

Bioaerosols

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1. Introduction

Bioaerosols are defined as living airborne particles or parts of them smaller than 100 μm , independent of their viability status. They form an important fraction of atmospheric particulate matter in rural as well as in urban areas. Bacteria and fungal spores dominate in central European PM₁₀ while airborne algae, pollen and moss spores are only very minor fractions in this size range. The importance of bioaerosols is not limited to health risks, viruses, bacteria and fungal spores can spread human, animal and plant diseases, fungal spores and pollen for example can and cause allergic reactions in sensitive people. Bioaerosols can also have effects on climate, acting as cloud condensation nuclei and or ice nuclei they can take part in cloud formation (e.g. Bauer et al. 2003, Deguillaume et al. 2008).

In Austria research on bioaerosols is carried out mainly at the University of Innsbruck, the Medical University of Graz and the Vienna University of Technology. In Vienna research focuses on aerosol carbon balance, analytical methods for the determination of bioaerosols and their nucleation properties. In Innsbruck the field of research is microbial communities in the cryosphere, while health related aspects in indoor and outdoor environments and at workplaces are investigated in Graz.

2. Analytical methods to determine airborne bacteria and fungal spores

Culture methods

For a long time airborne bacteria and fungi have been determined by culture methods after collection on filters, in sampling liquids or directly on different culture media. These methods allow the determination of viable and culturable microorganisms and their identification. Culture methods are widely used for hygienic investigations in exposure studies to airborne microorganisms in workplaces, hospitals, mouldy indoor environments and emission sources such as composting facilities, slaughter houses and wastewater treatment plants (Bauer et al. 2002, Haas et al. 1999, 2002, 2007, 2010a, 2010b, Kock et al. 1998, Posch et al. 2006, Reinthaler et al. 1999, Wuest et al. 1999).

The culturable part of airborne bacteria and fungi (concentrations expressed as colony-forming units CFU m^{-3}), however, represents only a limited part of

microorganisms suspended in the atmosphere. In case of bacteria the culturable fraction is less than 1% of the total count, in case of fungal spores the culturable part accounts for 40% or less of the airborne fungal spectrum (Lappalainen et al. 1996, Lee et al. 2006).

Microscopic enumeration

All bioaerosols – pathogenic or not, allergic or not, dead or alive – are contributors to PM₁₀ and to the aerosol organic carbon (OC), and were assessed in terms of their mass contribution to the PM aerosol in recent studies. Quantitative assessment of bioaerosols can be achieved by different approaches, one of them is counting the individual microorganisms under the microscope after staining with fluorescent dyes (Sattler et al. 2001, Bauer et al. 2002a, Bauer et al. 2008). To assess the biomass-carbon the number concentration of bacteria and fungal spores is multiplied with an average carbon content of 17 fg (Sattler et al. 2001) and 13 pg (Bauer et al. 2002b), respectively. The contribution of fungal spores to PM₁₀ can be derived by multiplying the number count with 33 pg to account for the spore mass (Bauer et al. 2002). The application of microscopic enumeration requires collection of aerosols with liquid impingement and does not allow distinguishing between viable and dead organisms, nor the identification of species.

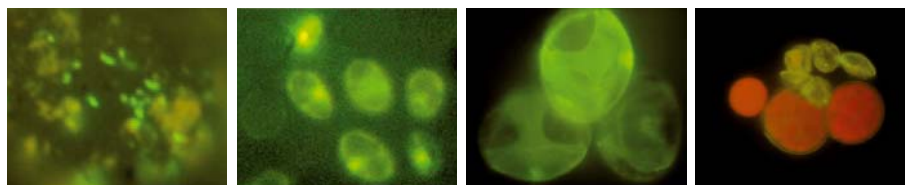


Figure 1: Bacteria, fungal spores, moss spores and pollen dyed with SYBR Gold® in the epifluorescence microscope (Bauer, 2008).

Analysis of chemical tracers

A second approach of quantifying total counts of bioaerosols is the determination of the concentration of chemical tracers. A specific tracer for fungal spores is the sugar alcohol arabinol which is a storage substance in fungi and has not been reported to be present in plants or other organisms in central Europe. The arabinol content varies in different species, but an average content per fungal spore, representative for the atmospheric fungal mixture was determined to be 1.2 pg spore⁻¹ (Bauer et al. 2008a). The arabinol tracer technique allows quantifying the concentration of fungal spores together with other organic and inorganic aerosol constituents using loaded filters.

