B.Sc. (H) Microbiology
SEC , Sem I

Unit 2

Air sample collection and analysis

Topics

- Bioaerosol sampling
- Air samplers
- Methods of analysis
- CFU
- Culture media for bacteria and fungi and identification characteristics

Bioaerosol sampling

- Bioaerosols are collected for a wide variety of purposes, for example,

1. To measure inhalation exposure,
2. To characterize indoor and outdoor environments,
3. To identify emissions from work activities,
4. To evaluate the effectiveness of control methods

Bioaerosol monitoring

- Bioaerosol monitoring is a rapidly emerging area of industrial hygiene.
- It includes the measurement of viable (culturable & non-culturable) and nonviable microorganisms in both indoor (industrial, office or residential) and outdoor (agricultural and general air quality) environments.

Indoor and Outdoor Bioaerosols

- Generally, Indoor microflora concentrations of a healthy work environment are lower than outdoor concentrations at the same location.
- If 1 or more genera are found at higher concentrations indoors than outdoors, then the source of amplification must be found and remedied.
- Bioaerosol sampling is often performed out of doors for pollen and fungi to assist allergists in their treatment of patients by identifying taxa distribution and concentration in air over time.
- Outdoor bioaerosol sampling may also be conducted in an occupational environment (agricultural investigations and sewage treatment plants).
- Indoor bioaerosol sampling is often conducted in occupational (industrial and office environments) and non-occupational (residential and educational buildings) settings.
- Sampling should be done before, during, and after the sampling area is occupied, including times when HVAC is activated and inactivated.

Viable and Nonviable Bioaerosols

- Viable microorganisms may be defined in two subgroups: culturable and nonculturable
- Viable bioaerosols sampling involves collecting a bioaerosol and culturing the collected particulate. Only culturable microorganisms are enumerated and identified, thus leading to an underestimation of bioaerosol concentration.
- Nonviable microorganisms are not living organisms; as such, they are not capable of reproduction. The bioaerosol is collected on a "greased" surface or a membrane filter.

The microorganisms are then enumerated and identified using microscopy, classical microbiology, molecular biological, or immunochemical techniques.

Challenges in Exposure assessment

Distinct challenges as compared to inorganic aerosols and chemical agents. For eg:

1. Pathogenic microorganisms may be hazardous at extremely low levels while other organisms may only become important health hazards only at higher concentrations.
2. Some organisms and spores are extremely resilient while others may be easily degraded in the sampling process.
3. Certain fungal spores are easily identified and counted while many bacteria are difficult to distinguish.
4. Sensitive and specific methods are available for the quantification of some biological agents while there are no good methods for others.

Challenges in Exposure assessment

5. Many of the newly developed methods [e.g. measurement of microbial agents such as β(1→3)-glucans or fungal extracellular polysaccharides, have not been well validated and are often not commercially available.
6. Some well-established methods (e.g. LAL assay), significant variations in exposure assessment between laboratories have been demonstrated.
7. Issues of storage and transport of bioaerosol samples have not been addressed despite knowing that these conditions may affect the activity of some biological agents, e.g. Endotoxin

Challenges in Exposure assessment

8. Many biological agents that may cause health effects are currently not identified.

   For instance, sewage treatment workers have an increased risk of developing a wide range of symptoms including respiratory, gastrointestinal and neurological symptoms, whereas causal agents have not conclusively been identified.

Environmental assessment

- Most of the national ambient air quality standards for aerosols are based on the mass concentrations for particles smaller than 10 µm in aerodynamic diameter (PM10).
- Recently, many countries have introduced similar standards for particles smaller than 2.5 µm in aerodynamic diameter (PM25). Therefore, particle mass concentrations are routinely measured in monitoring stations in various locations around the world

Principles of bioaerosol collection

Most aerosol sampling devices involve techniques that separate particles from the air stream and collect them in or on a preselected medium.

