# AIR MICROBIOLOGY

## DEFINITION:

Aerobiology can be defined as the study of passively airborne particles (of biological origin) like pollen and spores of microorganisms in air (both outdoor and indoor) with reference to their identity, origin, survival capacity and their behavior (transport, deposition, etc.) in the aerosol state in relation to meteorological conditions and their impact on human beings, plants and animals.(aerosol is a collection of particles suspended in the air).

## SCOPE OF AEROBIOLOGY:

The field of aerobiology is interdisciplinary with applications in subjects like respiratory allergology, immunology, medicine, public health, plant pathology, pest management, biological weathering, indoor air quality, air conditioning technology, industrial aerobiology, industrial and environmental hygiene, biological science, agriculture, atmospheric physics, environmental sciences etc. it is a rapidly developing science which also involves an interaction of engineering and meteorology. knowledge of biology, plant physiology, mycology, engineering and meteorology andaerosol physics is basic for studying production, release, transport and decomposition of biological particles.

## AIM OF AEROBIOLOGY:

Aerobiologists have tradiotionally been involved in measurement and reporting of airborne fungal spores and pollen as a service to allergy sufferers. They study the dispersal and effect of allergens and,human, plant and animal pathogens. Thus, the aim of aerobiology is

1) Qualitative and quantitative analysis of air for airborne microbes by air sampling techniques

2) Study of behavior of air microflora with regard to their survival capacity, dispersal, deposition and effect on humans, plants and animals and materials.

3) Development of improved air sampling techniques and equipment's.

4) Forecast of diseases and allergies and pollen emission.

5) Provision of clean dust and microbe free air whenever required.

6) Prevention and control of biodeterioration of materials like food and of air borne diseases and allergies using air sanitation and sterilization methods

## HISTORY:

Classical writers believed that the wind some times brought sickness to man, animals and crops. Hippocrates, the father medical science held that man was attacked by epidermic fevers when the inhaled air infected with such pollution which are hostile to the human race. Lucretius, in about

55B.C. was of the opinion that diseases originated from minute invisible particles in the air (which are carried in clouds by the wind) when the settle on wheat or when they inhaled from polluted atmospheres after Lucretius more than 1500 years passed before man became aware that air carries abundant microscopic living organisms. This discovery was possible due to the invention of the microscope and discovery of microorganisms by Anton van Leeuwenhoek in 1674. Leeuwenhoek believed that animalcules carried by air along with bits of floating dust were responsible for decaying animal and vegetable matter. he also accounted the ubiquitous occurrence of microorganisms to their air borne nature

Lazzaro Spallanzani showed, by a series of experiments that microbes were present in air and that they do not arise spontaneously in organic infusions. He developed the technique of hermetic scaling for exclusion of microbes in air from sterile infusions

Louis Pasteur used the gun cotton air filter and extracted the suspended dust in the air for microscopic explanation. He demonstrated in 1861 by microscopic and cultural methods, and with the help of the famous swan neck flask experiment, the existence of an air spora. He also made the 1st rough visual measurement of the concentration of the air spora in the atmosphere of city of Paris. He showed that the quantity of air borne germs differed in different places. He is considered to be the first experimental aerobiologist.

The most intensive sustained analysis of bacteria moulds in the atmosphere was made in Paris during the past quarter of 19 thcentury. Pierrre Miquel, a bacteriologist worked at the observatioreMontsouris,an institution responsible for studying the dust in the air of microscopic and cultural methods, Miquel, in the ____ was the 1st to make a long-term survey of the microbial content of the atmosphere by volumetric methods. He also studied air over seas and at high latitudes. He discovered diurnal periodicity in fungal spores and also in bacteria. He is considered as one of the father of aerobiology and the 1st professional aerobiologist he was interested in studying the influence of whether on bioaerosols and their impact on epidemics.

Charles blackley (1873), a physician, showed by means of his sticky slides that the air contains pollen which when inhaled can cause allergies such as hay fever.

Interest in airborne microbes quickened in the 20th century when techniques for trapping air borne particles were developed.

Fred C Meier of the united states department of agriculture (USDA) introduced the word aerobiology to describe a fare reaching project of research on microbial life in the upper air. The American association for the science published a symposium on extramural and intramural aerobiology and the new discipline was firmly launched. The international aerobiological association (IAA) was found in 1974 in Hague,Netherlands. aerobiology became a theme in 1964. When the international biological programme was established. in 1968 an international aerobiology working group was constituted to coordinate all the national aerobiological

programs. IAA holds seminars plenary sessions, scientific meetings and exhibitions on all aspects of aerobiology and publishes newsletters twice a year.

**Phillips herries Gregory (1907 -1986 ):** he coined the term *airspora* to describe microbes in air. He pioneered aerobiology as a topic for research, especially extramural studies. He was a versatile mycologist and phytopathologist and worked on understanding fungal spore dispersal, splash dispersal mechanism plant disease epidemiology and allergy. He was interested in natural history and meteorology and frequently suffered from asthma: He investigate the epidemiology of flower bulb disease and potato virus disease. He also worked on identifying the cause of farmer's lung disease. He worked at the Rothamsted Experimental Station UK and has authored a book Microbiology of the Atmosphere.

