AIRBORNE BACTERIA AND FUNGI LEVEL IN INDOOR AND OUTDOOR AREAS

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ABSTRACT

In this study, we investigated the relationship between indoor and outdoor concentrations of airborne actinomycetes, fungal spores, and Bacterial Species. Different types of indoor environments (Two different Canteen area, Institute’s auditorium, laboratory, Shopping Mall) and their outdoor environments (Institute’s Outer Building area) were investigated in terms of bio-aerosol contamination. A total of 120 samples were investigated in Indian Institute of Toxicology Research, Lucknow. The single-stage / Bio-stage sampler was used for viable bio-aerosol sampling. During the sampling, indoor and outdoor temperature, relative humidity, and CO2 concentration were measured. Total bacteria counts (TBC) and fungi concentrations varied on a large scale within and between the sampling site groups (10–103 CFU/m3). The highest TBC levels were measured in Humid less indoor area, while the highest mold levels were measured in Canteen, Auditorium. Micrococcus spp., Staphylococcus auricularis, and Bacillus spp. were predominant bacteria species and Penicillium spp., Aspergillus spp., and Cladosporium spp. were the most observed mould genera detected in the samples. Indoor-to-outdoor ratios of the observed fungi counts were calculated as approximately around one, and for bacteria counts these ratios were higher than one. There was no statistical difference between indoor and outdoor mould levels, while a significant difference was found between indoor and outdoor bacteria levels (p < 0.001). A significant correlation between indoor CO2 and bio-aerosols indicates insufficient ventilation.

Keywords: Contamination, Indoor Air; Bacteria, Outdoor; Aspergillum; Bio-aerosols; Conventional methods; Microorganisms

INTRODUCTION

A variety of contaminants responsible for adverse health effects may be found in indoor air. Since the beginning of the areas, airborne biological agents have attracted increased attention due to their diverse immunological activity and widespread occurrence indoors. Fungi are commonly present in indoor environments and cause of many diseases. Fungi and bacteria isolated from Canteen with moisture problems have shown both cytotoxic and immunotoxic characteristics. Many fungal species of Penicillium, Aspergillus, Alternaria and Cladosporium have been shown to trigger rhinitis, asthma and dermatitis. The major pathway of acquisition is inhalation of airborne fungal spores small enough in diameter to reach the deeper airways. The sources from which fungi and bacteria derive may have importance in their health effects. It has been established that most people spend over 90% of their lives indoors, offices, canteen for lunch / break-fast where they are exposed to some indoor environmental factors such as bioaerosols which could influence their health and physical condition. This has contributed to the growing interest in indoor microbial studies in recent years [1-5]. The ubiquitous nature of microorganisms in the atmosphere has contributed to the biological contamination of indoor environments, which is mostly caused by bacteria, moulds and yeast. They can be dangerous as pathogenic living cells but they can also secrete some substances harmful to health. These are different kinds of toxic airborne metabolism prod-
ucts, for example mycotoxins. The aerial spatial distribution of microorganisms varies according to environmental conditions, type of organism and location. Generally, a higher microbial concentration is found in the outdoor air of urban areas than surrounding rural areas \([7,8]\). Many activities like traffic, constructions and people gathering in urban areas contribute largely to outdoor microbial load \([1,2]\). Recent epidemiological studies have illustrated that extremely high concentration of microorganisms in the air can be allergenic, however sometimes even very low concentrations of some particular microorganisms can cause serious diseases. Moulds have been shown to react with humans causing 30% of health problems relevant to the indoor air quality \([3]\), particularly in rooms with heating, ventilation and air-conditioning systems \([11,6]\) and can breed allergies\([4]\), SBS symptoms (“sick building syndrome”) causing irritation of mucous membranes, bad physical condition, tiredness, headaches, vertigo, decrease of concentration, memory and intellectual work ability \([5,6]\), dermatosis, respiratory diseases (including asthma) \([7,8]\).

The proportion of pathogenic microorganisms is higher in indoor than outdoor air \([8]\). Environmental conditions such as relative humidity (RH), temperature and wind velocity exert significant effect on the type of population and amount of microorganisms in the air \([10,11]\). Generally, microbes enter into atmosphere from natural (vegetation and soil) and anthropogenic sources but their survival and distribution depend on the cell structure of microbes and meteorological conditions \([12,13,14]\). Many airborne microorganisms are either pathogenic or can cause sensitivities due to prolonged exposure.\([15]\) Airborne microbes attach to dust particles, condense and enter human body directly via inhalation or indirectly via ingestion of contaminated foods and water \([22]\) resulting in the development of disease \([22]\). Airborne bacteria can also affect visibility, climate and the quality of life \([16,18,20]\). It is important to know the distribution pattern of live bio-aerosols at different sites in the urban environment. The aim of this work is long-term observation of microbiological quality of indoor air in selected shops in two business locations in Ibadan metropolis, where thousands of people spend several hours working in enclosed spaces every day and where microbiological quality of indoor air can influence their health and physical condition. The study embraced a measurement of the concentration of bacteria and fungi in the air of selected shops and meteorological conditions such as temperature and relative humidity.