Three common sampling techniques used to separate and collect the bioaerosols are:

1. **Impaction**
2. **Filtration**
3. **Impingement**

Impaction

- Impaction is used to separate a particle from a gas stream based on the inertia of the particles to force their deposition onto a solid or semisolid collection surface.
- **Aerosol Impaction** is the process in which particles are removed from an airstream by forcing the gases to make a sharp bend. Particles above a certain size possess so much momentum that they can not follow the air stream and strike a collection surface which is available for later analysis of mass and composition.
- An impactor consists of a series of nozzles (circular/slot-shaped) and a target.
- Particles larger than a particular aerodynamic size will be impacted onto a collection surface while smaller particles proceed through the sampler.
- Impactors are selected so that the desired size particles will be collected
- The collection efficiency of the impactor approaches 100% when the aerodynamic diameter is greater than the d_{50}.
- Aerodynamic diameter (d_{ae}) is defined as the diameter of a hypothetical sphere of unit density (p = 1 g/cm^3) that has the same settling velocity as the particle

- The collection surface is usually an agar medium for culture-based analysis or an adhesive-coated surface that can be analyzed microscopically.
- The impaction process depends on th einertial properties of the particle (eg density, velocity) and on the physical parameters of the impactor (eg the inlet nozzle dimensions and the airflow pathway).
- Air enters the sampler thru an inlet nozzle, and the sampled air exits as the air is directed at the collection surface. Particles with sufficient inertia are impacted.
- Particles with lower inertia remain airborne with the air-flow.
- Centrifugal impaction also uses inertial forces to separate the particles from the air stream, but in a radial geometry.

Types of impactors

- **Stationary cascade impactors**
- **Individual impactors** are used in survey instruments, either as the primary collection mechanism, or as a preclassifier (for example to remove non-respirable particles from the sampled air stream).
- **Cascade impactors** consist of a stack of impaction stages: each stage consists of one or more nozzles (slits or holes) and a target or substrate (a greased plate, filter material, or growth media (agar) contained in petri dishes/ or it may be coated microscope slide, filter, or tape). Each succeeding stage collects smaller particles. A filter may be used as the final stage so that all particles not impacted on the previous stages are collected. The target may be weighed to determine the collected mass, or it may be washed and the wash solution analyzed. Filters may induce more particle bounce than greased or oiled plates.

- The particles impact onto growth medium with one or more bacterial or fungal colonies forming at some impaction sites. Multiple particles, each containing one or more organisms, passing through a single hole may be inaccurately counted as a single colony.

Advantages of impaction as a sampling method

1. An advantage of impaction as compared to filtration is that two key aerosol parameters, size and composition, can be simultaneously established.
2. Theoretical predictions can be made and empirically verified that give the cuts point and shape of the collection efficiency of an impaction stage.
3. Reduced desiccation & chemical transformations of the collected sample as air stream moves over the sample, not through it as in filtration
4. We can choose the type of surface on which the particles are impacted, as opposed to the limited choice of filter types.
5. By varying the speed of the air stream and the sharpness of the bend, one can separate particles into numerous size classifications while retaining a sample for analysis.

Disadvantages to impaction as a sampling method

1. Impactors are operationally complex, with key parameters that must be maintained by either maintenance or instrumentation.
2. lack of quality assurance experience as well as lack of experts in the application as impactors are not widely used
3. Interpretation of impactor data is often complex.
4. Costs of analysis can be high
5. High impact velocity can injure microorganisms, and bacterial and fungal survival has been found to decrease with increased impaction velocity or sampling time.
6. Only a limited amount of material is available for mass and compositional analysis, as one can not collect more than a few monolayers of particles before particle bounce and mis-sizing are a potential problem.

Examples

- Slit agar impactors (Mattson-Garvin air sampler)
- Single stage impactors (Anderson single stage, SAS)
- Burkard personal sampler and Burkard portable air sampler
- Cascade impactors (Anderson 2 and 6 stage sampler)
- Pollen, spore ,particle impactors

  Slide impactor, moving slide impactor, stationary slide impactor and cassette slide impactor

Filtration

- Used for collection of particles from a non-biological aerosol sample
- fibrous (typically glass) & membrane filters of pore size 0.01 -10 µm.
- Filtration achieves the separation of particles from the air stream by the passage of air through a porous medium.
- Collection of particles depends on their physical properties (eg size, shape , density), filter pore size and air flow rate.
- Inertial forces, interception, diffusion and electrostatic attraction result in the collection of particles on the surface of the filter.
- Thus, particles smaller than the pore size may be efficiently collected.

