

## **Microbiology of Aerosols**

# Microbiology of Aerosols

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**WILEY** Blackwell

This edition first published 2018

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*Library of Congress Cataloging-in-Publication Data*

Names: Delort, Anne-Marie, editor. | Amato, Pierre, editor.

Title: Microbiology of aerosols / edited by Anne-Marie Delort, Pierre Amato.

Description: Hoboken, NJ : John Wiley & Sons, 2017. | Includes bibliographical references and index. |

Identifiers: LCCN 2017023659 (print) | LCCN 2017035569 (ebook) | ISBN 9781119132295 (pdf) | ISBN 9781119132301 (epub) | ISBN 9781119132288 (cloth)

Subjects: LCSH: Air—Microbiology. | Atmospheric aerosols.

Classification: LCC QR101 (ebook) | LCC QR101 .M52 2017 (print) | DDC 579/.175—dc23

LC record available at <https://lcn.loc.gov/2017023659>

Cover Design: Wiley

Cover Image: Courtesy of Pierre Amato

Set in 10/12 pt, WarnockPro by SPi Global, Chennai, India

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## Preface

Despite its proximity, the air we breathe is an environment where the biology and its spatial and temporal variability remains poorly known and understood. Many airborne biological particles, bioaerosols, are transported in the atmosphere: animal and plant fragments, pollens, fungal spores, bacteria, viruses, proteins, etc. They are present among all particle size ranges in the atmosphere, from submicron to hundreds of micrometers, at concentrations of up to billions per cubic meter of air. Among the large variety of bioaerosols, microorganisms in particular are raising interest from the scientific community. Indoor and at short spatial scale, these are mostly regarded as potential allergens and pathogens to humans. Outdoors, they can be transported over long distances up to high altitudes, and their presence is also related to epidemiology and biogeography. In addition, airborne biological particles and microorganisms probably contribute to atmospheric physical and chemical processes, such as cloud formation, precipitation, and the processing of chemical compounds. The microbial cells surviving their travel in the atmosphere are also new incomers to natural and agricultural surface ecosystems, which they will eventually colonize or where they will compete with established communities.

In this volume, current scientific knowledge about the different aspects of the research on microbial aerosols is synthesized; this consists of four parts:

- the classical and latest developments of bioaerosol sampling and characterization methods
- the emission of bioaerosols and their dispersion on short to large scales
- the impacts of bioaerosols on microphysics and chemistry in the high atmosphere and in clouds
- the consequences of bioaerosols for human health and the environment.

We warmly acknowledge all the authors, an interdisciplinary and international panel of experts on the multiple facets of this fascinating topic, for their precious contribution and their effort in constructing this book collectively. The result is a high-level and up-to-date piece of work, which, we hope, will serve as a reference book for researchers and Master's degree to PhD students entering the emerging field of aerobiology. Finally, we are happy to thank two French artists for their humanistic bird's-eye-view contributions introducing this book.

*Anne-Marie Delort and  
Pierre Amato, editors*

## Hunting fog

At an early age, I started watching the rockets taking off from my grandfather's launch site. He didn't work for NASA though. At the age of 60, he had decided to trade his illegal hunting rifle for rockets.

The Pyrenean chamois that he had been chasing is not an easy animal to catch. One has to follow its track along craggy mountain paths. It is frail and light-footed, but also fearful and elusive. It always travels in groups. You can't ever let it see you—or smell you. You should know the territory better than your prey: the prevailing winds, and also the breezes, as well as their trajectories through the rocks.

I used to believe that the one they called the *dahu*<sup>1</sup> was this trophy, this chamois that one absent-minded day ended up getting shot by my grandfather, before being skinned and stretched out on a wooden board to be exposed in the living room.

When he could no longer attend to the fate of dahus, when he could no longer set off on exploration and measure himself against faster animals, my grandfather decided to stay with us.

On *his* territory.

He tended to our stomachs in a wiser, slower manner, taking care of the harvest that needed so much attention. Fortunately, he had acquired during the war a slower, tenacious type of endurance, carved out over months and maybe years, and he was now able to answer vigilantly to all of the soil's demands.

The love he felt for his land was quickly overpowered by the distrust and hatred toward everything that could harm it: caterpillars, lice, mildew, mushrooms . . .

But fighting all these diseases and vermin from the land was nothing compared with the swift and cataclysmic power of the weather. One summer in 1959, after a destructive rain of hail, my grandfather fell seriously ill.

He then joined another war, an insidious one, harder to comprehend. A war against anything that could come from the sky.

I don't mean a war of religion. I mean a war with no prey or ally. A war against the wind, the rain, and the importunate storms. A war for himself, which he justified by claiming it for mankind.

He would shoot rockets toward the sky as others throw bottles into the sea, because he wanted to be heard.

He attacked visible yet unpredictable targets, all potentially guilty by intent.

Alone, one knee to the ground, he would aim precisely for the heart of these masses, clouds, huge pachyderms and would set their evanescent and ephemeral souls free.

It took me a while to understand the enormous explosion that I could hear a few minutes after the launch. Even though I knew that it was all a game, I kept looking up, hoping to finally see the fireworks. But the missile would disappear in a loud and powerful discharge.

One day when I was watching him fire a rocket, the base moved during the launch, propelling it horizontally.

It spun, hit against the front of the building, bounced against a tree, and dug into the leaves before exploding over the lake a few yards away.

It was an astonishing vision when, running toward the lake, I saw the surface covered with silvery particles. The water seemed rough, thicker; as so many bubbles on a boiling pot of soup, fishes were coming up, one by one, revealing their bellies about to burst.

The lake was shiny, frozen, and lifeless. Immaculate.

My grandfather never recovered.

After that afternoon, we built him a chimney, small but firmly fixed, so he could keep on shooting at clouds.

Sitting on his chair near the pipe, fingers on the trigger, he is on the lookout: for the sky, the wind, and the humidity.

Only he knows when to shoot to avoid the hail or dry the storms.

Now he has gone, and the Météo-France<sup>2</sup> has taken over; they call to tell us when to light up the fireplace or kill the fire. But Météo-France is far less precise than my grandfather.

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---

1 The dahu is a legendary mountain creature.

2 Météo-France is the French national meteorological service.

## It all happens up there ...

It all happens up there, inside the large floating bellies of the clouds. Looking at them, what we believe we see is their ample motion. We follow their peaceful progression. Or are they disturbing? They scud along. We imagine them to be full of a remarkable accumulation of an uncertain substance. Sometimes we think they must be heavy, even though they may be inflated only with light water vapor, whose fine and dispersed moisture weighs little, and sometimes we sense that they are much weightier, laden with water that has already formed into droplets ready to fall back down on our heads, maturing into precipitations in the form of rain, snow, hailstones, maturing into our fogs and our monsoons.

We watch the passing clouds. Ascending and sliding sideways, they seem to come to life at the point where those two intersecting motions meet. We might notice also some changes in shape as they roll, twist, contract, or stretch out. Even when combined with these other changes, the sideways slipping motion still continues, and it is this vast translatory movement in the sky that we witness most frequently: with our nose in the air, we see them traveling along up there, dressed in their suit of light, bathed in more or less pronounced shades of grey, or sparkling tons of white. That is the sight that we see, and the motion that we follow, because the other movement, that is, the ascending motion, which is how they gradually come into being, is invisible to our eyes. At the start of the journey, there was a light moisture, a vapor, a breath, which managed to rise up, to detach itself from the ground, or to take flight from the foam of a wave. It ascends, the molecules bond together, and the huge floating mass comes into being. It is the source of our dreams, and the focus of our enchanted contemplation. It questions us too: who are they? But really, who are they?

At the end of the journey, up above, the moment finally arrives when the shape that we had been following with our eyes begins to dissipate. Whether by thinning out, by dislocation, or by dissolving, its gargantuan architecture is steadily dismantled, and its bulk, once so heavy, simply melts away. Like invisible balloons falling back down to Earth, the clouds then come to rest on the page where our children are drawing pictures, and are reborn beneath their chubby fingers, which press hard on paper as they delineate these puffy giants' cheeks.

We are able to capture virtually nothing of the clouds. They are but transition and transformation. We can never grasp the definition of their being. Terms belatedly applied to their shapes enable us to improve our way of looking at them. Yet we must also accept that, each time, these names refer to a kind of transient that enjoys only a brief existence—that of the ephemeral lifespan of a form also characterized by its color, however fleeting, and an altitude at which it lingers, however briefly ...

We have always found it difficult to follow clouds, and to *conceptualize* them. The airplane introduced us to their vertical dimension, whose existence we had not suspected from below, and to the gigantic stature developed by their upward billowing. In this way, we gained a more accurate picture of their vast bulk. We also came to appreciate that they evolve between two or three different states of being, quivering internally with droplets of water that hesitate here and there, either to continue life in liquid form or, on the contrary, to pack tightly together into small prism or needle-shaped crystals, unless the two states briefly coexist.

And so today, we understand that an invisible other is in action in the clouds—that we must now enter into their own being, deep into the grasping of a flux that we can see even less well from below, the flux which drives their inner life, this great *traffic of within*, these sweeping motions in which minute particles of matter bathe, combine, and recombine, where fragments of our deserts, our meadows, and our oceans can be found, and, who knows, where one day we may be able to identify a few specks of dust from our own skins, some atoms of our own breath, anything that the wind is strong enough to carry aloft, anything which, however tiny it may be down here, then coalesces in the sky into formations of gigantic proportions.

Perched atop conveniently located hills, we probe these bellies, and take samples of this medium in which ceaseless mutations are unfolding. Is it movement, is it both direction and speed of travel that control the current state of being of the clouds, for individuals have left one aggregate behind to go and establish themselves inside another?

Is it the change of components that builds up a new kingdom, as compounds break down? Is it the predominance of specific elements that prevails and reshapes the properties of a given chunk of a cloud? We would like to grasp them more clearly, we need something to match the wanderings of our minds, a chronology of these transformations perhaps, or, for one phase of the process, an indication of the forces that prevail, even temporarily, because chemical constituents obey their own laws, but, now, molecules will be affected if exposed to stronger light, and will also react if subjected to a fading away of radiation. And then the wind gets involved, smashes up the existing masses, divides clusters of dust particles, and binds others together. It would be useful to have some large machinery, some sort of giant MRI scans, that could provide us with sections of their inner state of being. For the moment, the large machinery we have at our disposal is that of measurements. With those curves, diagrams, average values, and sharpened analysis, we are gradually building up our interpretations ...

In view of the specific nature of this constantly changing object, the study of clouds, perhaps more than any other branch of research, requires us to engage in a peculiar mental exercise, and to undergo the experience of an elusiveness perpetually renewed. Yet this feeling of the *transient*, which troubles our minds, once we accept it, then becomes the spring for our lively questioning; it too is continuously restored. In the quest that drives us, we are conducting an exhilarating experiment.

Today, to be sure, Caspar David Friedrich, with one foot firmly planted on a rock, stands observing the flying clouds, admiring the infinite combinations of these celestial constructions, then, his lungs replete with ethereal air, he strides back down to his laboratory.

*Sara Chantal Saragoni,  
poet,  
Paris, France*

## Cela se passe là-haut ...

Cela se passe là-haut, dans les grands ventres flottants des nuages. Nous croyons voir d'eux d'amples déplacements, nous suivons leurs circulations tranquilles. Ou bien inquiétantes. Ils passent. Nous nous les figurons tout pleins d'une accumulation phénoménale d'une substance à la vérité incertaine. Tantôt nous les devinons lourds certes, pourtant seulement gonflés de vapeur encore légère, par le peu de poids d'une humidité dispersée, tantôt nous les pressentons bien plus pesants, chargés d'une eau déjà formée en gouttelettes prêtes à redescendre vers nous en devenant nos précipitations de pluie, de neige ou de grêlons, nos brouillards et nos moussons.

Nous observons leurs passages. Ascension, et translation, ils semblent prendre vie au croisement de ces deux mouvements. À quoi peut bien s'ajouter quelque figure d'enroulement, de torsion, de contracture ou d'étirement. Même combiné à ces autres figures, le glissement latéral se poursuit toujours, et c'est à ce mouvement majeur de translation dans le ciel que nous assistons le plus souvent : le nez en l'air, nous les voyons là-haut voyager en habit de lumière, dans ces gris plus ou moins prononcés, et ces blanches étincelantes. C'est cela que nous voyons, ce déplacement que nous suivons, car l'autre mouvement, le premier, celui de l'ascension par lequel ils se sont progressivement constitués, nous ne le voyons pas. Au tout début du voyage, il y eut une humidité légère, c'était une vapeur, une haleine, elle a pu monter, s'extraire de la terre ou bien prendre son envol depuis l'écume d'une vague. Elle s'élève, les molécules se rallient, et le grand être flottant se constitue, source de nos rêves, lieu de nos contemplations enchantées. De nos interrogations aussi : qui sont-ils, mais qui sont-ils donc ?

Au bout du voyage, là-haut, arrive pourtant le moment où la forme que l'on suivait des yeux s'efface. Par amenuisement, par dislocation, par dissolution, la formidable architecture se défait, son poids, un temps si lourd, s'évanouit. Invisibles ballons revenant au sol, les nuages viennent alors se poser sur la page où dessinent nos enfants, ils renaissent sous les doigts potelés qui appuient très fort sur la feuille pour cercler d'un trait les joues des géants.

Tout d'eux ou presque nous échappe. Ils ne sont que transition, transformation. Nous ne parvenons jamais à fixer la définition de leur être. Les dénominations si tardives de leurs formes nous aident un peu à progresser dans le regard que nous leur portons. Mais il faut accepter que ces noms désignent à chaque fois une sorte de « transitoire » qui ne se maintient qu'un temps, celui de la durée éphémère d'une forme que caractérisent également une couleur, mais passagère, une altitude de séjour, mais provisoire ... Nous avons des difficultés à les suivre, à les « penser ». L'avion nous avait découvert une dimension verticale que nous ne soupçonnions pas d'en bas, un gigantisme développé

dans la hauteur. Par là nous avons accédé à une connaissance un peu plus juste de leurs grands corps. Nous avons compris aussi qu'ils naviguent entre deux ou trois états de vie, tout tremblant intérieurement de gouttelettes d'eau qui hésitent ici et là sur le point de poursuivre leur vie sous forme liquide, ou au contraire de se resserrer en petits cristaux de prismes ou d'aiguilles, à moins que les deux états brièvement ne coexistent.

Et voilà qu'aujourd'hui nous comprenons qu'un autre invisible est en action dans les nuages, qu'il nous faut désormais entrer dans leur être propre, dans un mouvement que d'en bas nous voyons moins encore, et qui est celui qui anime leur vie intérieure, ce grand trafic du dedans, ces amples remuements où baignent et se combinent et se recombinent les infimes matières, où se rencontrent des parcelles de nos déserts de nos prairies ou de nos océans, et qui sait, où nous identifierons peut-être un jour quelques poussières de nos propres peaux, quelques atomes de nos souffles, tout ce que le vent a la force de hisser, tout ce qui, minuscule ici, s'accumule là-haut en formations alors gigantesques.

Perchés sur les dômes de reliefs bien situés, nous auscultons les ventres, nous prélevons des échantillons de ce milieu où se jouent d'inlassables mutations. Est-ce le mouvement, sont-ce les orientations et la vitesse des déplacements qui commandent l'état présent, des individus ayant quitté tel agrégat pour aller s'établir en un autre ?

Est-ce la modification des constituants qui, par dégradation des composés, édifie un nouveau règne ? Est-ce la présence en nombre d'éléments spécifiques qui l'emporte pour remanier les propriétés de telle portion de nuage ?

On voudrait y voir mieux, il nous faudrait on ne sait quoi qui réponde au balancement de nos esprits, une chronologie de ces transformations peut-être, ou encore, pour une phase, l'indication des forces qui prévalent, même temporairement, car les constituants chimiques vivent selon leur loi, mais voilà les molécules aux prises avec une lumière accrue qui agit, ou au contraire soumises à la diminution, tout aussi efficiente, de ce rayonnement, et puis le vent s'en mêle, bousculant les agglomérations existantes, dégradant tels amas de poussières, en recombinaison d'autres, On voudrait de grandes machines, des IRM géants nous donnant les coupes de leur état de vie.

Pour le moment, la grande machine dont nous disposons est celle des chiffres, avec leurs courbes, leurs moyennes, l'analyse qui s'affine, la construction progressive des interprétations...

Par la spécificité de son objet mouvant, l'étude des nuages, plus que d'autres recherches peut-être, commande que nous nous tenions dans un exercice singulier, que nous supportions l'expérience d'un éphémère perpétuellement reconduit. Mais ce sentiment du passager qui bouscule nos esprits, une fois admis, devient le formidable ressort d'une vitalité de questionnement elle aussi continuellement restaurée. Dans la quête qui nous anime, nous faisons cette expérience exaltante.

Aujourd'hui à n'en pas douter, Caspar David Friedrich, un pied sur le roc, regarde un temps les nuages, admire la combinatoire infinie des constructions du ciel, puis, les poumons gonflés d'air, il redescend à grandes enjambées vers son laboratoire.

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## Part I

### Bioaerosols, Sampling, and Characterization

## 1.1

### Main Biological Aerosols, Specificities, Abundance, and Diversity

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#### 1.1.1 Introduction

Biological aerosols, or bioaerosols, are ubiquitous in the Earth's atmosphere. By definition, the term "aerosol" refers to liquid or solid (or both) particles passively suspended in a gaseous medium. Bioaerosols are often defined broadly as material derived from biological systems, implying that they are composed of organic material (mainly C, H, O, and N), generally as a mixture of proteins, lipids, and sugars. Here, we will further restrict the discussion to primary biological aerosols (PBAs), i.e., biological material directly emitted to the atmosphere as particles from the surface (1, 2). This definition thus excludes so-called secondary particles, which form in the atmosphere through gas-to-particle conversion (e.g., condensation of semi-volatile organic compounds oxidized in the atmosphere). It is estimated that PBAs typically represent 5–50% of the total number of atmospheric particles >0.2  $\mu\text{m}$  in diameter (1, 3) and can constitute an even higher fraction of particulate mass in many environments. The diversity of bioaerosols reflects that of life. Thus, PBAs include a wide variety of objects with differing origins, shapes, and sizes, from a few nanometers to hundreds of microns: plant and animal debris, pollen grains, fragments of biofilm, spores and cells of bacteria and fungi, and viruses, as well as their fragments and excretions.

Whereas the transmission of human pathogens between individuals through breathing, coughing, and sneezing has long been known, recent findings have shown that humans release their own microbial cloud as they harbor diverse microbes in and on their bodies. Approximately  $10^6$  human-associated microbes are emitted into the surrounding air every hour by each individual, which can particularly influence air quality

in indoor environments (4–6). Outdoor, human activities such as composting facilities and wastewater treatment plants can generate locally high amounts of potentially hazardous biological aerosols (7–10), while plant canopies have been identified as the strongest natural source of PBAs, far exceeding oceans and seas (11–13).

In order to specifically detect, quantify, and eventually recover and characterize bioaerosols within a mixed population of airborne particles, it is important to have knowledge of some of their properties. Bioaerosols share certain features that allow them to be detected and categorized as biological particles, and they also have specificities that allow differentiation. For epidemiological and ecological reasons, the main, and most studied categories of bioaerosols are pollen, fungi, bacteria (and until recently archaea), and viruses. These are briefly described below; relevant references are provided therein for further details.

### 1.1.2 Pollen

Pollen (from the Greek *πάλη* (pale): flour or dust) is the male fertilizing element of the flowers of higher plants. It consists of ovoid-shaped grains with a diameter of a few tens of microns, contained in the anther at the end of the flower stamen. The counterpart of the pollen grain in lower plants (algae, mosses, ferns, prothalli) is the male gametophyte. Hence, pollen is not directly related to microbiology, i.e., the study of microscopic organisms, and is outside the scope of this book. Nevertheless, pollen grains are important biological components of the atmosphere and cannot be totally ignored, so they are briefly introduced here. The references provided can be consulted for more detail.

Pollen species that utilize wind for dispersion are referred to as anemophilous: these include all gymnosperm (fir, pine, etc.) and some angiosperm trees (notably oak, beech, birch, hazelnut, chestnut, willow, and poplar), and grasses. For anemophilous species, pollen grains must fall on a female gamete “by chance” to initiate fertilization and so plants must produce and release enormous quantities of pollen, which can be very abundant in the air during the flowering season.

The size of pollen grains is, on average, around 30–40  $\mu\text{m}$ , but this can vary widely by plant species. For example, pollen from *Myosotis* spp. can be as small as 7  $\mu\text{m}$ , whereas Cucurbitaceae pollen grains can reach diameters of more than 100  $\mu\text{m}$  (14). Many allergens are present in pollen grains of numerous plant species, such as birch, hornbeam, hazel, ash, olive, poplar, cypress, sycamore, alder, grasses, ragweed, plantain, and wall pelitory. Pollen grains are composed of one or several cells enclosed within two concentric layers. The outer wall, exposed to the environment (exine), is a very complex arrangement of sporopollenin, a biopolymer consisting primarily of short-chain dicarboxylic acids, fatty acids, and alkanes (15) that is extremely resistant to mechanical, physical, and chemical assault (15–17). This provides extreme longevity to pollen grains, which can maintain their integrity for thousands of years and be used for geological dating and paleontological investigations of past climates and ecosystems (18–21). The inner layer of the pollen wall (intine) is made of cellulose.