## AEROBIOLOGY IN INDIA

The first systematic work on airborne fungi was carried out by Cunningham (1873) at Calcutta Tail, which hepublished in the form of book titled Microscopic Examination of Air Later Prof. K.C.Mehta extensively investigated air for the presence of wheat dust spores and their epidemiology Other Indian Scientists like Joshi et al. (1972).Nagarajan and Singh (1973). have made aerobiological investigations in several crop fields and their results have been useful in forecasting of plant diseases and control

## DROPLET NUCLEI

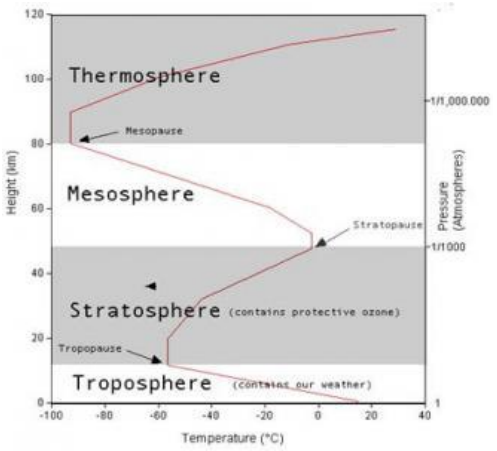
Sneeze droplets, small in size, ranging in diameter from a lower limit of 5-10µm and smaller than 50µm. couldevaporate instantaneously to droplet nuclei', and are then easily inhaled. The larger droplets carrying more inoculum, settle rapidly. The concept of droplet nuclei was developed by Wells (1955) Droplet nuclei are the particles formed from thesmallest droplets which evaporate before falling to the ground and so remain suspended in air. They consist of thesolid residue of the evaporated droplets. Together with any bacteria or virus particles and may be coated with Semidried up mucus which tends to preserve activity and viability. Most airborne bacteria are carried on ratio of dustparticles which settle rapidly and they are mostly harmless saprophytes. Droplet nuclei are a major means of transmission of many human diseases. Pathogenic bacteria are present only in special environments and are earned inthe droplet nuclei which are capable of entering and being retained in the alveoli of the lungs during inhalationex: Mycobacterium tuberculosis Indoor air spora is influenced by many factors such as ventilation rates, crowding nature and degree of activity of individuals living indoors and type of raw materials used in different apartments. the indoor of different environments namely dwelling houses, hospitals, laboratories, factories handling different raw materials, farms houses possess different microflora.

**Layers of the Atmosphere**

**What are the different layers of the atmosphere?**

The atmosphere can be divided into layers based on its temperature, as shown in the figure below. These layers are the troposphere, the stratosphere, the mesosphere and the thermosphere. A further region, beginning about 500 km above the earth's surface, is called the exosphere.

**THE REGIONS**




**The Troposphere**

This is the lowest part of the atmosphere - the part we live in.It contains most of our weather - clouds, rain, snow.The troposphere contains about 75% of all of the air in the atmosphere, and almost all of the water vapour (which forms clouds and rain).In this part of the atmosphere the temperature gets colder as the distance above the earth increases, by about 6.5°C per kilometre.

The decrease in temperature with height is a result of the decreasing pressure. If a parcel of air moves upwards it expands (because of the lower pressure). When air expands it cools. So air higher up is cooler than air lower down.The top of the troposphere is called the tropopause. This is lowest at the poles, where it is about 5 km above the earth's surface. It is highest (about 16 km) near the equator.

**The Stratosphere**

This extends upwards from the tropopause to about 50 km.It contains much of the ozone in the atmosphere.The increase in temperature with height occurs because of absorption of ultraviolet (UV) radiation from the sun by this ozone.By absorbing dangerous UV radiation, the ozone in the stratosphere protects us from skin cancer and other health damage.However chemicals (called CFCs or freons) which were once used in refridgerators and spray cans have reduced the amount of ozone in the stratosphere, particularly at polar latitudes, leading to the so-called "Antarctic ozone hole".Now humans have stopped making most of the harmful CFCs we expect the ozone hole will eventually recover, but this is a slow process.

**The Mesosphere**

The region above the stratosphere is called the mesosphere.

Here the temperature again decreases with height, reaching a minimum of about -90°C at the "mesopause".

**The Thermosphere and Ionosphere**

The thermosphere lies above the mesopause, and is a region in which temperatures again increase with height. This temperature increase is caused by the absorption of energetic ultraviolet and X-Ray radiation from the sun.

The region of the atmosphere above about 80 km is also caused the "ionosphere", since the energetic solar radiation knocks electrons off molecules and atoms, turning them into "ions" with a positive charge.

The ionosphere reflects and absorbs radio waves, allowing us to receive shortwave radio broadcasts in New Zealand from other parts of the world.

**The Exosphere**

The region above about 500 km is called the exosphere.

It contains mainly oxygen and hydrogen atoms, but there are so few of them that they rarely collide - they follow "ballistic" trajectories under the influence of gravity, and some of them escape right out into space.

## Composition of Air

The air in our atmosphere is composed of different gaseous molecules. The most common gases are nitrogen (78%), $CO_2$ (0.034%) oxygen (21%) argon (1%) and other molecules in trace level are present in the atmosphere.