Membrane filters

- Membrane filters available in a variety of pore sizes.
- polycarbonate, polytetrafluoroethylene (PTFE, Teflon®), mixed cellulose ester (MCE), and gelatin filters.
- Cellulose ester, polyvinyl chloride, and polycarbonate filters lack rigidity, must be used with a support pad.
- The choice of a filter medium depends on the contaminant of interest and the requirements of the analytical technique. Eg:

1. For gravimetric analysis, nonhygroscopic materials such as glass fibers, silver, or polyvinyl chloride membranes are selected.
2. For analysis by microscopy, cellulose ester or polycarbonate membranes are the usual choices.
3. Polycarbonate and PTFE filters typically are used when the collected material must be washed from a filter.

Membrane filter cassettes

- Filters (25,37, 47-mm dia.) are held in disposable three-piece plastic filter cassettes either in open- or closed-face modes.
- Open-face sampling is performed by removing the end plug and plastic cover, and is used when the particulate must be uniformly deposited (i.e., for microscopic analysis).
- Larger (47-mm) filter results in a lower pressure drop for a given volumetric flow rate as the pressure drop across a filter increases with the air velocity through the filter.
- Smaller (37-mm) filters results in deposition of contaminants onto a smaller total area, thus increasing the density of particles per unit area of filter. So, more useful for direct microscopic examination in low conc. of mos.
- At high concentration sites, the mos may have to be eluted, diluted & refiltered for microscopic analysis.

Membrane filtration

- The sampled organisms are washed from the surface of smooth-surface polycarbonate filters.
- Microorganisms in the wash solution are either cultured or refiltered to distribute the microorganisms uniformly on the membrane filter.

1. Used mainly because of its simplicity, low cost and versatility
2. Direct Culturing
3. Incubate membrane on petri plate,
4. Serial dilution and microscopic exmaination
5. Qualitative and quantitative estimation

- Loss of viability of vegetative cells due to desiccation

examples

- Filter holder (inhalable sampler for filter collection (button sampler, SKC Inc.)
- Membrane filter (0.025-8 um), Millipore corp.
- Nucleopore filter (0.05-12 um) from Millipore Inc. and (0.01 – 20 um) from Osmonics
- With air sample flow rates from 1 to 50 litres/min
- Sartorius MD 8 air sampler – a gelatin membrane filter, collected sample can be anlayzed by culturing only, gelation filter reduces dessication stress, can be used for high volume sampling, gelatin membrane can be incubated on the agar surface for culture anlaysis.

Impingement

- Impingers are a special type of impactors in that the inertial force of the particle is the principal force removing it from the air but the collection medium is a liquid (usually a dilute buffer solution, 0.3 mM phosphate-buffered water).
- The jet is positioned a set distance above the impinger base and consists of a short piece of capillary tube designed to reduce cell injury when the air is dispersed through the liquid and the particles are entrapped.
- The collected microrganisms move around freely in the bubbling medium causing the aggregates of cell to break apart.
- Impingers are useful for the collection of culturable aerosols. The collecion of the bioaerosol particles in a liquid medium allows divison of the sample and the potential application of several analysis methods.
- Additives such as proteins, antifoam, or antifreeze to aid in resuscitation of bacterial cells, prevent foaming and loss of the collection fluid, and minimize injury to the cells may be added.

Examples of impingers

1. **All glass impingers (AGI-1, AGI-4 and AGI-30), Millipore Inc.**

   AGI-30 samplers is a widely used liquid impinger sampler that has a curved inlet tube designed to simulate the nasal passage, making it useful for studying the respiratory infection potential of bioaerosols. The inlet tube may be washed with known volume of collection fluid to recover non-respirable airborne particles.

   -AGI have 12.5 litres/min airflow rate .