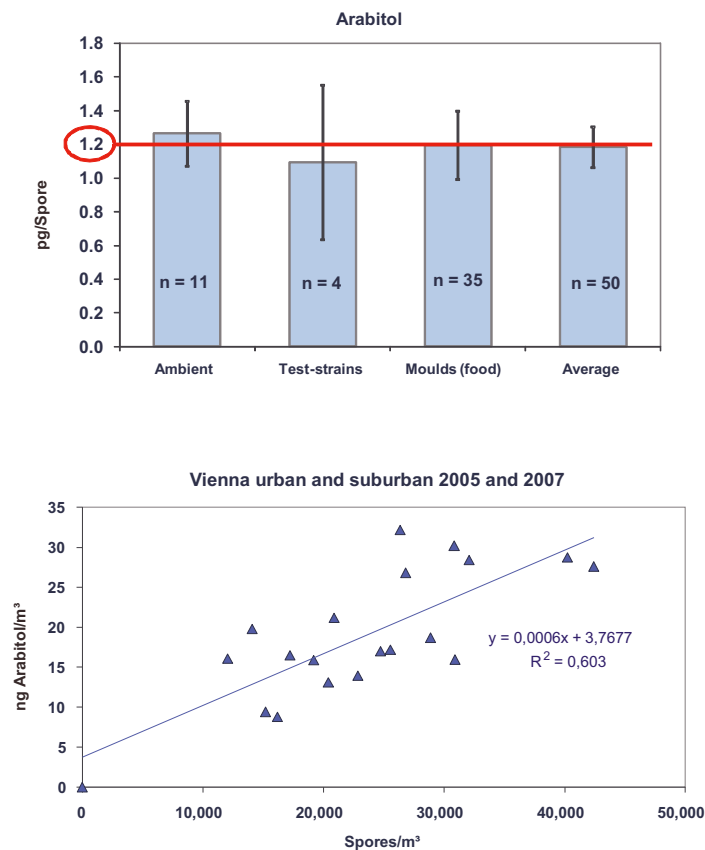


Figure 2: Arabitol – a suitable tracer for airborne fungal spores. Average arabinose content of fungal spores in source (upper graph) and ambient samples (lower graph) (from Bauer 2008).

In-situ analysis

A laser induced fluorescence excitation (L.I.F.E.) technique has been developed being capable to identify the presence of microbiological material in-situ in snow and ice environments (Sattler and Storrie-Lombardi 2010). The technique has been tested in the Antarctica and will be part of the ESA Mars mission scheduled for 2015.

3. Ambient occurrence of bioaerosols in Austria

Occurrence and seasonality of bioaerosols in Austria and their contribution to PM10 and OC10

Concentrations of airborne bacteria and fungal spores (total counts) were determined in extensive field studies in three Austrian cities, at suburban and rural sites and at a mountain site which was representative for the lower tropospheric background (Mt. Rax 1664 m.a.s.l.). Concentrations of airborne bacteria (total counts) determined at Mt. Rax (Bauer et al. 2002a) and in Vienna, were around 25% higher than those of fungal spores. The concentrations of airborne fungal spores showed high seasonal variations with around 35,000–40,000 spores m^{-3} in July at rural and urban sites, respectively. As a monthly average fungal spores OC contributed 15 and 10% to OC and fungal spores mass 7 and 8% to PM10 at urban and rural areas. At most sites a second peak could be observed in October. In winter the concentrations of airborne fungal spores amounted to around 4000 spores m^{-3} at rural and urban sites contributing around 1% to OC and 0.3% to PM10 (Figure 3).

Total emissions of fungal spores in Europe are estimated with 18 kg km^{-2} yr^{-1} , this means emissions of fungal spores in the magnitude of around 1400 tons yr^{-1} in Austria (Winiwarter et al. 2009). A first determination of airborne fungal species at an urban and a suburban site in Vienna indicates that around 20% of the detected spores belong to fungal species known to elicit hypersensitivity (Schüller 2008).