Despite the fact that pollen grains are more or less spherical, their aerodynamic diameter (i.e., the diameter equivalent of a spherical particle with unit density) is typically lower than their geometric diameter. Indeed, some species such as *Pinus* spp. have developed systems for helping flotation in the air, such as air bladders or aerostats, and

their surface is often sculpted with patterns of ridges and pores specific to the species. Hence, pollen size and shape and exine structure (stratification, surface sculptures and granules, number and arrangement of apertures, etc.) are used for identifying species by microscopic observation (22, 23). Their properties of fluorescence and light diffraction can also be used to specifically detect pollen in aerosols and to identify certain species (16, 24–30).

### 1.1.3 Fungi

Fungi can be unicellular or multicellular eukaryotic microorganisms, i.e., they have a complex intracellular organization with a well-delimited nucleus containing their genetic material and several types of organelles. They probably appeared 1.5 billion years ago. Around  $10^5$  species have been described but data acquired from several molecular methods have predicted that as many as 5.1 million fungal species may exist (31).

Fungi are ubiquitous organisms in the environment: in plants, soil, animals, water, indoors, etc. The majority of them are saprophytes living on dead organisms such as decaying plants or animals and on non-living organic substances such as food, paper, and fabrics. Many species are symbionts (endo- and ectomycorrhizae), meaning that they are important primary actors in the cycling of carbon, nitrogen, and other nutrients in the biosphere, and they are thus greatly involved in composting. Other fungi are important pathogens or parasites that obtain nutrients from their living host, such as species of *Ustilago* and *Urocystis* (smut), *Puccinia* (rust) and Erysiphales (mildews) on plants, and *Candida* and *Cryptococcus* species, among many others, on humans and animals. Thus the presence of fungi in the air has many epidemiological, agricultural, and ecological consequences, as well as meteorological impacts (see Sections 3 and 4).

Most vegetative forms of fungi are filamentous (hyphae aggregated, forming the mycelium). Their normal development comprises a vegetative phase of growth and nutrition, and, almost simultaneously, a reproductive phase in which spores are formed. Spore release can be part of the sexual or asexual stage of the life cycle. These spores are designed to be dispersed, often through the air, so they have developed resistance to desiccation and other environmental and atmospheric stresses. Their shape and size (typically between 2 and 56  $\mu\text{m}$  in diameter) vary between species. Under favorable water and nutrient conditions, spores deposited on surfaces in indoor environments will germinate, mycelia will develop, and the substrate will be colonized (32–34). This growth usually ends with a massive production of spores, released into the air through intrinsic, or natural, mechanisms and through external events such as human or animal activities (35). As outlined in Elbert et al. (36), certain types of fungal spores are preferably emitted under humid conditions such as actively wet discharged asco- and basidi-spores, which are emitted with the help of osmotic pressure or surface tension effects. Active discharge mechanisms also eject various organic molecules and inorganic ions that can be used as tracers of fungal spores in the atmosphere (37, 38). In contrast, dry discharged spores are preferably emitted under dry conditions and the emission is mostly wind-driven.

The global emission rate for fungal spores is estimated to be  $\sim 50 \text{ Tg a}^{-1}$  (2, 36). Most airborne fungal spores are within the breathable fraction of aerosols (e.g.,  $< 8 \mu\text{m}$ ) (39)

and 30–35% of species are estimated to be potentially allergenic for humans (40). In the air at near-ground level, the abundance of fungal spores typically ranges from  $\sim 10^3$  to  $\sim 10^6$   $\text{m}^{-3}$ , with large spatial and temporal variations linked in some cases with meteorological or environmental variables (39–49). Extremely high concentrations of up to  $\sim 10^9$  spores  $\text{m}^{-3}$  of air exist indoors and near strong sources of aerosols, such as composting facilities, farms, or greenhouses (50, 51), and the lowest concentrations ( $\sim 10^3$  spores  $\text{m}^{-3}$  or less) are observed at high altitudes and in polar areas (43, 45, 52, 53). Around 5% of the number of aerosols in the coarse mode ( $<10$   $\mu\text{m}$ ) are fungal spores (54). With an average carbon biomass of  $\sim 300$   $\text{ng m}^{-3}$ , they account for a significant fraction of the organic carbon in coarse aerosols at urban/suburban sites, i.e.,  $\sim 1$ –10% of the total organic carbon mass in  $\text{PM}_{10}$  aerosols (particulate matter  $<10$   $\mu\text{m}$ ), and up to 60% in the  $\text{PM}_{2-10}$  aerosols (54). At high altitude and in clouds ( $\sim 1500$  m. a.s.l.), the  $10^2$ – $10^4$  spores  $\text{m}^{-3}$  still represent around 1.5% of the organic carbon in aerosols larger than 0.2  $\mu\text{m}$  (43, 49).

Culture-based methods are often used to investigate airborne fungal diversity (40, 42). Indeed spores generally retain relatively high culturability in the atmosphere (often  $>10\%$  of the total fungal spore number), but only 17% of the known fungal species can be grown in culture (55). The application of DNA-based methods allows a better characterization of airborne fungal species richness, which is estimated to be around 1200 (56). There is a high similarity between the species found commonly outdoors and indoors (57). Frequent species include Ascomycota (*Cladosporium* spp., *Penicillium* spp., *Aspergillus* spp., *Botrytis* spp. are among the most abundant) and Basidiomycota (Agaricomycetes class, *Cryptococcus* spp., and *Dioszegia* spp. most notably) (44, 47, 49–53, 56, 58–66). Except from spores, infested materials can also release various fungal aerosols: hyphal fragments as well as toxic and allergenic particles (mycotoxins and beta-glucans adsorbed on particles of material and of fungi (67, 68) that can be smaller than spores); according to laboratory studies, their number is always higher than the number of intact spores released from contaminated surfaces (69–71). Fungi in the air can be detected through the presence of biomarkers, like ergosterol, arabinitol, or manitol, that enter their composition (37, 72, 73). Volatile organic compounds (VOCs) are also emitted by fungi during growth (74).

Many fungi potentially responsible for health issues in humans, animals, and plants are disseminated by atmospheric means (51, 60). The inhalation of fungal particles (spores, mycelium fragments) or their airborne metabolites (mycotoxins, VOCs) may lead to irritating and nonspecific symptoms in sensitive persons. Allergens can be released from spores under humid conditions, such as during thunderstorms or after cell damage (75–77). Moreover, prominent airborne fungi such as *Cladosporium herbarum* and *Alternaria alternata* have been found to release higher amounts of allergens after germination (78). Fungal fragments such as cell walls or cytoplasmic material are easily suspended in the air and inhaled as fine particulate matter (78). Secondary metabolites (e.g., mycotoxins), components of fungal cell walls (e.g., (1-3)- $\beta$ -D-glucan), and proteases have been reported to induce toxic, immunological, and inflammatory reactions (77).

In particular, fungal growth in indoor environments such as water-damaged homes, schools, children's daycare centers, offices, and hospitals creates severe sanitary problems and a potential human health risk. In northern Europe and North America, it is estimated that between 20% and 40% of buildings are contaminated by indoor molds (74). Flannigan (79) reviewed methods for indoor sampling of airborne fungi. Fungi can

secrete various hydrolytic enzymes, so they can colonize almost any damp or wet material, such as carpeting, upholstered furniture, gypsum wallboard, ceiling tiles, wood products, shower walls and curtains, and potted plants (80–82). Although central heating, ventilation, and air-conditioning systems with in-duct filters will remove many airborne spores, fungi can grow on air filters or on insulation lining the interior of air-handling units or air ducts. Long-term exposure to fungal propagules and allergens may cause severe, debilitating disease, and fatal infections, such as asthma, allergic diseases, alveolitis, and invasive pulmonary disease, and have an impact on other chronic pulmonary diseases, for instance chronic obstructive pulmonary disease. Mold allergies account for 25–30% of all allergic asthma cases (83). Between 3% and 10% of adults and children worldwide are affected by fungal allergies, as verified by skin tests (84).

### 1.1.4 Bacteria

Bacteria are unicellular prokaryotic microorganisms, i.e., their genetic material is not enclosed within a nucleus, and they have no or few organelles. Their shape varies from spherical in coccoid cells (*Micrococcus* spp., *Staphylococcus* spp.) to thin or thicker rods (*Pseudomonas* spp., *Bacillus* spp.). Cell diameter is typically around 1  $\mu\text{m}$ , but ultrasmall cells  $<0.1 \mu\text{m}$  in diameter exist in some species, notably some *Sphingomonas* spp. and *Arthrobacter* spp., retrieved from polar ice (85), and *Rickettsia* spp., intracellular parasites of eukaryotic cells. Giant bacteria also exist, such as filamentous bacteria (*Beggiatoa* spp.), which can be up to 120  $\mu\text{m}$  wide and several millimeters long; these have notably been found in anoxic deep-sea sediments (86). Some species of bacteria (*Bacillus* spp., most notably) can form spores intended to resist extreme conditions (temperature, ultraviolet, oxidation, chemical assault), allowing dormant survival for extended periods of time, potentially up to thousands years. These can “germinate” and develop when the conditions become favorable.

Bacteria cells are all composed of a lipid bilayer that surrounds the intracellular space, which contains the genetic material and most of the metabolic machinery. Transport proteins and electron transport systems like the respiratory chain producing biochemical energy, notably, are embedded within the membrane. Surrounding it, the cell wall protects cells against mechanical assault and osmotic variations. The cell wall is composed of peptidoglycan, a sugar polymer of *N*-acetyl-glucosamine and *N*-acetylmuramic acid linked together by peptide bonds constituted notably by D-amino acids, a unique feature in the living world. Two different categories of bacteria have been defined, depending on the structure of their wall, as revealed by their reaction to Gram differential staining. Gram-positive bacteria (e.g., Actinobacteria and Firmicutes phyla) have a thick peptidoglycan cell wall and no outer membrane, whereas Gram-negative bacteria (e.g., all Proteobacteria and Bacteroidetes phyla) have a thinner layer of peptidoglycan, surrounded by the outer membrane, a lipid bilayer in contact with the extracellular environment.

The genetic material of bacteria consists of a single circular chromosome, and eventually plasmids that individuals of some species can exchange with others by conjugation. Horizontal gene transfer in bacteria can also be achieved by transformation (integration of exogenous genetic material from the environment) or transduction (acquisition of genetic material through the intervention of a bacteriophage virus). The

size of the genome of bacteria ranges from ~110 kbp to ~10 Mbp, which is around 1000 times smaller than the human genome (~3.2 Gbp). Nevertheless, the trophic modes exhibited by bacteria for generating biochemical energy and biomass from their environment are extremely diverse and include all the known modes of functioning: chemotrophs oxidizing inorganic (chemolithotrophs) or organic (chemoorganotrophs) molecules as sources of electrons and energy and phototrophs taking energy from light and oxidizing organic or inorganic substrates as sources of electrons (photoorganotrophs or photolithotrophs, respectively). The source of carbon also defines trophic groups: autotrophy when the source is carbon dioxide (CO<sub>2</sub>) or heterotrophy when carbon is taken up from organic compounds (as a reference, humans are chemoorganoheterotrophic organisms, plants are photolithoautotrophic organisms). Anaerobic methanogens are chemolithoautotrophs that use CO<sub>2</sub> as a source of electrons and hydrogen (H<sub>2</sub>) as a source of energy, and releasing methane; nitrate reducers (denitrifiers) are chemotrophs that use nitrates as the terminal acceptors of electrons, i.e., they respire nitrates. Because of their versatility, bacteria have colonized all the environmental niches of the planet, including the most extreme: deep oceans, glaciers, hot springs, etc. The total number of bacteria on Earth was estimated to be ~10<sup>30</sup> cells, an amount of carbon nearly equivalent to that of plants (87). In the atmosphere at the global scale, the total number of bacteria aloft within the first 3 km of altitude was estimated to be around ~10<sup>19</sup> (87). Despite the small fraction of the organic carbon they represent in aerosols (<~0.01% (43)), they have important environmental and epidemiological impacts (88–95) (see Sections 3 and 4).

In nature, bacteria form biofilms on surfaces. Biofilms are composed of exopolysaccharide matrices where cells are embedded; these matrices protect cells against environmental assault and facilitate adhesion and molecular dialog (quorum-sensing) (96, 97). Biofilm formation can be problematic for industry and medicine notably, and biofilms are often responsible for health issues (98, 99). The surface of plants is also covered by biofilms of commensal and phytopathogenic organisms (100–102). The canopy is thus a major source of microbial aerosols outdoors (12). As most airborne bacterial cells are generally found aggregated together, they probably derive from biofilms (103, 104), which likely favor survival (105, 106).

The typical concentration of bacteria in the air near the ground ranges from ~10<sup>2</sup> to ~10<sup>6</sup> cells m<sup>-3</sup> (e.g., 107–115). As for fungi, there are large spatial variations: the lowest concentrations are found at high altitude and in polar regions (43, 45, 116–118), while the highest concentrations are detected indoors and in areas disturbed by human activities (51, 119–121). There is a very high temporal variability in bacteria number and composition in the air following diurnal and seasonal periodicities: their concentration is in general higher during the warm periods of the year than in winter, and during the day than during the night due to upward fluxes lofting cells from surfaces (11, 39, 46, 47, 58, 108, 113, 122–128). The influence of meteorological factors (wind speed, humidity, or temperature) on bacteria abundance in the air was reported in some studies (e.g., 46, 60, 111, 114, 129), but this seems to be highly dependent on the sampling site. No general relationship with meteorological variables, i.e., applicable anywhere on the planet, has been identified so far.

Aloft for typically 2–10 days (130), bacteria cells can travel over thousands of kilometers (127, 131–136) (see Section 2). Living specimens were recovered from altitudes of several tens of kilometers above ground level (137, 138). This attests to the high

resistance of certain species or strains to cold, ultraviolet, and other stresses that can be encountered in the atmosphere (139, 140). Many airborne bacteria outdoors originate from plants and soils (58, 89, 141, 142), where they probably acquired some level of adaptation to atmospheric stresses. However, the vision of the biodiversity differs from one study to another, partly due to differences in methods. So far, the patterns of biodiversity in the airborne communities appear to be very variable and have not been clearly linked to environmental variables. Among the groups frequently identified outdoors, Proteobacteria often dominate (45, 47, 49, 109, 114, 116, 118, 141, 143, 144), notably *Pseudomonas* spp. (Gammaproteobacteria), *Sphingomonas* spp. (Alphaproteobacteria), and *Methylobacterium* spp. (Alphaproteobacteria). *Pseudomonas* spp. are very versatile Gammaproteobacteria that include species of interest in aerobiology: human pathogens, plant pathogens, and species involved in ice nucleation such as *P. syringae* (11, 60, 92, 145–147) (see Chapter 3.1). Gram-positive species, Actinobacteria (*Micrococcus* spp., etc.), and Firmicutes, such as *Bacillus* spp. (spore-forming species) and *Staphylococcus* spp. among others, are also often reported, in particular indoors and in urban areas (45, 51, 89, 110, 111, 121, 144, 148–150). Finally, the presence of bacteria linked with severe human health issues were reported airborne, such as *Legionella* spp., *Salmonella* spp., and *Bacillus anthracis* (151–153).

### 1.1.5 Archaea

Owing to their apparent resemblance to bacteria (usually similar shape, size of the order of  $\sim 1 \mu\text{m}$ , cells containing neither a nucleus nor an organelle), archaea were long considered as extremophilic or odd species of bacteria. They are actually unicellular prokaryotic organisms and, since the 1960s, have represented a domain of life distinct from bacteria and eukarya, but sharing traits with both. Their existence was discovered more than a century ago, but, probably because of the difficulty in cultivating them, their functioning, abundance, and fundamental importance in ecosystems remained largely unknown until the recent development of molecular methods. Archaea provide fundamental metabolic functions of organic matter conversion in extreme environments, notably in deep marine sediments, hypersaline seas, or the digestive system of animals, notably methanogenesis. Traditional groups of archaea include methanogens, halophiles, and thermoacidophiles. Even though they were often considered specific inhabitants of environments seen as extreme for other organisms in terms of acidity, salinity, or temperature, archaea are in reality more ubiquitous than previously believed and have been found in oceans, freshwaters, soil, etc., where they also thrive owing to their high metabolic diversity. The presence of archaea has also recently been reported in the atmosphere as aerosols (126, 136). Previously unknown metabolic functions have been discovered specifically in archaea, such as the aerobic oxidation of ammonia. It appears now that their main specificities compared with the other domains of life are: (i) the structure of their membrane (absence of peptidoglycans in contrast to bacteria, glycerol with ether (as opposed to ester)-linked isoprenoid (rather than fatty acids) lipids); (ii) they use RNA polymerases and proteins in DNA replication and repair, resembling those of eukaryotic organisms; and (iii) a genome consisting of a circular chromosome such as that in bacteria, but organized more closely to that of eukaryotes, with the notable presence of histones in some species.

Among the relatively few recognized experts of these still very special microorganisms, Professors R. Cavicchioli (154) and N. Pace (155) have each authored review articles from which most of the information reported here was extracted. We recommend referring to these publications and references therein for more details.

### 1.1.6 Viruses

Viruses are obligate infectious entities with a single type of nucleic acid (DNA or RNA) as genetic material: they use the host cell machinery to replicate by copying their genome and capsid and generating new individuals. Viruses also exist in extracellular form consisting of at least one nucleic acid included in a protein capsid, in which case they can disseminate by air and be infectious, but not multiply. The presence of infectious viruses in the air near sick people has been widely studied, coughing and sneezing being responsible for aerosolization. For example in emergency services (156), active viruses were measured at several meters from their human reservoir and after more than 1 h (157). Other work showed airborne transmission of viruses in commercial air transport (156, 158, 159). Very few studies concerning the presence of viruses in the air have been published. The review by Griffin (133) in 2007 showed that a number of viruses in soil (polio virus, bacteriophage MS2) can be aerosolized and transported over long distances.

Viruses are the smallest class of bioaerosols. As a consequence, they can be found associated with particles of all sizes including nanometers, but they are in general found in complex aggregates of several microns wide (160–162). Their average concentration indoors is around  $10^3$ – $10^4$   $\text{m}^{-3}$ , although extreme spatial and temporal variability exists and extends the range from  $\sim 10^2$  to  $\sim 10^8$  viruses  $\text{m}^{-3}$  (160, 161). No link has yet been made between the size of aerosols and the infectivity of viruses (163). However, it is hypothesized that a virus aerosolized with other particles will be less overwhelmed by environmental conditions such as ultraviolet radiation and temperature as the other particles will act as shields. The nature of the material aerosolized along with viruses will thus play a role in their survival (164–166). If we were to define a virus-containing aerosol with characteristics optimized for infectivity, it would be surrounded by molecules protecting it from environmental damage, and small enough to travel long distances and spread far away from its source.

Viral transmission between living beings plays an important role in many spheres of our lives as viruses can infect all known living organisms. There have been many cases of viral illnesses transmitted between animals or humans proven to not have been caused by direct contact or by fomites. Some viruses, for instance the foot-and-mouth disease virus (FMDV) that can be fatal to cattle, have been reported to be transmitted through the air as far as 60 km downwind from the source (163). Recently, the aerosol transmission of a novel Tembusu virus causing death in ducks was also demonstrated during winter conditions (167). Moreover, many studies have highlighted the presence of viral genetic material in the air of hospitals and medical centers (156, 168–170), strongly suggesting that viruses can spread via the air in healthcare environments. Airborne viruses can cause significant financial losses, as mentioned above concerning FMDV. Airborne lactic phages can also spread into the air of dairy factories (157) and potentially affect the production of cheese, leading to a decreased production and

financial losses. The avian flu has also been responsible for economic losses during outbreaks over several continents (158). In some extreme cases, zoonosis can be observed from airborne avian flu viruses (159). This is possible because viruses undergo recognition by the immune system of organisms because of their high mutation rate (157). Airborne transmission of viruses is now well known and should be taken seriously more than ever because governments are not prepared for the treatment of highly dangerous emerging viruses such as Ebola and Zika viruses, which could potentially cause global pandemics. Although the study of airborne viruses requires robust sampling and analysis protocols, no standard approach is available, and several attempts have been made to efficiently quantify or even only detect airborne viruses in indoor and outdoor air (see Chapters 1.2 and 1.3).