   - AGI liquid is water or a liquid with same viscosity as water

2. BioSampler – same inlet geometry, same airflow rate as of AGI but combines impingement into a liquid with centrifugal motion, can be used with buffer or viscous collection fluid (mineral oil)
3. Burkard multistage liquid sampler – is a stainless sampler which collects particles in three size fractions (>10, 4-10 and <4 um)

Advantages/disadvantages

- Liquid samples may be concentrated or diluted for analysis depending on concentration of mos.
- Different culture media may be inoculated side by side for assessment of microbes with different culture requirements
- Amenable to biochemical, molecular or immunological assays to detect presence of specific mos
- Suitable for both culturable and non-culturable mos.
- Disdvantages:

  Efficient collection of microrganisms require very high sampling velocity which usually results in a violent motion of the collection fluid, causing re-aerosolization of initially collected bioparticles and stress causing loss of viability.

Electrostatic Precipitation

- Commonly used sampling methods (Impaction, impingement, filtration) adversely affect the viability of microorganisms being sampled.

- In electrostatic precipitation, bioaerosol particles are charged in the sampler's inlet resulting in their cross-sectional migration in an electric field inside the sampler and subsequent deposition on the charged plate, from which microorganisms can be extracted and analyzed.

Electrostatic Precipitation

- Collection efficiency for bacteria onto rectangular agar dishes was observed to vary from 50% to 90% depending on air flow rate and applied voltage.

- Recently, a **Wet Electrostatic Precipitator (WEP)** was developed for bioaerosol collection. An electrostatic field charges particles in an air stream and forces them to the wall of the WEP collection tube from which they are washed and concentrated by a recirculating liquid. An **Electrostatic Precipitator with Superhydrophobic Surface (EPSS)** achieved a concentration rate of 10^6 for latex particles.

- The EPSS subsequently was found to be compatible with PCR-based sample analysis and to reach a collection efficiency of 72% for *P. fluorescens and B. Subtilis.*

Gravitational sedimentation

- Gravitational settling/ depositional settling is a non quantitative collection method in which an medium is exposed to the environment and airborne microorgansims are collected mainly by gravity.
- Advantages:

  Easy to perform, inexpensive, widely used method
- Disadvantages:

1. Collection of airborne microorganisms is affected by size & shape of the particles as well as the motion of surrounding air. So large particles are deposited onto the collection surface, leading to misinterpretation of prevalence data.
2. Concentration of airborne microorganisms can't be determined by this method since the volume of air from which the particles originate is unknown.
3. Don't compare favourably with other sampling methods.
4. Not accurate quantitatively as well qualitatively.

Future Directions in Bioaerosol Sampling

- real-time instruments for bioaerosol measurement
- As good as advanced analytical methods
- A model **(UV-APS, time-of-flight spectrometer)**, ultraviolet aerodynamic particle size spectrometer
- The biological origin of the particle is confirmed by detection of ultraviolet irradiation-induced fluorescence.
- UV-APS relies on optical detection of particles; thus, its use is not feasible for particles <0.1 μm.
- Fluorophores decay after microbial death, thus, UV-APS is best suited for measurement of viable microorganisms