Exposure to bio-aerosols, containing airborne microorganisms and their by-products, can result in respiratory disorders and other adverse health effects such as infections, hypersensitivity pneumonitis and toxic reactions. Fungi are common in indoor and outdoor environments and nearly 10% of people worldwide have fungal allergy. In many environments including hospitals, animal sheds, clean-rooms, pharmaceutical facilities and spacecraft environments, the presence of bio-aerosols can compromise normal activities, making efficient monitoring crucial. Microbial damage in indoor/outdoor areas, is caused most frequently by molds and bacteria. These micro-organisms have a very important role in the biogeochemical cycle, as their task consists of disintegrating organic mass to reusable metabolites. In the environment spores of molds and bacteria may become airborne and are therefore ubiquitous. They can enter indoor areas either by means of passive ventilation or by means of ventilation systems. Many genera are also emitted by indoor sources like animals, flowerpots and wastebaskets.

In most cases \([21]\) normal flora is not harmful. However, growth conditions like excessive humidity and/or a high water content of building materials are encountered on a more frequent basis, which in most cases can be described as the limiting factor for microbial growth. This is caused by shortcomings of the buildings such as the lack of thermal insulation, as well as the incorrect behavior of users of rooms. The relative humidity and or the moisture content of the materials determines that to what extent different micro-organisms are able to grow on indoor or outdoor materials. These may cause destruction, adverse health effects and unpleasant odors. Therefore, the task of microbial examinations is to differentiate between normal indoor micro-organisms, airborne or adherent to walls and floors and fast growing species, attaching
itself to building materials and producing micro-
bial products and ultimately causing adverse 
health effects. Air sampling of microorganisms 
is a popular method of conducting microbial ex-
aminations, as it allows a direct toxicological 
evaluation. These results can be related to a 
concentration expressed in colony forming units 
per cubic meter. Sometimes information might 
even be available on a particle which allows for 
an estimation of how deep those particles may 
penetrate into the lungs of a human being.

Micro-organisms are generally not equally 
distributed in indoor air. They mostly occur in 
clouds and are often overlooked in air measure-
ments, especially if the microbial damage is hid-

den by paneling, walls, etc. Another reason for 
false-negative results obtained by air measure-
ments is that fungal spores are not released dur-
ing all the stages of its growth. In this case, other 
techniques are helpful, for example, the sam-
ping of household dust, the sedimentation 
method or direct sampling from surfaces. The 
differentiation of bacteria is performed by a bio-
chemical method as a rule, whereas in most 
cases the differentiation of molds is done micro-
scopically, especially when the forms of spores 
need to be detected.

On many occasions, the growth behavior 
and patterns on different nutrient agars also 
have to be evaluated. Non-sporulation species 
have to be triggered to produce spores, other-
wise "sterile mycelium" will result, which means 
they cannot be named by genera or even spe-
cies. Methods of genetic fingerprinting are still 
in their early stages and only available for some 
genera or species. In the meantime enzymatic 
tests have become available to decide between 
mold growth and normal quantities on building 
surfaces. Searching for hidden mold growth can 
be a very difficult task. An example of this is if 
adverse health effects like the fungal syndrome 
is observed. The fungal syndrome is character-
ized by the occurrence of unspecific symptoms. 
The analysis of microbial volatile organic com-
ounds or even the use of specially trained 
niffer dogs are some of the methods used to 
detect hidden mold growth. However, these meth-
ods have not been scientifically evaluated [22].

The extermination of microorganisms is of-
ten carried out, but this procedure is not suffi-
cient because non-viable spores for example, 
keep their allergenic potential. The acuteness 
of the rehabilitation procedures is normally con-
sidered according to the extent of the microbial 
damage. Adverse health effects are supposed 
to be linked with microbial growth in indoor ar-
eas and are mostly related with mold growth. 
Allergies is a predominant condition which has 
to be mentioned, followed by toxic alveolitis and 
reactions like (allergic) bronchitis, chronic ob-
structive pulmonary disease, as well as the ag-
gravation of asthma. Infections by molds and 
bacteria are very rare, but persons with immu-
nodeficiency are especially susceptible to fun-
gal infections. It has been found that spores of 
fungi contain fungal toxins (mycotoxins), which 
are well known from food contaminations. It has 
however not been confirmed whether these my-
cotoxins show toxic effects if fungal spores are 
hhaled.