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## 1.2

### Sampling Techniques

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#### 1.2.1 Introduction

Each sampling technique has associated benefits, requirements, and constraints, and it is important to keep them in mind when designing new sampling protocols and applying these techniques to specific projects: sampling duration, particle types and size(s) targeted, environmental constraints, etc. each influence the type, size, number, and viability of the bioaerosols collected. Sampling mixed populations of particles suspended in the air is challenging. This can be done passively (no pump or power source needed), e.g., by letting the particles settle on a Petri dish for example, or actively, by pulling the airstream using air movers (e.g., pumps) and recovering the particles deposited or embedded on or in a sampling medium: filter, impaction surface, or liquid. Active sampling takes advantage of particle inertia (for larger particles) and diffusion (for smaller particles), leading to their deposition. The sampler inlet must be designed to avoid disturbing the airflow as it enters the sampler by creating turbulence, i.e., the air should enter the sampler isokinetically to reflect accurately the particle size composition in the sampled air. Other problems such as the electrostatic charge can induce bias in the particle composition of the sample, but this can also be used as a means to selectively collect certain aerosols. Many collection mechanisms can damage or kill living microorganisms, and so analytical methods requiring cell viability (e.g., plate culturing) often require a different method of collection. Finally, there are unavoidable, but manageable, biases occurring during sampling due to particle losses in the sampling train (tubing, sampling apparatus walls, etc.) and particle bounce and re-aerosolization.

The method employed must, of course, be guided by the scientific question(s), and it also needs to be compatible with the downstream analytical procedures chosen, which can have specific requirements. Here we present examples of aerosol sampling techniques and indicate some analyses that can be associated with them for quantification and characterization of biological particles, presented in Chapter 1.3. Collection methods paired directly with real-time, or online, analysis methods will be dealt with separately in Chapter 1.4.

## 1.2.2 Passive and surface sampling

Most of the passive sampling methods described below are based on gravitational settling and are not, in principle, designed for the collection of bioaerosols in particular, but for sampling settled dust in general. Nevertheless, the use of these low-cost methods has a long history in environmental microbiology, and they continue to be used in some specific applications.

To collect aerosols, one of the most obvious methods is sampling by natural deposition. Outdoors, precipitation is a major means of aerosol deposition. Microorganisms in hailstones as atmospheric samples were investigated in the 1970s by culture (1), and more recently also by modern molecular methods (2, 3). Careful decontamination procedures are required in the case of material deposited on the ground. Rain and snow can be collected as they fall directly into sterile rain collectors or funnels (2, 4–7). In addition, in many studies fresh snow is collected from the ground after removing the surface layer, which will have been exposed to contamination (7, 8). In polar and mountain sites, the accumulating snow carries a temporal record of the material deposited with precipitation. Similarly to the collection of ice or sediment cores, this allows past atmospheric periods and situations to be investigated (e.g., 8, 9).

Passive sampling of settleable microorganisms in indoor environments in most cases is based on gravitational settling, historically on agar surfaces (sedimentation plates). This method consists of exposing Petri dishes to the ambient air for, say, 15 min to 1 h. After exposure, the plates are incubated at a suitable temperature and the resulting colonies are counted and identified.

In general, the number of cultivable microorganisms collected passively cannot be used to determine the cultivable microorganism concentration (colony-forming units (CFU)  $\text{m}^{-3}$ ) since the volume of air from which they deposited is unknown or uncertain. This is one of the disadvantages of this method, as described by Ghosh et al. in their review (10). These authors also stressed the great dependence of passive sampling on air currents (wind speed and direction), and the bias toward the largest particles. Despite the quantitative weaknesses of this method, it was used outdoors for assessing the deposition rates of natural bacterial aerosols (11).

Non-nutritive collection surfaces have been proposed to assess exposure to airborne microbial and allergenic components, such as endotoxins in the home environment:

- A passive sampler, designed for direct microscope observation and estimation of average airborne particle concentrations (12).
- The dustfall collector, described by Würtz et al. (13) as being aluminum foil sheets placed on the bottom of open cardboard boxes and covered with a wire mesh to prevent the collection of objects larger than airborne dust.

- Electrostatic wiping cloths (14).
- The electrostatic dust fall collector (EDC), which consists of a custom-fabricated polypropylene sampler that has electrostatic cloths attached to it to provide a sampling surface. Airborne dust settles on this surface and is captured by the electrostatic properties of the cloth (15). Since its design, the EDC has been used in several studies to detect endotoxins (16, 17), mite antigens (18), and other biological dusts. For instance this device has been used to estimate the cultivable microflora (fungi and bacteria) in farm buildings and rural dwellings (19, 20), in the bedrooms of newborns (21), and in European schools (22). Noss et al. (17) demonstrated that the EDC can also be used for glucan exposure estimation.

Note that Yamamoto and co-workers (23–25) have designed a personal aeroallergen sampler.

A novel passive electrostatic sampler that utilizes permanently polarized films for the capture of airborne microorganisms has been recently introduced (26). In this sampler, passive collection by electrostatic attraction is enhanced by particle settling and diffusion. Field studies with this sampler have shown that it achieves equivalent sampling flow rates of 1.2–2.5 L min<sup>-1</sup>, i.e., it captured the same number of microorganisms as a 100% efficient filter sampler operating at those flow rates.

### 1.2.3 Filtration

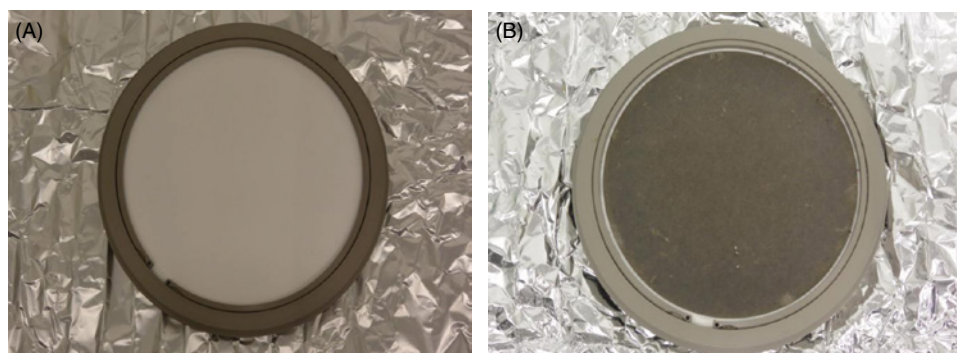
Filtration is a widely used method for bioaerosol sampling and air cleaning. The filtration method separates bioaerosols from an airstream as air passes through a filter. Filter sampling is an easy-to-use method, relatively inexpensive, and the samples are suitable for many types of downstream analyses such as microscopy, cultivation, DNA analysis, etc. Samples can be taken over long periods of time to increase total aerosol mass collected, and sample handling is generally simple (27–29).

A wide range of filter samplers and a variety of filter materials are available that allow the collection of samples with different characteristics (e.g., total suspended particles or certain size-fractions). The collection efficiency depends on the inlet characteristics and the flow rate of the sampler, the wind velocity at the time of sampling, the orientation of the sampler during sampling, and the characteristics of the filter (28, 30–32). Filter samplers range from small, personal-cassette samplers worn in an individual's breathing zone to determine personal exposure to large, high-volume samplers processing thousands of liters of air per hour (33, 34). Figure 1.2.1 shows a Digital DHA-80 sampler as an example of a high-volume filter sampler positioned to collect ambient aerosols in a remote location. This type of sampler can draw 1000 L min<sup>-1</sup> and has been utilized to collect sufficient aerosol mass to analyze DNA and molecular tracers characteristic of key bioaerosol classes (35, 36). Figure 1.2.2 shows a quartz fiber filter before and after sampling using this high-volume sampler.

The choice of the filter depends on the method of sample analysis as different types of filter materials have their own advantages and disadvantages. Fibrous filters, membrane filters, or flat filters are used for bioaerosol sampling (37). Fibrous filters such as glass fiber or cellulose have randomly oriented fibers and pores in a range of sizes. The microorganism elution for fibrous filters might not be consistent as particles are



**Figure 1.2.1** Digitel DHA-80 high-volume filter sampler operating from the roof of a building. (Photos: Anna Kunert, Max Planck Institute for Chemistry, Mainz, Germany.) (See color plate section for the color representation of this figure.)



**Figure 1.2.2** Quartz fiber filter in its holder before (A) and after 1 week of aerosol sampling (B) with the Digitel DHA-80 high-volume sampler. (Photos: Anna Kunert, Max Planck Institute for Chemistry, Mainz, Germany.) (See color plate section for the color representation of this figure.)

trapped in the fibrous matrix. Membrane filters such as cellulose acetate, cellulose nitrate, polytetrafluoroethylene (Teflon, PTFE), polyvinyl chloride (PVC), nylon, or gelatin gels are available in a variety of pore sizes. On membrane filters particles are trapped within the filter matrix and on the surface. In contrast, flat filters, such as the polycarbonate Nucleopore™ filter, collect the particles at the surface and air is channeled through pores or channels through the membrane (37).

To avoid or at least decrease contamination, the filters, and sampler parts that come into contact with the filters, should be sterilized or disinfected before sampling. Certain filters can be baked overnight at high temperatures (e.g., as high as 300–500°C for quartz fiber filters; 35, 36). This treatment destroys molecules of biological interest such as proteins, nucleic acids, endotoxins, (1→3)- $\beta$ -D-glucan, etc. (28). Additionally, field and dynamic blanks should be taken before, during, and after sampling and analyzed to detect possible contamination during sampling and sample handling (38).

Depending on the method of analysis the particles are either removed from the filter or analyzed directly on the filter. For instance, for microscopic analysis, cellulose ester or polycarbonate membranes are used as these filters can be analyzed by direct light, fluorescence, or scanning electron microscopy (39–41). For other analyses, such as polymerase chain reaction (PCR), particles should be eluted first since the filter material can inhibit PCR (37). Several different filter types, such as glass fiber (37, 42); quartz fiber (43, 44); polypropylene (37, 44) cellulose nitrate (37, 45); Celanex<sup>®</sup> polyethylene terephthalate (46); borosilicate (47); polyethersulfone (48); Teflon (PTFE) (49), etc., have already successfully been used for DNA analyses, but they have not been compared quantitatively with each other.

For the cultivation of microorganisms, filters can be placed directly on agar plates or be washed and the suspension or its proper dilution can be used as an inoculum. However, not all filter samples are suitable for culture analysis. As described above, microbial cells may be trapped within the pore matrix of fibrous and many membrane filters and the elution rates may be inconsistent (40). Flat filters, such as polycarbonate filters, can be used, and the particles can be washed from the surface (50). Moreover, dehydration effects and loss of viability of vegetative cells can occur when large volumes of air pass through the filter after these cells have been deposited (27–29, 51, 52). Gelatin filters or wetted porous foams can be used to reduce the desiccation effects (29, 53). Gelatin filters can be melted or dissolved in a buffer, and the resulting suspension can be used, or they can be placed directly onto the surface of agar plates, where they melt and are absorbed by the agar medium (27). Gelatin filters are also efficient for sampling airborne viruses as they do not significantly affect viral infectivity (54). However, the disadvantage of gelatin filters is that they are influenced by relative humidity. Using them at low humidity can cause them to desiccate, whereas high humidity can cause them to dissolve (54). Alternatively, PTFE filters with 0.3  $\mu$ m pore size have been found to be efficient for collecting airborne viruses and other biological particles in the range of 10–900 nm (30). Some recent devices also use charged filters to attract airborne particles by electrostatic forces: electret charged filters. Even if they normally present lower fiber density than conventional filters, particles smaller than 1  $\mu$ m will be collected more efficiently because of the electret forces. A reduced resistance will also be less likely to cause structural damage to cells and viruses. However, electret filters lose their charge over time, sometimes in a relatively short period of time.

As bioaerosol and total particle mass is often hard to predict, filters can be overloaded when sampling in a highly contaminated environment, making direct enumeration of bioaerosols by microscopic techniques or cultivation impossible (55). However, these samples may be analyzed for endotoxin or  $\beta$ -(1, 3)-D-glucan, or by cultivation by dilution plating after suspending the collected material into a liquid (28, 55).

## 1.2.4 Inertia-based samplers: sedimentation samplers, impactors, cyclones

The function of inertia-based samplers is based on the differences in inertial properties between the air and the particles they collect (52, 56).

### 1.2.4.1 Sedimentation samplers

In sedimentation samplers or gravimetric sensors, the particles are separated by gravity based on their aerodynamic diameter. These samplers generally consist of a chamber in which aerosols are trapped at a defined flow rate and allowed to settle at their terminal velocity in a sedimentation chamber where the airflow is negligible. The terminal velocity ( $v_t$ ) of a particle is a function of its density, size, and shape, and can be expressed as follows:

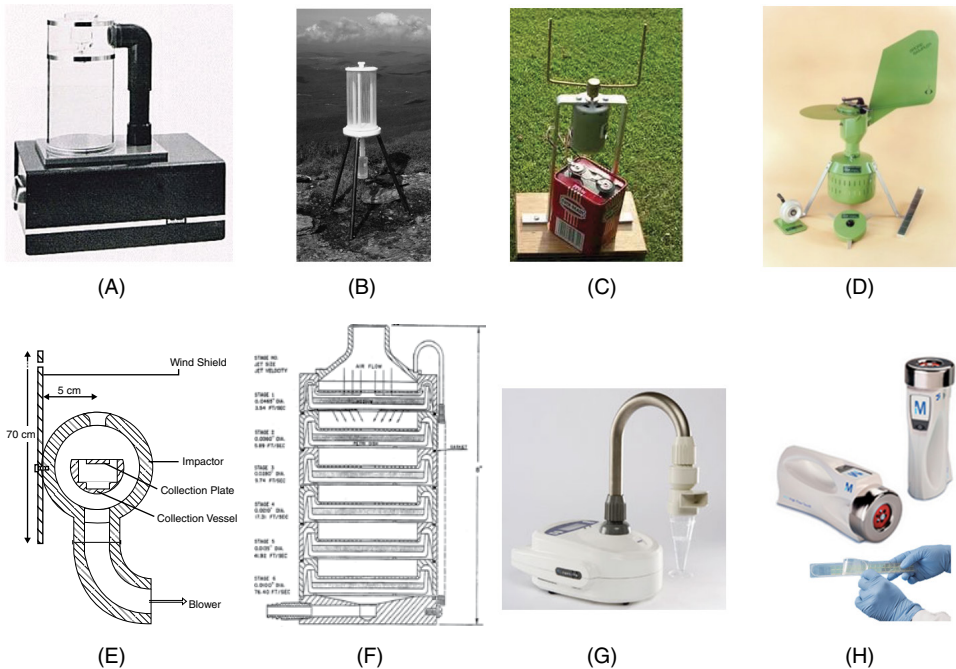
$$v_t = \frac{d_v^2 \times (\rho_p - \rho_0) \times \chi}{18\mu}$$

where  $d_v$  is the spherical volume equivalent diameter of the particle,  $\chi$  is the particle dynamic shape factor (=1 for spheres),  $\rho_p$  and  $\rho_0$  are the particle and air density, respectively ( $\rho_p \gg \rho_0$ ), and  $\mu$  is the gas viscosity ( $1.85 \times 10^{-5} \text{ kg m}^{-1} \text{ s}^{-1}$  for air at 300 K).

The sedimentation sampler Burkard™ high throughput “jet” spore and particle sampler (Figure 1.2.3), for example, is designed for collecting particles of 5–50  $\mu\text{m}$  diameter, the size range of fungal spores and pollen grains, with 95% efficiency at a rate of 850 L of air  $\text{min}^{-1}$ . The particles are pumped with air and then settle within a sedimentation chamber, where a Petri dish filled with a culture medium, a microscope slide, or any other surface can be placed for direct enumeration and/or characterization. For quantitative results, the surface analyzed will also have to be related to the entire surface section of the sedimentation chamber corresponding to the total volume of air sampled.

### 1.2.4.2 Impactors

In impaction methods, the particles are separated from air based on their size, or more precisely on their momentum. The airstream is forced to turn suddenly, and the particles that cannot follow the flux because of their size and/or mass and velocity impact a surface, from which they can be collected. Consequently, in such an apparatus, the collection efficiency increases with the particle’s diameter. The cut-off diameter,  $d_{50}$ , is defined as the diameter for which a collection efficiency of 50% is reached, i.e., 50% of the particles with a diameter equal to the cut-off diameter are impacted whereas the other 50% continues to be carried by the airflow. The cut-off diameter is directly linked to the distance between the inlet and the impaction plate and depends on the flow rate, which thus needs to be precisely controlled for optimal functioning of active impactors. Wittmaack et al. (60) developed impactors for observing bioaerosols by electron microscopy and detailed the calculation of the cut-off diameter in their report. In



**Figure 1.2.3** Examples of inertia-based samplers used for collecting bioaerosols. A, High-throughput “jet” spore and particle sampler: this sedimentation sampler is used for collecting spores and pollens (Burkard Scientific). (Courtesy of Burkard Scientific.) B, String collector: a passive impactor used for collecting cloudwater droplets (57). (Reproduced with permission from the Royal Society of Chemistry.) C, Rotating arm collector: an active impactor used for collecting pollens and spores. (Courtesy of Jim Deacon, The University of Edinburgh.) D, Seven day volumetric spore trap: an active impactor used for monitoring spores and pollens. (Courtesy of Burkard Scientific.) E, Cloud droplet impactor: an active impactor designed for collecting cloudwater at high wind speeds (58). (Reproduced with the permission of Backhuys Biological Books.) F, Six-stage Andersen sampler: an active cascade impactor with impaction plates consisting of nutritive agar medium (59). (Reproduced with the permission of American Society for Microbiology.) G, Coriolis<sup>®</sup>  $\mu$ : a centrifugal liquid impactor. (Courtesy of Bertin Technologies.) H, Reuter Cyclone Sampler (RCS<sup>®</sup>) and agar strips: a centrifugal impactor on agar medium. (Courtesy of Merck-Millipore.) See text for details on the different categories of samplers. (See color plate section for the color representation of this figure.)

cascade impactors several impaction plates are mounted in consecutive series, called stages. These are designed for separating different size ranges of particles by collecting the largest remaining fraction at each stage. Each stage interrupts particles of decreasing diameter that are carried by an increasing airflow rate. Finally, a filter can be mounted at the outlet.

In passive impactors, the airflow necessary for separating the particles from the air is created either by displacement, for example when sampling from an airplane, or by natural wind or turbulence. However, passive impactors have the major inconvenience that the airflow is not precisely controlled, so the cut-off diameter remains unknown and varies according to the wind conditions, as well as the volume of air actually sampled. In the late 1970s, Imshenetsky et al. (61) sampled stratospheric aerosols using a rocket as a passive impactor (see also Section 2.3). The upper part of the rocket contained an

impaction surface made of Teflon and was covered with a viscous nutrient medium that could be opened at the desired altitude. The motion of the rocket created the airflow necessary for the particles to be impacted on the impaction surface. Similarly, an impactor composed of five Teflon rods connected to a high-density polyethylene bottle was used for collecting cloudwater droplets from an aircraft (62). Other examples are the largely used string collectors (Figure 1.2.3) designed for collecting fog or cloudwater droplets from the ground on strings, like spider webs: these consist of metal or Teflon strings drawn, in general, vertically and connected to the bottom of a bottle. Droplets carried by wind and turbulence impact them and then flow down the strings and accumulate in the bottle, where they can be recovered for analysis (57). This principle of, very simple, collection can be used for recovering drinkable water from mountain clouds in dry regions, notably in South America where large nets are deployed and can recover several liters of water per day. In aerobiology, string collectors were used notably in the Po Valley, Italy, for studying cultivable bacteria in fog (63). However, owing to a lack of control on the cut-off diameter and the airflow rate, passive string collectors were demonstrated to be less efficient in cloud droplet collection than active impactors (see below) (64). Some instruments are equipped with fans to force the flow toward the strings; these are not strictly, by definition, passive impactors, as the airflow rate, and so the cut-off diameter, is still not precisely defined, but they have greater collection efficiency than the active rotating arm collectors, in particular for small droplets (65).

For precise control of the diameter of the particles collected by impaction, the airflow rate must be precisely controlled using a vacuum pump. Active impactors allow particle populations of defined size ranges to be separated owing to control of the airflow rate. These are widely used in aerosol studies, including biological aerosols, notably because they allow specific aerosol size ranges to be focused on. In the most basic versions of such impactors, called rotating arm collectors (Figure 1.2.3), or rotorods, the impaction plates, which are coated with a sticky substance, rotate at a defined speed around an axis, like wings. Such devices require low amounts of energy (12 V), which can be particularly convenient in the field in remote areas, but they are affected by the natural wind speed, which can alter their nominal collection efficiency and cut-off diameter. However, they have the advantage of being insensitive to changes in wind direction. Because of the limited speed of rotation, these can be adapted to large particles such as fungal spores or pollen, but not to bacteria or smaller particles (65). Some have been used notably in sub-Antarctica for collecting fungal spores (66), and for collecting cloud droplets and to observe biological residues (67).

