Experiment 21	**Occurrence of culturable bacteria and fungi in indoor and outdoor air**
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Reading	Chapter in BBOM 10th: 25.11
Objectives	Quantitative determination of culturable bacteria and fungi from indoor and outdoor air samples Correlation of numbers of airborne organisms (bacteria and fungi) to non-living particles
Background	The presence of viable microorganisms in the air can be determined quantitatively by a number of methods. However, no single sampling and analysis method is suitable for the collection and analysis of all types of bioaerosols, and no standardized protocols are available. Bioaerosols comprise of a series of microbial agents such as viruses, bacteria, fungi, algae, and protozoa as well as bacterial and fungal toxins. Regarding indoor environments, bacteria and algae grow in areas with standing water (air condition, humidification systems condensation pans). Fungi differ significantly, in certain respects, from most other airborne microorganisms such as bacteria, viruses, and protozoa. Fungi can exist outdoors and enter the building through the air intakes. No other respiratory pathogens can exist outdoors - viruses and bacteria are carried and transmitted indoors by human or animal hosts, with anthrax being perhaps the one exception. Fungi are normally harmless and non-parasitic. Fungal infections inevitably result from fungi being in the wrong place, often as the result of poor cleanliness or improper design of ventilation system components. The selection of an analysis method is a critical component of the sampling plan. Traditionally, airborne microorganisms have been analyzed by culturable and total count (microscopic) determinations. However, limitations to both of theses methods have led to the development of (biochemical and molecular) techniques that can increase the sensitivity and accuracy of bioaerosol monitoring. Many of the currently available bioaerosol sampling methods rely on cultivation. Microorganisms that are collected on a nutrient agar surface by impaction can be cultured directly, while organisms that are collected in a liquid or on a filter have to be transferred to a culture medium. Only those cells which survive and reproduce under the culture conditions to form visible colonies are enumerated. Generally, it is necessary to perform replicate sampling. Several broad-spectrum media have been recommended. We will use nutrient agar (NA) to determine the total number of bacteria and malt extract agar (MEA) for fungi. Viable counts are determined after the appropriate incubation period by enumerating the cfu (colony forming units). Enumeration errors are usually associated with cfu that are very low or very high. Colony counts that are too low can be non-representative of the population. At high colony counts errors are occurring because of overlap of colonies and inhibitory effects of microorganisms on one another. For statistical accuracy, only those plates with counts between 30 and 300 cfu (per 100 mm Petri dish) are enumerated.

Literature	Brandl H. et al. (2005) Generation of bioaerosols during manual mail unpacking and sorting. Journal of Applied Microbiology 99:1099-1107 Lighthart B. (1997) The ecology of bacteria in the alfresco atmosphere. FEMS Microbiology Ecology 23:263-274
www Links	http://www.iha.bepr.ethz.ch/pages/forschung/Publikationen/Erdregister.pdf http://helios.bto.ed.ac.uk/bto/microbes/airborne.htm
Practical work	We will enumerate airborne microorganisms grown on selective growth media
Materials and Experimental Protocols	Incubated Petri dishes (agar growth media) are observed for microbial growth. Colonies are counted either manually or automatically with an computerized imaging system. Eventually, picks from cultures are observed microscopically.
Experiences gained	Knowledge on variability of airborne microorganisms with respect to sampling location and organismic group.
Timing	120 min
Reporting	Note in a table: date, sampling location, sampling volume, cfu (colony forming units) per volume sampled, cfu per m^3 air, average and standard deviation of replicates
Questions to be answered	What is the variation regarding sampling location? What is the dominating organismic group of airborne microorganisms? How are our results related to guidelines for indoor air quality?
Outlook	With the wide variety of bioaerosol sampling and analysis methods, the investigator must have a understanding of objectives of bioaerosol sampling before sampling is conducted. This information will assist in the selection of appropriate sampling and analysis methodology for collecting meaningful bioaerosol data.