**Table 9.1:** Composition of Air

| Gas | Symbol | Content | |
|---|---|---|---|
| Nitrogen | $N_2$ | 78.084% | 99.99% |
| Oxygen | $O_2$ | 20.947% | |
| Argon | Ar | 0.934% | |
| Carbon dioxide | $CO_2$ | 0.033% | |
| Neon | Ne | 18.20 ppm | |
| Helium | He | 5.20 ppm | |
| Krypton | Kr | 1.10 ppm | |
| Sulphur dioxide | $SO_2$ | 1.00 ppm | |
| Methane | $CH_4$ | 2.00 ppm | |
| Hydrogen | $H_2$ | 0.50 ppm | |
| Nitrogen oxide | $N_2O$ | 0.50 ppm | |
| Xenon | Xe | 0.09 ppm | |
| Ozone | $O_3$ | 0.07 ppm | |
| Nitrogen dioxide | $NO_2$ | 0.02 ppm | |
| Iodine | $I_2$ | 0.01 ppm | |

**Air-borne Microorganisms**

1. Bacteria. Bacteria comprise a heterogeneous group varying in size from 0.3 to 10-15 ilm and those bacteria that are capable of withstanding prolonged desication only can persist in air for long periods. Fortunately, such forms are saprophytic. Among these are sporulating species of bacilli, cocci like Sarcina, and non-sporulating rods like Serratia. However, it is common to find throat and mouth inhabiting bacteria examplified by Streptococcus, Staphylococcus, Pneumococcus, etc. in air.

Without any active dispersal mechanism the bacteria are carried in air via mechanical disturbances of particulate matter, and on clothing and surgical dressings, etc. Bacteria-laden minute droplets are continuously thrown into air by rainsplashes, breakers or sea sprays, sneezing or coughing. Indoor source of airborne bacteria are mainly the man and animals.

**2. Protozoa**. Most of the protozoans are transported as cysts; the cysts range from 2-50 um in diameter. When protozoa are not in cyst condition, they vary in shape from irregular, plasmodial to rigid flattened or ovoid.

**3. Microalgae.** Some common airborne microalgae are Chlamydomonas, Chlorococcum, Gongrosira, Phormidium, Protococcus, Diatoms, Spirogyra, Oscillatoria, Chlorella, Amphora. Some cyanobacteria (former blue green algae) like Aulosira, Lyngbya, Nostoc have also been reported in air.

The microalgae are airborne mainly due to air currents even in the absence of adaptation for take off into air. A regular constituent of airborne microalgae comprises of algal cysts or other resting stages. The microalgae in the range of 1-90 um are significant in respiratory diseases and allergies as these are retained by the mucous membrane of respiratory tract.

**4. Microfungi.** Fungal spores are the propagative phase of microfungi and many of which are adapted to air transport. The density of fungal spores in still air is approximately 1.0. However, they are able to remain suspended in the atmosphere and become disseminated through the action of turbulent air movement even though they are subjected to gravity. Their fall rate is governed by Stroke's Law.

**Stroke's Law**

Fungal spores present in air sediment generally have different velocity, The sedimentation of such spores can be calculated by Stroke's Law. According to this law :

$$EV \{ 3.10^{-8}, \lambda\, d^2$$

where, E is density ($kc/m^3$)
V is terminal velocity, and
$d^2$ is the diameter in µm

Hyphal Fragments of microfungi constitute a significant population of air spora; these are primarily the conidiophores of asexual forms. Culturing of these hyphal fragments revealed that they mostly belong to Cladosporium, Alternaria, and Penicillium. These usually inhabit leaves of many plants and are dispersed due to scrubbing action of the infected plants which occurs during air currents. Hyphal fragments constitute an important airborne microflora. Tilak and Bhalke (1981) have described and classified various airborne hyphal fragments of fungi.

**Bioaerosols**

The term bioaerosol is used to describe living airborne particles or those originating form living organisms. Bioaerosols vary considerably in size (Fig. 2.1). Their composition depends on several factors including the type of microbe or toxin, type of particles they are associated with (as mist or dust), and the gases in which the bioaerosol is suspended. In general, bioaerosols range from less than 0.01 um to more than 100 um in diameter and are classified on the basis of their size into different modes as follows:

| Mode type | | Size range (µm in diameter) |
|---|---|---|
| Nuclei | Fine particles | <0.1 |
| Accumulation | | 0.1 to 2.0 |
| Coarse | | > 2.00 |

Bioaerosols in the respirable size range (s 10 um) are of particular concern to human health. The composition of bioaerosols can be liquid or solid or a mixture of the two and should be thought of as microorganisms associated with airborne particles containing microorganisms. It is rare to find microbes not associated with dust or mist or other airborne particles in the atmosphere.

**Aeromicrobiological (AMB) pathway**

Aeromicrobiological (AMB) pathway describes the launching of bioaerosols into air, the subsequent transport via diffusion and dispersion of these particles, and finally their deposition. An example of AMB pathway is the liquid aerosol containing influenza virus launched into the air through cough, sneeze or even talking. The virus-containing aerosols are dispersed by a cough or sneeze, transported through the air, inhaled, and deposited in the lungs of a nearby person initiating new infection

**Launching** is the process whereby particles become suspended within the earth's atmosphere. Though launching of bioaerosls occurs from both, terrestrial and aquatic sources, atmosphere becomes more loaded from the former one. Launching into the surface boundary layers occurs by various mechanisms, such as air turbulence created by the from movement of humans,

animals and machines; generation, storage, treatment and disposal of waste materials, natural mechanical process as action of water and wind on contaminated solid or liquid surfaces; and the release of fungal spores in natural life cycles. Airborne particles can be launched from either point, linear or area sources. Point source, as a pile of biosolid matter, is an isolated and well-defined site of launching, whereas linear and area sources involve larger, less well-defined areas.