The momentum of particles is also particularly useful for collecting non-precipitating cloudwater droplets, which are in general  $>10\ \mu\text{m}$  in diameter. A cloud droplet impactor specially designed for working at high wind speeds at mountain sites was developed (58) (Figure 1.2.3); its cut-off diameter is  $7\ \mu\text{m}$  for an air-sampling rate of  $83\ \text{m}^3\ \text{h}^{-1}$ . This has been used worldwide for investigating cloudborne biological particles, notably at Mount Sonnblick in Austria (3106 m a.s.l.; (68)), at Mount Rax in Germany (1644 m a.s.l.; (69)), and at Puy de Dôme in France (1465 m a.s.l.; (70–72)). From the water samples collected, analyses included notably microscopy, cultures, biological activity measurements, and ice nucleation assays.

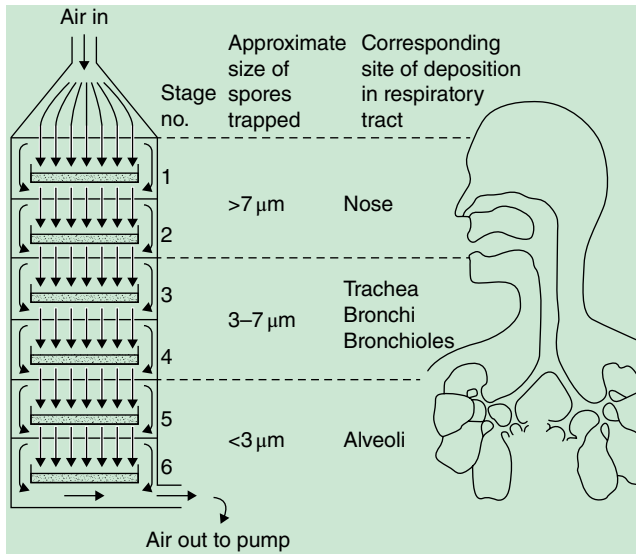
Hirst spore traps (73) are impactors largely used for automatic collection of large particles such as pollens and fungal spores. The impaction plate consists of an adhesive tape supported by a clockwork-driven drum, so it is progressively exposed, thus

keeping a spatial record of the particles collected over time. These function at low flow rates ( $\sim 10 \text{ L min}^{-1}$ ) and their collection efficiency is highly wind speed dependent, with better results for winds  $< 2 \text{ m s}^{-1}$  or  $> 9 \text{ m s}^{-1}$  (73). Some alternative versions of such impactors have been developed for pollen monitoring. Notably, the Burkard<sup>TM</sup> 7 day volumetric spore trap (Figure 1.2.3) includes interchangeable orifices for modifying the trapping efficiency of different particle sizes. This has been used widely for collecting outdoor fungal spores in the approximate range  $1\text{--}10 \mu\text{m}$  (74) and pollen up to diameters of tens of microns (75, 76). The impaction tape is typically replaced weekly; to analyze the tape, it is cut into 1–2 h segments, mounted on microscope slides, and stained with basic fuchsin for counting and taxonomic identification.

Some impactors used widely by aerobiologists permit aerosols to be collected directly onto an impaction surface consisting of a nutrient medium of the user's choice. Living airborne microorganisms are immediately put into culture medium upon sampling. Similarly to rotating drums that can record particles on an adhesive tape, slit impactors record viable microorganisms over time by impaction on a slowly rotating surface (agar medium, adhesive surface, etc.), or a rotating cover, thus exposing a small surface to the particle sampled. This was used to determine the temporal diurnal variability of culturable bacteria in the air near the ground at high resolution (1 h), by counting colonies on  $12^\circ$  segments of agar medium (corresponding to  $0.057 \text{ m}^3$  of air each) (77). Matthias-Maser and Jaenicke (78) used a combination of two impactors for collecting aerosols and to perform optic or electronic microscope observations depending on the size of the particles: an isokinetic two-stage slit impactor for particles  $0.2 < r < 10 \mu\text{m}$ , with particles impacting a glycerol jelly as the adhesive surface, and a free-wing impactor for larger particles.

Another impactor, probably the most widely used of all samplers in aerobiology, utilizes nutrient medium as the impaction plate: known as the Andersen sampler (59), this is often demonstrated to be the most efficient sampler for viable airborne microorganisms (e.g., (79)). The preferred versions of it are cascade impactors made of two or six stages holding standard 90 mm Petri dishes filled with nutritive agar. This works at a flow rate of  $28.3 \text{ L min}^{-1}$  maintained constant by a vacuum pump, typically for sampling periods of a few minutes. In Figure 1.2.3 a six-stage version of the Andersen sampler is depicted. Each stage consists of a defined number of input nozzle holes of precise diameter, which directly influences the airflow rate at the stage and, consequently, the size of the particles collected. The six stages have cut-off aerodynamic diameters of  $>7.0 \mu\text{m}$ ,  $4.5 \mu\text{m}$ ,  $3.3 \mu\text{m}$ ,  $2.1 \mu\text{m}$ ,  $1.1 \mu\text{m}$ , and  $0.65 \mu\text{m}$ , respectively. The analogy between these stages and the deposition of airborne particles in the human respiratory tract is one of the reasons for the timeless popularity of the Andersen sampler (Figure 1.2.4). The deeper in the cascade a particle is found, the deeper in the respiratory tract it can be deposited. Particles smaller than  $10 \mu\text{m}$  will more likely stick in the region between the trachea and the bronchi, and respirable particles, smaller than  $3.5 \mu\text{m}$ , can reach the alveoli. Knowing the cut-off aerodynamic diameter of each stage, it is possible to combine the sizes of the particles and the likelihood that they may cause human diseases by converting where they are deposited in the respiratory system.

Because of possible overlapping of the colonies formed on the agar impaction plates underneath the nozzle holes, a correction factor must be applied when the number of particles collected exceeds certain values (depending on the stage). This was called the



**Figure 1.2.4** Representation of the cut-off diameters of each stage of the cascade Andersen sampler, and corresponding sites of deposition in the human respiratory tract. (Courtesy of Jim Deacon, The University of Edinburgh.) (See color plate section for the color representation of this figure.)

“positive hole” method by the creators of the instrument; a table recapitulating the corrections that need to be made on the CFU counts is provided in (59). Owing to their simplicity of operation, Andersen samplers were used in a large number of studies. The capacity of particle size segregation enabled the demonstration of the fact that, for example, most culturable bacteria in the air have an aerodynamic diameter larger than 2.1  $\mu\text{m}$  (larger than the size of their own individual cells), so that they are attached to particles or aggregated together (80). These samplers were also frequently used to investigate the temporal variability of airborne microbial cell number concentrations in the short term (diurnal) (81) and in the long term (seasonal) (82–84) and to study the influence of the sampling site environment, showing in general fewer airborne culturable microorganisms at coastal sites than at rural and urban sites (81, 83, 85). Lindemann and Upper (86) used an Andersen sampler to sample vertical fluxes of bacterial aerosols from plants, covering the sampler with a metal shield as protection against precipitation.

The MOUDI™ Cascade Impactor (MSP Corporation) is based on the same mechanism as the Andersen sampler, but it has from three to 13 stages and a lower cut-off point of 56 nm. Its cut points are very sharp, meaning that very few particles with an aerodynamic diameter over the cut-off of a stage will pass to the stage below. It also allows the rotation of collection plates, leading to a more uniform deposition of particles (87, 88). The impaction substrates can vary, but generally filters with grease or oil are used to reduce the bouncing of particles maximally. Two pressure gauges measure the pressure inside the column and assure the airflow is still going through all stages. If not, it indicates when to clean the holes of the lower stages. Stages may have up to 2000 nozzles, increasing the possible flowrate to 30 L min<sup>-1</sup>. Another interesting feature of the MOUDI™ is the possibility to prepare and insert collection plates on each stage

aseptically hours before sampling. The design is such that the impaction plates are interchangeable, making it easier and faster to manipulate in the field. The column is sealed and therefore not contaminated during transportation from the laboratory to the field and vice versa. MOUDI™ impactors have been utilized widely to collect aerosols for many purposes, including recent efforts to correlate size-resolved ice-nucleating particles with biological sources (35, 89, 90).

Portable versions of cascade impactors have also been developed for air sampling. The eight-stage Marple (Thermo Fisher Scientific Inc.) is a personal sampler that impacts particles by accelerating air through smaller radial slots instead of holes. Connected to a small pump, the set-up is light enough to be worn by an individual for a day or so to evaluate its exposure to viral aerosols. Particles are collected on filters placed on each stage aseptically before the sampling. Once again, particles from 0.5 to 21  $\mu\text{m}$  are separated between the stages based on their aerodynamic diameter.

### 1.2.4.3 Centrifugal impactors

Centrifugal impactors, or cyclones, are also frequently used: they allow sampling at high flow rates with good efficiency, and they are thus adapted to low biomass environments or analytical methods requiring relatively high biomass. The principle of centrifugal impaction consists of rotating air to expel the particles onto a surface placed around the axis of rotation. In some cyclones, the particles are collected directly into a liquid. For example, a wetted wall cyclone sampler (DSTL MK1, Biotrace International) sampling air at a rate of 800  $\text{L min}^{-1}$  was used to collect air samples as large as 144  $\text{m}^3$  and concentrate the aerosols into 100 mL of water/Tween 20, allowing one of the first DNA-based studies of microbial aerosols and revealing a highly diverse and variable community structure (91).

A miniaturized cyclone with liquid impaction has been developed and is commercially available from Bertin Technologies: the Coriolis®  $\mu$  sampler (Figure 1.2.3). This collects airborne particles directly into a microtube, at a rate of 300  $\text{L min}^{-1}$ . Particles from large volumes of air can thus be concentrated many times in a small volume of liquid. Chen et al. (92) designed a personal sampler based on cyclone principles and using a 1.5 mL microcentrifuge tube as a particle collection receptacle, which allows samples to be readily analyzed by PCR and immunoassays. This device operates at a flow rate of 4  $\text{L min}^{-1}$ . At this flow rate the  $d_{50}$  is 1.5  $\mu\text{m}$  (92). This sampler has been modified to a two-stage cyclone for a personal bioaerosol with a  $d_{50}$  of 2.6  $\mu\text{m}$  and 1.6  $\mu\text{m}$  for, respectively, the first and the second cyclone stages at a flow rate of 2  $\text{L min}^{-1}$  and a  $d_{50}$  of 1.8  $\mu\text{m}$  and 1  $\mu\text{m}$ , respectively, at a flow rate of 3.5  $\text{L min}^{-1}$  (93).

Some other easy-to-use and portable (working on batteries) aerosol samplers have been developed for assaying microbiological air quality, in general by culture and for indoor sampling. Notably, the Reuter Centrifugal Sampler (RCS®) (Figure 1.2.3) is based on the principle of centrifugal impaction; it is almost ready to use even for inexperienced users, because the agar strips, which are also available commercially, simply need to be placed into the instrument. The air is pumped by a fan, the rotation of which forces the particles to impact the agar strip placed around it. According to the manufacturer, this is adapted to volumes of samples ranging from 10 L to 1  $\text{m}^3$ , at an airflow rate of around 50  $\text{L min}^{-1}$ . The volume of air to be sampled can be pre-set in the instrument for automatic operation. After removing the agar strip from the instrument and

incubating it under the desired conditions, colonies can be visually counted and related to the volume of air sampled. The ease of use of such samplers makes them convenient for controlling the presence of contaminants in environments such as hospitals or medical areas. There are some examples of outdoor aerobiology studies using the RCS<sup>®</sup>. Notably, culturable bacteria and fungi were investigated with this instrument in urban and rural areas in southern France and showed that large temporal variations exist in the concentration of airborne microorganisms (94). However, these types of samplers were demonstrated to be not as efficient in collecting microorganisms as the Andersen samplers (95, 96).

Active sampling methods are largely used to collect fungal aerosols. International standardization documents, ISO 16000-16: 2008 and ISO 16000-18: 2011 specify, respectively, the requirements for long-term (0.5 h to several hours) sampling of molds in indoor air by filtration (ISO 16000-16, 2009) (97) and the requirements for short-term (1–10 min) sampling of molds in indoor air by impaction on solid agar media (ISO 16000-18, 2011) (98).

### 1.2.5 Impingement

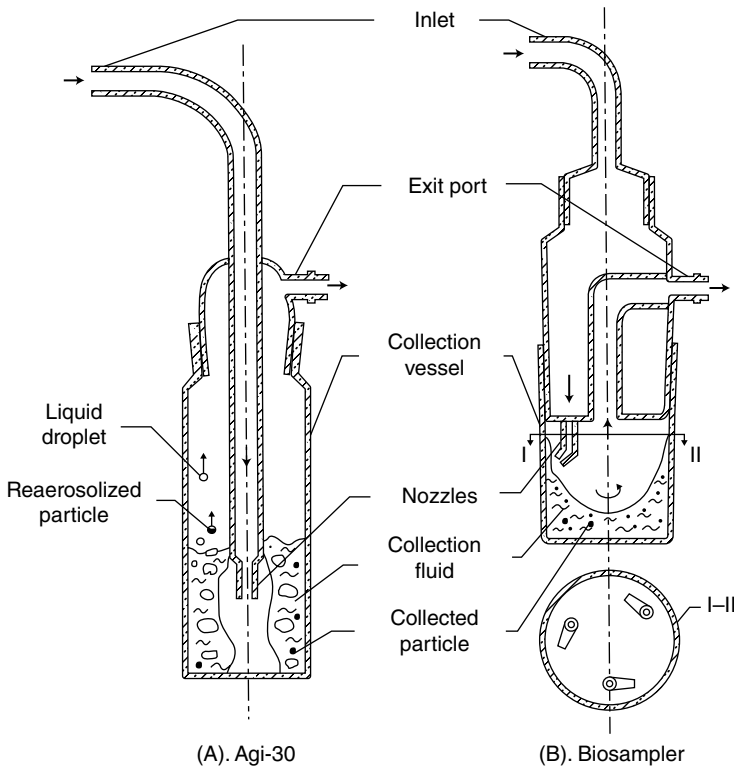
Impingers are impactors with liquid serving as the impaction medium. Sampling by impingement consists of bubbling air, using a vacuum, through a collection liquid to scavenge the airborne particles contained in it by (liquid) impaction. Liquid impaction is thought to be gentler for living microorganisms than filtration or impaction on solids, and it is thus the method of choice for investigating viable airborne microbial communities. In addition, depending on the collection liquid (water, phosphate buffer, etc.), the sample is suitable for a number of analytical procedures: cultures, microscopy, PCR, etc., with or without a preliminary step of concentration by filtration.

Typical set-ups for impingers are a battery of several of them placed at approximately 1.5 m above the ground. The collection liquids can then be pooled (99) or subdivided to be used in several analytical procedures (e.g., 100). The sampling rate with impingers is typically  $12.5 \text{ L min}^{-1}$  for a volume of collection liquid of 20 mL. Much larger versions are also used, notably in low-biomass environments such as at high altitude, where large volumes of sample need to be collected: 80 mL and a sampling rate of  $18 \text{ L min}^{-1}$  in (69), or a modified commercial vacuum cleaner Kärcher DS 5600 containing 2 L of collection liquid and working at an airflow of  $1.8 \text{ m}^3 \text{ min}^{-1}$  run from an airplane in (101).

Impingers are usually made of glass, rendering the decontamination procedure convenient by rinsing with, for example, either 10% bleach or ethanol, then deionized water, and heating at 180–500°C for several hours, and/or autoclaving (69, 102–104). The compatibility of bioaerosol sampling by impingement with the subsequent analytical methods depends on the collection fluid used for trapping aerosols. Of course, this needs to be sterile and as much as possible particle free, which is classically achieved by autoclave and/or filtration on 0.22  $\mu\text{m}$  porosity filter or finer. The collection liquid can be ultrapure water (103, 105, 106), a commercial mineral or a water-based solution (99, 106), NaCl solution (69), or, most of the time, a phosphate-buffered saline solution at pH 7.0–7.2 (79, 100–102, 104, 107). The last allows a wide range of analytical methods from cultures to DNA-based methods, while water will be preferred in the case of ice

nucleation assays, for example (103, 105). Additives in the collection liquid can improve the results: for example, the addition of catalase or of betaine significantly increased the recovery of bacteria collected by impingement (108, 109). Antifoam or antifreeze compatible with biological analyses can also be added if needed. To protect the collection liquid against freezing during sampling, notably at high altitude, the impinger can be wrapped in heating tape and maintained at around 10°C (69). Similarly, protection from direct sunlight can be achieved by wrapping in aluminum foil (110).

This volume of the collection liquid needs to be precisely taken into account, along with the air-sampling rate, to define *a priori* a sampling duration long enough to reach a cell concentration compatible with the analytical methods used, and to convert quantitative measurements made on the collection liquid to the original airborne community. The sampling time with impingers varies from a few minutes to several hours. Because of the evaporation inherent to the method, there are losses of collection liquid over the sampling time, which can be particularly dramatic over long sampling periods. The volume of the collection liquid should be corrected periodically by adding water or collection liquid to the original volume to maintain a satisfactory collection efficiency (101, 102). This can conveniently be achieved by weighing to reduce the manipulations and the chances of contamination (111). The particles lost in the curved inlet tube can be recovered afterwards by rinsing with a known volume of fresh collection liquid



**Figure 1.2.5** Schematic diagrams of (A) the conventional AGI-30 impinger and (B) the BioSampler<sup>®</sup>, improved for reducing re-aerosolization and evaporation (106).

(107, 112). Finally, in order to prevent potential adherence of bacteria on the surface of impingers, the inside can be coated with dimethylpolysiloxane (104).

Several impingers specially designed for bioaerosol collection are currently commercially available. For decades, the All-Glass Impinger AGI-30 was recognized as the reference sampler for bioaerosols in terms of collection efficiency and viable organism recovery rate (113). However, there is a chance of reaerosolization of particles sampled by bouncing, which is directly correlated with increasing flow rate (89–91). So, an improved modified version of it, the Swirling Aerosol Collector, has been developed (106, 114) and is available from SKC Inc. under the name of BioSampler<sup>®</sup> (Figure 1.2.5); today, it is probably the most widely used impinger. In the BioSampler<sup>®</sup>, three nozzles are oriented toward the walls of the collection vessel rather than one single nozzle placed perpendicular to the bottom. It creates a swirl by combining centrifugal motion with liquid impaction, which significantly improves the collection efficiency: ~80% for 300 nm particles, and >90% for particles of 1  $\mu\text{m}$  or larger (114). Using bioluminescence to measure adenosine triphosphate (ATP) concentration, collection efficiencies of 69% and 54% for bacterial aerosols of *Pseudomonas fluorescens* and *Bacillus subtilis*, respectively, were determined (112). This system also reduces rebounds, reaerosolization of particles from the collection liquid, and evaporation, which were major weaknesses of the original version of the AGI-30 impinger (114). Moreover, it allows the utilization of more viscous collection liquids, such as glycerol for reducing evaporation (106). The BioSampler<sup>®</sup> has been, and still is, largely used for sampling and characterizing an airborne microbial community by microscopy, cultures (100, 110), and DNA-based methods such as cloning–sequencing of rRNA genes (e.g., (102)) and metagenomics (99). Impingers are also often selected for sampling airborne viruses (115).

Apart from these commercially available samplers, some authors modify existing devices or design their own. For example, Liang et al. (116) modified the dimensions of glass impingers for their estimation of the total number concentration of fungal spores in the urban atmosphere (campus of Tsinghua University, Beijing (China)) by flow cytometry in combination with fluorescent stains.

Other liquid samplers rely on the volume of air filtered rather than the viability of microorganisms, as their flow rates are excessively high. For example, the SASS-2300 (Research International Inc.) has a flow rate of 325  $\text{L min}^{-1}$  and can sample more than 30  $\text{m}^3$  of air. This will lead to more concentrated samples, and should be prioritized if the aim is to detect viruses, for example, and if the preservation of viability or infectivity throughout the sampling process is not essential.

### 1.2.6 Electrostatic sampling

In electrostatic precipitators, air with particles is drawn into a sampler, where the particles acquire an electrostatic charge and are subjected to an electrostatic field that deposits them onto a collection surface. This method and various devices derived from it have been extensively used for air quality control purposes as well as for the evaluation of atmospheric contaminants (117, 118). Also, compared with particle deposition velocities in inertia-based bioaerosol collection methods, such as impaction and impingement, the particle deposition velocity in electrostatic precipitators is much lower, thus suggesting lower stress on the collected microorganisms (119). In addition,

electrostatic precipitators are typically open channel devices, which results in low power requirements. These features drive interest in the application of electrostatic collection techniques for bioaerosol capture. In this case, to facilitate analysis of captured biological particles, they can be deposited on different collection surfaces (agar, metal, filter, or water), removed, and analyzed by various techniques, including culture-based, microscopy, PCR, sequencing, ATP-based bioluminescence, and others.