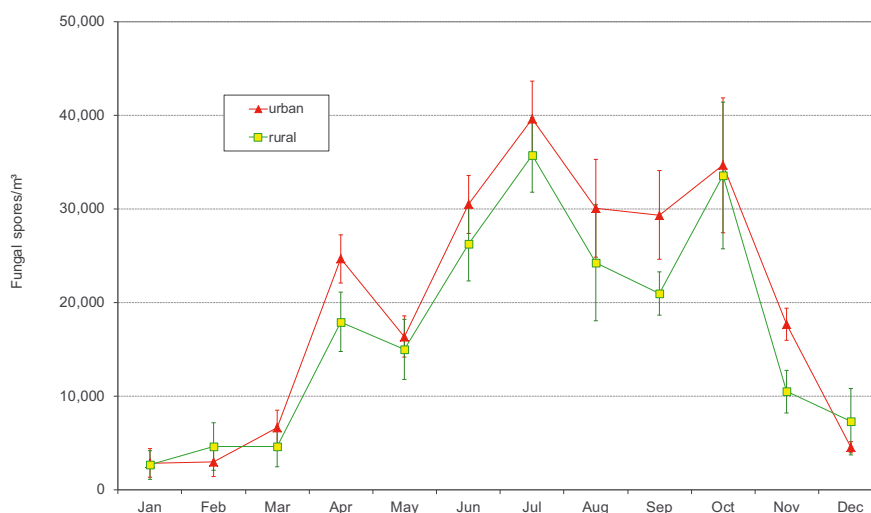


Figure 3: Seasonal variation of the concentration of fungal spores in PM10 in three Austrian cities in 2004 (Bauer et al. paper in preparation).

Exposure studies

Exposure studies were carried out to estimate the potential health risk. Measurements of culturable airborne bacteria and fungal spores were performed especially in waste treatment facilities such as waste water treatment plants (Bauer et al. 2002c, Haas et al. 2002, 2010a), composting facilities (Haas et al. 1999, Reinthaler et al. 1999). Culturable airborne microorganisms were assessed as well in metropolitan areas (Kock et al. 1998), in households (Haas et al. 2007, Gonçalves et al. 2010), wine cellars (Haas et al. 2010b), agriculture (Wust et al. 1999) and in food production (Posch et al. 2006).

Microbial cloud formation

For the Earth's weather and climate system, clouds are of major importance. On the one hand they cool by reflecting parts of the solar radiation and on the other they heat by absorbing solar radiation and by trapping the outgoing infrared radiation. In the discussions about climate change, however, clouds appear as one of the largest non-anthropogenic uncertainties of the earth's radiation balance (IPCC 2007). Not only inorganic atmospheric particles but also some bioaerosols are involved in cloud formation. Bacteria, dead or alive were found to be good cloud condensation nuclei (Bauer et al. 2003). Specific bacterial species and possibly larger bioaerosols are able to act as ice nuclei, lowering the freezing temperature of atmospheric water vapor. Investigations of potential ice nucleating pollen and fungal spores and the responsible microstructures are currently carried out at Vienna University of Technology.

4. Bioaerosols in the cryosphere

Research groups at the Institute of Zoology and Limnology, University of Innsbruck have a long tradition in investigations of microbial communities in the cryosphere. Starting with studies of microbiological life forms in ice and snow of high mountain lakes (Felipe et al. 1995) the group investigated the interactions of the eolian input of microbes and nutrients into the snow and ice forms of High Alpine lakes and glaciers (Psenner 1999, Battarbee et al. 2005) as well as of the effluents (Battin et al. 2001). Tieber et al. (2009) studied the input of radionuclides into cold ecosystems. Thiess et al. (2007) reported about an "Unexpected Response of High Alpine Lake Waters to Climate Warming".

The University of Innsbruck was also involved in a survey of the distribution of several trace elements in contemporary and pre-industrial sediments in 275 lakes in alpine and arctic lake districts across Europe including the Pyrenees, Alps, the Rila Mountains, Retezat, Julian Alps, Tatras, Scottish mountains, Central Norway and Greenland (Camarero et al. 2009). They conclude: "In general terms, the increase in trace elements in modern with respect to

pre-industrial sediments reflects the history of a long range contamination affecting the remotest locations in Europe." A methodological survey of the existing techniques for detection, quantification, physical and chemical analysis of biological particles is presented by Georgakopoulos et al. (2009).

