSI guidelines (2014). The bacterial slates which were prevalent and frequently reported were subjected to the test. The resistance pattern of *Bacillus* sp. was below 45.45 %, the highest resistance recorded for Penicillin G is >70% (Mao et al. 2019). Surprisingly, most of the isolates were found to be susceptible to the antibiotics used. The *Bacillus* isolates showed susceptibility of 100% against many antibiotics. However, in *Staphylococcus* sp. the resistance against Penicillin (60.24%) and Ampicillin (57.83%)

against was the highest among all he antibiotics. While chloramphenicol, methicillin and tetracycline showed 100% susceptibility. *Pseudomonas* sp. showed 13.63 % resistance against streptomycin and no resistance against norfloxacin. As it has also been studied that even very low concentrations of antibiotics are sufficient to provide a selective advantage for resistant microorganisms (Gullberg et al. 2011). There has been reports that the presence of heavy metals and biocides could co-select antibiotic resistance in the environment. Resistance genes from these environmental reservoirs could then be transferred to pathogenic bacteria (Allen et al. 2010). With the fact that such studies are essential for public health, apologetically, the source of these bacterial resistance however lower than the susceptibility cannot be established (Fair and Tor 2014; Peterson and Kaur 2018).

Ambient Air Quality Monitoring

Environmental pollution is one of the problems faced worldwide, and it is well known that pollutants are the byproduct of man's own action (Manisalidis et al. 2020). The environmental pollution is mainly caused by population explosion (Dong et al. 2020), unplanned dumping of wastes and sewage (Vergara and Tchobanoglous 2012) and unhygienic mode of living (Ghorani-Azam et al. 2016). As the problem of pollution has affected the entire world, Sikkim is also not free from pollution due to rapid urbanization, industrialization and population of vehicles. Although, the pollution level may not be as high as other States of India, the concern is rising as the rapid increase in industries contribute substantially to environmental issues.

The objective of the ambient air quality standard is to provide a basis for protecting public health from adverse effects of air pollution and for eliminating or reducing to a minimum, those contaminants of air that are likely to be hazardous to human being, animals, vegetation and the historical constructions (WHO 2021). Different standards have been laid down for industrial, residential and sensitive areas as given by National Ambient Air Quality Standards (NAAQS). The air quality surveillance and monitoring are undertaken to detect any deterioration in air quality arising from industrial, vehicular, residential and natural sources of pollution, as a large seasonal variation occur in the concentration of various pollutants. The annual average of the tested parameters PM10 during the study period ranged from 8.42 – 87.72 $\mu g/m^3$, the lowest was recorded at Chungthang, north Sikkim in December 2017 and the highest recorded in at Rangpo in January 2019. This data was not surprising as Chungthang is a small town in North Sikkim located at an elevation of 3498 m and lightly populated. While Rangpo is a town located in east Sikkim which lies at an elevation of 327 m, it joins the NH 31 A to the West Bengal. The population of Rangpo as per 2011 census is 10,450 and being a highway not has a heavy flow of vehicles and people. The value of PM10 recorded at Rangpo exceeded the annual average (60 $\mu g/m^3$) standard given by National Ambient Air quality standards (NAAQS). The annual average for PM10 exceeded the NAAQS standard many a times at Rangpo (66.95 and 60.69) in 2017 in January and February respectively and Singtam (60.69) in February 2017, 77.37 in Deorali in February 2017. In 2018 the pollutant level exceeding with much higher values, with the highest value recorded at Rangpo (90.61) in Rangpo in February, which implies that dust (due to vehicular movement and construction works) was the contributing factor for such a high value of PM10. All these sampling stations are densely populated than other stations and the season is dry and dusty which would add to the higher value of PM10.

The annual average of the pollutant SO_2 during the study period ranged from 0.73-19.95 $\mu g/m^3$ lowest in Mangan, North Sikkim. The value of SO_2 was recorded much lower than the NAAQ standards which is 50 $\mu g/m^3$. Subsequently, the value of the pollutant NO_2 ranged from 0-21.2 the lowest or below the detection level was at Ravangla. south Sikkim and the highest at Deorali east Sikkim. Gaseous pollutants present in such as (NO_2, SO_2, CO) and affect the heath of human (Chen et al. 2007; Manisalidas et al. 2020). However, such gaseous pollutants gaseous (NO_2 and SO_2) contributed less to the environmental pollution in the State, indicating not in high health.