**Transport** or dispersion is the process by which kinetic energy provided by the movement of air is transferred to airborne particles, with resultant movement from one point to another. Transport of bioaerosols can be defined in terms and distance. Submicroscale transport involves short periods of time, under 10 min. as well as relatively short distances, under 100 m. It is very common in buildings and other confined spaces. Microscale transport ranges from 10 min. to one hr, and from 100 m to one km and is the most common type. Mesoscale transport refers to transport in terms of days and distances up to 100 km. and in macroscale transport, both time and distance are still larger. As bioaerosols travel through time and space, different forces diffusion, inactivation and ultimately deposition act upon them,

**Deposition** is the final step in the AMB pathway. The airborne bioaerosol eventually leaves the turbulence of the suspending gas and ultimately becomes deposited on a surface by one combination of interrelated mechanisms. These mechanisms include gravitational settling, downward molecular diffusion, surface impaction or rain and electrostatic deposition.

It has now become possible to predict the airborne bioaerosol concentrations in the vicinity of a contaminated source through mathematical modelling Appropriate equations have been developed to simulate the aerobiological pathways i.e. how far the bioaerosols (launched into air) will travel and in what concentration.

## Microflora of Air

Human beings and animals are continuously inhaling the microbes present in the air that cause various infectious diseases. Most of the respiratory tract infections are acquired by inhaling the air pathogen. The microflora of air is studied under two categories such as indoor and outdoor microflora.

### Indoor microflora/Intramural airspora

The air found inside the closed environment (building/ room) is referred as indoor air and the microbes present in this region is called indoor microflora. Example: *Staphylococcus*, *Bacillus*, *Penicillium*. It involves home and work place environments in which air borne microbes create major public health concerns. Microbes can survive for extended period in indoors as they have relatively less exposure to radiations. Some of the indoor environments are described as following

### Private homes and office building:

Extent of bioaerosols development determines the health of any building. These include several factors that influence the formation of bioaerosols. this include the presence of air filtering systems designed and fitted in the building , the health and hygiene of the occupants, the amount of clean outdoor air circulated through the building, the type of lightning, the ambient temperature in the building and the relative humidity. In spite of all precautions some microbes may develop mechanism for survival and transmission.

### Hospital and laboratories:

These two indoor environments have such potential for the aerosolisation of pathogenic microbes. Microbiological laboratories are also a breeding center for pathogenic microbes.

### Space flight:

Microbes have been detected even from harsh environments. They are associated with every aspect of life even space craft. Microbes are also beneficial for us. Air purification is an example of a beneficial use of microbes in association with AMB pathway. Biological air filtration (BAF) is a method currently being investigated for use during aircraft. This method reduces more than 99 % of toluene, chlorobenzene and dichloromethane in the air stream.

### Public health:

AMP pathway is used for immunization against some disease like they are currently being used for influenza vaccines. However they are not widely used because they are painful.

**Outdoor Microflora/Extramural airspora**

The air in the exterior environment is called outdoor air and the microbes that reside there are called outdoor microflora. Example: *Bacillus, Aspergillus*. In outdoor or extramural environment, the expanse of space and the presence of air turbulence are the two controlling factors in the movement of bioaerosols. Other environmental factors, such as radiation, temperature and relative humidity are important in limiting the duration of viability of aerosolized microbes and hence modifying the effect of bioaerosols.

**Airborne Crop Pathogens**

Bioaerosols are of direct relevance to agriculture. Airborne microbial pathogens are responsible for a range of important diseases of crop plants. Bioaerosols contaminate the crops and thus have significant economic impact worldwide. Major airborne pathogens of crop like wheat are responsible for causing the outbreak of wheat rusts in different areas of the world. There are similar examples of other such epidemics caused by bioaerosols in other crop plants including rice. Not only in crop, but in vegetable plants like potato also, airborne pathogens are responsible for outbreaks of late blight disease.Besides plants bioaerosols are also important in animal husbandry. The occurrence of foot-and-mouth disease is an example of the role of bioaerosols in the spread of airborne disease. Besides respiratory pathogens bioaerosols are involved in transmission of gastrointestinal pathogens also. For instance the gastrointestinal pathogen of calves, *Salmonella typhimurium* is spread through bioaerosols. Similarily, aerosolised *Solmonella enteridis* could infect laying hens.

**Waste Disposal**

A range of pathogenic microbes-viruses, bacteria, protozoa and helminths associated with waste effluents bring about health hazard during their treatment and disposal handlings. For instance. waste water treatment plants utilizing activated sludge and trickling filters create large amount of aerosols during treatment processes. These aerosols are very rich in pathogenic microbes. Aerosols containing pathogenic microbes are also generated during other treatment processes, such as composting and land disposal etc.

**Biological Warfare (Germ warfare/ Bioterrorism)**

Bioterrorism has become the most dangerous hidden, inhuman weapon these days. However such a strategy in wars is very old and as early as 1346 A.D. Tartars besieging the walled city of Kaffa used catapults to launch plague-infested bodies into the city. In 1950s in U.S.A. field-scale experiments were made using inert substances like fluorescent dyes to simulate biological warfare agents. These aerosols were released into air circulation system of a subway system and

into the air off the coast of San Francisco. An accident at a biological warfare research institute in Russia caused the widespread exposure of nearby populations to a genetically modified strain of *Bacillus anthracis*. In 1998, in Japan, Tokyo police detected large quantities of *Clostridium botulinum* toxin during a raid on a terrorist-controlled facility. In Iraq there were investigations in 1990s for mass scale production facilities for biological warfare agents. Detection of biological warfare agents is an area that requires intensive training and sophisticated equipment to develop an advanced antibiological warfare defense.

