CHAPTER I

INTRODUCTION

1.1 Pollution

Environmental pollution is a problem both in developed and developing countries. Human intervention in science and technology had results the presence of contaminants into the natural environment that cause adverse change known as” Pollution”. Pollution can take the form of chemical substances or energy, such as noise, heat or light. The pollutants are primarily fouling the air, water and land. Primary pollutants are emitted directly from the source and secondary pollutants is not directly emitted as such, but forms when react with primary pollutants. Primary pollutants include Oxides of sulfur, oxides of nitrogen, ammonia, etc., emitted from burning of fossil fuels. Secondary pollutants like ozone, formaldehyde, smog and peroxo acetyl nitrate are formed from primary pollutants.

Biological pollutants like bacteria, molds, mildew, viruses, animal dander and cat saliva, house dust, mites, cockroaches, and pollen are primary pollutant is global threat which cause epidemic disease worldwide. Aerobiology is a branch of biology that deals with organic particles, such as bacteria, fungal spores, very small insects, pollen grains and viruses are present in the air both outdoor and indoor. Biological particles are carried in the air that affects the many aspects of our life. For example, many people have allergic reactions to inhaled biological particles and many human, animal and plant pathogens are transported by the air.

Some organisms are adapted for wind transport whilst others become airborne incidental, or only as debris. The study of airborne microorganism has expanded from the traditional arena of transmission of disease via the respiratory route to include not only
human pathogens but also plant pathogens, opportunistic and nonpathogenic organisms, and aerosolized microbial by products, airborne cultural and non culturable bacteria, saprophytic fungi, free living parasites, viruses, and algae that may result in adverse health effects of environmental impact are now studied within the field of aerobiology. The main incentive for the development of aerobiology as a scientific discipline has been the desire to understand the dispersal of diseases of man, animals and plants in order to try and prevent them.

1.2 Global scenarios

Globally, 3.7 million deaths were attributable to ambient air pollution (AAP) in 2012. About 88% of these deaths occur in low-and middle-income (LMI) countries, which represent 82% of the world population. The Western Pacific and South East Asian regions bear most of the burden with 1.67 million and 936,000 deaths, respectively. About 236,000 deaths occur in the Eastern Mediterranean region, 20,000 in Europe, 176,000 in Africa, and 58,000 in the Americas. The remaining deaths occur in high-income countries of Europe (280,000), Americas (94,000), Western Pacific (67,000), and 14, 000 in the Eastern Mediterranean (WHO, 2014).

Airborne culturable microorganisms, including bacteria, fungi and viruses (Bioaerosol) that may result in adverse health effects on humans and plants. Respiratory allergy is prevalent among all populations all over the world. Epidemiological studies carried out in different countries report the prevalence of respiratory allergy from 15% to 30%. A survey conducted in Finland shows a prevalence of around 14% allergic rhinitis and 2.5% asthma. In
Australia 27% of children are reported to have wheeze. Asthma is prevalent in about 9% Greek population.

1.3 Air pollution in India

Recent surveys carried out in India revealed 20 to 30% of the population suffering from allergic rhinitis and 15% develop asthma. An epidemiological study carried out 46 years ago (1964) in Delhi had reported only 10% population having allergic rhinitis and 1% asthma. Thus, it is well established that the incidence of allergy is increasing all around the world.

In a study conducted as a part of the European Community Respiratory Survey, the prevalence of asthma in adults aged 20 to 44 years in Mumbai was reported to be 3.5% using clinician diagnosis and 17% by a physician diagnosis and broncho-provocation test\textsuperscript{4}. A recent study\textsuperscript{5} in school children from Delhi reported asthma prevalence of 10–13%. Other reports indicate a geographical difference in the prevalence of asthma as well as differences in urban and rural population.

Developing countries like India, the affluent urban population following Western lifestyle appears to be more prone to allergic risk, resulting in increased incidence of allergic manifestations like asthma in children. It therefore appears that low socio-economic levels, high temperatures, poor housing conditions and lack of enough hygiene still prevalent in East Asian developing countries may predispose inhabitants to infections and helminthic infestations which protect against allergic sensitization. This is probably the reason why developing countries like India do not show a similar rise in allergic diseases as seen in the developed industrialized countries. Increase in air pollution, as a result of
changing lifestyle may have a role in asthma, which can worsen the clinical status, but the correlation between pollution and incidence of allergy or asthma has not been established (Sneller 1984, Jindal 2010, Agarwal et al., 2010, Ritesh 2011).

