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Bioaerosols in Indoor Environment - A Review with Special Reference to Residential and Occupational Locations

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Abstract: Bioaerosols such as bacterial and fungal cells and their spores are - along with non-biological particles - part of indoor airborne particulate matter and have been related since a long time to health issues of human beings as well as flora, and fauna. To identify the different risks and to establish exposure thresholds, microbiology of air samples from a series of indoor environments must be characterized, i.e. the different microorganisms (bacteria and fungi) must be identified and quantified. This review discusses the techniques of air sampling and sample analysis. In addition, a literature study has been performed regarding the levels of these microorganisms in various indoor occupational (e.g., schools, offices, hospitals, museums) and dwelling environments. These results will provide a significant scientific basis for indoor air quality control and help in elaborating risk prevention programs for workers and dwellers. This review shall contribute to the knowledge of identification and quantification of airborne microbial constituents in various indoor environments. Combining the indoor microbial load data with data from studies focusing on health effects caused by inhalation of specific airborne microorganisms will allow the evaluation of various risks to which inhabitants are exposed.

Keywords: Bioaerosols, microbial diversity, sampling, monitoring, occupational environment, maximum acceptable values.

INTRODUCTION

Aerosols are liquid or solid particles suspended in a gaseous medium with size ranges from 0.001 to 100 μm [1]. Bioaerosols consists of aerosols containing microorganisms (bacteria, fungi, viruses) or organic compounds derived from microorganisms (endotoxins, metabolites, toxins and other microbial fragments) [2]. Aerosol particles of biological origin (cells, cell fractions or organic matter of animal, plant and microbial origin) form a significant portion of atmospheric aerosols, sometimes reaching close to 50% numerically of all aerosol particles [3]. Bioaerosols vary in size (20 nm to >100 μm) and composition depending on the source, aerosolization mechanisms, and environmental conditions prevailing at the site [4]. The inhalable fraction (PM 2.5) is of primary concern because it is the most susceptible portion of the bioaerosols to reach the deeper parts of the respiratory system [5]. Because of their light weight, airborne particles are readily transported, transferred, and displaced from one environment to the other. Indoor air contains a complex mixture of bioaerosols such as fungi, bacteria and allergens along with non-biological particles (e.g., dust, smoke, particles generated by cooking, organic and inorganic gases) [6]. Airborne microorganisms might pose an environmental hazard when present in high concentrations in indoor environments resulting in health problems [7].

When bioaerosols are measured at sampling sites, monitoring of environmental factors can be a useful tool to explain possible bioaerosol sources. There are some evidences that show the significant associations between bioaerosols levels and some environmental factors, such as temperature and relative humidity [7]. Since most of the bacteria and fungi need specific environmental conditions to grow and propagate, their levels are strongly affected by these factors. In some cases, heating, air-conditioning or ventilating systems may provoke fluctuations of temperature and relative humidity, such as in museums, which can cause serious harm [8].

In non-industrial indoor environments, one of the most important sources of airborne bacteria is the presence of human beings [9]. In particular activities like talking, sneezing, coughing, walking, washing and toilet flushing can generate airborne biological particulate matter. Food stuffs, house plants and flower pots, house dust, pets and their beddings, textiles, carpets, wood material and furniture stuffing, occasionally release of various fungal spores into the air [10, 11]. According to several studies, the moisture content of building material, relative humidity and temperature [12, 13], outdoor concentrations, air exchange rates [14] and number of people and pets [15] significantly affect the levels of indoor bioaerosols. Generally higher concentrations of bioaerosols have been reported from warmer than cooler climates. Moreover, housing conditions, the activities and life style of occupants considerably contribute to the varying concentrations [16]. Under normal conditions, bacteria and fungi do not notably grow in building materials or structures or on indoor surfaces, mainly because of lack of moisture [17].

The indoor air is a very dynamic system in which particles of biological and non-biological origin are distributed and displaced. Studies have been carried out to check

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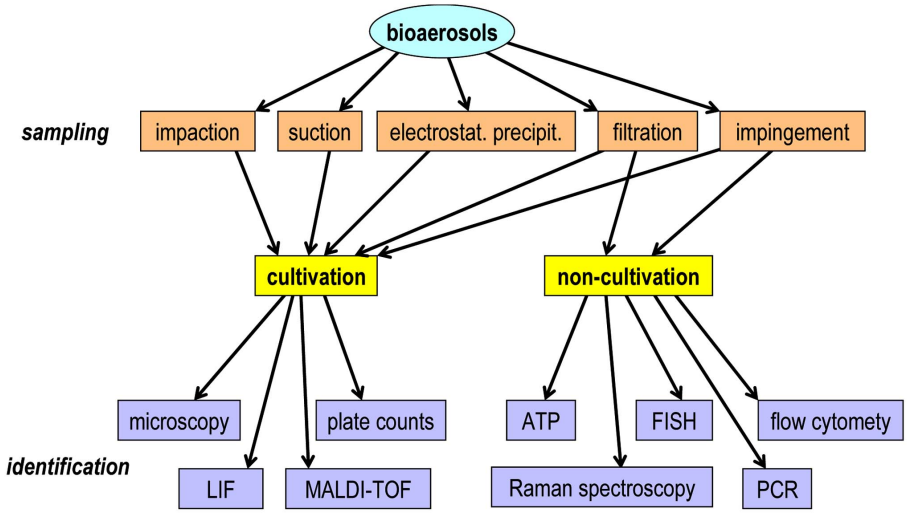

Fig. (1). Flow chart indicating selected examples of fungal and bacterial bioaerosol sampling methods and identification techniques described in the text (see text for abbreviations) in relation to sample processing (i.e., cultivation or non-cultivation).

the indoor air quality (IAQ) as it is an increasingly important issue for occupational and public health [18]. The sampling and analysis of airborne microorganisms in indoor air has received attention in recent years [19-21]. Bioaersosols contribute to about 5 to 34% of indoor air pollution [22]. The source of bioaerosols in indoor air includes furnishing and building materials, microbiological contamination within the walls and ceilings and floor activities. Another significant source of airborne indoor bacteria are occupants [21, 23]. Sources of indoor bioaerosols are often located outdoors and particles are transferred to the inside through openings of the building envelope (windows, doors). However, one of the most important factors affecting indoor air quality is how the building is heated, ventilated, air-conditioned [24] and its occupancy [25]. These factors can be used to model and predict indoor bioaerosol concentration [14, 26].