On the whole, the dose relationship be-
tween the concentration of microbial particles 
already mentioned and the adverse health ef-
fects described, is not very well established. 
When sanitary effects are observed, the sus-
ceptibility of the individual is very often crucial. 
The result of this is that guidelines concerning 
microbial products in indoor areas are sparse 
and mostly not scientifically sound. In non-indus-
trial indoor environments, the most important 
source of airborne bacteria is the presence of 
human. Specific activities like talking, sneezing, 
coughing, walking, washing and toilet flushing 
can generate airborne biological particulate 
matter. In addition food stuffs, house plants and 
flower pots, house dust, pets and their bedding, 
textiles, carpets, wood material and furniture 
stuffing, occasionally release spores of Alternaria, 
Aspergillus, Botrytis, Cladosporium, Penicil-
lium, Scopulariopsis into the air. Although indoor 
environments are considered to be protected, 
they can become contaminated with particles that 
present different and sometimes more serious 
risks when their concentrations exceed recom-
mended maximum limits than those related to 
outdoor exposures. Human beings build the 
home to be protected in the environment.

Indoor air pollution can be as much more 

terrible than that of outdoor air, it can cause a
wide range of health problems. Mold, mildew, fungi, bacteria, viruses, microorganisms, chemical fumes, organic odors, dust pollen and other floating particles are potential threats in many households. Most people assume that this particular problem is addressed if they filter the air. The truth is that filters will not remove all the particles from the air.

Even if a high-efficiency particulate air filter (HEPA) is used, the problem will not be effectively addressed. HEPA filters will only remove particles the size of 3 microns or larger. Consequently, dust particles smaller than 3 microns will pass through unhindered. Unfortunately, filters can also become breeding grounds for mold and bacteria. A filter only collects and does not kill toxic particles. For a filter to work effectively, air has to pass through it. If a person inhales air prior to it passing through a filter, the particles would have already entered the person’s lungs. In addition, if a filter collects only mold spores, it does not solve the problem. Effectively, the mold that created the spores is still alive and continues to generate mold spores. A filter is not designed to eliminate the source itself. Ultraviolet lights (UV) are claimed to kill 99.9 % of all organisms. Even though UV has the potential to kill 99.9 % of all organisms, it will only kill that which passes through the light. In addition not all UV rays have the same potential to kill organisms.

Airborne bacterial concentrations were usually higher than fungi. Bacteria and fungi had similar diurnal variation patterns. The objective of this study was to investigate the airborne fungi and bacteria collected in indoor and outdoor environment. The study was carried out in four areas, using conventional enumeration of airborne micro-organisms and relied on a culture-based method for bio-aerosol sampling, aimed at generating an exposure database and examines the relationship between the in- and outdoor culturability of fungi and bacteria. The primary goal of the bio-aerosol sampling was the quantitative evaluation of the viable airborne bacteria and fungi. Besides the standard enumeration of culturable microbes as CFU/m³, this study attempted to identify and evaluate the colonies through their specific color, turbidity or other characteristics that appear when grown on selective media.

MATERIALS AND METHODS

Air Sampler Performances

(a) Air Sampling

Total fifty air samples were collected from indoor areas as canteen, auditorium, office and from outdoor area as outdoor near building I.I.T.R. (Indian Institute of Toxicology Research) area of Institute building. These all samples were taken at various time throughout eight working hours. Airborne concentration of microorganisms can be studied by counting propagules in air samples or settled dust particles. Sampling of culturable microorganisms is based on impaction (in which microorganisms are collected from the air stream due to an inertial force that deposits them on to solid or semi solid selection surface), liquid impingement ( separation of microorganism from the airstream by passage through a porous medium such as filter).

After sample collection colonies of bacteria and fungi are grown on culture media at a defined temperature for the length of time required for colony development (usually 3-7 days). Colonies are counted manually or by image analysis techniques. To date, no standard methods are available for detecting and enumerating fungi in indoor environments, which significantly limit the potential for comparing data from different studies. These include poor reproducibility selection of certain species for example the choice of sampling method, culture media or temperature chosen and lack of detection of non-culturable and dead microorganisms and microbial components, although they too may have toxic or allergic properties. Sampling methods for airborne particles can be subdivided into passive sampler using natural aerosol conviction, diffusion or gravity, and active samplers using stationary or personal pumps. The stationary sampling is the most widely used method for conducting microbial measurements in indoor/outdoor environments. The results obtained with different devices are not easily comparable with each other due to differences in sampling times, volumes and principles.