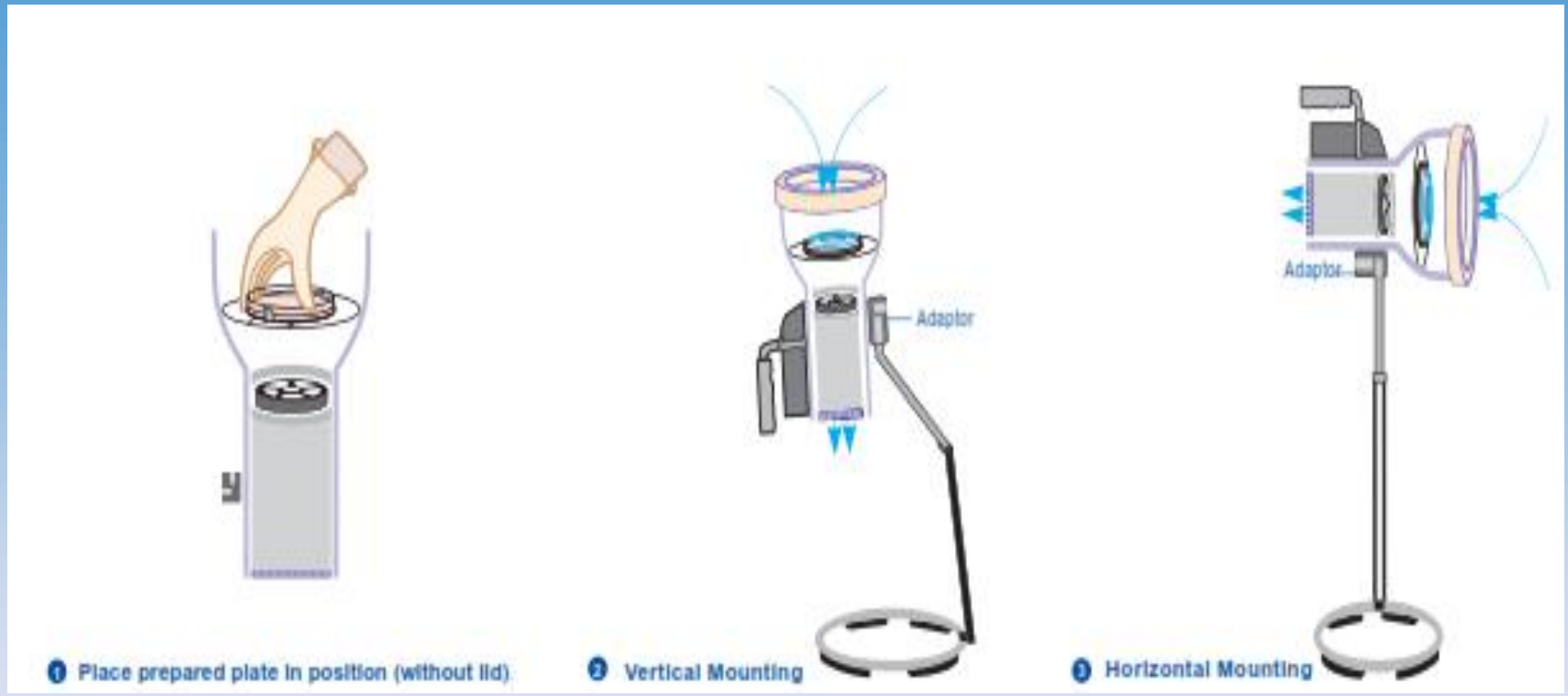
- **FLAPS-III** measures particle size using light scattering and fluorescence emission at two distinct wavelengths, which increases specificity for biological particles.
- **The IBAC** has been made rugged and packaged for military applications. It can be connected to a concentrator and used as a trigger for a secondary aerosol sampler with subsequent identification of biological particles.
- The **Vero Tect Bio-detector** measures particle shape in addition to measuring the particle size and fluorescence emission at two wavelengths.