Microbiological air quality is an important criterion that must be taken in to account when indoor workplaces places are designed to provide a safe environment. This review provides information on what is currently known on various indoor air concentration of microorganisms and describes bacterial and fungal loads for different kinds of indoor environment (such as in occupational and dwelling places). A brief description of various sampling and analysis methods used to characterize airborne microorganisms is also given.

COLLECTION OF AIR SAMPLES

The devices used to sample airborne fungi and bacteria mainly rely on three different principles namely, impaction, impingement and filtration which are described below (Fig. **1**).

Impactors - Solid media such as agar are used to collect bioaerosols by impaction sampling. Cheap costs of samplers and their easiness to handle are major advantages [27]. Typically, samplers are equipped with a fan transferring air through a perforated template (sieve samplers) or a narrow slit (slit samplers) directly onto standard agar plate containing a suitable agar growth medium. Impaction velocity is determined by the flow rate and nozzle diameter or the width of the slit and is the range of 40 km/h. When hitting the collection surface, the air sampled changes direction perpendicularly and any suspended particles are tangentially impacting onto the agar surface. Agar plates can be removed when appropriate volumes of air have been sampled and incubated directly under appropriate conditions without further treatment. The number of visible colonies can be counted by visual inspection after incubation resulting in a direct quantitative estimate of the number of culturable microorganisms in the sampled air.

Rotorod sampler [28] is used to know the particles quantitatively recovered per unit of air sampled. The rotorod sampler [29] is a volumetric, rotation impaction device capable of quantitatively sampling airborne particles in the size range of 1 to 100 µm at sampling rates up to 120 liters per minute. Its trapping efficiency is nearly 100% for particle size larger than 15 µm in diameter in still air. Rotorod sampler from Sampling Technologies Inc. USA is popularly used.

The “Andersen sampler” is one of the best known impactors. It consists of a multi-stage cascade sieve unit that uses perforated plates with progressively smaller holes at each stage, allowing particles to be separated according to size. A statistical “positive hole correction” is needed to evaluate highly loaded plates [30, 31]. Another well known instrument is the Casella slit sampler. A turntable - on which an agar plate is placed - is positioned below a slit. When air is drawn through the slit, the agar plate rotates, so that particles are evenly dispersed over the agar surface [32].

MAS-100eco single stage impaction samplers are used for the collection of bioaerosols by some authors [11, 33]. An amount of 50 to 500 l of air (or less depending on the sampling location) can be collected in time intervals of 3 to 5 minutes. Standard 90mm Petri dishes containing different solid growth media can be used with the impaction sampler [27]. Nutrient agar is used for the determination of culturable bacterial strains. For determination of total number of culturable bacteria, tryptic soy agar is used. MacConkey Agar is use to determine Gram-negative bacteria [34]. For the determination of fungi (moulds and yeasts) malt extract agar has been frequently used.

Impingers – In contrast to impactors, particle collection by impingement is based on liquid media. Typically, sampled air is drawn by suction through a narrow inlet tube into a small flask containing the collection medium accelerating the air collected towards the surface of the collection medium. Flow rate is determined by the diameter of the inlet nozzles. When the air hits the surface of the liquid any suspended particles are impinged into the collection liquid. Once the sampling is complete, aliquots of the collection liquid can be cultivated in appropriate growth media to enumerate viable microorganisms. Since the sample volumes and sampling times can be defined, results allow quantitative determinations.

The "BioSampler" liquid impinger (SKC, Eight Four, PA, USA) is popularly used. The sampler is an all-glass, swirling aerosol collector consisting of an air inlet, three tangentially arranged nozzles and a collection vessel [35]. The AGI-30 sampler (Ace Glass Inc., N.J., USA) is a cheap, but less efficient impinger developed to sample bioaerosols [36, 37].

Suction sampler - Suction samplers are based on the suction of a certain volume of air according to a known velocity and for a chosen duration on each trapping. Ogden [38] designed a volumetric sampler, based on aerodynamic principles. There are several suction samplers available like Hirst automatic volumetric sampler [39], Burkard seven day volumetric sampler, Burkard personal slide sampler, Burkard Petriplate sampler (Burkard Inc., Burkard Manufacturing Co. Ltd., England) [40].

Filtration samplers - With this method, particles are removed from the air by suction filters of definite pore mesh size, which offers volumetric potential, appropriate for smaller aerosol classes and where ambient velocities are low. Air is drawn by a vacuum line through a membrane filter made of glass fibre, polyvinylchloride (PVC), polycarbonate or cellulose acetate (which can be incubated directly by transferring onto the surface of agar growth media), or gelatine which can be dissolved liquid cultures. However, filtration is less convenient than impaction-based sampling and may cause dehydration stress in the trapped microorganisms. Dehydration stress depends on sampling time and while gelatine filters offer a more "friendly" environment for the microorganisms, microorganisms can still suffer from dehydration stress compared to impactors [41]. Use of polyurethane foam inserts allows collection of bioaerosols according to the size fractions [42]. Filter samples allow sampling for longer times without the loss of collection efficiency compared to impactors and impingers. Dehydration due to long-term sampling may prevent from determining colony forming units (CFUs), but one can use molecular analysis techniques.