In a commentary article in Science about a paper of Priscu et al. (1998) "Perennial antarctic lake ice: an oasis for life in a polar desert", Psenner and Sattler (1998) compare the newly observed occurrence of microbial communities in "water pockets" in "slush ice" in some meters depth during austral summer with microbial life in snow layers of high alpine lakes. They state:

"A comparison of freshwater ice systems (...) suggests that the example described by Priscu et al. represents the most extreme community one can imagine, both regarding physico-chemical factors and organisms involved; namely, an assemblage of autotrophic cyanobacteria (blue-green algae) and heterotrophic bacteria, encapsulated in the permanent ice cover of lakes. The term "oasis" used by the authors" (Priscu et al. 1998) "refers to the fact that pockets of liquid water form around dust particles and microorganisms blown in from a cold desert, allowing active growth and reproduction of predominantly prokaryotic organisms during the Austral summer."

In that instance microorganisms are not only transported as aerosols, but they also obtain nutrients from aerosol particles and, being enclosed in ice, the particles' ability of sunlight absorption provides opportunity for liquid water formation in Antarctic summer sunlight, so much needed for any life processes. Psenner and Sattler (1998) further state: "The vision of a cold origin of life may lay the foundation for a model of past conditions on Mars and of present-day conditions on Europa".

In a pioneering paper Sattler et al. (2001) showed that bacteria isolated from supercooled droplets at 3 km elevation duplicate at a similar rate as bacteria in temperate freshwater lakes. They concluded that clouds do not act as conveyors of microbes, but support actively reproduction of microbial life due to the presence of nutrients, acting as a habitat and seeders of life (Sattler et al. 2002a, b).

The studies were extended to the colder climate of Antarctica, where "it has become evident that much of the microbial life within remote environments is transported by air currents" (Pearce et al. 2009). They summarize that many microbial genera commonly encountered in air samples were also found in the Antarctic by various groups, bacterial genera found in both environments included for example *Staphylococcus*, *Bacillus* and *Corynebacterium*, among

fungal genera *Penicillium*, *Aspergillus* and *Cladosporium* were observed. There is also evidence of microbes living in Antarctic "slush" habitats. Similarly to the Alpine situation microbial life forms were studied in Antarctic cryoconites (Foreman et al. 2007) and glacial discharges (Mikucki et al. 2004, Mindl et al. 2007).

The observation of proteobacterial strains in very cold as well as in very hot environments led to the hypothesis, that primitive life forms may have developed in extraterrestrial cold environments e.g. at Mars or at Titan. For a mission of probing the surface crust for the existence of prebiotic or microbiological tracers a suitable in-situ method had to be developed. Such a method, based on the exemplary work of Pinnick et al. (1995) of a fluorescence particle counter for detecting airborne bacteria and other biological particles aiming at detecting biological warfare species is currently tested for identifying microbial or prebiotic material on a Mars mission (Storrie-Lombardi et al. 2009a). The ExoMars mission is scheduled for 2016 with a primary goal of surveying the Martian subsurface for evidence of polycyclic aromatic hydrocarbons and other organic material. A test of the laser induced fluorescence excitation technique (L.I.F.E.) has been performed for in situ nondestructive detection of microbial life in the ice covers of Antarctic lakes (Storrie-Lombardi et al. 2009b). The authors conclude: "L.I.F.E. survey strategies described may be of interest for orbital monitoring of photosynthetic primary productivity in polar and alpine glaciers, ice sheets, snow, and lake ice of Earth's cryosphere. The findings open up the possibility of searching from either a rover or from orbit for signs of life in the polar regions of Mars and the frozen regions of exoplanets in neighboring star systems." A review on this issue is given by Sattler and Storrie-Lombardi (2010). An intriguing problem of the search for prebiotic or microbiological features on other planets is the prevention of biological contamination from the explorers. Microbes or related material may adhere on the astronauts' coveralls. Groemer et al. (2011) report about methods for reducing potential contamination of the astronauts' suits from microbes using the L.I.F.E. technique.

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