CONCLUSION

It is well known that bacteria and fungi present in the air can affect human health and can be more harmful for immunocompromised people. Therefore, it is important to monitor the indoor as well as environmental microbial air composition from time to time. The approach of this study was to study both the prevalent bacteria and fungi and it was observed that the load of bacteria was dominant in majority of the samplings. As we have collected the samples by culture dependent method there are both the advantages and challenges. In the former method the organisms will grow and produce a typical characteristic within a definite period of time, while the culture dependent method would remain accurate and time saving. In order to overcome the difficulty of traditional culture-based method, rapid, accurate techniques for airborne microorganisms should be considered. However, even in culture dependent method we found that for lower level of contamination simple settle plate or gravitational method was highly effective. As it is difficult to find the source of the airborne microorganisms, the variation in microbial load in rural and urban area were not significant. Gram positive cocci were the most prevalent among which *Bacillus* sp. was frequently isolated. Among fungal genera *Fusarium* sp. and *Pencillium* sp. were highly prevalent which are mostly a plant pathogen and allergy causing spores. There has been reports that seasonal variation affects the composition of air microbial flora, however in our study the variation was almost negligible. In this study, most of the bacteria and fungi isolated from air samples were nonpathogenic in nature and or either only harmful for immunocompromised people. The antibiotic resistance pattern of the bacteria isolated were minimal to the antibiotics tested. The ambient air quality of Sikkim throughout the study period remained below the National Ambient Air Quality Standards, many a times going below detection level to very few times going above the standard level (exceptionally the

pollutant Particulate Matter 10), which signifies that the air quality of Sikkim is less polluted and less contaminated.

SUMMARY

The main objectives of this work were to study the prevalent microbes (bacteria and fungi) in Sikkim, a small mountainous State of India, distribution pattern of microbes, their identification and antimicrobial susceptibility patterns. The sample collection sites were pre-decided considering the factors such as vegetation, elevation and population. Air sample were collected from four districts of the State viz. North, South, East and West districts and from ten different locations (Deorali, Singtam, KSC, Tadong, Changu Lake, Pelling, Lachung, Chungthang, Mangan and Ravangla) which fell into these districts. The filed survey irrespective of any criteria related to age or gender was also conducted along with sample collection hoping to find a coordination between the prevalent microbes and the diseases among the populations. Total of 561 individuals were surveyed during the period. The ambient air monitoring of seven stations (Deorali, Singtam, Rangpo, Pelling, Chungthang, Mangan and Ravangla) were also monitored to study the health risks of people in the State, in which three pollutants (PM10, NO_2 and SO_2) were measured. The air sampling from various sampling sites was carried out by two methods, (Plate sedimentation method) and active method (Impaction method) considering dry and wet seasons. The logCFU/m^3 of the bacteria and fungi were calculated which ranged from 1.41- 3.78 log CFU/m^3 and 1.32- 3.71 log CFU/m^3, respectively. Further, the phenotypic characterisation of the microbes was done. A total of 570 bacteria isolates were isolated from 188 air samples, of which 226 survived sub-culturing and retained its morphological and cultural characteristics, which was considered for further study. Out of 226 bacterial isolates 68 were Gram-positive rod (30.88%), 91 (40.26%) Gram-positive cocci and 67 (29.64%) Gram-negative rod, which was further confirmed by the KOH test. During phenotypic characterization, *Bacillus* sp., *Staphylococcus* sp., *Micrococcus* sp., *Pseudomonas* sp., *E. coli*, *Proteus* sp., *Serratia* sp., *Citrobacter* sp.., and *Enterobacter* sp., were tentatively