In the past few years, portable (battery-operated) impactors have become popular for the collection of culturable bioaerosols. Such devices do not require heavy external pumps and feature high sampling flow rates. Various performance parameters of a series of portable impactors have been compared when collecting polystyrene latex particles and biological particles under controlled laboratory conditions [43-45]. Results suggested that when impactors are used for the collection of airborne bacteria and fungi, sampling times should be kept as short as possible to minimize under-representation of airborne microorganism concentration. In a field study involving the same portable impactors it was found that a majority of them underperformed compared to a BioStage impactor (SKC Inc., Eighty Four, PA), which is an equivalent to the Andersen N-6 viable impactor [41].

Electrostatic methods- Mainelis *et al.* [46] developed a bioaeorosol sampler, called electrostatic precipitator, which utilizes an electric field to deposit charges on bacterial samples and a solid agar as bacterial growth media. In this device, two ionizers in the inlet charge the incoming biological particles if they carry an insufficient charge for efficient collection. The particles are then subjected to a precipitating electric field and are collected onto two square agar plates placed one after the other along the flow axis. In electrostatic precipitators, the particle velocity component perpendicular to the collection medium is two to four orders of magnitude lower than that in bioaerosol impactors and impingers operating at comparable sampling flow rates [47]. Therefore, the electrostatic precipitation technique is potentially less damaging to the microorganisms. In addition, instruments based on this technique can operate at low power input. Low-power bioaerosol collectors are of interest to bioaerosol monitor developers and field practitioners, especially in situations where low-power-consuming monitors are placed in and around buildings and installations to serve as warning devices against bioterrorism [48].

The recovery efficiency varies depending on the air sampler used. It has been found approximately 75% of the Gram-negative bacterium *Pantoea agglomerans* is re-aerosolized and displaced from the sampler during use of an AGI-30 sampler, whereas only 20% is lost using the SKC Biosampler [49]. This was also shown with standardized particles of non-biological origin such as monodisperse polystyrene beads [50]. In swirling airflow collectors (e.g. BioSampler) re-aerosolization is reduced and minimized due to the nozzle-guided tangential air flow in the sampling vessel resulting in reduced shear forces [50]. It has been shown that collecting air samples by filtration usually resulted in a recovery efficiency of only approximately 50% [49]. However, a differentiation of sampling efficiency and culturability of microbes collected is needed. In addition, other studies also demonstrated that recovery strongly depended on the target organism [51]. As example, *E. coli* could not be recovered by filtration because of desiccation, whereas sampling efficiency for *Bacillus subtilis* was comparable to efficiencies of impingement or impaction samplers. The culturability of yeast cells was much better after collection by impingement rather than filtration on nuclepore or gelatine filters [52]. However, a dependence on environmental parameters such as relative humidity was observed. It is generally accepted that prolonged sampling times (e.g. >60 min) usually decrease recovery efficiencies in both impactors and impingers due to several factors such as desiccation, shear forces, or re-aerosolization [49, 52]. Recent research shows that even short sampling times affect the recovery of collected microorganisms when sampled with impactors [53]. As conclusion, it is therefore of fundamental importance that when comparing culturable bioaerosol concentrations determined in different studies, air sampling techniques as well as the methods used for identification (e.g. growth medium for

cultivation) have to be similar or even identical [54]. In addition, multi-investigator round-robin testing might be carried out for better understanding of sampling biases.

PARTICLE COUNTING

Laser particle counters are used to determine particle numbers [55, 56]. Particle size determination is based on optical particle counting by light scattering (refraction, reflection, and diffraction) from single particles flowing out of a nozzle. Both the number and size of particles can be simultaneously determined.

Several studies have demonstrated that there is a correlation between the total particle numbers of a specific size (e.g. 1 to 5 μm) and the number of fungal or bacterial colony forming units [55-58]. Particle counting can be done on a fast basis (using appropriate equipment) without the need of applying air sampling and microbial identification techniques (e.g. cultivation, DNA extraction and sequencing). The simple counting of particles of a certain size class might give a first "quick and dirty" approximation of a possible microbial contamination of the air. It has been stated that "total particles might be used to trace the viable bioaerosol particles" [57]. Bacteria might have correlation with numbers of all particle size ranges assessed, whereas fungal colony forming units were correlated only to size range 1 to 5 μm [58]. The number of culturable fungi correlated well with total number of particles <2.5 μm [6]. However, other studies showed that also bacterial numbers are only correlated to size rages of 1 to 5 μm and >5 μm [56].

IDENTIFICATION OF AIRBORNE BACTERIA AND FUNGI

A wide range of analytical methods is used to determine the presence of airborne microorganisms and to characterize composition and activities of these microbial communities, many of the methods covering well-proven classical microbial techniques such as e.g. microscopy or cultivation (Fig. **1**) [59, 60]. Current methods have been applied both on a non-molecular and molecular (DNA- or RNA-based) level. In addition, spectroscopic techniques based e.g. on the mass of fragmented biomolecules, on molecular vibrations of chemical bonds of biomolecules, or on fluorescence of cellular constituents, all in combination with chemometric data analysis have been introduced.

Microscopy - Microscopic examination and enumeration of airborne biological particles are done with air samples that are drawn on glass slides or filters fitted on to samplers. For most microorganisms, species identification is not possible without processing the sample with a technique designed to identify taxa or species. To facilitate the description of fungal spores several stains that differentiate fungal spores from debris are available [61]. They are identified by morphology and a certain level of expertise is also required. In combination with classical microscopy, fluorescent probes are applied to stain and determine specific bacterial groups or even species in a sample [62, 63]. Total number of bacteria are normally determined after staining with a fluorescent dye such as DAPI (4, 6 diamidino-2-phenylindol) or SYBR Green (asymmetrical cyanine dye) that bind to DNA. Acridine Orange is used to detect viable cells. As example, fluorescence *in situ* hybridisation (FISH) using specific molecular probes binding to ribosomal RNA of intact cells has been used to detect airborne microbes such as eubacteria or - more specifically - *Pseudomonas aeruginosa, P. fluorescens, P. mendocina* and *Comamonas acidovorans* in swine barns [64].