**Sources of micro-organisms in air:** Air is not a favorable environment for the microbial growth as it does not contain enough moisture and nutrients to support the growth and reproduction and there is no indigenous growth of flora in the air as well. Quite a number of sources have been found and studied that are responsible for the introduction of microbes in the air. The most common is soil. The soil microbes with the wind flow suspended in air and stay there and sometimes accumulate. Man activities like digging, sloughing, and running also introduce microbes in the air. Air currents and splashing of water also introduces microbes in the air. Besides that air currents take away plant and animal pathogens from their surfaces and spread them in the atmosphere. Plant pathogens can spread more rapidly as compared to animal pathogens .For example: spores of Puccini a gamines travel over a thousand kilometers. The main source of the introduction of microbes in the air is human beings. Human activity is the biggest source. The pathogenic microbes present in the respiratory tract of human being and the microbes present in the mouth are constantly released in the air but coughing, sneezing and laughing. The microbes released in the air are in three forms depending upon on the size and moisture content.
These three forms are: 1. Droplets 2. Droplets Nuclei 3. Infectious dust

**Droplets:** Millions of Droplets are released when we sneeze, and mucus is expelled at about 200 miles away. These droplets are water droplets that carry micro-organisms if a diseased person released them. These droplets contain saliva and mucus. The microorganisms they carry are mostly of respiratory tract. The size of droplet determines for how long micro-organisms stay on the droplets. Droplets of large size settle in the air rapidly. These droplets carry microorganism are source might be a source of infectious disease.

**Droplets Nuclei:** Particles of liquid, 1 -5 micrograms in diameter released during sneezing and coughing. Droplet nuclei are considered to be the raw material for the respiratory disorders. It contains saliva and mucus on its surface. Due to their small size they suspend in the air for a longer period of time. Droplet nuclei are considered to be the constant source of bacterial infections if the bacteria present on its surface remains viable. The viability of bacteria depends on the physical factored i.e. humidity, sunlight, moisture and the size of droplets as well.

**Infected dust particles:** These dust particles introduced in the air by bed making, handling a hand kerchief ,dealing with a patient having dried secretion and digging and ploughing. Microorganisms stick to the surface of these droplets and get dried then they suspended by the methods given above. Dust particles laden with microbes are larger in size and they settle down in the air. Airborne diseases caused by two types of droplets.

1. Droplet infection caused by the droplets larger than 100micronmeter in diameter.
2. Airborne infections are caused by some dried residues of droplets.

Droplet infection remains localized and concentrated whereas airborne infection may carry long distance. Microorganisms can grow for a longer period on dust particles. This is proved hazardous in hospitals and labs when closed dried specimens bottles are open and cotton plugs are removed from the bottles.

**Factors Affecting Growth of Microorganism in Air**

There are several factors which influence the ability of a bioaerosol to survive in air:

- Particular resistance for a given microorganism (morphological characteristics)
- Meteorological conditions (inter alia, air humidity, solar radiation),
- Air pollution,
- The length of time in air.

**Resistance of microorganisms:**

It is a species dependent feature, which relies on the microorganism's morphology and physiology.

**Relative humidity:** The content of water in air is one of the major factors determining the ability to survive. At a very low humidity and high temperature cells face dehydration, whereas high humidity may give cells protection against the solar radiation. Microorganisms react differently to humidity variations in air, but nevertheless most of them prefer high humidity. The morphology and biochemistry of cell-surrounding structures, which may change its conformation depending on the amount of water in air, are crucial. Actually, an exact mechanism of this is not known. Forms of resting spores with thick envelopes (e.g. bacterial endospores) are not particularly susceptible to humidity variations. Gram-negative bacteria and enveloped viruses (e.g. influenza virus, myxo) deal better with low air humidity which is contrary to gram-positive bacteria and non-enveloped viruses (e.g. enteroviruses) that have higher survival rates in high air humidity.

**Temperature:** Temperature can indirectly affect cells by changing the relative-air humidity (the higher the temperature, the lower the relative humidity) or a direct affect, causing, in some extreme situations, cell dehydration and protein denaturation (high temperatures) or

crystallization of water contained within cells (temperatures below 0°C). Therefore, it can be concluded that low temperatures (but above 0°C) are optimal for the bioaerosol. According to some researchers the optimal temperatures are above 15°C.

**Solar radiation:**

Solar radiation has a negative affect on the survival rate of the bioaerosol, both visible as well as ultraviolet (UV) and infrared radiation due to the following factors:

- Causes mutation,
- Leads to the formation of free radicals, which damage important macromolecules.
- Creates a danger of dehydration.

Visible light rays of about 400-700 nm wavelength, create the so-called photodynamic effect, which produces free radicals within cells, especially compounds such as peroxy and hydroxyl radicals. These radicals demonstrate strong oxidizing activities and may cause damage to crucial macromolecules, e.g. DNA or proteins.

UV radiation has a much larger affect on cells than visible light does, especially the rays of 230-275 nm wavelengths. The mechanism of this effect is based on changes to DNA, both directly (e.g. by creating thymine dimer and consequently causing mutation), as well as indirectly, by creating free radicals as in the case of the visible light.

In addition, infrared (IR) radiation may have a negative effect upon cells contained in air - heating up and consequently dehydration.