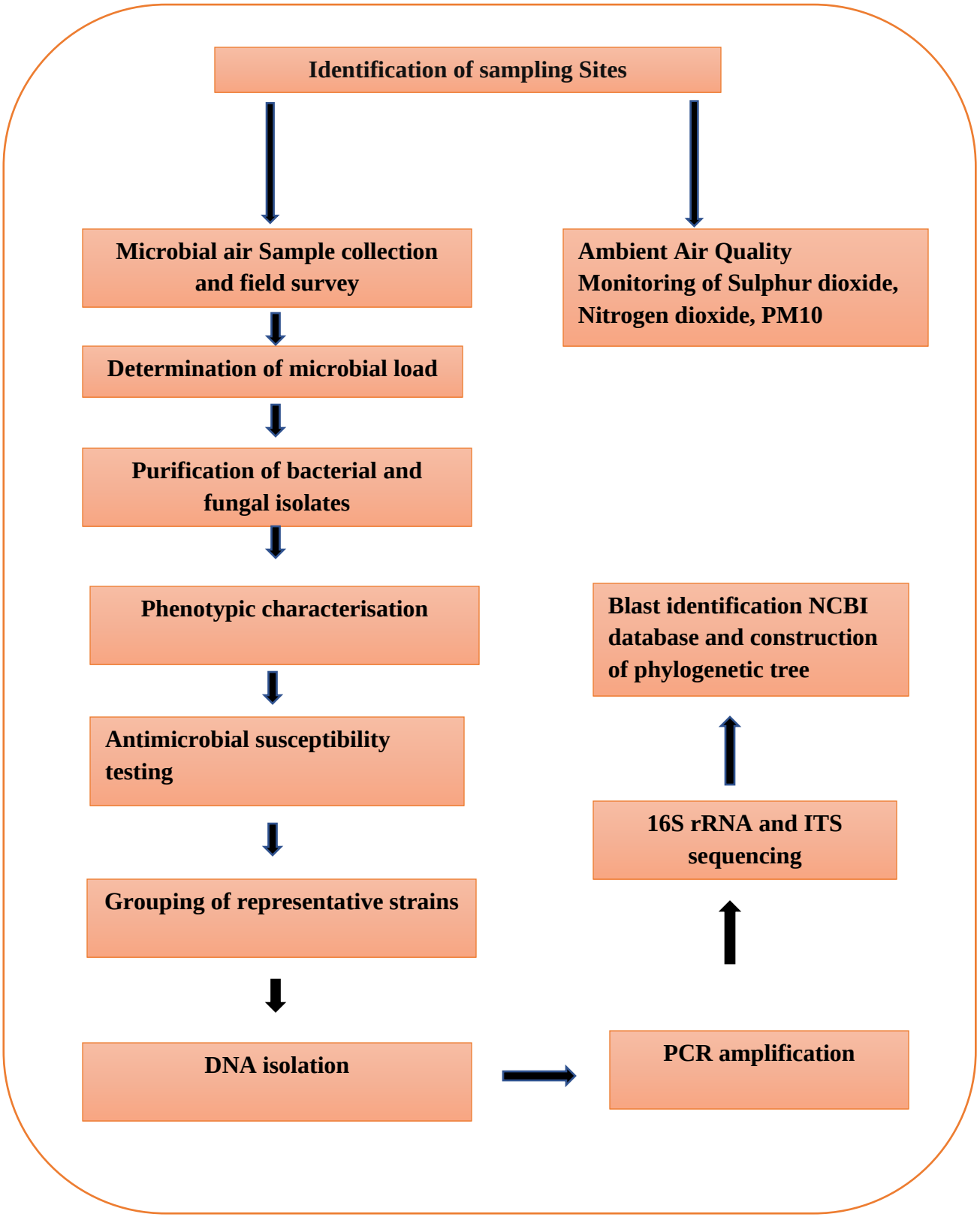
identified after performing the standard biochemical tests. Phenotypic characterization of selected bacterial isolates was also conducted using Omnilog Plus system (BIOLOG), a fully automated high throughput aerobic identification system. In this method, four bacterial species were identified *Staphylococcus haemolyticus Lysynibacillus sphaerius, Corynebacterium sphaerius and Bacillus flexus.* Antibiotic Susceptibility test was conducted for the bacterial isolates which showed frequent occurrences. Hence, the species of *Bacillus, Staphylococcus* and *Pseudomonas* were tested for antibiotic susceptibility against 12 antibiotics; ampicillin, amikacin, amoxycillin, chloramphenicol, clindamycin, erythromycin, methicillin, norfloxacin, penicillin G, streptomycin, tetracycline and vancomycin. While majority of the isolates showed susceptibility to the tested antibiotics, *Bacillus* sp. and Staphylococcus sp. showed highest resistance (45.45%) and (60.24 %), respectively to Penicillin-G, while *Pseudomonas* showed greater amount of resistance to Erythromycin (7%). The 16S rRNA gene sequencing result based on Sanger method showed a complex microbial community with four genera, six different species viz. *Bacillus safensis* subsp. *Safensis, Bacillus aryabhattai, Bacillus altitudinis, Staphylococcus sciuri, Pseudomonas stutzeri, Cellulosimicrobium cellulans.* Nine representatives of fungal strains were identified using ITS region viz. *Penicillium brevicompactum, Fusarium proliferatum, Fusarium solani, Fusarium oxysporum, Trichoderma reesei, Colletotrichum graminicola, Colletotrichum graminicola, Phaeosphaeria avenaria f. sp. Avenaria, Metarhizium granulomatis.* The annual average of the tested parameters PM10 during the study period ranged from 8.42 – 87.72 µg/m^3, the highest recorded at Rangpo even exceeding annual average the standard limit of (60 µg/m^3). The annual average of the pollutant SO_2 during the study period ranged from 0.73-19.95 µg/m^3 lowest in Mangan, North Sikkim. The value of SO_2 recorded was much lower than the NAAQ standards which is 50 µg/m^3.