Cultivation - Studying microbial biodiversity in air samples is mostly relied upon on culturing for the quantification and identification of airborne bacteria and fungi. Microorganisms that are collected on a nutrient agar surface by impaction can be cultured directly, while organisms collected on liquid or on a filter are transferred to a culture medium. Colony-forming units (cfu) on solid growth media are counted after visual inspection. However, since microorganisms exhibit a wide range of nutritional requirements, no formulation is capable of culturing every type of organism [65]. Therefore, a common strategy in bioaerosol monitoring is to use general media which promotes the growth of many diverse species. Another strategy is to use several media and incubation conditions (temperature, incubation time, pH, nutrients, antibiotics, etc.) specific to the particular microorganisms to be analysed [66]. Many investigators have conducted studies for which the goal was to evaluate microbial load in various indoor environments such as indoor occupational, indoor agricultural, and in dwelling places. The majority of these studies used culture based techniques to isolate, quantify and identify airborne microorganisms. Table **1** gives the concentrations of various bacteria, fungi and viruses obtained in these indoor environments. Generally it is necessary to perform replicate sampling using different culture media or to divide samples for inoculation on to multiple types of nutrient media. Several broad spectrum media have been evaluated for culturable airborne fungi such as malt extract agar, Rose Bengal agar and DG-18 agar [33, 67-69]. For the cultivation of bacteria, several broad spectrum media such as tryptic soy agar or nutrient agar are commonly used [27, 56]. It has to be stressed, however, that only a small fraction of airborne microbes in a sample can be cultivated, resulting in numbers usually one or two orders less than determined by cultivation-independent methods [70]. This cultivable fraction is a part of the live microbes in a sample, whereas total numbers include dead microbes too. These can be identified by staining with specific dyes.

Flow cytometry - Flow cytometric analysis on air samples is usually performed after air collection by impingement. In flow cytometry a suspension of cells is passed rapidly through a capillary in front of a measuring window. Light emitted from a source is scattered by particles in the liquid and several particles such as size, shape, biological and chemical properties can be measured simultaneously. Autofluorescence or indirect fluorescence of cells affector labelling is also used to detect cells. In addition, specific dyes such as e.g. DAPI, Acridine Orange, SYTO, TO-PRO or wheat germ agglutinin (WGA) are applied to determine total number and live/dead-ratios of microorganisms, respectively [71]. Fluorescence *in situ* hybridisation (FISH) and flow cytometry might be combined resulting in a more powerful analysis of air samples [70].

Polymerase chain reaction (PCR) - PCR technique has been used to detect and quantify microorganisms from

Table 1. Airborne Microorganisms (Bacteria and Fungi) and their Concentrations in Various Selected Indoor Locations. SM: Sampling Method; ID: Identification Method; GM: Growth Medium (1 Blood Agar; 2 Czapek-Dox Agar; 3 DG-18 Agar: Dichloran Glycerol-18 Agar; 4 Endo Agar; 5 MacConkey Agar; 6 MEA: Malt Extract Agar; 7 NA: Nutrient Agar; 8 Peptone Dextrose Agar; 9 Potato Dextrose Agar; 10 PYA: Potato Yeast Agar; 11 PCA: Plate Count Agar; 12 Rose Bengal Agar; 13 Sabouraud Dextrose Agar; 14 Sheep Blood Agar; 15 TSA: Tryptic Soy Agar; 16 yeast extract agar); Temp: Temperature at sampling site (ºC); RH: Relative Humidity in %; cfu: Colony Forming Units; BD: Below Detection Limit; NA: Not Applicable; ND: Not Determined; NS: Not Specified

Location	SM	ID	GM	Temp (ºC)	RH (%)	Bacterial Counts (cfu/m^3)				Fungal Counts (cfu/m^3)				
						Ave	Min	Max	Dominant Genus	Ave	Min	Max	Dominant Genus	Ref.
Hospital	Single stage Andersen sampler	Cultivation	6, 15	NS	NS	ND	2	423	NA	ND	1	3115	ND	[106]
Hospital	MAS-100 sampler, single stage Anderson sampler	Cultivation	6, 10	NS	NS	ND	ND	ND	NA	200	10	85	ND	[161]
Hospital	MAS-100 sampler	Cultivation	6, 7	NS	NS	ND	ND	ND	NA	96	ND	ND	*Alternaria* *Aspergillus* *Cladosporium* *Penicillium*	[123]
Hospital	6-stage Andersen sampler	Cultivation Microscopy	7	23-28	72-80	ND	35	728	NA	ND	ND	ND	NA	[107]
Hospital	Six stage Andersen sampler	Cultivation Molecular identification	1, 12, 15	NS	NS	ND	38	131	*Bacillus* *Micrococcus* *Staphylococcus*	ND	14	611	*Alternaria* *Aspergillus* *Cladosporium* *Penicillium*	[68]
Hospital	6-stage cascade impactor	Cultivation Biochemical identification	6, 15	NS	NS	372	ND	ND	*Bacillus* *Corynebacterium* *Micrococcus* *Staphylococcus*	156	ND	ND	*Alternaria* *Aspergillus* *Cladosporium* *Penicillium*	[108]
Hospital	Burkard personal Petri plate sampler	Microscopy	12	NS	NS	ND	ND	ND	NA	5437	3419	7701	*Aspergillus* *Cladosporium* *Geotrichum* *Penicillium*	[69]
Museum	6-stage Andersen sampler	Cultivation	6, 15	NS	NS	714	545	883	*Bacillus* *Corynebacterium* *Micrococcus* *Staphylococcus*	39	28	49	*Acremonium* *Aspergillus* *Penicillium* *Rhizopus*	[109]
Museum	Gravitational sedimentation	Cultivation	7, 8	NS	NS	50	ND	ND	*Arthrobacte* *Bacillus* *Micrococcus* *Pseudomonas* *Staphylococcus*	30	ND	ND	*Alternaria* *Aspergillus* *Penicillium*	[110]
Office	Single stage Andersen sampler	Cultivation, microscpy	3, 6	18-23	9-60	ND	ND	ND	NA	22	1	618	*Alternaria* *Aspergillus* *Cladosporium* *Penicillium*	[67]
Office	Burkard portable sampler, 2-stage Andersen impactors	NS	NS	21-35	37-50	ND	ND	ND	ND	431	106	1113	*Alternaria* *Aspergillus* *Cladosporium* *Curvularia* *Penicillium*	[67]
Office	Single stage Andersen N-6 samplers	Cultivation, microscopy	3, 6	23	33	ND	ND	ND	NA	42	1.1	618	*Alternaria* *Aspergillus* *Cladosporium* *Penicillium*	[132]
Office	2-stage Anderson sampler	Cultivation	10	21-23	25-29	1987	900	3100	*Arthrobacter* *Bacillus* *Micrococcus*	ND	ND	ND	NA	[113]
Office	Single stage, multiple hole impactors	NS	NS	NS	NS	ND	ND	116	Gram-positive cocci	ND	ND	ND	NA	[112]