1.4 Air pollution in Bengaluru

In recent decades, air pollution has become one of the most important problems of megacities. Initially, the main air pollutants of concern were sulfur compounds, which were generated mostly by burning coal. Problems of Outdoor and indoor air quality are recognized as important risk factors for human health in both low-income and middle- and high-income countries. Out Door air is important also because populations spend a substantial fraction of the time. In Shopping centers, vegetable market, residential area and other special environments, outdoor air pollution affects population groups that are particularly vulnerable due to their health status or age. Microbial pollution involves hundreds of species of bacteria and fungi that grow outdoor and indoor when sufficient moisture is available. Exposure to microbial contaminants is clinically associated with respiratory symptoms, allergies, asthma and immunological reactions. Outdoor air pollution containing particulate matter, CO, CO2, SO2, NO2 and particulates of biological origin (e.g. Microorganisms and pollen) are from potential source of motor vehicle exhaust, garbage and open biomass burning.

It is important to note that geography and climate play an important role in determining the outdoor air microbial concentrations because the transport of bioaerosol is primarily governed by hydrodynamic and kinetic factors, while their fate is
dependent on their specific chemical makeup and the meteorological factors to which they are exposed.

Rapid urbanization, dense vehicular movement worsening the Bangalore air quality. RSPM and NOx values are exceeded to the national limit of 60.0 and 40.0 μg/M$^3$ respectively, for the period of 2007-12. During the year 2012-13, RSPM has exceeded the limit and SO2, NOx, and CO values are within the national limit. At City Railway Station Metro work is under progress, hence only RSPM has exceeded and traffic has decreased meanwhile pollutants like SO2, NO2 and CO are showing a decreasing trend. Today, photochemical smog, Oxides of nitrogen, Corban monoxide, heavy metals, particulate matter-induced primarily from traffic, but also from industrial activities, power generation, and solvents-has become the main source of concern for air quality. It is reported that air pollutants are still a major problem in many cities of the developing world. Air pollution has serious impacts on public health.

1.5 Prevalence of asthma in Bangalore

Prevalence of asthma in Bangalore, hospital based study in a general pediatric outpatient by a pediatric pulmonologist on international guidelines on 20,000 children under the age of 18 years in 2 decades from 1979, 1984, 1989, 1992 and 1999 in the metropolitan city showed 9%, 10.5%, 18.5%, 24.5% and 29.5% respectively. The steady rise in prevalence correlated with demographic changes in the city (Paramesh, 2000) like increase in number of industries, increased density in population from the migration of rural population in search of jobs and increases the number of automobiles to commute resulting in air pollution.
1.6 Aerobiology

Airborne or are transmitted biological particles have registered historical records during the 13th century. An Unknown airborne disease caused by virus and bacteria registered the massive death in the early 13th century. The Black Death (black plague) was one of the most devastating pandemics in human history caused by *Yersinia pestis* resulted in the deaths of an estimated 75 to 200 million people and peaking in Europe in the years 1346–1353. *Yersinia pestis* is a bacterium that normally infects rodents such as rats, rock squirrels, wood rats, prairie dogs and transmitted to humans via air, flea bites and contact with infected animal. Over the year, the plague were speeded every Europe’s, central Asia and reduced the world population from an estimated 450 million down to 350–375 million in the 14th century (BBC News 2008, ABC/Reuters 2008, Haensch et al., 2010).

Another major death roll taken by Smallpox was an infectious disease caused by either of two virus variants, Variola major and Variola. The disease killed an estimated 400,000 Europeans annually during the end of the 18th century and was responsible for a third of all blindness.

Such kind of airborne disease, disaster influenced the Louis Pasteur (French chemist) to carry out the research on microbial species in water, air and soil. Between 1857 and 1859, Pasteur convinced that the liquids he had studied were being contaminated with microbes that floated in the air. The air contained living organisms can produce putrefaction. From this first impetus, many researchers have dedicated themselves to this challenge. But none had the caliber of Pierre Miquel (French pharmacist) who has literally taken Pasteur's wish at his words, referring to the above
citation on the first pages of his monumental work of 1883. Pierre Miquel was one of the fathers of aerobiology (Comtois, 1997). This brought a large number of aerobiological studies and it grew up following the major advancement in “Allergology”. The term “Aerobiology” was defined in the 1930s by Fred Campbell Meier during 1893-1938 (Sofiev and Bergmann, 2012).

1.7 Microbes and earth atmosphere

Earth’s atmosphere is known to team with airborne microorganisms, though the high light intensities, extreme temperature variations, low concentrations of organic matter, and a scarcity of water, make the atmosphere as unsuitable environment habitat for microbial growth (Atlas, 1984).