One of the first attempts to utilize electrostatic precipitation for bioaerosol collection was made in 1941, when this technique was used to collect *Serratia marcescens* (120). An electrostatic air collector operating at a flow rate of  $1000 \text{ L min}^{-1}$  was designed and used to recover airborne viruses in the 1960s (121). Then, the availability and ease of use of other bioaerosol sampling methods led to a reduced interest in the application of the electrostatic technique for bioaerosol collection. However, over the past 15–20 years, this technique has seen a renewed interest and revival of its application, primarily due to concerns over the negative effects of inertia-based sampling on microbial viability and metabolic activity. In one such attempt, an existing Electrostatic Aerosol Sampler (model 3100, TSI Inc.) was modified to hold agar containers and then used to capture several different microorganisms in a laboratory setting (119). The study served as a stepping stone for the development and application of a stand-alone electrostatic collector for bioaerosols in the laboratory and field studies (122–124). In a separate development, Gast et al. (125) developed and used an electrostatic collector for the detection of airborne *Salmonella enteritidis* in the environment of experimentally infected laying hens.

Over the past 10 years or so, the development and application of electrostatic samplers for bioaerosol collection primarily focused on three areas: samplers for improved detection sensitivity, personal or high-portability samplers, and sampling applications utilizing native microorganism charge.

#### 1.2.6.1 Electrostatic samplers for improved detection sensitivity

Bioaerosol detection sensitivity can be improved by sampling a larger volume of air over a short period or by concentrating the collected sample in a small amount of collection medium (liquid) so that the entire sample can be analyzed at once without the necessity for extra sample preparation steps. In the latter case, sampler performance is described by its concentration rate ( $\text{min}^{-1}$ ), which is directly proportional to the sampling flow rate ( $\text{L min}^{-1}$ ) and collection efficiency, and inversely proportional to the volume of the final liquid sample (L) (126). A collection of microorganisms into liquid allows sample analysis by multiple techniques, including microscopy, quantitative PCR, ATP-based bioluminescence (126–128), PCR denaturing gradient gel electrophoresis (129), or the use of tracers such as arabinol (130).

An electrostatic collector capable of sampling flow rates as high as  $3500 \text{ L min}^{-1}$  has been used to capture dust for the detection of total fungi, bacteria, endotoxin, and actinomycetes (131). A wet electrostatic precipitator (WEP-2) capable of sampling biological agents at a rate of about  $500 \text{ L min}^{-1}$  is offered by Zaromb Research Corporation.

In an attempt to increase the detection sensitivity by minimizing the volume of liquid, several new electrostatic sampler concepts have been presented. In one such concept, the combination of a narrow collection electrode covered by a superhydrophobic substance (“lotus leaf” type) and a specifically-shaped ground electrode enables efficient

collection, removal, and concentration of bacteria and fungi in liquid droplets ranging from 5 to 40  $\mu\text{L}$  (126, 127, 132). The field-deployable version of the sampler achieved sample concentration rates approaching  $0.5 \times 10^6 \text{ min}^{-1}$  and, when combined with an ATP-based detection technique, allowed airborne microorganisms to be detected approximately 40 times more rapidly than traditional impingement or filtration (133). In a similar concept, an electrostatic collector featuring circulating liquid as the collection substrate has also been proposed (134). Park et al. (128) presented a hand-held electrostatic sampler consisting of a wire-rod-type charger and a cylindrical collector. The sampler could be operated at flow rates up to  $151 \text{ L min}^{-1}$  and when used in conjunction with an ATP bioluminescence assay allowed for fast detection of *Staphylococcus epidermidis* in a laboratory setting: 3 min sampling, followed by 1 min detection.

As its name indicates, the sampler ELPI<sup>®</sup> (Electrical Low Pressure Impactor, and the upgraded version ELPI<sup>®</sup>+; Dekati Ltd) creates a low-pressure environment inside its column to capture smaller particles that are not normally amenable to inertial impaction. Particles from 6 nm to 10  $\mu\text{m}$  are collected from an airflow rate of  $10 \text{ L min}^{-1}$ . First, particles entering the column pass through a unipolar corona charger, which gives them a known charge and pre-separates them. Once they are charged, they pass into the low-pressure cascade of 14 stages. Each stage is electrically insulated and collects particles bigger than its cut-off size of aerodynamic diameter. The importance of charging the particles before the measurements is to record, in real time, the impaction of molecules by the current read every 10 Hz. Indeed, particle number size and concentration are directly proportional to the current signal on each stage. Particles are normally impacted on greased porous metallic filters to eliminate bouncing. These filters can be removed afterwards for further analysis. A front panel indicates the electrical measurements in real time and a USB port is available to save the measurements if needed. Another advantage of the ELPI<sup>®</sup>+ is the possibility of preparing more than one column aseptically before going to the field and of taking many samples easily by changing the columns, as for the MOUDI<sup>™</sup> impactor. The possible applications of the ELPI sampler to biological aerosols are still limited in number, but promising: for example, characterization of endotoxin-containing particles in aerosols in an industrial environment (135), and investigation of the size distribution of airborne bacteria outdoors at a composting facility, using an 11-stage ELPI and analysis by quantitative PCR (136).

### 1.2.6.2 Personal or portable samplers

Particle collectors based on the electrostatic principle also lend themselves to miniaturization and increased portability. In addition, electrostatic collectors feature low-pressure drops that allow the use of miniature pumps or even ionic wind to pull particles into a sampler. These features encouraged the development of personal bioaerosol samplers based on the electrostatic principle. Roux et al. (137) described a personal electrostatic sampler in which ionic winds create a sampling flow rate of up to  $5 \text{ L min}^{-1}$ . The sampler features a collection efficiency above 90% for particles between 10 nm and several microns. The fungal spores collected on a dry collection surface were analyzed via arabinol detection and showed a good correlation with arabinol collected on filters. An updated version of the sampler was able to operate at  $10 \text{ L min}^{-1}$  (138) to better simulate the human breathing rate. Foat et al. (139) introduced a personal

sampler based on electrostatic precipitation with a laboratory-based electrowetting-on-dielectric concentrator. When sampling *Bacillus atrophaeus* spores at  $20 \text{ L min}^{-1}$ , the sampler showed a collection efficiency of 2.7%, but its concentration rate was  $1.9 \times 10^5 \text{ min}^{-1}$ . Han and Mainelis (140) presented a personal electrostatic bioaerosol sampler, in which the air mover (fan) and power supplies are integrated into one unit and the airborne particles are deposited onto a stainless-steel plate for easy removal. This sampler features a novel charger design to minimize ozone production and has a collection efficiency exceeding 70%.

### 1.2.6.3 Utilization of native microorganism charges

While the electrical charge on most aerosol particles is eventually reduced to a Boltzmann equilibrium charge distribution (141), bioaerosol particles seem to have higher electrostatic charge than non-biological airborne particles, because of the charge imposed by particle dispersion processes as well as a high natural charge associated with their cellular metabolic activity (142). Wei et al. (143) showed that typical indoor and outdoor bioaerosol particles have a high net charge (up to 92 elementary charge units) with most bioaerosol particles typically exhibiting a net negative charge.

Utilization of native bioaerosol charge allowed the design of an electrostatic sampler without a charging section, i.e., the native bioaerosol charge was sufficient for their capture (144). When used outdoors, this sampler collected five to nine times more culturable bacteria and fungi than an Andersen N-6 equivalent impactor. Native bioaerosol charge is also used with passive bioaerosol sampling techniques, leading to electrostatic dustfall collectors (145–147). It was concluded that this method allows settled dust to be evaluated over longer periods of exposure. However, traditional sampling substrates in such collectors lose their charge rather quickly (within hours to days, depending on the temperature) (148), thus reducing the effectiveness of this method. As mentioned in Section 1.2.2, a novel passive bioaerosol sampler, where the collection substrate maintains its charge indefinitely, has been introduced (26). The bioaerosol particles are captured by electrostatic attraction, which is enhanced by particle settling and diffusion.

### 1.2.6.4 Concerns regarding electrostatic collectors

One of the concerns with electrostatic samplers for bioaerosols is the production of ozone during particle charging and the potential effect of ozone on microorganism viability. In fact, this concern was raised during one of the earliest applications of this technique in 1941 by Berry (120). While some data on ozone interaction with airborne microorganisms are available, owing to the different configurations of the tested electrostatic samplers and varying microorganism test species, the overall picture is incomplete. At the same time, there is a consensus that ozone production should be kept to a minimum. However, there is no clear definition of “minimum” or acceptable microorganism exposure (ozone concentration  $\times$  exposure time) and generalized information regarding the effect of ozone on microorganism properties during their collection remains elusive. In 1925, it was thought that ozone concentrations have to reach 6500 ppm to be germicidal to airborne microorganisms (149). However, exposure of agar plates with bacteria and fungi to 0.01 ppm (10 ppb) ozone for 1 h showed a reduction in culturability of 50% and 75%, respectively (150). Application of 1–3 ppm ozone

to plates with *Escherichia coli* saw a 2-log reduction in 30 min. However, data on the interaction of ozone and microorganisms in the airborne phase are limited.

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## 1.3

### Quantification and Characterization of Bioaerosols (offline techniques)

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#### 1.3.1 Cultures and metabolic/phenotypic characterization of microbial isolates

For a long time, cultures were the most common methods used to investigate microbial communities, including airborne ones (see, for example, the analytical methods used for characterizing bioaerosols in the late 1990s in (1)). Since the end of the 1990s, these have tended to be replaced by molecular approaches, which are largely accepted as more exhaustive and sensitive descriptors of the microbiological content of environmental samples. Indeed, cultures are restricted to the viable and culturable fraction of the microbial community, which represents in general less than 1% of the total number of cells in bacteria but a much larger fraction (up to approximately 40%) in fungi (e.g., (2, 3)). Despite this limitation, cultures provide evidence of viability and give the unique possibility of maintaining living specimens in the laboratory, notably for investigating their physiology, metabolic functioning, and so on. In addition, environmental isolates are a potential reservoir for biotechnologies (enzymes, pigments, etc.).

Cultures consist in providing nutrients, energy sources, and favorable environmental conditions to the microorganisms present in a sample, allowing them to multiply. Cultures thus select a fraction of the microorganisms that are viable, and, given the diversity of functioning of microorganisms in a community, those that are able to grow on the nutrients provided under the conditions at which the culture is incubated: temperature, level of oxygen, light, specific carbon source(s), inhibitors (fungicides or antibiotics, for example), etc. A direct consequence is that cultures are necessarily selective, both because of the intrinsic culture conditions and also because microorganisms, notably bacteria, can remain viable but not culturable for unclear reasons, in a so-called viable-but-not-culturable (VBNC) state. Many studies refer to the microorganisms that develop in or on a culture medium as “the viable fraction,” which is not strictly exact considering the VBNC state, so this should rather be termed “culturable,” i.e., a subfraction of the viable community.

Practically, cultures are realized either in a liquid medium, by mixing or supplementing a sample with a culture medium, or on solid agar in Petri dishes. The concentration of culturable microorganisms can be estimated from liquid cultures by dilution-to-extinction culturing assays associated with statistics, as did Falhgren et al. (4), for example, from aerosol samples collected on gelatin filters. Briefly, the sample is serially (in general 10-fold) diluted in sterile water, a buffer, or a saline solution, and known volumes of these are used to inoculate tubes of liquid nutritive medium. The most probable number (MPN) of culturable microorganisms in the volume of sample assayed is 1 multiplied by the dilution factor in the tube containing the most diluted inoculum where microbial growth occurred. This assumes that the most diluted volume of sample contained one to 10 (in the case of decimal dilutions) culturable cells. For accurate results, the MPN has to be determined from a large number of replicates, ideally in 96-well microplates, for example. Better quantification is obtained from cultures on solid agar: a volume of sample (typically 0.1 mL for 9 cm diameter Petri dishes), or dilutions of it, is spread on the surface of the agar medium, and generally after a few days culturable microorganisms have multiplied and formed colonies of clones of the original cell—so-called colony-forming-units (CFUs) (Figure 1.3.1). CFUs can be counted to approximate the number of culturable cells originally present in the volume of sample spread on the agar; “approximate” since several cells aggregated together will likely generate indistinguishable colonies that will be considered as one colony. It is generally accepted that plates must contain fewer than 300 CFUs for a reliable count, in order to avoid overlap of colonies. In addition to counting CFUs, culturing methods also allow (i.e., subculturing) microorganisms to be isolated from specimens living in the sample,



**Figure 1.3.1** Colony-forming units on potato-dextrose-yeast extract agar (PDYA) medium from air sampled with a Casella CEL Airborne Bacteria Sampler (slit sampler). (Photo: T.C.J. Hill, Colorado State University, Colorado, USA.) (See color plate section for the color representation of this figure.)

by transferring single CFUs to a fresh culture medium for further investigation (identification and phenotypic characterization notably).

In aerobiological studies, samplers such as the Andersen impactor are used as reference samplers to allow direct inoculation of airborne biological aerosols on culture plates filled with agar medium (see Section 1.2.4) (5–7).

As for liquid samples like cloudwater and precipitations (8–10), the collection liquid of impingers (see Section 1.2.5) can serve as the inoculum for liquid or agar cultures (11, 12), after dilution in a buffer or after concentration by filtration if needed. In contrast, aerosols collected on impaction surfaces or on filters can be extracted into a liquid then used as the inoculum, put directly in a liquid culture medium, or deposited at the surface of the agar medium (4, 13, 14). A variety of culture media are used in atmospheric studies, in general for seeking mesophilic aerobic chemoorganoheterotrophic aerobic microorganisms, i.e., growing on organic substrates in the presence of oxygen at temperatures between approximately 20°C and 40°C, often at room temperature. Some studies focused on psychrophilic (optimum growth temperature <20°C) and thermophilic (optimum growth temperature >40°C) organisms (14–17). Although we know with certainty that they are present, there is to our knowledge no report yet published (2017) of cultures of photosynthetic microorganisms from aerosols.

Among the most widely used, undefined (containing digests or extracts of mixed composition not precisely known), and non-selective media in aerobiology, trypticase soy agar (TSA), based on tryptone (a digest of casein), is a nutrient-rich medium adapted to a large spectrum of organisms, bacteria, and fungi (11, 18–20). Although it is not selective, it seems particularly adapted to Gram-positive bacteria such as *Staphylococcus* spp., *Micrococcus* spp., *Bacillus* spp. etc. Another undefined nutrient-rich medium, but based on peptone (digest of proteins) and yeast extract, and containing relatively high amounts of NaCl, Luria–Bertani (LB) medium (21) is also largely used in aerobiology as a non-selective medium, although it is generally used to grow the bacterium *Escherichia coli*. In this medium, other bacterial species such as *Bacillus* spp., *Curtobacterium* spp., *Clavibacter* spp., *Arthrobacter* spp., *Psychrobacter* spp. were notably grown from atmospheric samples (7, 22–24). Nutrient broth or agar, also based on peptone, led to the recovery of, mostly Gram-positive, bacteria (*Staphylococcus* spp., *Micrococcus* spp., *Bacillus* spp., etc.) (6, 25). The oligotrophic medium R2A, containing less peptone and pyruvate as the main carbon source, was originally developed for isolating bacteria from freshwater environments (26); it is also particularly well adapted for cultures of bacteria and yeasts from atmospheric samples: *Pseudomonas* spp., *Sphingomonas* spp., *Methylobacterium* spp., *Bacillus* spp., etc. (8, 9, 12, 27, 28). Zobell's medium is a salty medium for the isolation of marine bacteria. It was also used to grow *Pseudomonas* spp. and *Sphingomonas* spp. from aerosols (4, 29). Among the semi-selective media used in aerobiology for targeting groups of microorganisms, one can also cite Drigalski and MacConkey media for Gram-negative rods and enteric bacteria, respectively (12, 30), and King's B medium for pseudomonads (31), with added boric acid and cephalixin for *Pseudomonas syringae* in particular (10).

For cultures of fungi, Sabouraud's medium, potato dextrose agar, and malt extract agar are reference media on which *Aspergillus* spp., *Cladosporium* spp., and *Penicillium* spp. notably have been grown from atmospheric samples (9, 11, 14, 27, 32, 33).

In order to render non-selective media more selective toward certain groups of organisms by excluding others, the media can be supplemented with additives: for example,

cycloheximide (in general 200–500  $\mu\text{g mL}^{-1}$ ) or actidione (0.04%) to prevent the growth of fungi and yeasts (5, 7, 10, 19, 31), or chloramphenicol (0.01%), penicillin, streptomycin, natamycin (0.05  $\mu\text{g mL}^{-1}$ ), or other antibiotics as antibacterial agents for the selective isolation of eukaryotic species (11, 14, 19).

A combination of several culture media for targeting various organism types in the same sample is often used. For example, Durand et al. (16) used simultaneously R2A incubated at 30°C for seeking mesophilic bacteria, malt extract agar incubated at room temperature or at 45°C for the growth of mesophilic and thermophilic fungi, respectively, and TSA incubated at 56°C for thermophilic bacteria. Radon et al. (14) used a more complex association: malt extract added with penicillin and streptomycin for mesophilic fungi (21°C), DG18 agar added with chloramphenicol for thermophilic fungi (45°C), and tryptone glucose extract agar added with delvolid (i.e., natamycin) for mesophilic (21°C) and thermophilic (55°C) bacteria. A common set-up that permits bacteria to be distinguished from fungi is to involve a non-selective medium for bacteria (TSA or R2A) supplemented with cycloheximide to inhibit fungal growth, associated with a non-selective medium for fungi (malt extract agar or Sabouraud's medium) added with chloramphenicol to prevent the growth of bacteria (11, 19).

After CFU counts, colonies can be isolated into pure colonies for identification and/or investigation of their phenotypic/metabolic capacities. For identification, a common, inexpensive, and frequently used method is based on the hypervariable ribosomal gene sequence (16S for bacteria, and 18S or 25S–28S for fungi) (3, 4, 34, 35). Briefly, DNA is extracted from pure cultures and the ribosomal region of interest is amplified by polymerase chain reaction (PCR), purified, and sequenced (see Section 1.3.3). The sequence obtained is finally compared with known sequences in databases using alignment software such as BLAST (Basic Local Alignment Search Tool, accessible notably from the NCBI website at <https://blast.ncbi.nlm.nih.gov/Blast.cgi>) to determine the most probable taxonomic affiliation of the organism (36). Targeted PCR, using taxonomy-specific primers, can also help to determine whether an isolate belongs to a specific group of organisms (species, genus, clade, or other taxonomic group) (see, for example, (37)). Finally, although this is less stringent and restricted to well-described taxonomic lineages, fungal isolates can also be identified by microscopy, along with metabolic and phenotypic profiling using discriminative criteria (10, 19, 20, 25, 27, 32, 38–40). Bergey's Manual of Determinative Biology (41) gives reference species characteristics and provides relevant criteria for the taxonomic affiliation of bacteria; this has been updated periodically since its first edition in 1923. Preconditioned phenotyping commercial assays such as API™ galleries or Biolog™ plates are also used in environmental aerobiology studies for identifying isolates (6, 24, 42). Phenotyping and metabolic profiling have the advantage of furnishing information about the metabolic functioning of the isolates, and so potentially also about their ecological significance in the environment sampled (9, 18, 43).

For viruses, which cannot be isolated using general culture media like bacteria and fungi as they are obligate parasites of their hosts, one must determine which viruses are to be isolated in order to make them grow under the right conditions. When working with bacteriophages, culture is feasible directly by adding samples on bacterial lawns corresponding to their host strains. As human or animal viruses infect more complex living beings, their growth requires more specific and difficult manipulations. One way to isolate and quantify viruses *ex vivo* is the cell culture. Although this technique is

relatively simple, there are certain restrictions. Several viruses have not been grown on known cell lines yet. For example, murine norovirus is the only serotype of norovirus able to grow *in vitro* into macrophages or dendritic cells (44, 45); it is therefore impossible to isolate any other serotypes (such as human serotypes) of noroviruses by cell culture. In the same vein, *in vitro* studies of the viability of human or porcine noroviruses need to be carried out with surrogate murine viruses.

Another alternative for the culture of infectious viruses is culture in eggs. This technique is, however, more difficult. Eggs are particularly delicate to grow and to inoculate. An inappropriate temperature gap or a needle inserted a few millimeters deeper than needed will result in dead embryos. Culture in eggs is a long process and the death ratios are variable. Proper preparation is required in order to realize successful culture in eggs. Once again, not all serotypes of viruses can grow in eggs. This method is essentially used to produce influenza vaccines worldwide (46).

## 1.3.2 Microscopy and flow cytometry

Looking at a sample, either visually using light or epifluorescence microscopes or by means of technology with electronic microscopes or flow cytometry, seems instinctively to be the first analysis to perform. It can help to determine the microbiological composition of a sample and its history.