**Biological aerosols**

Microorganisms in air occur in a form of colloidal system or the so-called bioaerosol. Every colloid is a system where, inside its dispersion medium, particles of dispersed phase occur whose size is halfway between molecules and particles visible with the naked eye. In the case of biological aerosols, it's the air (or other gases) that has the function of the dispersion medium, whereas microorganisms are its dispersed phase. However, it is quite rare to have microbes independently occurring in air. Usually, they are bound with dust particles or liquid droplets (water, saliva etc.), thus the particles of the bioaerosol often exceed microorganisms in size and may occur in two phases:

- Dust phase (e.g. bacterial dust) or
- Droplet phase (e.g. formed as the result of water-vapour condensation or uring sneezing).

The dust particles are usually larger than the droplets and they settle faster. The difference in their ability to penetrate the respiratory tract is dependent on the size of the particles; particles of the droplet phase can reach the alveoli, but dust particles are usually retained in the upper

respiratory tract. The number of microorganisms associated with one dust particle is greater than in the droplet phase.

The average size of bioaerosols ranges from about 0.02 μm to 100 μm. The sizes of certain particles may change under the influence of outside factors (mainly humidity and temperature) or as a result of larger aggregates forming. By using size criterion, the biological aerosol can be subdivided into the following:

- Fine particles (less than 1μm) and
- Coarse particles (more than 1μm)

Fine particles are mainly viruses, endospores and cell fragments. They possess hygroscopic properties and make-up the so-called nucleus of condensation of water vapour. At high humidity water collects around these particles creating a droplet phase.

Then, the diameter of the particles increases. Coarse particles consist mainly of bacteria and fungi, usually associated with dust particles or with water droplets.

Biological aerosols as a human hazard source .

- What types of dangers are connected to the presence of microorganisms in air?
- Infectious diseases (viral, bacterial, fungal and protozoan),
- Allergic diseases,
- Poisoning (exotoxins, endotoxins, mycotoxins).

Bioaerosols may carry microorganisms other than those which evoke respiratory system diseases. The intestinal microorganisms contained in aerosols may, after settling down, get into the digestive system (e.g. by hands) causing various intestinal illnesses.

**Air Borne Diseases**

Many microbial diseases are transmitted through the air (Table 9.2). The incidence of diseases caused by airborne transmission can be reduced by covering one's nose and mouth during coughing or sneezing and by the use of face masks.

**Table 9.2:** Important airborne human diseases and causative agents (pathogens)

| Human Diseases | Pathogens |
|---|---|
| **Bacterial diseases** | |
| Pulmonary tuberculosis | *Mycobacterium tuberculosis* |
| Pneumonia | *Klebsiella pneumoniae* |
| Streptococcal respiratory infections | *Streptococcus pyogenes* |
| **Fungal diseases** | |
| Aspergillosis | *Aspergillus fumigatus* |
| Cryptococcosis | *Cryptococcus neoformans* |
| **Viral diseases** | |
| Influenza | *Influenza Virus* |
| Common cold | *Picorna Virus* |
| **Protozoal diseases** | |
| Pneumocystosis | *Pneumocystis carinii* |

**Nosocomial infection**

Hospital acquired infection are also known as a nosocomial infection. It is acquired in a hospital or other health care facility. Infection is spread to the susceptible patient in the clinical setting by various means, one of them being air droplets. The infection can originate from another infected patient, staff, or in some cases, the source of the infection cannot be determined. The most common pathogens that cause nosocomial infections are *Staphylococcus aureus, Pseudomonas aeruginosa,* and *Escherichia coli.* One of the common nosocomial infections is respiratory pneumonia.

**TECHNIQUES OF TRAPPING AIRBORNE MICROBES/ AIR-SAMPLING METHODS:**
Air sampling is used routinely to monitor the populations of airborne particles, and to inform the public about air quality and pollen/spore counts through public broadcasting(weather reports, etc.). It is used by major hospitals to monitor the populations of specific allergenic particles (fungal spores, etc.). so that the causes of patient's allergies canbe determined. It is also used in crop pathology for disease-forecasting, so that grower can apply fungicides as and when required.Knowledge of the air spora has depended on development of sound Techniques of air sampling. Microbes are trapped on a surface using an apparatus and then observed directly by microscopy or after growth in cultures. Visual methods(microscopy) are useful to the allergists who are normally interested in the number of particles (dead or alive) in agiven volume of air, whereas cultural methods are useful to the plant pathologists andplant breeders who aremoreinterested in the deposition of viable spores or pollen grains. The various kinds of apparatus for sampling the air spora are grouped according to the principle of construction and the physical processes by which they remove (or trap) particles from the air and deposit them on surfaces of Examination, as follows:

1. **Gravity sedimentation methods**:
   Deposition of microbes is under the influence of gravity alone. The angle of presentation of the substrate is zero i.e., horizontal E.g.: gravity slide, nutrient agar /PDA plate exposed horizontally (gravity petri dish exposure)
2. **Impaction methods;**
   An air stream is allowed to impact on to a flat surface so that the microbes present in the air get impacted on to the Surface by head- on collision Eg. A sticky slide or nutrient agar plate held at an angle (450) to the horizontal.

   Impaction can be achieved mainly in three ways:
   A. By keeping still objects in still/moving air eg.a sticky slide at vertical position /450 vertical cylinder sporetrap, aeroscope.

B.By keeping amoving object in still/moving air eg Rotorod sampler C. Suction through an orifice eg: Andersen sampler, Hirst spore trap Burkard spore trap, sieve device.