Each year, several millions of metric tons of dust, water, and anthropogenic pollutants make their way into the atmosphere, often traveling between continents on jet streams. Biological material may contribute about 20% to 22% of the total airborne particulate by volume in remote continental, populated continental and remote maritime environments respectively. Some microbes make the trip with them, seeding the skies with billions of bacteria and other organisms. Thousands of airborne microorganisms include bacteria and fungi floating in the troposphere, over 60% of all microbes are still alive about 10 kilometers over (Matthais, et al., 2000; AAAS Science News, 2013).

1.8 Bioaerosol

A collection of airborne biological particles is called a bio aerosol. Generally bioaerosol generated as poly dispersed droplets or particles of different sizes ranging from 0.5-30 μm in diameter.
Particles over 100 μm in diameter are generally referred to as droplets. Particles from 1 to 2 μm can deposit deep into the alveoli of the lungs, while particles greater than 3.5 μm in diameter are trapped in the upper respiratory tract. Particles between 2 and 3.5 μm in diameter generally do both.

Source: Nandini, 2014

Most pathogens target the alveoli, but there is evidence that primary infection for both Variola virus (i.e., smallpox) and Yersinia pestis bacilli (i.e., pneumonic plague) can occur in either the alveolar region or the upper respiratory tract. Air serves as a mode of transport for the dispersal of bioaerosol from one location to another location. Numerous anthropogenic activities, especially agricultural practice and wastewater treatment process, serve as the origin of bioaerosol in outdoor environments. The release of biotechnology products (e.g. Genetically engineered microorganisms and microbial pest control) developed to enhance agricultural productivity, mineral recovery, oil spill cleanup, and toxic waste disposal, sanitary landfill operations and reuse water irrigation practice can also a source of airborne bacterial and viral pollution in the air (Christopher and Cox, 1995; Nandini, 2014).
1.9 Air spora

The population of airborne particles of plant or animal origin called as the air ‘spora’ contains spores and pollens of various shapes ranging in size from 100 μ in diameter for some tree pollens down to 3-5 μ with some of the smallest fungus spores. In addition, air spora may also comprise biological particles can include viruses, bacteria, actinomycetes, fragments of fungi and fungal spores, lichen fragments and their spores, protists (e.g. protozoa, algae and diatoms), spores of plants (e.g. mosses and ferns), pollen, plant fragments and small seeds, invertebrates (e.g. nematodes, mites, spiders and insects) and their fragments and faecal material, plus skin, hair, dried mucus and excrement from larger animals. These particles range in size from 1 to >200 μm (Lacey and West, 2006).

1.10 Fungi as Allergenic Agents

Fungi are ubiquitous in Earth’s biosphere, and fungi (including molds) contribute a vital role to our ecology found in both outdoor and indoor air samples, although the two main natural reservoirs are organic debris and soil. Generally, fungi are not a common cause of human pathology, although well-described illnesses are caused by fungi for example, allergies, hypersensitivity pneumonitis, infections (usually in immune compromised individuals), and toxicity from mycotoxin exposure after excessive occupational or agricultural exposure. The fungi exhibit a remarkable ability to use almost any carbon source as a nutrient. Nevertheless, substrata a specific species can use as food to a large extent depends on what digestive enzymes it is capable of producing and releasing into the environment. In addition to the
availability of a suitable nutrient source, other factors that influence growth and spore release are moisture, temperature, pH, and oxygen availability (Deacon 1997, Domsch et al., 1980).

The prevalence of respiratory allergy to fungi is estimated at 20 to 30% among atopic individuals and up to 6% in the general population. The major allergic manifestations induced by fungi are asthma, rhinitis, allergic broncho pulmonary mycoses, and hypersensitivity pneumonitis. These diseases can result from exposure to:

1. Spores
2. Vegetative cells
3. Fungal metabolites

More than 80 genera of the major fungal groups have been associated with symptoms of respiratory tract allergy. In addition, as the fungal spores are small (usually less than 10 μm) a majority of them are capable of penetrating the lower airways of the lung and mediate allergic reactions. The condo and fungal spores associated with the immediate type of hypersensitivity are usually larger than 5 μm, while those associated with delayed type of hypersensitivity are considerably smaller (approximately 1 μm) and can penetrate the smaller airways. The site of deposition of spores is dependent on, whether spores enter the respiratory tract as individual propagules or as aggregates for e.g. the clusters of small conidia of *Aspergillus* / *Penicillium* are usually deposited in the upper respiratory tract, while the smaller individual spores reach the lower airways (Artaxo and Hansson 1995; Lindemann and Upper 1985).
1.11 Airborne Bacteria as allergic agents