Himedia air sampler

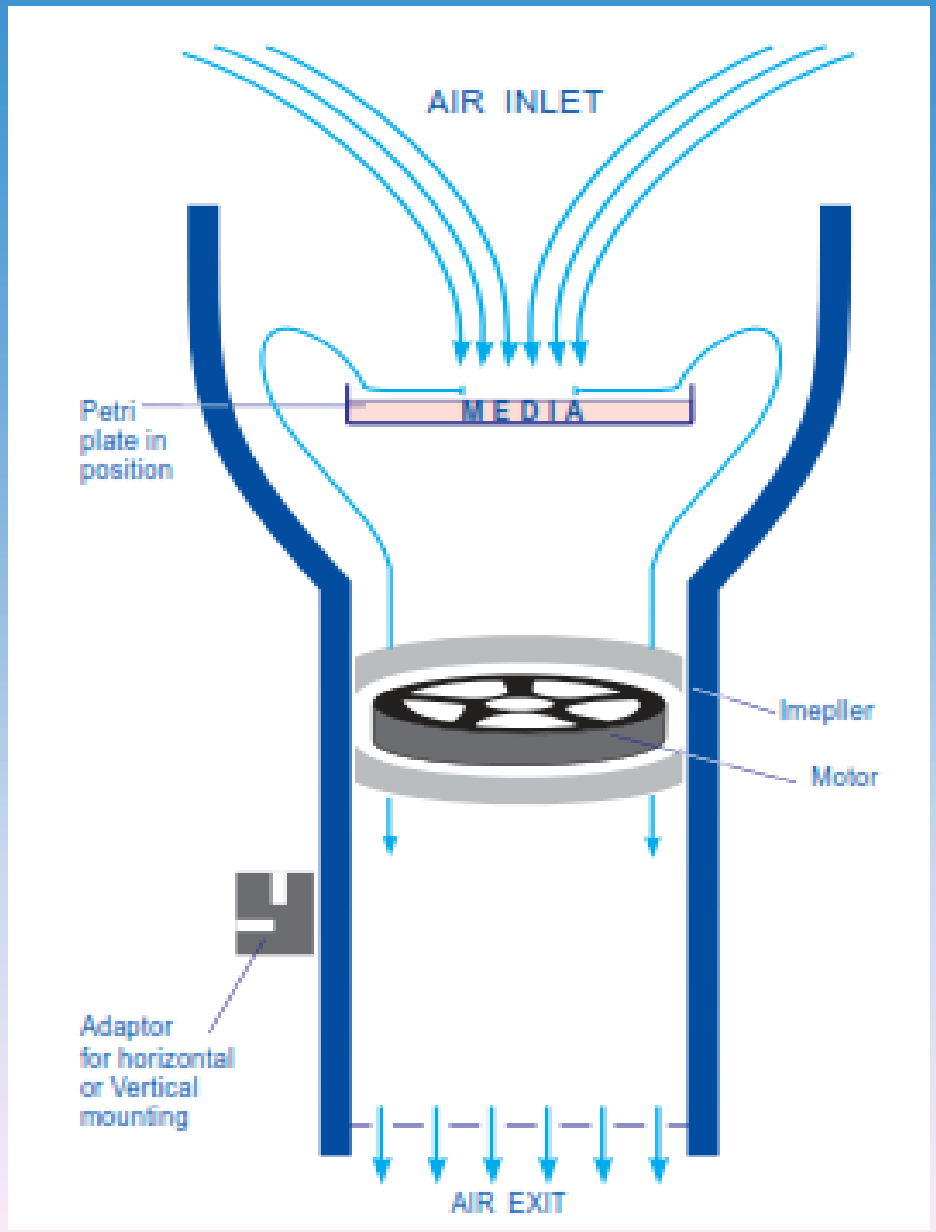
- The Air Petri sampling system is based upon the principles of sieve impactor as described by Andersen, which aspirates air through a perforated plate. The resulting air-stream, which contains particles, is directed onto the agar surface of a standard Petri dish.
- After a collection cycle, cfu/m is noted after incubation of the Petri dish.
- The instrument consist of a container designed to accommodate a Petri dish containing a nutrient agar or any other desired medium.

Features of air sampler himedia

- User friendly, fully automatic.
- Rechargeable Battery operation and Low Battery Indication in LA637.
- Micro controller based silent fan to assure maximum reliability of the air volume sampled
- Remote control operation and delay start function to avoid cross contamination.
- Two sampling position 90 & 180 with the help of built in adaptor for stand.
- Holes designed to optimize colony distribution and reduce colony overlapping.
- Perforation designed to precipitate contaminant carrying particle practically having cutt-off size d < 3 50 m
- Suitable for sampling in clean room.
- Produce highly precise and reproducible results.
- Conversion table provided for easy enumeration of microbial count.


Adaptor
Adaptor
1 Place prepared plate in position (without lid)
2 Vertical Mounting
3 Horizontal Mounting

Schematic diagram



Personal samplers

- Personal sampling is becoming more convenient for individual exposure measurement. For example, personal samplers have been used to quantify viruses with real-time PCR.
- An unconventional personal sampler fits into a test subject's nostrils (Intra-nasal air sampler, INAS) and has been used to measure allergen and fungal exposures

ultrafine particles (UFPs)

- 10-100 nm size
- UFP represent less than 1% of the total airborne particulate volume and mass. Thus, they have not generally been regarded as important contributors to the toxic effects of inhaled ambient air.
- In this size range, particle aerodynamic behaviour is dominated by Brownian diffusion
- Gravitational and inertial effects are of little importance at UFP size range, (upto 150 nm), when particles are inhaled. Thus, UFP behaviour in human airways is dominated by diffusion.
- UFPs deposit in the respiratory tract primarily by diffusion, with deposition increasing as particle size decreases. When the smallest UFPs are inhaled, they deposit very efficiently in the nasal and oral passages.