3. **Impingement techniques**:

In this technique, air is bubbled through a sterile broth medium and the medium is incubated and observed for microbes eg: Multistage impingers

**4 Other methods** Centrifugation, electrostatic precipitation, wash-off by rain, filtration..

Of these, the widely used trapping techniques are based on sedimentation and impaction.

1.Sedimentation methods

**A. Gravity Slide**:

It was used by Pouchel for dust examination Blackley (1873) first made use of the gravity slide to study pollen and spore content of air. It was a routine method for investigation of pollen and spore content since early days of Hay fever studies. Scheppegrell (1922) used 70mm x 22mm microscope slides and exposed them without any protection from rain. Wodehouse (1945). Hyde and Williams (1950) exposed horizontal slides with some kind of protection from rain. Durham (1946) designed a shelter for the protection of the slide from precipitation (rain)

**Principle:**

This method is based on the principle of deposition by sedimentation under the influence of gravity alone. The angle of presentation is zero..A microscope slide is made Sticky using petroleum jelly/Vaseline/ silicone grease and kept horizontally at a desired place of sampling, under a Durham's shelter for 24 hours. The sticky slide is taken to the place of sampling in a closed container and after exposing to air it is brought back to the laboratory under cover for microscopic examination.

The slide is observed under the microscope after staining with cotton blue and covering it with a long cover glass (22mm breadth)

**Durham's shelter**:The Durham's shelter consists of two circular stainless-steel plates of 12 inches diameter separated by three 9 inches long rodson the lower plate, at the center, a small platform is raised by about an inch and the sticky slide is placed on it.

**Impaction methods / inertial methods**:

**a) VERTICAL CYLINDER SPORE TRAP(VCST):**

Principle:

In this method, impaction of microbial particles in the air on to a trapping surface is used. Here impaction is on a still object in still moving air. It was designed by Rempe in 1937. He used Sticky glass cylinders of 2-5 cm diameter for trapping air borne pollen. Later Gregory and

Steadman used o.5cm diameter glass rods and found them to be more efficient . The glass rods weremade sticky in a particular portion and kept upright allowing impaction to take place. They made the rod sticky using cellophane strip. The above design was modified by Ramalingam in 1967 which is widely used at present. It is similar to the old design except for the projection of the vertical cylinder which is held hanging by means of a screw from a circular protective shield of about 3 diameters. A 20mm2 cellophane strip is fixed at the bottom edge of the rod. The shelter protects the cylinder from atmospheric precipitation without altering the impaction efficiency. The cellophane strip is exposed for24hrs at the desired place and then carried to the lab in a test tube provided with a single-holed rubber cork. The cellophane strip is removed, mounted on a slide using suitable mountant and observed microscopically for the presence of air borne particles.

The vertical cylinder spore trap can be used particularly in agricultural monitoring for aerobiological surveys toforecastdiseases. It is mainly used in extramural aerobiological experiments

**Advantages**:

It is cheap. convenient and easy to handle

**Disadvantages:**

It is dependent on wind velocity. There would be zero catch of large particles in still air and small particles even atordinary wind speed. Its efficiency changesgreatly with changes in wind speeds. Hence there are chances ofmisrepresentation of air spora

**ROTOROD SAMPLER**:

The Rolorod sampler was designed by Perkinym 1957 (Pexkins Rotorod samples) It is based on the principle ofimpaction on a moving object in still, moving air . It consists of a Ushaped flat brass rod of 2mm (or 1.6mm inwhirling Rotorod Sampler) cross-section which is connected to a shafton a motor of Knownrpm (rotations perminute) by means of a hollow screw system

The rod is bent in such a way that the arms are 6cms high and the ends of the rod at the top are separated by a 6cmdistance so that during revolution, it expands (due to centrifugal force) to form a perfect circle of 8cm diameter. Themotor usually rotates at 2520rpm using 9-15V dry batteries. To the flat vertical rods, two pieces of 2mmx80mmcellophane strips are fixed on opposite sides of the two rods. The strips are smeared with petroleum jelly. When the motor is on the rods rotate with a high speed and it can be operated only for a short duration of about 5-15 mins.It samples air at 120 l/min. After putting off the motor, the strips are removed and observed by mounting them on a micro slide using glycerine jelly under a cover glass.

This type of sampler is most effective for trapping relatively large particles (upwards of about 7 micrometres) such as the larger fungal spores and pollen grains

**Advantages**:
1. is a highly efficient method and independent of wind velocity and direction Even the fall out spores are trapped

2. It gives both qualitative and quantitative data on diversity of microbes. Volume of air sampled can be easily determined and therefore concentration of microbes on the strips can be expressed in terms of number of microbes per $cm^3$ of air

3. The equipment is portable and can be used both for indoor and outdoor purposes.
4. It can be used to precisely locate a source of spores of a particular fungus.
5. It is cheap and simple.

**Disadvantages.**
1.It does not give continuous sampling for 24 hours and gives only spot sampling data. It cannot be routinely.

2. It cannot give an idea of the bacterial population in the air as it is not a cultural technique.

**HIRST SPORE TRAP/HIRST VOLUMETRIC SUCTION TRAP:**

J.M Hirst) (1952) designed it and is still widely used for sampling air bome microbes. It is based on the principle of impaction by suction through an orifice. It is a power-driven trap designed for operating continuously in the fields

It is simple in construction. It has a single impaction slit(14x2mm) through which air is sucked into the apparatus which is made air tight . Behind the orifice, a sticky trapping surface eg: a micro slide is placed. The air flow rate is 10L/min. The slide is drawn upwards by a Clockwork mechanism at the rate of 2mm/hr. Particles in the air are sampledor deposited by impaction on the slide which is changed everyday

A transverse bar of deposition is obtained in the course of 24hrs and a trace is deposited in a band. Hirst spore ideal for field survey work.