Bacteria are prokaryotic organisms with size ranging from 0.3 to 10 micrometer. Bacterial aerosolization can occur from the surface of soil, water bodies, vegetation and by anthropogenic sources. Bioaerosol constituents of the atmosphere have long been reorganized, and large numbers of bacteria were found even at high altitudes and remote oceanic and polar region. The presence of bacteria in the atmosphere is significant for several reasons: they may spread diseases, play a role in ice nucleation in the atmosphere and could nucleate cloud and fog droplets.

Bacterial aerosol in the air is the most potent activators of airway Macrophage which cause the allergic-asthma problems in most of the cases. Constituents of the cell wall or membrane of both Gram-positive and Gram -negative bacteria stimulate the release of a variety of inflammatory mediators which are involved in the recruitment of inflammatory cells to the airways. Most of them originate from natural sources such as soil, lakes, animals and humans. Moreover, agricultural practices, health care units and industrial operations such as sewage treatment, animal rendering, fermentation processes, and food processing plants also emit viable microorganisms into the air (Cox 1989, Fuzzi et al., 1997, Schnell and Vali 1976, Chandramouli et al., 2005, Posfai et al., 2007).

1.12 Involvement of Microbial components and Immune Response to Air Pollutants

Microbial agents, including bacteria, viruses, and fungi, are the most potent activators of antibodies. The cell wall or membrane constituents present in the both Gram-positive and Gram-negative
bacteria stimulate the release of a variety of inflammatory mediators which are involved in the recruitment of inflammatory cells to the airways. In severe infection these mediators e.g., tumor necrosis factor (TNF), interleukin (IL)-1, IL-8, IL-6, and various chemokines are involved in septic shock. Ambient air has variable numbers of bacteria. Most bacteria in the air are nonviable or do not grow on conventional substrates. Therefore, the actual amount of microbial material in the air is underestimated. Gram positive and Gram negative bacteria and their degradation products are part of particulate matter of outdoor air, and are found to be associated mainly with inhalable particulate matter. Inhalation of dust-containing bacterial components, especially lipopolysaccharide (LPS), may cause symptoms involving airways inflammation, fever, decrements in lung function, and airways hyper reactivity (Hodgson, et al., 2001).

### 1.13 Effects of Air Pollution on Children

Children are more vulnerable to the adverse effects of air pollution than are adults. Eighty percent alveoli are formed postnatally, and changes in the lung continue through adolescence. During the early post neonatal period, the developing lung is highly susceptible to damage after exposure to environmental toxicants.

Children have an increased exposure to many air pollutants compared with adults because of higher minute ventilation and higher levels of physical activity. Because children spend more time outdoors than do adults, they have increased exposure to outdoor air pollution. Exposure to bioaerosols at workplace and residential area is associated with adverse like respiratory irritation similar to mucous membrane irritation (Hassan et al., 2002).
1.14 Role of Meteorological Parameters

Meteorological parameters such as Temperature, Sunshine, Humidity, Snow, and Wind speed and direction are therefore important in the distribution and deposition of outdoor airborne fungal spores (Al-Doory and Domson, 1984). The fungal phenomenon involves nonspecific ailments and health complaints associated with some indoor fungi or molds, often identified only by the involved individual as the cause of their health problems. This condition is reminiscent of the Sick Building Syndrome, which apparently is no longer a problem or an issue.

The presence of many biological agents in outdoor environments is attributable to dampness and organic waste disposal. Excess moisture in almost all outdoor materials leads to the growth of microbes, such as mould, fungi and bacteria, which subsequently emit spores, cells, fragments and volatile organic compounds into the outdoor air. Moreover, dampness initiates chemical or biological degradation of materials, which also pollute air. The dampness has therefore been suggested to be strong, consistent indicator of risk of asthma and respiratory symptoms (e.g. Cough and wheeze). The health risks of biological contaminants of outdoor air could thus be addressed by considering dampness as the risk indicator.

1.15 Association of bioaerosol and air pollutants

Bioaerosol in ambient air is not only related to meteorological parameters. In addition, some associations exist between bioaerosols and air pollutants. In the summer of Cincinatti, airborne viable bacteria and carbon monoxide had a strong correlation during summer (Lee et al., 1973). In France, viable bacteria and nitric oxide, nitrogen dioxide, and suspended
particulate pollutants had a correlation (Mancellini and Shulls 1978).