In addition, no good methods for sampling personal air for culturable microorganisms are available, and air sampling for more than 15 min-
utes is often not possible, whereas air concentrations usually vary widely over time. Nevertheless, counting culturable microorganisms is potentially a very sensitive technique, allowing the identification of many different species. Traditional culture methods have proven to be of limited use for quantitative assessment of exposure. Culture-based techniques thus usually provide qualitative rather than quantitative data. The former can, however, be important in risk assessment, as not all fungal and bacterial species pose the same hazard. Furthermore, a qualitative comparison of indoor and outdoor micro biota (in samples collected at the same time) may provide important information about potential indoor sources of contamination. More extensive reviews of techniques for sampling and culturing microorganisms are available. The Sample is collected from Bio stage Sampler, at a flow rate of 28.3 liter / minute. There were collected 50 liters of air with Bio stage sampler by using for two minutes. The instruments were placed one meter above the floor and in two meter distance in every sampling in various times.

Nutrient agar media (peptone-0.5% beef extract/ yeast extract- 0.5%, agar-1.5%, NaCl-0.5%) is also used for the bacterial growth. The malt extract agar was incubated at 35°C for 24-48 hrs and the nutrient agar and Luria Bertani agar is to be incubated at 37°C for 48-70 hrs. For inhibiting the contamination of fungi, during making media especially bacterial media, the anti- fungal AMPHOTERICIN-B is used in 10 micro-liters in 200 ml of media sample, to be taken for highly concentrated.

The institute, IITR-CDRI (outdoor) building, where the sample was taken is situated on the bank of Gomati river. The climate is warm and humid in March to May and a normally cool in January to February. The temperatures fluctuate between 32°C to 45°C in summer, and in 15°C to 24°C in early winter. Each stage of sampler contains 200 spores, each having a diameter of 1.5 mm in all stages, for fungi, a viable spores captured by samples were also isolated on 2% malt extract agar medium supplemented with Streptomycin Sulphate (40 microgram per ml) following incubation for 48-72 hrs at 27°C.

The colonies were identified based on their colony characteristics such as color, shape and other morphological features of the mycelia and spores to the lowest taxonomic rank possible. The most efficient methods of removing suspended particles from the air, example, filtration through fine pore matrices, might be adequate for resistant forms of microorganisms, such as spores, but can be less damaged environmentally resistant vegetative cells.

Determination of concentration of Microbes in air samples

The concentration of Microorganism counted from Petri plates or on slides for spores is calculated as per the given formula:

\[ \text{Number of CFU} = \frac{\text{Total number of microbes' colonies}}{\text{Total volume of air sampled (l)}} \times 1000 \]

Total volume of air sampled the counts are expressed as number of colony forming units (CFU/m³) or number of spores per colony (spores / m³).

After isolation of microbes in another media by streak plate method, spread plate method by using Inoculation loop, they have to dyeing for the detection of their biochemically identifi-
cation. Gram’s staining is used for the identification and especially for classification of bacteria in their gram positive, or gram negative bacteria. For the slide preparation a drop of stain were put on the clean slide then picked-up a small tuff of fungus. Then gently teased the tuff to separated the hyphae by cool needle and mix with the Lacto phenol cotton blue stain properly for Fungi.

RESULT AND DISCUSSION

The results obtained from indoor area and outdoor area monitoring reveals that the microbial concentration, bacteria and fungi were lower in morning time in comparison to those in the evening time. In addition to bacteria and fungi reduction of heterotroph were also observed from morning to evening time in canteen, office and auditorium samples. In the afternoon these counts rise gradually up to a maximum level at the end of working period, which indicates that an indoor environment provides more favorable conditions for the survival of aerosolized fungi. The highest in- and outdoor culturability of fungi was observed in the humid condition. Cladosporium had the highest median value of culturability (38 % and 33 % for indoor and outdoor, respectively) followed by Aspergillus/Penicillium (9% and 2 %) among predominant genera of fungi.