**Advantages**: it is robust, simple, continuously operating and needs minimum servicing
**Disadvantages**: Major limitation is the high cost. Since it is a visual technique and not a cultural technique, identification is difficult. Identification is based mainly on morphology.

**ANDERSEN SAMPLER**

The Andersen sampler was designed by Andersen in 1958. It is a cultural technique ideal for bacteriological identification.

It is based on the principle of impaction by suction through an orifice. It consists of two units (a) Suction motor and (b) Fractionating column.

The fractionating column consists mainly of eight separable metallic unit out of which the first six have perforated metallic plates. Each of these plates has about 400 pores, the diameter of which goes on decreasing from plate one to plate six. The whole fractionating column is made airtight by ring seals or gaskets. The electic suction motor sucks air through the bottom unit of the column and is calibrated to suck 28.3 l/min. when air is sucked from the top of the column, air carrying microbes gets impacted on the sterile medium in open Petri plates plates under the perforated metal plates. Air sucked in at the top of the column travels at relatively-low speed towards the first agar plate. and so only the largest particles impact onto the agar surface. The air then travels round the edge ofthe agar plate and through the perforations to the Second agar plate. and so on. As this process continues down the stack. The same volume of air is forced to travel through successively Smaller perforations. and so, the air speed is progressively increased. The progressively increased air speed lower down the column raises the momentum at the air-borne particles. So that even the very Smallest particles (less than 3µm diameter) can impact on to the lower aga rplates. Thus, larger particles get deposited on the upper plates and Smallest particles in the last plates.When the Sampler has run for 5-15 minutes or more the metal plates are separated and the petridishes are remove for incubation to identify the colonies that develop

**Advantages:**

1. Its Maximum application is intramural aerobiology
2. It is more efficient than gravity Petri dish for enumeration of air borne fungi.
3. It automatically separates airborne particles into SIX aerodynamic sizes and indicates respiratory tractpenetration and hazards.
4. Air spora trapped on the medium can be identifiedbased oncultural methods.
5. Amount of air sampled is known based on period of exposure and hence volumetric data is obtained.
6. Walls losses are claimed to be negligible
7. Retention is said to be 100%

**Disadvantages:**

1. Time interval of exposure is short (30sec-5 minutes).
2. It cannot be used for continuous sampling
3. Only Spot sampling can be done for small duration
4. Requires infra-Structure (well-developed lab with laminar airflow chamber, etc)
5. it is time consuming to prepare and Sterilize media

One of the Interesting features of the Andersen Sampler is that it mimics the deposition of spores (or other airborne particles) in the human respiratory tract. For Example, relatively large fungal spores and pollen grains tend to be trapped on the Mucus-covered hairs of our nostrils, where they can cause hay fever symptoms in sensitized individuals. Smaller particles are not trapped in the nostrils but instead are carried down into the bronchioles and alveoli. Here the air speed is very low, because the successive branching of the respiratory tract has reduced the air speed to a minimum. But spores of about 2-4 micrometres diameter can settle onto the mucosal surfaces of the alveoli. Some of these spores are important in initiating infections of the lungs. However, it is important to note that the underlying mechanisms of spore deposition in the Andersen sampler are entirely different from those in the human respiratory tract - the Andersen sampler traps spores by impaction whereas spores are deposited in the human respiratory tract mainly by sedimentation.

## SIGNIFICANCE OF MICROORGANISMS IN AIR /ROLE OF MICROORGANISMS IN AIR

When microorganisms are present in air, they are of no significance to man. Only when they come in contact withliving or non-living objects. they may produce beneficial or harmful effects They are as follows:

**aerobiology and human health**:

**In human and plant health- Air-borne diseases and allergies:**

Micro flora of the air causes various types of diseases in man, animals and plants. The main Sources of Microorganisms in the air are saprophytic soil organisms or from the body tissues introduced into the air during Coughing, sneezing ,talking and singing. About 1%of the airborne bacteria are pathogenicThe major diseases transmitted by air-borne bacteria are pneumonia, whooping cough tuberculosis,diptheria, Scarletfever, Q fever , Sore throat,meningitis,etc. The common air-borne fungal diseases are Systemic mycosis, aspergillosis, Candidiasis, etc.The major Viral diseases are common cold, chicken pox, Small pox, German Measles ,mumps, influenza foot and mouth disease, etc.

**Some important diseases of humans transmitted from person to person by inhaled airborne particles**

| Virus diseases (virus type in brackets) | Bacterial diseases (bacteria name in brackets) |
|---|---|
| Chicken pox (varicella) | Whooping cough ( Bordetella pertussis) |
| Flu (influenza) | Meningitis (neisseriaspecieas, haemophillusinfluenzae |
| Measles ( rubeola) | Diphtheria ( corynebacteriumdiphtheriae) |
| German measles (rubella) | Pneumonia (mycoplasma pneumoniae, streptococcus species) |
| Mumps (mumps) | Tuberculosis (mycobacterium tuberculosis) |
| Small pox (variola) | Scarlet fever (streptococcus pyogenes) |
| Common cold (rhinovirus) | |

**REFERENCE:**