Table 1. count….

Location	SM	ID	GM	Temp (ºC)	RH (%)	Bacterial Counts (cfu/m^3)				Fungal Counts (cfu/m^3)				
						Ave	Min	Max	Dominant Genus	Ave	Min	Max	Dominant Genus	Ref.
Office	MAS-100 samplers, single stage Anderson sampler	Cultivation	6, 10	NS	NS	ND	ND	ND	NA	ND	10	700	ND	[161]
Office	MAS-100	Cultivation	4, 9	24	63	ND	400	500	NA	ND	ND	ND	NA	[11]
Office	Andersen sampler	Semi automa-tic counter	10, 13, 14	21	30.7	135	44	283	*Bacillus* *Micrococcus* *Staphylococcus*	113	18	274	*Aspergillus* *Cladosporium* *Penicillium* *Ulocladium*	[111]
Office	Impactor Sampler	Metabolic fingerprinting analysis	NS	22	NS	176	240	200	*Micrococcus* *Staphylococcus*	44	10	75	*Aspergillus* *Cladosporium* *Penicillium*	[18]
Office	SAS Super 90 Impactor	Cultivation	9, 15	NS	NS	414	ND	ND	ND	ND	235	805	*Cladosporium* *Hyalodendron* *Penicillium*	[124]
Residence (apartment)	Six stage Andersen sampler	Cultivation	6, 15	17-27	35-85	NA	0	2039	*Aeromonas* *Bacillus* *Kocuria* *Micrococcus* *Nocardia* *Pseudomonas* *Staphylococcus*	NA	0	896	*Aspergillus* *Penicillium*	[115]
Residence	6-stage Andersen sampler, gravitational sampler, RCS plus aeroscope	Cultivation	NS	NS	NS	NA	88	4751	*Aeromonas* *Bacillus* *Kocuria* *Micrococcus* *Nocardia* *Pseudomonas* *Staphylococcus*	NA	2	16968	*Aspergillus* *Penicillium* yeasts	[114]
Residence	Reuter centrifugal air sampler	Cultivation	15	NS	NS	ND	ND	ND	NA	1133	463	3125	*Alternaria* *Cladosporium* *Curvularia*	[6]
Residence	Slit-to-agar single stage impactor	Microscopy, Cultivation	6, 13	NS	NS	ND	ND	ND	NA			12640	*Aspergillus* *Cladosporium* *Penicillium* *Rhizopus*	[124]
Residence (high rise apartments)	NS	NS	NS	NS	NS	ND	10	103	ND	ND	10	103	*Alternaria* *Aspergillus* *Cladosporium* *Penicillium*	[122]
Residence	MAS-100	Cultivation	3, 6	NS	NS	ND	ND	ND	NA	250	310	1700	*Aspergillus* *Cladosporium* *Penicillium*	[133]
Residence	Six stage Andersen sampler	Cultivation	6, 7	22	47	ND	1557	5036	ND	ND	925	2124	ND	[16]
School (classroom)	Andersen sampler	Cultivation	NS	NS	NS	782	ND	ND	ND	811	ND	ND	*Aspergillus* *Cladosporium* *Penicillium* yeasts	[139]
School (classroom)	Andersen sampler	Cultivation	15	NS	NS	ND	65	425	*Bacillus* *Corynebacterium* *Micrococcus* *Staphylococcus*	ND	ND	ND	ND	[25]
School (classroom)	Andersen sampler	Cultivation	6, 15	11-21	17-40	1002	269	1621	ND	415	324	616	*Alternaria* *Aspergillus* *Cladosporium* *Penicillium*	[148]