Microbial flora of indoor air depends on several factors including the number and hygienic standard of people present, in indoor space and mechanical movement within the enclosed space. In poor quality and crowded domiciles, the higher number of residents confined to a small space result in the build-up of airborne microbes shed by the human body. For both the in and outdoor air samples, the concentration of total bacteria were lower than the concentration of total fungi for all the areas. For individual fungi species the concentration of both the indoor and outdoor air was Cladosporium, Penicillium, Aspergillus and Alternaria in descending order. For both the total bacteria and the total fungi, the outdoor concentration for the four different areas was usually higher compared to the indoor concentrations. The indoor concentrations of Aspergillus and Penicillium were usually lower than the outdoor concentration near office building due to the fact that open area naturally have higher temperatures, more humidity, and more nutrients available for bacteria to exist naturally.

Data recorded for the concentration of microbes, bacteria and fungi in Indoor and Outdoor area

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<th>Sr. no.</th>
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<th>Fungal concentration (Colony/plate)</th>
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<th>Fungal concentration (Colony/plate)</th>
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In this study, an attempt was made to determine the composition and variability of airborne fungal spores in a comprehensive manner by synchronous use of non-viable and viable sampling method. The study was of a kind that first volumetrically assessed the cultivable airborne fungal spore in the region and determined its relationship with the meteorological factors.

Non-viable sampling of total airborne spore

The total airborne fungi monitored by the bio stage sampler showed a perennial occurrence pattern and attained the main peak in early winter and subsidiary peak in April-May. The relative humidity and temperature in moderate temperature in moderate range, very low wind speed, and minimal dew, during early winter might have facilitated the release in dispersion of dry spore mass; these factors presumably contributed toward achieving the main peak during these months. In January, whereas ascospores and basidiospores were the major components of airborne fungi which peaked in May.

The total cultivable airborne fungi attained two peaks in the first month of January-last week to May-first week, total 50 samples were taken for four months. Among 13 cultivable fungal genera (comprising 26 species) recorded by the viable sampler, the predominant fungal genus was Aspergillus spp., though Cladosporium cladosporioides occupied the first position, other prevalent genera included Curvularia spp., Alternaria spp., Penicillium spp., Nigrospora spheroids. Sterile mycelia (non-sporulating colonies), which may represent the vegetative phase of either Ascomycota or Basidiomycota, contributed a significant fraction to the total viable spore count. The remainder such as, Absidia corymbifera, Mucor hiemalis, and Syncephalastrum racemosum was observed in considerably low concentration throughout the six months. Eight species of Aspergillus were isolated of which Aspergillus niger occupied the first position in order of dominance, followed by Aspergillus nidulans, Aspergillus fumigates, Aspergillus ochraceus, Aspergillus japonicas, Aspergillus ustus, and Aspergillus sydowi.

Several cultivable fungal spores (e.g. Curvularia hunata & Curvularia pallescenes, Aspergillus japonicas, Aspergillus sydowi). During February, only Penicillium spp., achieved an elevated concentration level. In a handful cultivable spores (e.g. Absidea corymbifera, A. flavus, A. fumigates, A. nidulans, Cladosporium spp) only three genera of bacteria and six genera of fungi as, Alternaria, Aspergillus, Penicillium, Cladosporium, Curvularia, Drechslera and Nigrospora and in bacteria especially, Escherichia coli, Streptococcus spp., Staphylococcus
spp. etc. were found during sampling. The ascospores and basidiospores that constitute a significant part of the airborne fungi were recorded only by the non-viable sampling. The ratio of total airborne fungal spores to cultivable mold was obtained in the range of 0.19 to 4.16. The spores of Ascomycetes and Basidiomycetes members are visible in light microscope, but they are unable to grow in laboratory culture media. For this reason, decrease in concentration of cultivable mold compared to total fungal spores is generally anticipated while analyzed by light microscopy.

CONCLUSION

This study clearly indicates that there is significant assessment of the indoor and outdoor airborne bacteria and fungi. Airborne fungal and bacterial concentrations in auditorium, offices, canteen were lower than in the other facilities of the outdoor area of building.

A large range of different bacteria including Actinomycetes, mesophilic bacteria, Mycobacteria, gram-negative bacteria, and thermophile bacteria were present in canteen, which is specific for indoor air quality. The moisture damage affects diversity of microbial concentration. In the moisture-damaged building, the microbial diversity was higher in canteen, offices and other indoor area. The measurements of airborne microbial concentrations during four consecutive months showed the variation of microbial levels due to climatic conditions.

The results showed that the average fungal concentration was 80 CFU/m³, similarly the average concentration of bacteria was 60 CFU/m³ which was lower than the fungi concentration in outdoor area and in indoor area the average fungal concentration is 90 CFU/m³ in comparison to bacterial concentration is 65 CFU/m³. So the result is the concentration of fungi is higher than the bacteria in indoor and outdoor area.

REFERENCES

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