Subsequently, the value of the pollutant NO_2 ranged from 0-21.2 which was also way below the standard limit. Giving a general impression that the air of Sikkim is clean considering the current havoc of air pollution around the globe.

Highlights of findings

- Based on 16S rRNA gene sequencing result, *Bacillus safensis* subsp. *Safensis, Bacillus aryabhattai, Bacillus altitudinis, Staphylococcus sciuri, Pseudomonas stutzeri, Cellulosimicrobium cellulans* were identified form the ambient air of Sikkim.
- Phenotypic characterization of selected bacterial isolates using Omnilog Plus system (BIOLOG), identified the bacteria genera *Staphylococcus haemolyticus Lysynibacillus sphaerius, Corynebacterium sphaerius and Bacillus flexus.*
- The high throughput gene sequencing should be preferred for studying ambient air microorganisms as the conventional culture-dependent methods for identification of organisms for environmental studies could be challenging and laborious.
- The bacterial load found in air environment was much higher than the fungal load.
- Most of the bacteria and fungi isolated from air samples of different regions and places of Sikkim were non-pathogenic and non-infectious, which indicate the possibly of quality of air or less microbial contaminated air.

Schematic Diagram and Pictorial Presentation of Complete PhD Work



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QUALIFICATION

Degree	University	Month and year of joining	Month and year of passing	Division/ Grade
M.Phil (Microbiology)	Sikkim University	07/2011	12/2014	First
MSc (Microbiology)	HNB Garhwal University	08/2009	11/2011	First

Research Experience: Experience in Microbiological Analysis (Isolation, Identification, Characterization) and Maintenance of air, water and clinical samples. Bacterial identification with Culture-dependent method, DNA extraction, PCR analysis, Phylogeny Tree Constructions and ELISA. Antimicrobial susceptibility testing with disc diffusion and Minimum inhibitory Concentration method. Work experience on ambient air monitoring System and analysis.

Awards: Awarded and attended 15 days NER training programme on **"Gene Cloning, Protein Biochemistry, Structure Biology & Bioinformatics"** from February 4-15, 2019 at Tata Memorial Centre, Advanced Centre for Treatment, Research and Education in Cancer, Kharghar, Navi Mumbai.

Workshop/ Conferences attended at National/International Conferences: 8

Publications

1. Singh, A.K., Das, S., Singh, S., **Pradhan, N**., Gajamer, V.R., Kumar, S., Lepcha, Y.D. and Tiwari, H.K. (2019). Physicochemical parameters and alarming coliform count of the potable water of Eastern Himalayan state Sikkim: An indication of severe fecal contamination and immediate health risk. *Frontiers in public health 7* 174.
2. Singh, A.K., Das, S., Singh, S., Gajamer, V.R., **Pradhan, N.,** Lepcha, Y.D. and Tiwari, H.K. (2018). Prevalence of antibiotic resistance in commensal Escherichia coli among the children in rural hill communities of Northeast India. *PloS one 13*(6): e0199179.