Table 1. count……

Location	SM	ID	GM	Temp (ºC)	RH (%)	Bacterial Counts (cfu/m^3)				Fungal Counts (cfu/m^3)				
						Ave	Min	Max	Dominant Genus	Ave	Min	Max	Dominant Genus	Ref.
School	Petri plate gravitational	Cultivation	2, 6, 7, 8	28	65	259	ND	ND	*Corynebacterium Pseudomonas Staphylococcus*	371	ND	ND	*Aspergillus Penicillium*	[135]
School (classroom)	Air-O-cell	Microscopy	ND	20-24	23-57	ND	ND	ND	NA	505	0	6370	*Alternaria Aspergillus Bipolaris Cladosporium Penicillium*	[134]
School (university)	Gravitational sedimentation	Cultivation, Microscopy	2, 16	NS	NS	ND	390	630	*Micrococcus Staphylococcus*		330	520	*Aspergillus Cladosporium Penicillium*	[137]
School (atrium)	MAS-100eco	cultivation	6	22-26	33-44	562	290	1270	ND	213	70	615	ND	[56]
School (university)	Burkard single stage sampler	Identification kit	10	NS	NS	225	ND	ND	*Bacillus Flavobacterium Micrococcus Neisseria Staphylococcus*	ND	ND	ND	ND	[120]
School (classroom)	Andersen sampler	VITEK32	10, 13, 14	11-28	15-64	633	62	1696	*Bacillus Micrococcus Staphylococcus*	100	BD	574	*Aspergillus Cadida Penicillium Rhizopus*	[111]
School (classroom)	Impinger	NS	ND	19-21	52-61	ND	480	1634	ND	ND	100	660	ND	[118]
School (child care)	Gravitational sedimentation	Cultivation, Microscopy	6,8	23	70	ND	ND	ND	ND	ND	ND	ND	*Alternaria Aspergillus Cladosporium Penicillium*	[136]
School (university)	Settle plate method	Cultivation	7, 13	24-25	50-60	ND	ND	ND	*Bacillus Staphylococcus Actinomyces*	ND	ND	ND	*Aspergillus Cladosporium Mucor*	[117]
School	MAS-100	Cultivation	2, 6, 9	NS	NS	ND	ND	ND	ND	ND	ND	ND	*Alternaria Aspergillus Cladosporium Penicillium*	[138]
School (child care)	Gravitational sedimentation	Cultivation, Microscopy	1	24	60	9	26	ND	*Bacillus Corynebacterium Staphylococcus Streptococcus*	ND	ND	ND	ND	[119]

various environments [68, 72-74]. It is used to copy and amplify many million-fold specific regions (typically <1000 bases) of the genome, providing enough for analyses [1]. This method is very rapid compared to that with culture techniques and is sensitive enough for the detection of specific microorganisms which are slow growing and difficult to culture. Mainly outdoor microorganisms have been investigated using this method [75-77]. The conventional PCR assay has been applied to analyse air samples for the presence of airborne mycobacteria [78] and fungi commonly associated with adverse health effects [79]. PCR allows the detection and identification of non-culturable airborne microorganisms; it does not allow distinguishing between non-viable and viable microorganisms [80]. Currently, the real-time PCR (RT-PCR) is evolving into a promising tool capable of reproducible and accurate measurements of total microorganism concentrations in environmental samples. The advantage of RT-PCR is the capacity of rapid sample quantification as well as species-specific identification [81]. A recent study has demonstrated that viable *Legionella pneumophila* can be efficiently sampled by the AGI-30 and BioSampler and successfully quantified by with quantitative PCR [82]. As examples, real-time quantitative PCR to determine airborne bacterial loads or - more specifically - *Salmonella* in poultry houses was only applied recently [83, 84].

ATP-bioluminescence - ATP is the most important biological fuel in living organisms. An assay using ATP (adenosine triphosphate), which plays a central role as an intermediate carrier of chemical energy linking the catabolism and biosynthesis within microbial cells, has long been recognized as "the most convenient and reliable method for estimating the total microbial biomass in most environmental samples" [85-87]. ATP bioluminescence is based on a light generating

reaction with luciferin and firefly luciferase. Since the light emitted from the reaction is proportional to the ATP concentration contained in the total biological contaminants, hygiene monitoring techniques using the ATP bioluminescence have been used to quickly access and monitor microbial contamination on surfaces, such as in food industries [88]. The method is fast, robust easy to perform, affordable and detects both cultivable and uncultivable organisms [89]. Using an aerosol condensation system in combination with an ATP-bioluminescence transducer system, a biosensor has been developed to detect ATP from aerosols in real-time and to determine the existence of airborne microbes within 10 min [90]. The ATP bioluminescence method has also been applied to characterize the performance of bioaerosol sampling devices [91]. Detecting ATP originating in air could thus be an important method for detecting living organisms like airborne pathogens.

Spectroscopic techniques such as matrix assisted laser desorption/ionization time of flight - mass spectrometry (MALDI-TOF-MS) or Raman-spectroscopy have been recently introduced for the analysis of bioaerosols. However, some of them are still depending on cultivation prior to investigation.

Matrix assisted laser desorption/ionization time of flight mass spectrometry - MALDI-TOF mass spectra can be used for the identification microorganisms such as bacteria or fungi. A microbial is transferred from a nutrient plate directly onto the sample target. Spectra generated are analyzed by dedicated software and compared with stored known profiles. Species diagnosis by this procedure is much faster, more accurate, and cheaper than up-to-now standard procedures based on immunological or biochemical tests. In combination with an impactor sampler, bioaerosols directly deposited on a target have been analysed [92]. Using instrumental improvements it is even possible to obtain a mass spectrum of a single airborne particle allowing on-line measurements and analysis without the need of prior cultivation [93].

Raman spectroscopy – Identification of a single bacterial cell and the differentiation between spores and vegetative growth states was possible using micro-Raman spectroscopy [94]. Raman spectroscopy (together with infrared spectroscopy) is based on vibrations between the chemical bonds of the atoms making up the material representing, therefore, the chemical composition of a cell and resulting in a typical vibrational "fingerprint".

Laser-induced fluorescence – Fluorescence spectra of bacteria obtained after excitation by laser light can result in a differentiation of the organisms. Air samples were collected by impaction, subsequently cultivated and classified by the fluorescence signals which were correlated to morphology, Gram staining or family [95]. A differentiation on the species level was not possible.