Seasonal, Diurnal, and Geographic Variability in Ambient Bioaerosol Concentrations

- Bioaerosol prevalence and fluctuations in concentration are influenced by climate and weather (resulting in diurnal and seasonal cycles) and by local sources (resulting in regional variation)

1. Time of the day:

- Diurnal patterns in bioaerosol concentrations due to changes in air and surface temperatures, RH, fluctuations in wind speed and turbulence, all of which affect the emission, suspension, and removal of pollen grains, fungal spores, and other bioaerosols from the atmosphere.
- Each biological species responds differently to external conditions.

2. Time of the year

- Seasonal patterns in bioaerosol concentrations are caused by temperature, moisture availability, and hours of daylight.
- Airborne pollen are found predominantly during their respective pollination periods from early spring to fall when plants are producing pollen and releasing whole grains.

3. Geographic region

- bacterial concentrations, are generally higher in urban than rural atmospheres in the absence of local sources such as animal houses, agricultural operations, waste treatment plants, or composting facilities.
- The distribution of pollen-producing plants is a result of natural floristic patterns, but landscaping has significantly changed the air biota in many parts of the world
- Ragweed (*Ambrosia species),* a dominant pollen allergen in the United States,
- *Parietaria (pellitory)* is an important allergen in areas of Southern Europe and Australia with moderate-to-warm maritime climates.

Bioaerosol samplers considerations

- Include culturable, nonculturable and nonviable bioaerosol sampling
- Subcategories include free bacteria (i.e., mostly single cells), free fungi (i.e., mostly single spores), clumped bacteria & fungi with MMAD 4 µm or mos attached to another particle such as a skin scale or piece of lint.
- Culturable bioaerosol sampling instruments must minimize injury during the collection process and maintain the culturability of the collected mos.
- In case of culturable bioaerosol sampler, sampling time should be selected so that ~ 30-100 colonies (upto 300) develop per plate.
- A qualitative estimation may be done at sites of extremely low levels of culturable bioaerosols.
- Collection efficiency should be the primary concern when nonviable microorganisms are sampled or when culturability is not of concern.
- Limitations of each type of samplers should be considered while sampling.

Extra info

Impaction

- Impaction is used to separate a particle from a gas stream based on the inertia of the particle.
- An impactor consists of a series of nozzles (circular- or slot-shaped) and a target. Perfect impactors have a "sharp cutoff" or step-function efficiency curve.
- Particles larger than a particular aerodynamic size will be impacted onto a collection surface while smaller particles proceed through the sampler.
- Impactors are selected so that the desired size particles will be collected
- In other words, the mass of the particles smaller than the d_{50} that are collected equals the mass of the particles larger than the d_{50} that pass through the impactor. The collection efficiency of the impactor approaches 100% when the aerodynamic diameter is greater than the d_{50}.
- Aerodynamic diameter (d_{ae}) is defined as the diameter of a hypothetical sphere of unit density (p = 1 g/cm3) that has the same settling velocity as the particle

Types of impactors

- **Stationary cascade impactors**
- **Individual impactors** are used in survey instruments, either as the primary collection mechanism, or as a preclassifier (for example to remove non-respirable particles from the sampled air stream).
- **Cascade impactors** consist of a stack of impaction stages: each stage consists of one or more nozzles (slits or holes) and a target or substrate (a greased plate, filter material, or growth media (agar) contained in petri dishes). Each succeeding stage collects smaller particles. A filter may be used as the final stage so that all particles not impacted on the previous stages are collected. The target may be weighed to determine the collected mass, or it may be washed and the wash solution analyzed. Filters may induce more particle bounce than greased or oiled plates.

- The particles impact onto growth medium with one or more bacterial or fungal colonies forming at some impaction sites. Multiple particles, each containing one or more organisms, passing through a single hole may be inaccurately counted as a single colony.