### 1.3.2.1 Light microscopy

Optic microscopy under white light at 400× or 1000× magnification allows bioaerosols to be observed, counted, and identified. Specific stains can be used to reveal molecular structures and to facilitate the detection of bioaerosols among other materials and count them. For example, it was demonstrated that around one-third of aerosols >4 μm were biological by impacting aerosols on a glycerol jelly containing a dye specific for proteins, and observing samples under light microscopy. A similar method was applied for counting pollens in aerosols stained with basic fuchsin (39). Light microscopy can also be useful for partial identification of microorganisms. Fungi are generally observed under phase contrast for identification from morphological criteria (38). Bacteria can classically be stained using the differential Gram staining procedure to characterize the composition of their cell wall under light microscopy. This consists in applying two dyes consecutively: crystal violet, then Safranin, with an intermediary step of washing with alcohol. The cells retaining crystal violet, so appearing purple under light microscopy, have a thick peptidoglycan layer in their cell wall (Gram-positive cells), whereas those stained only with the counterdye Safranin appear red and are characterized by a thin peptidoglycan layer between two membranes (Gram-negative cells). Gram-positive bacteria include Actinobacteria and Firmicutes; Proteobacteria and Bacteroidetes are notably Gram-negative bacteria.

The number concentration of pollens, fungal cells, or bacteria in a sample can be obtained by counting them on a limited number of microscope fields taken as subsamples (e.g., 30–50, for statistical significance) and extrapolated. Malassez cells are grids of defined size specifically designed for counting the largest cells. To count bacteria and fungi, liquid samples (atmospheric waters or impingement liquid) are preferentially

stained then filtered through polycarbonate or cellulose nitrate with a porosity of 0.45 or 0.22  $\mu\text{m}$  (17, 32, 47).

### 1.3.2.2 Epifluorescence microscopy

Epifluorescence microscopy uses the properties of fluorescence, either intrinsic or gained through specific staining, of bioaerosols or of some of their specific content. The sample is illuminated by ultraviolet (UV) or blue light and the particles that are fluorescing are thus easily distinguishable from others due to the presence of the fluorochrome. The nature of the cells observed (bacteria, fungi, etc.) can be determined from their shape and size characteristics.

The sample, in general a liquid suspension (collection liquid from an impinger, or aerosols in solution), is first buffered with Tris-EDTA or Tris-borate-EDTA as both the fluorescence quantum yield and cell integrity are dependent on the pH. Then, the addition of a fixative such as glutaraldehyde 0.5–2.5% (final concentration) allows cell multiplication to be avoided and maintains cell integrity for up to a few weeks at 4°C (preferably not frozen to avoid cell burst), before analysis by microscopy of flow cytometry; obviously, viable cell counts cannot be performed on fixed samples. Cells are then specifically stained by incubating the sample for a few minutes in the presence of the dye chosen, and finally filtered and rinsed through a black polycarbonate filter mounted on a microscope slide. Most of the time an antifading agent such as phenylenediamine in the mounting oil is recommended as fluorochromes tend to bleach under exposure to light, i.e., when viewed under a microscope. The principle for counting cells is very similar to that for light microscopy, i.e., the cells are counted on a statistically relevant number of observation fields taken as subsamples, and the number concentration in the original sample is extrapolated by proportionality. A variety of specific fluorescent stains are commercially available; some will indistinctly stain live and dead cells while others allow viable cells to be distinguished from non-viable cells. The most frequently used fluorochromes in aerobiology are the following.

- *DAPI (4', 6-diamidino-2-phenylindole)*: this is probably the most frequently used stain in epifluorescence microscopy for counting total bacteria and fungi in environmental samples, and aerosols are no exception (12, 15, 48–53); however, it is usually now replaced by less toxic and more specific molecules. It binds to A–T-rich regions in DNA and has a maximum absorption at 358 nm (UV) and a maximum emission at 461 nm (blue). Under UV light excitation, DAPI stains in blue all cells containing DNA, either viable or dead, and also organic matter in yellow (54, 55).
- *SYBR<sup>®</sup> Green I and II, SYBR<sup>®</sup> GOLD, SYBR<sup>®</sup> SAFE*: this family of commercial dyes binds to nucleic acids (double- or single-stranded DNA and/or RNA depending on the dye); they are commonly used for staining nucleic acids in gel electrophoresis, instead of the harmful ethidium bromide, or in quantitative PCR applications. As for DAPI, these dyes stain dead cells indistinctly from living cells. The maxima of absorption are ~300 nm (UV) and ~495 nm (blue), and the maximum of emission is around 535 nm (green).
- *CTC (5-cyano-2, 3-ditolyl tetrazolium chloride)* is intended to specifically dye living and respiring cells. The molecule is reduced by the respiratory chain of cells into

formazan, an insoluble compound accumulating inside cells and fluorescing in red ( $\lambda_{em} = \sim 630 \text{ nm}$ ) under UV light excitation ( $\lambda_{exc} = \sim 450 \text{ nm}$ ) (56). CTC was notably used on cloudwater samples for quantifying viable cells in bioaerosols (57).

- *Acridine orange (AO)* binds to both DNA and RNA with distinct fluorescence properties. In theory, this allows viable to be differentiated from non-viable cells, but AO is mostly used for quantifying the total number of cells following the direct count method developed by Daley and Hobbie (58). When bonded to double-stranded DNA, AO fluoresces in green ( $\lambda_{em} = \sim 525 \text{ nm}$ ) under blue light excitation ( $\lambda_{exc} = \sim 502 \text{ nm}$ ); when bonded to single-stranded DNA or RNA it fluoresces in red ( $\lambda_{em} = \sim 650 \text{ nm}$ ) under excitation at 460 nm (blue). AO has been used to quantify the total number of cells in clouds and precipitation (57, 59).
- *Live/Dead<sup>®</sup> (Baclight<sup>™</sup>)*: this commercial staining mixture is a combination of two dyes that bind to DNA. It is used to distinguish between viable and dead bacteria based on their membrane integrity. The principle is based on dual staining by SYTO9 and propidium iodide: all cells are permeable to SYTO9 (green fluorochrome), while propidium iodide (red fluorochrome) can only cross compromised membranes. Hence, under UV light excitation at 488 nm, viable cells only contain SYTO9 and fluoresce in green ( $\lambda_{em} = \sim 530 \text{ nm}$ ), while both dyes enter dead cells, which thus appear red ( $\lambda_{em} = \sim 630 \text{ nm}$ ). Despite the fact that viability is not limited to cell integrity, Live/Dead staining is widely used in environmental microbiology studies to estimate the proportion of viable cells (49, 60). Also, based on the selectivity of staining following membrane integrity, Sytox<sup>®</sup> Green stains only dead cells, at the same excitation/emission wavelengths as SYTO9.

### 1.3.2.3 Electron microscopy

*Electron microscopy* provides images that can be used to look at aerosol particles at very high magnification (up to 10 000 000 $\times$ ). Briefly, the functioning involves a high-energy electron beam focused by magnetic or electrostatic lenses on the sample adsorbed on a surface; the interactions between the electron beam and the sample are recorded and used to generate high-resolution images of structures down to the submicrometer scale. Electron microscopy requires specific sample preparation protocols, some of which can be found in, for example, (61–63).

Both *transmission and scanning electron microscopy* (TEM and SEM, respectively) observations of aerosols have been reported. Coupled with energy-dispersive X-ray (EDX) spectrometers, they allow the number fraction of biological particles to be determined in insoluble aerosols, based on their shape and elemental composition (47, 64). EDX provides elemental analysis of the particle under observation and helps to determine its biological nature: relatively high amounts of phosphorus compared with mineral particles, which are characterized essentially by high silica and calcium contents. It was found that about 25% of aerosols, dry or as insoluble residues in rain- and cloudwater, were biological. SEM and SEM-EDX observations showed that bioaerosols are composed of a wide range of materials: insect brochosomes and scales, fungal spores and fragments, pollen, bacteria, protists, or plant debris (65, 66). High-resolution images of each type of bioaerosol are shown in (65, 66).

#### 1.3.2.4 Flow cytometry

Similar to epifluorescence microscopy, *flow cytometry* detects particles based on their properties of fluorescence, either natural or gained from specific staining. The same fluorochromes used in epifluorescence microscopy can be used in flow cytometry, depending on the instrument capabilities (available excitation wavelengths). Typically, this analysis requires much less volume than microscopy, and cells can be quantified from samples <1 mL in a few minutes. The particles are individualized in a microfluidic tubing system that contains a sheath fluid (in general, deionized particle-free water) running at laminar flow, and a laser beam is used for high-throughput ( $\sim 50 \mu\text{L min}^{-1}$ ) online excitation of the particles being transported. The fluorescence emitted and the light dispersed by each particle are then measured by online photon detectors. Particles are characterized based on their properties of fluorescence in the wavelength chosen (depending on the fluorochrome targeted) through back-scattering (BSC) and side-scattering (SSC) light intensities. These are used to define the populations of cells. BSC is linked to the amount of fluorochrome in the cell, so it is a proxy for its nucleic acid content, i.e., its relative level of activity when a fluorochrome binding to both DNA and RNA is used (e.g., SYBR Green). SSC increases with cell size and can thus be used to discriminate larger cells (fungi) from small cells (bacteria). An absolute reference for size can be provided by synthetic autofluorescent latex beads of known and precise size introduced in the sample at a known concentration. In some environments such as oceans, flow cytometry can allow populations of cells of distinct taxonomy to be distinguished (67–69). In bioaerosols, Chen and Li (70) compared the performance of fluorochromes for total and viable cell quantification by flow cytometry. They concluded that SYTO-13 and YOPRO-1 were the most appropriate dyes, respectively. Live/Dead staining can also be adapted to flow cytometry to count viable and non-viable cells distinctly (71).

### 1.3.3 Nucleic acid-based methods

The development of nucleic acid-based methods has improved the comprehensive characterization of biological aerosol particles/bioaerosols. DNA-based methods enable the detection, identification, and quantification of living and dead, culturable and unculturable microorganisms, and fragmented plant or animal material and viruses (48, 70–85).

#### 1.3.3.1 DNA extraction and amplification

For DNA extraction, bioaerosols are collected either in liquid samples (impingement) or on different types of filters (50, 72, 73, 75, 80, 88, 89). Depending on the sample and the aims of the analysis, various DNA extraction methods and kits are available.

Extraction kits originally designed for soil samples are often used for atmospheric samples (78, 80, 83, 90) as they are designed for the parallel extraction of various types of organism, e.g., bacteria, fungal spores, and pollen, which are present in air samples. Furthermore, these extraction kits are optimized to deal with different kinds of inhibitory substances (e.g., soot, sea salt, humic acids) that might be present in the air samples

as well (91). Inhibitory substances could prevent DNA amplification, thus it is necessary to remove or minimize these inhibitors. In addition, the choice of polymerase influences the success of the amplification in the presence of inhibitors (91). In many cases, inhibition can be reduced by diluting the purified DNA extract, which at the same time dilutes inhibitors (74).

When studying viruses from environmental water samples, filtration is usually performed to eliminate larger particles and organisms, as waterborne viruses are generally found as single particles in a liquid environment (92). However, airborne viruses are *bona fide* found in aerosol particles. Once collected in liquids, those clusters might not all dissolve even during mechanical agitation. The liquid must be shaken well in order to maximally disrupt aerosol clusters.

To detect and identify single species, genera, or groups of organisms in a sample, amplification of the characteristic regions of the genome by *polymerase chain reaction* (PCR) is used. Theoretically, a single gene copy serving as the template is required for amplification, using primers that are complementary to and specific for the target region. However, in environmental samples the practical detection limit is higher because of losses of DNA during DNA extraction, and PCR inhibition from aerosol contaminants (74). Primers must be complementary and as specific as possible in order to avoid amplification of non-target regions of the genome. This is particularly important in amplification reactions from environmental samples, where genomes of different organism groups are present and where the aim is to amplify a single given gene originating from one or several distinct species. Primer sets, depending on their sequence (in general around 20 bp for PCR), which defines their specificity, can target large groups of organisms, for example bacteria, to investigate community composition or focus on a particular species or gene. However, there is a risk of amplifying contaminant DNA. To decrease contaminant DNA levels before sampling, filters can be baked at 300–500°C for 12 h to decontaminate them (75, 78). During filter handling, gloves and tweezers should be used. Also, the sampling, sample handling, DNA extraction, and amplification procedures should be carefully controlled by analyzing background and blank samples to detect possible contamination (75, 78).

*Multiple displacement amplification* (MDA) is a newly developed untargeted DNA amplification method. This is used to amplify large fragments from very small amounts of DNA with high coverage to perform, most notably, metagenomic sequencing analyses (93, 94). It has been used in combination with next-generation sequencing (NGS) techniques to investigate bioaerosols (see Section 1.3.3.4).

### 1.3.3.2 Quantification

The number of copies of a target gene in a sample can be determined by *quantitative PCR* (*qPCR*) by measuring the increasing amount of PCR products in real time using fluorescent dye (SYBR Green) or TaqMan probes. If the number of copies of the target gene per cell is known, an estimation of the number of individuals of a certain species is possible. However, quantifying genes such as the 16S RNA gene gives only an approximate estimate as, among different species of bacteria, the number of 16S RNA gene copies varies from one to 15 per cell (95). Despite uncertainties due to the multicopy character, 16S and internal transcribed spacer (ITS) regions provide advantages

compared with single-copy target regions. 16S and ITS sequences for many species are available in public databases, making it easy to design genus- or species-specific primer pairs. qPCR has been successfully applied on air samples to quantify individual species, genera (90, 96–99), or groups such as fungi (90, 100), bacteria (60, 87, 100), and archaea (87).

### 1.3.3.3 Analysis of the diversity

For diversity studies multicopy genes coding for ribosomal subunits (16s RNA for prokaryotes, 18s RNA for eukaryotes) or *internal transcribed spacers (ITS)* are often used as targets for PCR amplification (4, 72, 75, 77, 78, 87, 101–104). Ribosomes are essential cell organelles that are responsible for protein synthesis. Some parts of the ribosomal gene sequences are conserved and can be used as primer binding sites while other sequence parts are variable and can be used for identification of the organisms. The diversity of the community is reflected by the variation between the homologous sequences. Another advantage of targeting multicopy regions is that the likelihood of amplification of samples containing small quantities of the target organisms is improved (105).

For identification of species the PCR products can be *cloned* and then sequenced by Sanger sequencing. Cloning enables the separation of different PCR products when universal primers are used for amplification, as one vector can ligate only one PCR product. To confirm the insertion of the PCR product into the cloning vector the inserted product can be amplified by using vector-specific primers in a colony PCR. Ribosomal amplicons from DNA extracts are often cloned before sequencing to assess the taxonomic diversity in a sample (4, 12, 28, 29, 50, 51, 53, 73, 86, 89, 106).

For a broader community characterization and selection of colony PCR products for sequencing, *fingerprint techniques* based on PCR amplification and restriction digests can be used. The diversity of the amplified products is resolved by electrophoretic migration on agarose or polyacrylamide gels, depending on the size or on the sequence of the PCR products (107). To increase the likelihood of sequencing as many different colony PCR products as possible a restriction digestion can be applied. The resulting *restriction fragments length polymorphism (RFLP)* can be used to select representatives for sequencing and to analyze communities' structures (78, 108, 109). Fluorescence-labeled primers for PCR amplification make it possible to detect only terminal fragments after restriction digest and separation by gel electrophoresis. Differences in sizes reflect differences in the sequences, and the peak size gives an estimate of the relative abundance. *Terminal restriction fragment length polymorphism (T-RFLP)* has been applied to air samples and can be used to monitor changes in the structure and composition of microbial communities (75, 100). The diversity represented by the T-RF pattern depends on the restriction enzyme used and on the gene and primers used for PCR amplification (91, 110). When combined with sequencing of the PCR products T-RF peaks can be taxonomically assigned after calculating the theoretical T-RF peak of a sequence. However, one T-RF peak may consist of terminal fragments of different species that have the same restriction site.

Other separation methods applied on PCR products are *denaturing or thermal gradient gel electrophoresis (DGGE/TGGE)*. This is a gradient of either chemicals (urea, formamide) or temperature on a gel where the PCR products are electrophoresed.

Fragments of the same size are influenced in their melting behavior by their sequence. The resulting band patterns allow a comparison of the diversity in different samples (103, 111, 112). The method is particularly useful for samples with less diverse communities, as diverse samples may produce too many bands. A limitation is that only PCR products up to 500 bp can be efficiently separated. In addition, PCR products of a single cell might produce several bands and fragments with different sequences might migrate to the same position on the gel (107). The high diversity of bacteria existing in clouds observed on the limited fraction of culturable organisms was later revealed by DGGE (113)

*Amplified ribosomal DNA restriction analysis (ARDRA)* involves the analysis of 16S ribosomal gene restriction fragments. The choice of enzyme is crucial for optimal resolution and has been evaluated systematically (107, 114). This was notably used to investigate the diversity of bacteria in hailstones (42).

*Ribosomal intergenic spacer analysis (RISA)* analyzes the length of the intergenic spacer between the 16S and 23S ribosomal genes. Maron et al. (73) used automated RISA to compare the community structure of bacteria in two air samples. Beside a high diversity they found distinct patterns of community structure, suggesting temporal changes in the genetic structure of airborne bacterial communities. As with T-RFLP a peak might represent the products of several species. Furthermore, single species might produce more than one peak as the spacer region can differ in different ribosomal RNA operons in a bacterium genome (91, 115).

#### 1.3.3.4 Sequencing

In the past, many bioaerosol studies used Sanger sequencing as this provides sequences long enough to identify individual genera or species by comparison with sequences that are available in online databases, such as GenBank and the NIH genetic sequence database (72, 73, 75, 77, 78, 86, 87, 102, 116, 117). However, Sanger sequencing-based bioaerosol analysis is currently being replaced by modern NGS technologies. In the past, the length of the sequences has often been a limiting factor for identification at the genus or species level, as the reads were much shorter than sequences obtained by Sanger sequencing. NGS technologies are continuously improving and are now able to provide longer reads (117, 118). These technologies also allow the generation of millions of reads from air samples in order to analyze the genomes and transcriptomes, and have been successfully applied in several recent bioaerosol studies (50, 80–83, 85, 88, 117, 120). One approach is the sequencing of a particular target region, which is amplified by PCR before sequencing. Many NGS studies have focused on the characterization of diversity of bacteria using 16S ribosomal RNA gene-based sequencing (60, 83, 88, 121, 122). Species from other domains (archaea, eukarya) are often not analyzed or only appear as coamplification from chloroplast sequences as 16S rRNA gene primers also bind to chloroplast DNA because of the bacterial origin of these organelles (116). Bowers et al. (123) used a multidomain PCR primer set to also determine diversity and abundances of fungi and plant aerosols.

In contrast, metagenomics focuses not only on a particular region of the genome, but on the whole genome. It consists in the sequencing of all nucleic acid sequences of a complex sample by whole genome shotgun sequencing, after metagenomic amplification by MDA. Sequences are then reconstructed and identified by comparison with

databases. The greatest advantage is that every DNA sequence present in the sample will theoretically be sequenced, allowing the taxonomic composition and metabolic potential to be determined (80, 81, 124). MDA was employed on aerosols to investigate active and total airborne fungal communities over the Amazon forest (125), and to describe bioaerosols biodiversity in urban environments (126). Metagenomic approaches are powerful tools for the characterization of airborne viral diversity and dynamics as viruses are genetically highly variable and do not possess conserved genes, which makes PCR-based applications challenging (84). Viral metagenomics studies have been conducted of different environmental sample types such as water, air, and fecal samples (84, 127). Since the culture of viruses is very difficult and nucleic acid profiling is nearly impossible, there is a serious lack of information in viral databases, which still greatly limits viral sequence attribution.

Gene activity can also be analyzed at the transcriptome level, by looking at the transcribed genes rather than at the genes themselves, and so, by extension, at the functions expressed by microorganisms at a given time. However, this requires the metabolic state of the microorganisms to be frozen upon sampling in order to reflect their state while airborne; this can be done by direct freezing in liquid nitrogen or by using fixative agents such as RNA Later<sup>®</sup>, a saturated ammonium sulfate solution. RNA is then retrotranscribed into DNA and processed similarly. Transcriptome analysis targeting ribosomal genes to identify active groups was achieved on fungal communities collected above the Amazon forest by impingement, using a preservation solution as the collection liquid (125). Metatranscriptomic analyses open new avenues to ecosystem and health-related studies.

#### 1.3.3.5 Microarrays

For the characterization of bioaerosols microarrays have been used in several studies. Species- or group-specific probes on the chip can hybridize with the fluorescently labeled DNA from air samples. From the position of the fluorescence on the chip their identity can be determined. Brodie et al. (89) used bacterial 16S rRNA genes to analyze the diversity and changes in the bacterial population in urban aerosol samples, whereas Wilson et al. (128) and DeSantis et al. (129) used a chip containing prokaryotic and eukaryotic probes.

### 1.3.4 Chemical and biological tracers

The methods for identifying and detecting bioaerosols are challenging owing to the complexity of the biological material. Many different primary biological aerosol particles (PBAPs) can introduce significant detection biases (130). Quantification of specific microbial cell envelope constituents, such as protein from bacteria or ergosterol from fungal biomass, exemplifies the concept of biomarkers as an alternative approach to measuring bioaerosols (131, 132). Liquid and gas chromatography coupled with mass spectrometry and spectroscopy (nuclear magnetic resonance (NMR), Fourier transform infrared (FTIR), etc.) represent the main techniques used to assess the quantity of biological aerosol.