In Brisbane, there was a negative correlation between daily fungal counts and mean daily average air pollutants, including NOx, CO, SO2, and PM (Glikson, et al., 1995). Particulate matter PM10 (10 mm) and PM2.5 (2.5 mm), are associated with respiratory allergic diseases, asthma and mortality. Particulate matter is diesel exhaust can act as a carrier of allergens and adjuvant with bioaerosols enhancing IgE production. Intact pollen cannot reach the small airways, however, PM2.5 and bioaerosol can easily penetrate aggravate respiratory allergy and other pulmonary diseases (Monn 2001, Parnia et al., 2002).

1.16 Bio aerosol sampling techniques

Sampling of bioaerosols involves separating the airborne viable particles from the air by various samplers utilizing different physical separation principles. Bioaerosol samplers can be divided into inertial devices and filters. Inertial devices include impactors, impingers, and centrifuges. Filters device includes a high volume sampler and a respirable dust sampler.

i. Inertial impactors

An inertial impactor sampler collects the bioaerosols onto solid media surfaces using inertial forces. The air stream is forced to change direction in the collection stage of the impactor, and particles with high inertia are impacted on a solid collecting surface (Henningson and Ahlberg 1994, Thompson et al., 1994). Impactors with multiple collection stages, e.g. the Andersen six-stage sampler,
provide information of the particle size distribution (PSD) of bioaerosols.

Several types of impactors have been used for bioaerosol sampling, including cascade impactors with one, two or six stages; slit samplers; and the ‘surface air system’ sampler (Thompson et al., 1993, Nevalainen et al., 1993). Among them, the Andersen six-stage sampler is the most commonly used for bioaerosol sampling in livestock buildings and often served as a reference sampler by some researchers in evaluating other sampling devices (Predicala et al., 2001, Cox and Wathes 1995, Henningson and Ahlberg 1994).

ii. Liquid impingers

A liquid impinger sampler works in a similar way as impactors, except that the collection surface of an impinger is liquid nutrient broth and that of impactors is solid culture media (Henningson and Ahlberg, 1994). There are different types of impingers: all-glass impinger-30 (AGS-30), all-glass impinger-4 (AGS-40), SKC biosampler, and multistage all-glass liquid impingers (Thompson et al., 1993). The all-glass impinger method has been widely used since 1920s when it was firstly introduced (Thorne et al., 1992).

iii. Centrifugal samplers

This type of samplers also uses the inertial behavior of the particles. In centrifugal samplers, aerosol is forced into a centrifugal motion, and the particles with high inertia will deposit on the sampler wall (Henningson and Ahlberg 1994). A centrifuge can be used to obtain the aerodynamic size distribution of irregular shaped particles, aggregate particles, and fiber as well as their
concentrations (Baron and Willeke 2001). On the other hand, centrifuge sampler is very expensive.

iv. Filter sampler

Infiltration, inertial force also works to separate the particles from the airstream, but besides that, other mechanisms, such as interception, diffusion, and electrostatic attraction contribute to the deposition of particles onto the filter (Nevalainen et al., 1992, Henningson and Ahlberg 1994). Filtration samplers are relatively inexpensive and easy to use, and have been suggested for sampling some organisms within certain environmental limits for its simplicity and its suitability for a variety of environments (Thorne et al., 1992). The major problem associated with this method is the inactivation of microorganisms due to the dehydration effects caused by the large volume of air passing over the filter and the damage or loss of parasols when they are being removed from filters for lab analysis (Thorne et al., 1992, Cox and Wathes 1995).

The study was focused on seasonal variations of fungal and bacterial aerosol which is essential to frame, the control measures of allergic disease in Bangalore. In addition, addressing the bioaerosol, air pollutants, weather change of Bangalore city could be useful for preventing epidemiological disease in future.
The aim of this research was to fill the knowledge gaps in quantifying bioaerosols and seasonal changes with meteorological parameters. The newly gained knowledge will provide fundamental information for studies of fate and transport of bioaerosols emitted from various sources as well as for assessment of potential health effect of residents of Bengaluru urban. The specific objectives of this study were as follows;

1. To study the sources of Microbial air pollution

2. To study microbial diversity in ambient air and population variation with change of Meteorological Parameters during different seasons

3. To study the influence of meteorological parameters on bioaerosolization

4. To study the relationship between air pollutants and Microbial pollutants

5. To Study the effect of biological pollutants on Human Health