COMPOSITION OF BIOAEROSOLS

Bacteria - Typical and most important bacterial strains found in an indoor atmosphere are representatives of the genera *Bacillus*, *Micrococcus*, *Kocuria* and *Staphylococcus*. Bacilli have the ability to form spores which are characterized by their resistance to harsh environmental conditions such as UV radiation, desiccation, lack of nutrients, or extreme temperatures. These metabolic capabilities facilitate the distribution and survival. *Micrococcus* and *Kocuria* are able to produce colored (pink, yellow, orange, and red) pigments, which is typical for a large portion of airborne microorganisms [96]. It is assumed that these pigments act as "sun screen" for the organisms to protect them from UV radiation. However, pigments are also formed during long term storage of bacterial cultures at 4°C in the dark (Brandl, unpublished observation), what assumes a function as "anti-freezing agent". In addition, there are a few reports on the relationship between pigmentation and pathogenicity, virulescence, and resistance to antibiotics [97, 98].

Several studies have reported that exposure to large concentrations of airborne microbes is often associated with asthma and rhinitis [99], hypersensitivity pneumonitis [100] and sick building syndrome [101]. In addition, exposure to microbes has also been associated with a number of other health effects, including infections [102]. Among the various indoor environment various bacteria such as *Bacillus* sp., *Streptomyces albus*, *Pantoea agglomerans*, *Pseudomonas chlororaphis*, *Arthrobacter globiformis*, *Thermoactinomycetes vulgaris*, and *Corynebacterium* sp. were identified which were known to have allergenic or immunotoxic effects on human health (Table **1**), [103-105]. Results have shown that the occurrence of distinct and reproducible short term dynamics (on a time scale of minutes) of total particles and bioaerosols related to periods of anthropogenic activity (presence/absence of people) in the University hallway, i.e. when lectures are held in lecture rooms and the intermissions in between [64]. In general when monitoring air quality of indoor environments for the occurrence of both biological and total particles, these short-term temporal dynamics need to be considered. The predominant genera of airborne bacteria in a general hospital were *Staphylococcus*, *Bacillus*, *Micrococcus*, *Corynebacterium* [76, 106-108]. The bacterial counts ranged from 35 to 728 cfu/m^3. The principal factors to affect the level of airborne microorganisms might be not cleanliness of hospital but the activity of people, organic materials derived from the outdoors and ventilation efficiency applied to hospital. Exposure of the immune-compromised people like patients to airborne bacteria and fungi distributed in the air of general hospital can be potentially associated with respiratory diseases although most of airborne microorganisms identified in general hospital do not have pathogenicity [108].

Airborne microbes were detected in museums by various workers and bacterial counts ranged from 545 to 883 cfu/m^3 [109, 110]. The five dominant groups include *Staphylococcus*, *Arthrobacter*, *Bacillus*, *Pseudomonas* and *Micrococcus*. The museum environment is of critical importance for the preventive conversation (for the storage and display of objects) and for the visitors health. Due to a correctly operated and maintained air-conditioning systems and limited number of visitors, good air quality of the studied storerooms and low levels of microbial contamination of museum collections were observed [109]. Considering the bacterial species present in office buildings, predominance of Micrococcus and Staphylococcus genera is seen, which could be due to the human presence [18, 111, 112]. The bacterial counts ranged

from 900 to 3100 cfu/m^3 [11, 113]. In residential appartements the dominant bacterial genus were *Aeromonas, Bacillus, Kocuria, Micrococcus, Nocardia, Pseudomonas and Staphylococcus*. The bacterial counts ranged from 88 to 4750 cfu/m^3 [114, 115]. In schools, predominance of *Bacillus, Corynebacterium, Micrococcus, Staphylococcus* were seen and bacterial count was high as 1696 cfu/m^3 [111, 116-120].

Fungi - Fungal strains which are proportionally of importance in indoor air samples are comprised of the genera *Alternaria*, *Aspergillus*, *Cladosporium*, and *Penicillium* [121-126]. All fungal strains are able to form spores which are resistant to changing environmental conditions. In all the studies surveyed for this review, fungi are always characterized using culture dependent techniques followed by standard identification protocols (Table **1**). For individual fungi, the threshold concentrations for evoking allergic symptoms have been estimated as 100 *Alternaria* spores per cubic meter air [127], while *Aspergillus sp.* spore concentrations above 50 cfu per m^3 have been potentially associated with a higher prevalence of sick building syndrome [128]. Evidence from both epidemiological and experimental studies supports the fact that these are highly allergenic fungi. They are known to cause allergic diseases of the respiratory system such as bronchial asthma and allergic rhinitis [129-131]. High fungal concentration was observed in hospital environment ranging from 3419-7701 cfu/m^3 [77]. In offices the range was much lower from 106 to 1113 cfu/m^3 [18, 75, 111, 132]. Higher mould concentrations (463-3125 cfu/m^3) were observed in dwelling places since these environments may promote mould growth due to high relative humidity and the existence of potential substrates [6, 16, 133]. In schools the fungal count ranged from 70 to 6370 cfu/m^3 [111, 118, 134-139]. Fungal growth in indoor air depends on mainly on moisture and available carbon sources. Therefore, most important strategies for the reduction or the elimination of fungal growth is controlling moisture present and reducing indoor organic contaminants [77]. Mechanized ventilation, forced air-heating systems, dehumidifiers, air filters, and air conditioners reduce indoor fungal count [140]. The design, construction, and maintenance of building envelopes is crucial for the prevention of excess moisture and subsequent fungal growth [141].