### 1.3.4.1 Biomarkers

As an alternative approach to investigate bioaerosols, the constituents or metabolites of microorganisms can be measured as proxies, i.e., molecular “signatures” of the biological nature, in the atmosphere. These compounds, referred to as biomarkers, are specific, i.e., they are produced by only a limited group of organisms. The biomarker can, therefore, be used to characterize a specific microbial community in environmental samples. For biomass determination, the biomarker should be present in relatively constant amounts in the organisms of interest (131, 133, 134). Different molecular tracers can be used to assess different microbial groups, such as protein for total bacteria, 3-hydroxy fatty acids (3-OH FAs) for Gram-negative bacteria, and ergosterol for fungal biomass. Many groups are still working on developing new specific molecular tracers to characterize different types of biological aerosols more accurately (135).

#### Ergosterol and mannitol/arabitol

Ergosterol is a primary fungal membrane sterol, and is found almost exclusively in fungi. Hence, it provides an efficient biomarker for determining the fungal biomass in ambient aerosols (50, 135–137). Ergosterol is commonly determined by gas chromatography–mass spectrometry (GC-MS), but is known to weakly fluoresce (138, 139). Sugar alcohols, such as mannitol and arabitol, provide energy reserve materials in fungi. Both have also been proposed as suitable biomarkers for fungal spores, and measured by gas or ion chromatography (136, 137).

#### Endotoxins

Endotoxins are also used as proxies for airborne bacteria (134, 135, 140). Endotoxin—lipopolysaccharide—is a collective term designating a characteristic group of constituents of the outer membranes of Gram-negative bacteria (132, 141). The lipid portion of the endotoxin is chemically distinct from all other lipids in biological membranes and consists of characteristic 3-OH FAs. Lee et al. (131) measured 3-OH FAs by GC-MS to estimate the amount of endotoxin from bacteria in atmospheric aerosols.

Measurement of exposure to environmental endotoxin is frequently performed using a *Limulus* amoebocyte lysate (LAL) test (141–144). In the LAL test, Gram-negative bacteria are detected through a cascade reaction in which endotoxin catalyzes the activation of coagulase (endotoxin → proenzyme → coagulase → coagulogen → coagulin). Endotoxin concentration determines the initial rate of activation. The molecule (1, 3)- $\beta$ -D-glucan, a major component of fungal cell walls, can also induce a similar reaction. Menetrez et al. (144) measured both endotoxin and  $\beta$ -glucan concentrations in bioaerosols using the LAL test. The LAL test is rapid, sensitive, and highly standardized, but is not necessarily well adapted for microorganism quantification (135, 141).

#### Biofluorophores

A variety of biological molecules constituting bioaerosols exhibit intrinsic fluorescence, or autofluorescence: aromatic amino acids (and therefore the proteins composed of them), reduced nicotinamide adenine dinucleotide (NADH), chlorophyll, etc. This property is notably used by a number of online laser-based instruments for the specific detection and quantification of bioaerosols (see Chapter 1.4). It is generally admitted

that bioaerosols contain proteinaceous material, although this is not strictly exact since a solid lipid aggregate derived from a biological system, for example, can also be considered a bioaerosol *sensu stricto* as soon as it is aloft. Viruses also do not systematically contain proteins.

Fluorescent biological aerosol particles (FBAPs) fluoresce at different wavelengths. Thus, it was suggested that FBAPs can be seen as an effective lower bound for PBAPs (145). Certain amino acids and coenzymes are among the most commonly analyzed biofluorophores, but numerous other metabolites and biogenic molecules emit fluorescence. Three amino acids with aromatic side chains—tryptophan (Trp) and, to a lesser extent, tyrosine (Tyr) and phenylalanine (Phe)—are considered as universal markers for biological materials since these amino acids will be present in nearly all proteins. These fluorophores are known to have characteristic emission bands between 300 and 400 nm when excited around 280 nm (139, 146, 147). At longer wavelengths, the most probable biological molecules that fluoresce are coenzymes involved in energy metabolism such as flavin mononucleotide phosphate and NADH. Both exhibit blue–green fluorescence (450 and 560 nm, respectively) under ultraviolet A (UVA) excitation (~300–400 nm) (148, 149). Similarly, chlorophyll *a* fluoresces in red (wavelengths of 630–800 nm) when excited by UV light (139, 140, 150, 151). It was estimated that species-level identification based on fluorescence was unlikely (146). Nevertheless, with the recent improvements in sophistication, this property is widely used in very sensitive and online spectroscopic methods, e.g., laser-induced fluorescence (see Chapter 1.4), for the specific detection and quantification of bioaerosols (134, 152).

#### 1.3.4.2 Ice nucleation activity

At temperatures above homogeneous freezing of water (i.e., approximately  $-39^{\circ}\text{C}$ ), ice is formed by heterogeneous nucleation of water molecules triggered by foreign particles, called ice nuclei (IN). The temperature at which IN are active, i.e., at which they initiate the formation of ice from supercooled water, is related to their composition and structure. The discovery in the early 1970s that the most efficient IN are biological (153) and associated with bacteria (154) led to the hypothesis that they are involved in atmospheric processes (155). Since then, many studies have demonstrated that bacterial and other biological IN are widespread in the atmosphere (156–159) and participate in cloud freezing and precipitation (e.g., 155–164) (see Chapter 3.1). Hence IN activity is currently a major topic in the study of bioaerosols. Some of the best biological IN identified so far are membrane-bound proteins produced by some Gram-negative bacterial species (e.g., 165–167). Fungi and lichen mycobionts are other organisms found airborne exhibiting IN activity (79, 168–176). Hence, assaying biological ice nucleation activity in atmospheric samples is common in outdoor aerobiology studies. The most frequently used method for measuring the concentration of IN in a sample was originally proposed by Vali in 1971 (177). In a so-called droplet-freezing assay a liquid sample is distributed in small drops of approximately 10–400  $\mu\text{L}$  on a surface or in 0.2–0.5 mL microtubes or 96-well plates, and incubated at decreasing negative temperatures in a cryobath or cooling block from  $0^{\circ}\text{C}$  to around  $-15^{\circ}\text{C}$ . The frozen droplets are counted at each temperature step. The atmospheric samples suitable for IN analyses can be, for example, aerosols extracted from a filter into high purity grade water (50), aerosols in

solution collected with impingers (178), rain, melted snow (156), or cloudwater (157), or grown cultures isolated from air sampling on agar plates (37, 179, 180). Whether the drops are frozen or unfrozen can be ascertained visually, or with the help of a camera coupled to image analysis software (181), or any other method allowing this distinction. The cumulative concentration of IN,  $C_{\text{IN}}(\text{N mL}^{-1})$ , at temperature  $T$  is then calculated as follows:

$$C_{\text{IN}}(T) = \frac{(\ln N_0 - \ln N_T)}{V}$$

where  $N_0$  is the total number of drops assayed,  $V$  is their volume in milliliters, and  $N_T$  is the number of drops still unfrozen at temperature  $T$ . The function  $C_{\text{IN}} = f(T)$  generally increases exponentially with decreasing temperature. In this method of quantification, the concentration of IN is sensitive to both the volume and the number of drops studied, with higher values obtained for large drops than for the same total volume of sample distributed into more drops. The review in (182) provides a large overview of laboratory investigations of atmospheric ice nucleation by particles.

Heating samples at 95°C for at least 10 min prior to performing IN measurements allows biological structures to be denatured and distinguishes between biologically and non-biologically driven ice nucleation, by comparing the ice-nucleation profiles of heat-treated and intact samples (156, 157, 178, 183). Organic matter can also be removed selectively by a number of targeted treatments, such as the addition of hydrogen peroxide at a high concentration (35%) before IN assays (184, 185). The addition of lysozyme at 3 mg mL<sup>-1</sup> final concentration and incubation for 72 h at 4°C was used as a method to specifically suppress, and so quantify, bacterial IN in precipitation samples (156). In the last decade, the importance of IN in atmospheric sciences has led to the development of online instruments for IN quantification (e.g., 145). These are presented in Chapter 1.4.

### 1.3.4.3 Mass spectrometry

One of the most widely used methods for microorganism identification is pyrolysis mass spectrometry. The sample is heated, generating volatile metabolites such as ubiquinones and phospholipids, which are biomarkers of bacteria (187–189). Proteins from viruses can be detected and characterized using electrospray MS (190). Matrix-assisted laser desorption/ionization (MALDI) has been central to the MS of large biological molecules (191–193). MALDI has shown considerable success in the detection of proteins with an absolute detection limit of a few zeptomoles (10<sup>-21</sup> mol) (189). Coupled with a time-of-flight analyzer, MALDI can enable the identification of bacteria and fungi. However, it can only analyze compounds greater than 600 Da (134). Jackson and Murray (188) observed bioaerosols with on-the-fly MALDI.

Fatty acids were evaluated early on as biomarkers, and then so were phospholipids, glycolipids, and lipopeptides. Bloom et al. (194) analyzed fungal chemical markers such as ergosterol by GC-MS, and mycotoxins by high-performance liquid chromatography (HPLC) MS. Parker et al. (195) developed an instrument that utilized ion-trap MS for the detection and analysis of pollen, bacteria, and other aerosol types. However, the

most reliable MS biomarkers are considered to be amino acids, in particular tryptophan because of its fluorescence (139, 196, 197).

GC-MS is utilized for the most notably in environmental samples (194, 202). Wady and Larsson (203) used solid-phase microextraction combined with GC-MS to study the adsorption and desorption of microbial volatile organic compounds on house dust particles. With the recent advances in analytical techniques, HPLC and GC can also be coupled with isotope ratio mass spectrometry (IRMS). Both techniques have traditionally been used to measure the natural variation in isotope ratios of single compounds due to isotope fractionation during primary production, respiration, and assimilation (204) (see Section 1.3.5). Liquid chromatography (LC)-IRMS broadens the types of biomarkers that can be analyzed (133, 205). With the development of gas chromatography–combustion–isotope ratio mass spectrometry (GC-c-IRMS), it is now possible to analyze stable isotope ratios of specific compounds, including a number of biomarkers with excellent sensitivity (133). Finally, the differentiation of proteins and viruses can be performed using pyrolysis gas chromatography differential mobility spectrometry (PY/GC/DMS) (206).

#### 1.3.4.4 Spectroscopy

FTIR and NMR spectroscopies can be used jointly for bioaerosol analysis, because the information available from both methods, and thus their strengths and drawbacks, are similar (207). NMR, in particular, was considered to be a time-consuming technique with low sensitivity (207–209). However, the potential of the NMR technique to investigate aerosol composition (209), or biomarkers such as chlorophyll (200), was demonstrated. Owing to recent improvements in sophistication, such as high-field instruments, inverse probes, cryogenic probes, or more sensitive solid-state probes, the characterization of bioaerosols by NMR is improving (210–212). FTIR spectroscopy also plays an important role in searching for biomarkers. This spectroscopy technique has been widely used for pollen identification (140). This allows the detection of biological aerosols with passive FTIR sensors, combined with data treatment *in silico* (213, 214). Parodi et al. (215) suggested that bioaerosols such as pollens can be identified by an alternative spectral method: FTIR photoacoustic spectroscopy (FT-IRPAS), which requires no sample preparation.

Manoharan et al. (216) pointed out that many biologically important molecules have electronic transitions in the deep UV, and obtained resonance Raman spectra for biological material which contains fluorophores such as tryptophan or NADH. This vibrational spectroscopy is firmly established as a tool to investigate and characterize bacteria and pollen grains (140, 217). The addition of nanostructures to the suspension greatly augments the Raman scattering (218). Surface-enhanced Raman spectroscopy (SERS) enabled bacterial identification (219) and the characterization of pollen (220). SERS can be incorporated into a real-time bioaerosol detection apparatus (221).

Pastuszka et al. (222) applied X-ray photoelectron spectroscopy (XPS) for identification of airborne bacterial strains and species, in conjunction with complementary techniques, such as FTIR spectroscopy, to offset its less sensitive detection ability. XPS, as do the following techniques, provides an elemental analysis of the minor constituents and trace elements (223). Pepponi et al. (224) investigated pollen as an indicator for atmospheric pollution by means of total reflection X-ray fluorescence (TXRF)

analysis. Recently, they characterized a wide range of bioaerosol particles by using SEM coupled to energy-dispersion X-ray (SEM/EDX) spectroscopy (225). Pöhlker et al. (226) associated scanning TEM with near-edge X-ray absorption fine structure (STXM-NEXAFS) spectroscopy for the characterization of biological aerosols.

#### 1.3.4.5 Immunoassay method

The immunoassay method is a special type of biochemical test in which a specific binding antibody is used to evaluate the presence and amount of the corresponding antigen. It is therefore necessary to produce antibodies with specificity toward a single species. Monoclonal antibodies offer increased specificity and may be useful for the characterization of bioaerosols (135, 223). Enzyme-linked immunosorbent assays allow the sophisticated detection and enumeration of components in bioaerosols based on antibodies specific for antigenic components of microorganisms (227).

### 1.3.5 Biological activity-based methods

Living airborne microorganisms can be vectors of sanitary problems on plants, animals, or humans (see Chapter 4.1). In addition, metabolic activity likely impacts atmospheric chemistry (see Section 4.2). The interactions existing between living organisms and their environment have led to methods intended to detect them. Alive biological aerosols can thus be distinguished from inert particles (dead biological particles or minerals), and eventually quantified.

#### 1.3.5.1 Supplementation with nutrients

One of the most obvious methods used for decades for detecting living microorganisms based on their activity is to look for their multiplication. The formation of colonies on culture media attests to biological nutrient uptake and transformation (see Section 1.3.1). Cultures give an overview of the microorganisms able to develop under the conditions provided and of their number, i.e., in general a small fraction of the total living organisms. Monitoring the multiplication of cells over time in a sample supplemented or not with nutrients is also an indirect method of detecting viable cells based on biological activity (228). Supplementing a sample with a specific compound potentially used as a nutrient by cells can allow particular groups of interest to be detected. Such enrichment cultures were used to demonstrate the presence of living methanotrophs in the atmosphere (43). However, the principle itself of these methods based on a modification of the biomass does not permit precise quantification of viable cells in the original sample.

#### 1.3.5.2 Supplementation with radiolabeled precursors of anabolism

Similarly, but necessitating no significant biomass increase, a common method used for tracking biological activity is to supplement samples with radiolabeled compounds. The insoluble material is recovered from the samples after a short incubation period and the radioactivity is measured, in general by scintillometry after addition of a chemical

developer. The temporal increase in radioactivity in the biological material or in the headspace of microcosms can be used to estimate biologically driven processes such as biomass production or respiration. Radiolabeled precursors of protein and DNA synthesis, [ $^3\text{H}$ ]- or [ $^{14}\text{C}$ ]leucine and thymidine, respectively, or carbon uptake, using tracers such as [ $^{14}\text{C}$ ]acetate and glucose for heterotrophy or [ $^{14}\text{C}$ ]NaHCO<sub>3</sub> for autotrophy, can be used to estimate the rates of microbial biomass production, cell multiplication, protein or lipid synthesis, respiration, or photosynthesis using the corresponding conversion factors (see, for example, (229–233)). This method demonstrated the bacterial activity in cloudwater and was used to estimate biomass carbon production and cell generation time (52).

### 1.3.5.3 Enzymatic activity

In order to detect viable cells broadly, independently of their capacity to take up molecules, samples can be supplemented with markers of metabolic activity. For example, the dye CTC is biologically reduced to a fluorescent molecule by the respiratory chain, which allows respiring cells to be detected and quantified by microscopy or flow cytometry (234) (see Section 1.3.2). This has been used to investigate viable biomass in clouds (57). Other fluorescent dyes can testify to enzymatic activity as they accumulate in the intracellular space in direct proportion to reaction rates: for example, fluorescein diacetate (FDA) is reduced to fluorescein by esterases, and resazurin is reduced to resorufin by NADH and NADPH, and so are used to measure intracellular oxidoreduction reactions and respiration rates (235–238). Recently, FDA was used for investigating microbial activity in bioaerosols in China (239).

*Isotope ratio measurements* can also be used to detect enzymatic activity, notably in microorganisms. Indeed, enzymes are generally selective for lighter isotopes ( $^{12}\text{C}$  is preferred to  $^{13}\text{C}$ ), resulting in substrates enriched in  $^{13}\text{C}$  and metabolites enriched in  $^{12}\text{C}$  compared with the original pool of substrate molecules. This property is often used to detect and characterize biological activity through the relative quantification of isotopes by mass spectrometry (e.g., 240–243) (see also Section 1.3.4).

### 1.3.5.4 Adenosine 5'-triphosphate

Adenosine 5'-triphosphate (ATP) universally carries chemical energy in cells through its high-energy phosphate bonds. Its dephosphorylation into adenosine 5'-diphosphate (ADP) and 5'-monophosphate (AMP) releases the energy necessary for most endergonic enzymatic reactions and transport in cells. Furthermore, the measurement of ATP quantities can be very sensitive (theoretically down to one cell with sensitive detectors) because the assay involves the enzyme luciferase from fireflies (note that ATP concentration can also be measured by HPLC (244)). Luciferase oxidizes D-luciferin and emits photons in the presence of ATP, at a stoichiometric ratio (one photon per ATP molecule). The reaction is almost instantaneous and decreases relatively fast, so a luminometer equipped with autoinjectors is needed. Since the intensity of light produced is directly proportional to the ATP content (which is proportional to biomass), it is possible to quantify the microbial biomass even at very low concentrations by measuring the number of photons emitted from a known volume of sample containing

luciferase and its substrate luciferin. Using conversion factors ( $\sim 10^{-18}$  mol ATP per bacterial cell;  $10^{-15}$  g ATP per bacterial cell (245)), the amount of ATP measured can be converted into the number of potentially viable cells. Current luminometers and reagents are in theory capable of detecting the light emitted by the ATP of one single bacterial cell. Eydal and Pedersen (246) compared microbial counts in groundwater samples obtained using commercial ATP assays with traditional microscopic and culture-based counting methods; they found the assay to be reliable and robust in estimating microbial concentration.

ATP measurements have long been used for detecting viable biomass and microbial contaminants in clinical, environmental, and food-processing settings (245–250), and this method has been applied to atmospheric samples (15, 251, 252).

### 1.3.5.5 Virus infectivity

Animal exposure to viral aerosols is also used to characterize their infectivity and virulence. Tests to determine the effect of aerosolization, the minimal dose exposure via the air pathway, or other characteristics are conducted on animals. As some strains are transmissible from animals to human, such experiments require a higher level of confinement. Exposure to viral aerosols can be done via the nose only, the head only, or in a whole-body chamber. Tests on animals as large as monkeys are currently carried out with highly lethal viruses such as Ebola and Marburg (253–255).

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## 1.4

### Online Techniques for Quantification and Characterization of Biological Aerosols

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#### 1.4.1 Introduction

As has been discussed in previous chapters, a host of techniques for sampling and analysis of primary biological aerosol particles (PBAPs) in the atmosphere have been available to the scientific community for many decades. Until relatively recently, however, all bioaerosol sampling technologies required some kind of offline analysis, often microscopy or culture based, to be performed after sampling. This limits the immediacy of the impact an analysis can have on the understanding of a particular environment and also typically limits the time and size resolution a given analysis can provide. Time resolution from a few hours to days is typical for most collection techniques paired with offline bioaerosol methods. As a result, offline analyses may not be able to analytically separate aerosol emission processes with fast timescales from those with slower trends or with very narrow size distributions from wider features. Offline analyses can also be costly and inconvenient, owing to the high number of person-hours required to collect and analyze a given sample (1). To overcome these challenges, several classes of real-time bioaerosol characterization techniques have been developed and applied recently, with increasing attention in the last decade. These techniques typically utilize physical and chemical properties (e.g., spectroscopic information) to differentiate and analyze sampled particles and are proving increasingly powerful tools, especially when used in conjunction with offline analyses such as those discussed in Chapter 1.3 (2–5). As will be pointed out later in this chapter, support from national defense-related agencies triggered the development of many of the real-time bioaerosol detection technologies in an effort to be able to rapidly detect airborne biowarfare threat agents (BWAs) (e.g., anthrax spores). In addition to developments aided by defense-related funding, improvements in technological instrument components over the last two decades have accelerated development. For example, improved photomultiplier tubes (PMTs), development of laser diodes and light emitting diodes (LEDs) at shorter wavelengths, higher power, and reduced cost, and improved particle-focusing lens technology (6) include a few of the key technological advancements that have allowed bioaerosol instrumentation to flourish recently. A wide spectrum of technologies have been proposed and developed. Many

are still utilized primarily by military or defense agencies (7); however, an increasing number of instruments have become commercially available and are now routinely applied by civilian research groups toward a variety of non-BWA questions.