- Impactors use a variety of film and foil substrates to collect particles classified according to their aerodynamic diameters on a series of stages, each with a successively smaller cut size. The chemical composition of the size-resolved aerosol samples can then be determined using a variety of analytical techniques. Classifying particles according to aerodynamic diameter is ideal for health effects studies since lung deposition of particles larger than a few tenths of a micron depends on aerodynamic diameter. Impactors that collect particles down to 0.05 µm are now used routinely, examples of which include the Berner impactor [Berner et al., 1979] and the MOUDI cascade impactor (the Micro Orifice Uniform Deposit Impactor, Applied Physics Co., Niwot, CO, USA). Particle bounce is an inherent problem with impactors. Coated substrates largely eliminate bounce and are commonly used for atmospheric sampling [McMurry, 2000 and references therein]. While coatings that do not interfere with some types of chemical analysis have been found, no available coating is compatible with measurements of the particulate organic carbon content.
- Aluminium foil is often used for samples that are going to be analysed for organic and elemental carbon (OC/EC), because these substrates can be pre-cleaned to reduce the carbon blank to very low levels.
- Pre-cleaned Teflon or Mylar film is used when ion chromatography is the analysis technique, as ion blanks can be made very low on these surfaces. Teflon membrane filters have also been used as impaction surfaces. Although these are more expensive than film or foil substrates, they

Air samplers

- The bioaerosol of interest categories include culturable bioaerosol sampling, and nonculturable and nonviable bioaerosol sampling. Subcategories include free bacteria (i.e., mostly single cells), free fungi (i.e., mostly single spores), and clumped bacteria and fungi with MMAD 4 µm.

- Culturable bioaerosol sampling instruments must minimize injury during the collection process and maintain the culturability of the collected microorganisms.
- Free bacteria and fungi are the bioaerosols of interest in some environmental investigations, and the sampler must collect these small aerosols.
- Clumps of microorganisms or microorganisms attached to another particle such as a skin scale or piece of lint. When using any culturable bioaerosol sampler, the investigator must select sampling time, considering estimated concentration, such that 30-100 colonies (up to 300 in some situations) develop per plate.
- The lower limit (30 colonies) is necessary to obtain sufficient statistical power for comparison purposes. However, when a clean room or other environment having extremely low levels of culturable bioaerosols is sampled, the lower limit of 30 colonies may not be achievable. In such a situation, a qualitative representation must be used without accommodation of statistical validity.
- The upper limit (100-300 colonies) is the maximum range in which one can easily count and differentiate colonies.
- When nonviable microorganisms are sampled or when culturability is not of concern, collection efficiency is the overriding concern.
- Limitations of each type of bioersol samplers should be considered while sampling.

ultrafine particles (UFPs)

- 10-100 nm size
- In this size range, particle aerodynamic behavior is dominated by Brownian diffusion, and particle size is adequately described by a thermodynamic diameter.
- The thermodynamic diameter is the diameter of a sphere of unit density that would have the same diffusion coefficient in air as the particle of interest.
- At an upper limit value of 150 nm for the particle size, gravitational and inertial effects are of little importance, when particles are inhaled. Thus, UFP behavior in human airways is dominated by diffusion.
- UFPs deposit in the respiratory tract primarily by diffusion, with deposition increasing as particle size decreases. When the smallest UFPs are inhaled, they deposit very efficiently in the nasal and oral passages.

ultrafine particles (UFPs)

- These particles, on average, represent only about one half of 1% of the total airborne particulate volume and mass. Thus, they have not generally been regarded as important contributors to the toxic effects of inhaled ambient air.
- However, recent evidence suggests that they may have an important role in health decrements associated with ambient particulate matter (PM). Other evidence suggests that particle number may also be a better metric than mass on which to base risk estimates for certain occupational diseases.
- More than 90% of all airborne particles are generally found in nuclei less than 150 nm in diameter
- Number concentrations in an urban environment vary, depending on local sources of particles and gaseous precursors, season, and weather.
- Measured concentrations may range from104–105 particles cm–3 in quiet rural area to 1000 cm–3 in an area with vehicular traffic.
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Examples of impingers

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