MAXIMUM ACCEPTABLE LEVELS OF BACTERIAL AND FUNGAL BIOAEROSOLS

There is no uniform international standard available on levels and acceptable maximum bioaerosol loads, what has been pointed out already earlier [142]. Even terms and wording is different in different countries and include e.g., "threshold limit value, TLV" [143-144], "acceptable airborne bacteria levels" [145], "guideline value" [116, 146, 147, 148], "orientation value" [149], "acceptable maximum value, AMV [118], "maximum acceptable value" [150], "maximum allowable concentration" [151], "contamination indicator" [152].

The American Conference of Governmental Industrial Hygienists (ACGIH) is stating that "a general TLV for culturable or countable bioaerosol concentrations is not scientifically supported" due to the lack of data describing exposure-response relationships [144]. Additionally, ACGIH is stressing that "bioaerosols in occupational settings are generally complex mixtures of many different microbial, animal and plant particles". At the moment, there is no uniform standardized method available for the collection and the analysis of bacterial and fungal biaerosols, which makes the establishment of exposure limits difficult [144, 149]. Attempts have been made to model and predict acceptable airborne bacteria levels in indoor environments such as e.g., air-conditioned spaces including airborne bacterial counts (cfu m^{-3}), temperature and relative humidity [145].

Published values for acceptable bacterial and fungal bioaerosol concentrations vary from country to country and are exemplarily shown in Table **2** [153-158]. Values are related in some cases to specific indoor sampling locations (Chinese guidelines, guidelines of the European Commission). In addition, guideline values tolerated have been established in relation to specified groups of microorganisms such as Gram-negative bacteria or a specific microbial strain such as e.g., *Penicillium canescens* (Russian and Swiss Guidelines). However, neither air sampling techniques nor identification and cultivation methods have been internationally standardized reducing, therefore, the comparability of data.

CONCLUSION

Exposure to bioaerosols has already been associated with a wide range of health effects such as e.g., infectious diseases, acute toxic effects or allergies. Owing to the ubiquitous presence of airborne microbes in nature, they are essentially present in most enclosed environments [159, 160]. With every breath we inhale approximately 0.5 litres of air. By making 15 breaths per minute, 10 m^3 of air are transferred to our lungs during the course of one day. It can be assumed that 1000 cfu (colony forming units, i.e. microbial (bacterial or fungal) cells able to growth on solid nutrient media) are present per m^3. However, this represents only a fraction of approximately 1% of all microorganisms present, a value typically found by a series studies in soil, aquatic sediments, or water [e.g., 162, 163]; and also applicable for air samples. This low percentage is due to our limited abilities to mimic natural nutrient requirements and other environmental parameters needed for cultivation.. In total, therefore, we inhale approximately 10^6 microbial cells per day.

As observed in several studies [159], the high bacterial count within buildings compared to that observed outdoor could be associated with various internal sources, including human activities. A programmed and periodical cleaning operation and maintenance activities of various indoor environment should be organised as a preventive measure. Increasing the ventilation rate by means of mechanical or natural systems can play a role in improving the indoor air quality. High fungal concentrations in various indoor environments and the presence of allergenic genera point out a potential health risk such as respiratory diseases. Combining the indoor microbial load data with data from studies focusing on health effects caused by inhalation of specific airborne microorganisms will allow the evaluation of various risks to which inhabitants are exposed.

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Table 2. "Guideline Values" of Bacterial and Fungal Bioaerosols (Bacteria, Fungi) in Different Selected Countries. [a]for a Mixture of Species; [b]Depending on Location Such as e.g. Hotels, Movie Theatres, Libraries, Museums; [c]NS: not Specified, but "no Visible Damage or Odour Should Occur"; [d]Depending on Specific Fungal Species; [e]for Aerobic Mesophilic Bacteria; [f]for Gram-Negative Bacteria; [g]TLV (Threshold Limit Value) is not Supportable; [h]for Private Homes; [i]for Non-Industrial Indoor Locations

Country, Organization	Number of Culturable Organisms as Colony Forming Units (cfu m^{-3})			References
	Bacteria	Fungi	Total Bioaerosols (Bacteria + Fungi)	
Brazil		750		[150, 161]
Canada		150[a]		[116]
China	2500 to 7000[b]			[115]
Finland	4500			[156]
Germany	10000	10000		[153, 154]
Korea			800	[148]
Portugal		500		[118]
Netherlands	10000		10000	[157]
Norway		NS[c]		[146]
Russia		2000 to 10000[d]		[151]
Switzerland	10000[e] 1000[f]	1000		[149, 158]
USA	---[g]	---[g] 1000	---[g]	[144, 152]
WHO	500			[147, 155]
European Union	10000[h] 2000[i]	10000[h] 2000[i]		[143]

ABBREVIATIONS

ACGIH	=	American conference of governmental industrial hygienists
AMV	=	Acceptable maximum value
ATP	=	Adenosine triphosphate
CFU	=	Colony forming units
DAPI	=	4, 6 diamidino-2-phenylindol
DG-18 Agar	=	Dichloran glycerol-18 agar
ELISA	=	Enzyme linked immunosorbent assay
EMA-qPCR	=	Ethidium monoazide with quantitative polymerase chain reaction
ESP	=	Electrostatic precipitators
FISH	=	Fluorescence *in situ* hybridisation
LIF	=	Laser induced fluorescence
MALIDI-TOF-MS	=	Matrix assisted laser desorption/ionization time of flight mass spectrometry
MAS	=	Microbiological air sampler
MEA	=	Malt extract agar
NA	=	Nutrient agar
PCR	=	Polymerase chain reaction
PM2.5	=	Particulate matter <2.5 μm
PVC	=	Polyvinylchloride
RIA	=	Radio immunoassay
RT-PCR	=	Real time polymerase chain reaction
TLV	=	Threshold limit value
TSA	=	Tryptic soy agar
WGA	=	Wheat germ agglutinin

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