Some nomenclature should be noted at this point for readers across broad disciplines. The term “real time” here denotes that an analysis is performed immediately (on the scale of seconds or less) after sampling a particle directly from the air. In this chapter we use the term interchangeably with “online,” which is often also used to denote an autonomous collection and analysis process. In contrast, the term “offline” implies that, while collection may or may not be performed automatically for some period of time, the process of taking these particles (individual or as an ensemble) to an analytical detection method requires human input. As a result of the autonomous nature of “real-time” or “online” techniques, time resolution, data continuity, and convenience are usually improved substantially. This often leads to dramatically increased data volume, which can produce a bottleneck at the analysis or interpretation stage, which always requires some level of rational human input.

This chapter will provide an overview of key technologies associated with real-time techniques for the quantification and characterization of biological aerosols, with emphasis on commercially available technologies. Many resources are available that highlight other subsections of real-time bioaerosol analysis and should be considered in addition to this chapter (e.g., 3, 4, 8–16).

### 1.4.2 Single-particle fluorescence spectroscopy

Single-particle fluorescence spectroscopy is probably the most widely used technique at the present time for real-time detection and characterization of bioaerosols. The principle of utilizing fluorescence spectroscopy to detect and characterize bioaerosol hinges on the idea that most biological material contains a reasonably small set of characteristic fluorophore classes that can be used to differentiate it from non-biological material (16). Fluorescence microscopy has been utilized widely by all facets of the biological research community for many decades. The addition of fluorescent stains or tags to biological tissues, cells, organelles, fluids, or excretions is particularly powerful in the characterization of material and is routinely applied to the offline analysis of bioaerosol material (e.g., 15). Most online analysis of bioaerosols using fluorescence spectroscopy, however, requires some existing components of the aerosol to exhibit intrinsic fluorescence, or autofluorescence. A full review of the compounds that fluoresce within commonly observed biological particles is beyond the scope of this review, and can be found via the resources cited here and linked references therein (16–22).

The broad classes of biofluorophores most commonly probed include: proteins, amino acids, coenzymes, cellular metabolites, and structural material (16, 20–25). While excitation sources of nearly all wavelengths have been utilized on some level for bioaerosol analysis, three wavelength regions have been utilized most commonly: nominally 280 nm, 350 nm, and 405 nm. Excitation at approximately 260–280 nm has been shown to promote fluorescence from certain amino acid residues common to proteins. For example, tryptophan, with an excitation maximum at 286 nm, is used as a key fluorophore for bioaerosol detection (21, 26, 27), and many technologies utilize Nd:YAG (Nd:Y<sub>3</sub>Al<sub>5</sub>O<sub>12</sub>; yttrium aluminum garnet doped with neodymium) lasers and other

optical sources to probe this wavelength of the electromagnetic spectrum. Observed fluorescence from excitation near 270 nm has often been used as a general marker for biological material, stemming from the assumption that fluorescence promoted at this wavelength comes as a result of proteins. Excitation at approximately 340–360 nm has been shown to promote fluorescence from the ubiquitous biological coenzyme NAD(P)H (nicotinamide adenine dinucleotide (phosphate)) (16, 28). Because of its role in cellular metabolism, the presence of this molecule, and by extension the observation of fluorescence promoted from this excitation region, has frequently been assumed to be useful as a marker for living or viable biological organisms. There is no doubt that fluorescence from NAD(P)H in controlled settings and from known microorganism types indeed correlates with cell viability. Extrapolating these connections to the ambient atmosphere or mixtures of unknown particle types is less clear, however, and mounting evidence in recent years has challenged early assumptions about the utility of applying fluorescence information toward conclusions about airborne microorganism viability. Lastly, excitation sources at 405 nm have been increasingly utilized within the last decade. This has been driven largely by the dramatic decrease in cost of 405 nm laser diodes, whose commercial development was revolutionized because of their application as the light source in Blu-Ray™ disk players. This has led to a growing list of instruments that apply 405 nm sources for bioaerosol detection. The preceding sentences are intended as a summary of the key source wavelengths that have been applied for real-time bioaerosol detection, but it is important to note two things. First is that sources at many other wavelengths have been utilized, and are able to promote fluorescence of biofluorophores at varying efficiencies. Second, the fluorophores listed above are simply those most commonly assumed to be detectable at these wavelengths. There are thousands of biofluorophores and also non-biological materials in the atmosphere that can fluoresce, and so it is important for individual instrument users to exert caution when interpreting real-world data. Fluorescence observed in ambient particles can be influenced not only by the presence of biological material, but also by certain interfering species such as some mineral dusts, humic-like substances (HULIS), and other breakdown products from biological material, as well as polycyclic aromatic hydrocarbons (PAHs), and some secondary organic aerosol species that include substituted aromatic hydrocarbons (16, 22, 29–33). Despite these complications, evidence suggests that real-time fluorescence detectors provide an important first approximation of the physical properties of many classes of airborne bioaerosol particles.

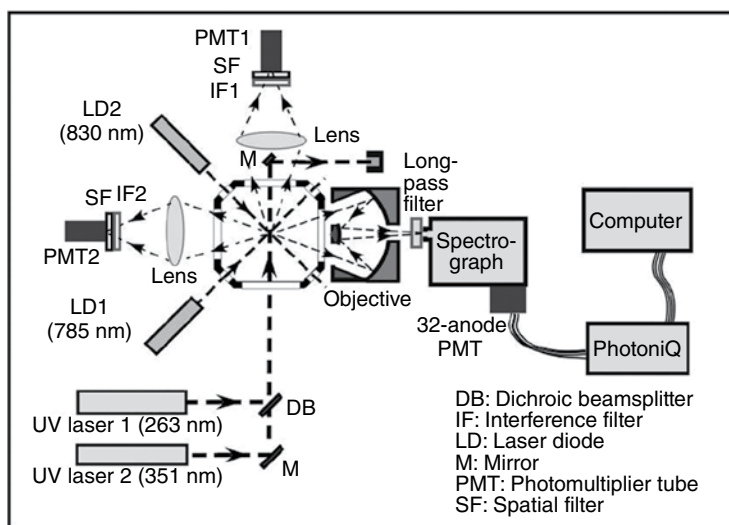
Correct interpretation of ambient biological aerosol measurements using fluorescence techniques must also necessarily include an understanding of the limitations of fluorescence to measure these particles. Fluorescence intensity increases strongly as a function of particle size and is especially dependent on the surface properties of the particle (e.g., membrane opacity) (34–38). Detector sensitivity is often also not usually flat as a function of wavelength, and so particle detection can be heavily influenced by technical details that influence whether a given particle will be above the noise threshold and thus categorized as fluorescent or not in a given spectroscopic channel. Different instrument types and different operators may employ very different thresholding strategies, which can dramatically influence the interpretation of results. As a result of these factors, results from different instrument types are often challenging to compare and it is critical that end-users properly keep these factors in mind when drawing conclusions from fluorescence-based bioaerosol spectrometers.

In addition to instrument considerations, changes that biological particles can undergo as they are exposed to atmospheric chemical processes have been shown to impact their fluorescence properties. To date, however, there has been limited study of these mechanisms, and the application of this understanding to the measurements is difficult. The impact of ozone and water vapor on the fluorescence of biological aerosols has been investigated for a number of types of aerosolized biological materials, for example *Bacillus thuringiensis* (39,40), and MS2 bacteriophage, an 8-mer peptide (25, 41). The studies with whole biological organisms showed decreases in fluorescence and indicated that an ozonolysis–hydrolysis process may be responsible for the observations. Spectra from 266 nm and 351 nm excitation of the peptide particles confirmed that this process impacted observations of particles by demonstrating decreases in 266 nm fluorescence coincident with increases in 351 nm fluorescence when these particles were exposed to both ozone and high relative humidity. It was proposed that this indicates the formation of *N*-formyl kynurenine (NFK) and kynurenine (KU) from the reaction of tryptophan with ozone and water vapor (e.g., 42). Similar to the effect that ozone has on biological compounds, proteinaceous material has been shown to exhibit significantly reduced fluorescence intensity upon nitration (43), although relatively little has been done on atmospheric samples. The ultraviolet (UV) component of sunlight is also likely to degrade biological fluorescence at all relevant wavelengths. Studies of particles exposed to ambient outdoor conditions in chambers at Adelphi, MD, USA, show a strong correlation between increasing UV radiation and degradation in the fluorescence of *Bacillus thuringiensis* and MS2 bacteriophage at 266 and 365 nm (25). These changes, as well as those from atmospheric chemical processes that have not been fully investigated, should be considered for any study of ambient biological aerosol, since they may negatively impact the ability to measure and characterize these particles with existing instrumentation.

The following sections will highlight the technologies and applications of some of the instruments most widely used within the civilian bioaerosol research field and those that have been discussed most often within peer-reviewed manuscripts. Many instruments now exist to measure the fluorescence of single particles that are both deployed in military conflict zones for threat detection and sold commercially for environmental and health research applications. It should also be noted that there is a significant amount of research, and even advanced technologies, that has remained primarily within military communities and that will not be dealt with here. Academic and other civilian researchers, however, have benefited significantly from the instrument development and initial research programs of defense-oriented communities.

#### 1.4.2.1 Single-particle fluorescence spectrometer

The single-particle fluorescence spectrometer (SPFS), developed at the United States Army Research Laboratory and Yale University, has been used to measure high-resolution spectral fluorescence from single aerosol particles at a single wavelength (263, 266, 351, or 355 nm) (44,45) or at two wavelengths (266 and 365 nm) (46,47). In these systems, particles are drawn into a small airtight chamber and focused into a laminar jet using sheath air. Particles larger than 1  $\mu\text{m}$  flowing through a triggering region at the intersection of two diode-laser beams (650 nm and 685 nm) are detected and subsequently excited by a single pulse generated by either a 263 nm (44,45) or 351 nm laser,



**Figure 1.4.1** General schematic overview of the single-particle fluorescence spectrometer SPFS instrument. (Reproduced from Pan et al. (47) with the permission of Elsevier.)

or by two pulses (one at 263 nm, another at 351 nm) in rapid succession (46,47). Rather than being collected into fixed bands as many competing instruments are, the emitted fluorescence within the SPFS is collected by a reflective objective and dispersed by a spectrograph to yield spectra of individual particle fluorescence, with 2 nm resolution. In more recent improvements on this device (41), particle size is also estimated using near-forward scattering from a 705 nm diode. These detailed spectral measurements allow the SPFS to help discriminate between mold, humic acid, and bacteria. This technique has also been demonstrated to help illuminate how reactions with ozone and water may affect bioaerosol fluorescence (25,41). Figure 1.4.1 shows one iteration of the SPFS. The instrument and its predecessors (48,49) have been utilized for extensive laboratory investigations (47–49), and several times for field observations (44–46).

#### 1.4.2.2 Two-wavelength single-particle fluorescence analyzer

Another one-of-a-kind instrument used for multiwavelength excitation and measurement of bioaerosols has been developed by the United States Naval Research Laboratory. Sivaprakasam et al. (50) describe a two-wavelength excitation, single-particle fluorescence analyzer (2-SPFA) that utilizes a five-channel emission band system to characterize biological particle types. This system uses the output of two pulsed Nd:YAG lasers at 266 nm and 355 nm to sequentially excite aerosol particles as they pass through the measurement region. Outputs of the 266 nm excitation pulses are measured over three bands: a 70 nm wide band centered at 350 nm, an 80 nm wide band centered at 450 nm, and a 100 nm wide band centered at 550 nm. The output of the 355 nm excitation is measured over the bands centered at 450 nm and 550 nm. Particle size is estimated by using elastic scattering from a continuous wave (CW) 785 nm diode laser. This work demonstrated that the fungal spores and bacterial spores studied could be easily

discriminated from the rest of the biological particles by using this multiwavelength technique. A refinement of this technique was described by Sivaprakasam et al. (51), who showed improved discrimination of bioaerosols from non-biological background particles, particularly against diesel soot, a prevalent interferent for UV fluorescence sensors. A modified version of this instrument enables selective targeting, electrical charging, and collection of single particles of interest, based on their spectral signature, onto a conductive substrate to provide a sample for further analysis (29). This system permits correlation of ambient aerosol spectral signatures with chemical or biological compositional analysis.

### 1.4.2.3 Fluorescence aerodynamic particle sizer (FLAPS)/ultraviolet aerodynamic particle sizer (UV-APS)

#### Instrumentation

The fluorescence aerodynamic particle sizer (FLAPS) instrument represents a smaller and generally simpler instrument than the instruments described in the previous sections. Original FLAPS development was commissioned by the Canadian Department of National Defense at Suffield, Canada, to assess the feasibility of single-particle fluorescence sensing for live biological threat agent detection, and resulted in a breadboard instrument based on a TSI, Inc. aerodynamic particle sizer (APS) with a 354 nm HeCad CW laser added to excite fluorescence of the sized particles (52–54). Particle sizing is provided as an aerodynamic diameter, which convolves the particle shape, density, and size (55), and is achieved by measuring the particle time of flight between two red He-Ne lasers at 633 nm (54). The size of each particle is recorded in one of 52 logarithmically spaced size bins, or alternatively into one of 30 linearly spaced bins (56).

In the years following the initial FLAPS development, technology was licensed and sold by TSI, Inc. as FLAPS II and also as the ultraviolet aerodynamic particle sizer (UV-APS<sup>TM</sup>), models 3312 and later 3314 (57,58). These instruments are built on the optical design of a more recent APS design using a 780 nm diode laser to create the time-of-flight measurement beams, and a 355 nm third-harmonic Nd:YAG laser for excitation of fluorescence. The APS instrument has typically been reported to reliably size particles of 0.5–15  $\mu\text{m}$ , although the manual for the commercially available 3314 UV-APS states an upper range of 20  $\mu\text{m}$  (59). One benefit of the UV-APS is that the aerodynamic size generally allows the instrument to provide a more narrowly resolved particle size measurement than instruments that detect size optically. The pulsed fluorescence excitation laser is actively Q-switched and is triggered to illuminate each particle when it reaches the location of the 355 nm beam. The fluorescence emission is recorded for each particle as the total intensity detected by a single photomultiplier tube (PMT) filtered to detect in the wavelength range 420–575 nm (64-bin intensity resolution), so the instrument cannot spectrally resolve fluorescence emission. In addition to the aerodynamic size and fluorescence intensity, the intensity of light scattered by the near-infrared sizing beams is also recorded and in principle can be used as a measure of optical diameter; however, data are only recorded as ensemble distributions of particle size and fluorescence over a user-defined period (minimum 1 s). This limits the ability of a user to compare aerodynamic and optical size for discrete particles. Without the ability to investigate and compare the properties of individual particles, the broad applicability of the UV-APS for continuous measurements of ambient aerosol is significantly

diminished. Another practical limitation of the UV-APS is that it was sold by TSI, Inc. without data analysis software, rendering the complex data files difficult to interpret in detail. Several groups have written user-driven analysis code that can handle the large datasets (e.g., 57). As of June 2014, according to the TSI, Inc. website the model 3314 UV-APS has been discontinued and is no longer sold (59).

TSI, Inc. now sells a model 3317 FLAPS III<sup>™</sup>, spelled out as “fluorescent aerosol particle sensor,” in contrast to the original “sizer” version. This instrument evolved from the first FLAPS I (52) and from the model 3314 UV-APS. The FLAPS III can be combined with a particle concentration unit sold by Dycor, Inc. for a hyphenated instrument referred to as C-FLAPS (60,61). In comparison with the UV-APS, the FLAPS III is smaller and more robust by using a 405 nm diode laser for fluorescence excitation and two channels of fluorescence emission (430–500 nm and 500–600 nm), which aid in discerning the approximate emission spectra. The reported particle sizing is limited to a maximum of 10  $\mu\text{m}$ , and the unit weight was reduced significantly from 35 kg for the model 3314 UV-APS to 10.5 kg for the model 3317 FLAPS (62). Also significant is that the aerosol dynamic sizing feature was replaced in the FLAPS III with optical particle sizing, a change which eliminates the small accelerating nozzle that could clog.

The FLAPS III technology is also incorporated in another TSI, Inc. instrument, the BioTrak<sup>™</sup>, first offered in 2013 (63). The BioTrak is designed to meet standards for monitoring pharmaceutical clean rooms. It is configured so the aerosol is first drawn through a conventional optical particle counter at 28.3 L  $\text{min}^{-1}$ , then enters a 28:1 aerosol concentrator with a 50% lower efficiency cut point of 2.0  $\mu\text{m}$  before entering the FLAPS III detector at 1 L  $\text{min}^{-1}$ . Embedded software in the BioTrak reports particles as “viable” based on the FLAPS III measurements of light scatter and the two wavelength bands of fluorescence intensity.

### Application

The FLAPS and UV-APS instruments have seen significant application toward detection of BWAs and as bioaerosol monitors for industrial applications. These applications often do not appear in the peer-reviewed literature, however. The instruments have found basic research application in the last 15 years, mostly within the fields of indoor and outdoor aerosol science. Agranovski, Kanaani and co-workers published a series of papers (64–69) reporting performance evaluations with respect to fungal spores and bacteria aerosolized in the laboratory, analyzing aspects such as the link of viability with respect to emitted fluorescence intensity. More recently Saari et al. (70) reported a UV-APS performance evaluation in comparison with a second fluorescence-based bioaerosol sensor. Ratnesar-Shumate et al. (25) utilized the UV-APS alongside the Army SPFS instrument for testing of outdoor aerosols modified by urban pollution. The instrument has also been applied to investigate bioaerosol sources, emission properties, and aerodynamics in the built environment (71–74). The Bae group in the Republic of Korea has utilized the UV-APS for several studies involving bacteria aerosolized in the laboratory (75–78). Various other reports have investigated the utility of applying the UV-APS in a variety of settings, such as for detection of bioaerosol transmission in a hospital (79) and via automobile air-conditioning units (80).

Several groups have reported changes to the physical instrument or to the analysis process in order to gain more detailed information. Ma et al. (81) divided the emission channel into two detectors to roughly differentiate fluorescence emission by wavelength.

Kaliszewski et al. (82) applied a multivariate cluster analysis technique, as will be discussed briefly in Section 1.4.2.6. Utilizing the user-written software to allow investigation of ambient analysis, several papers have been published in the last 5 years using the UV-APS to monitor fluorescent aerosols in a variety of ambient outdoor settings. For example, the instrument was used to monitor semi-urban aerosols in Germany (56), in the pristine Brazilian rainforest (83–85) (Figure 1.4.2), southern India (86), and in several northern hemisphere forest locations (87,88). At a rural site in Colorado, USA, the fluorescent aerosol measurements from the UV-APS were shown to correlate strongly with aerosol associated with rainfall and also with ice-nucleating aerosol particles (89–91).

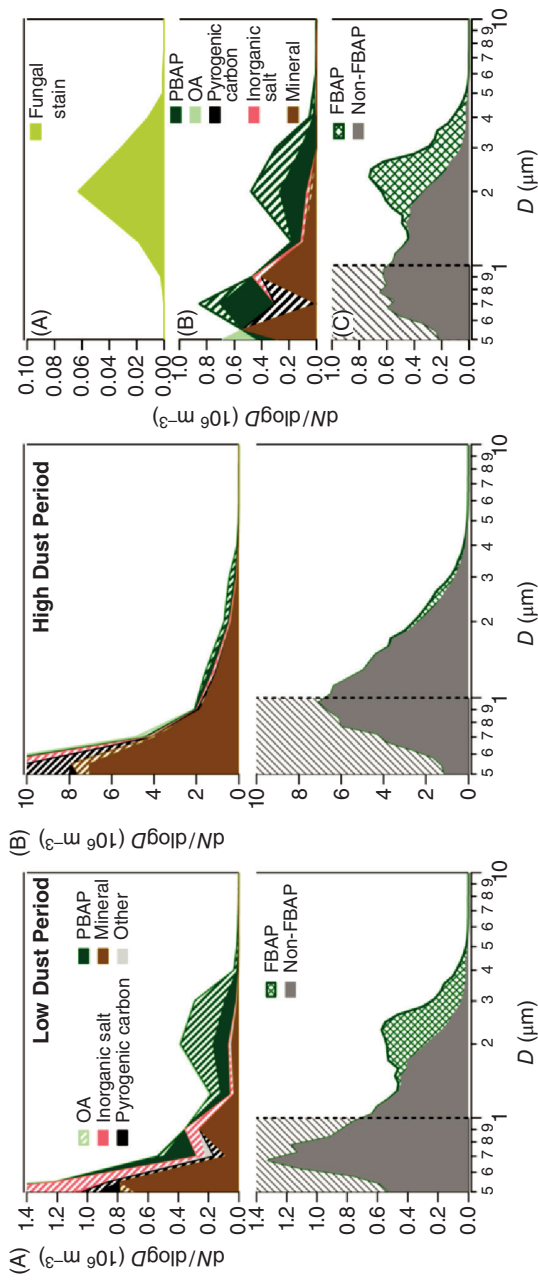
The main application of the FLAPS III/C-FLAPS is toward the rapid detection of biological agents (92,93); however, it has also been utilized for investigation of low levels of bacterial contamination in clean room air (94).

#### 1.4.2.4 Wideband integrated bioaerosol sensor (WIBS+) and spectral intensity bioaerosol sensor (SIBS)

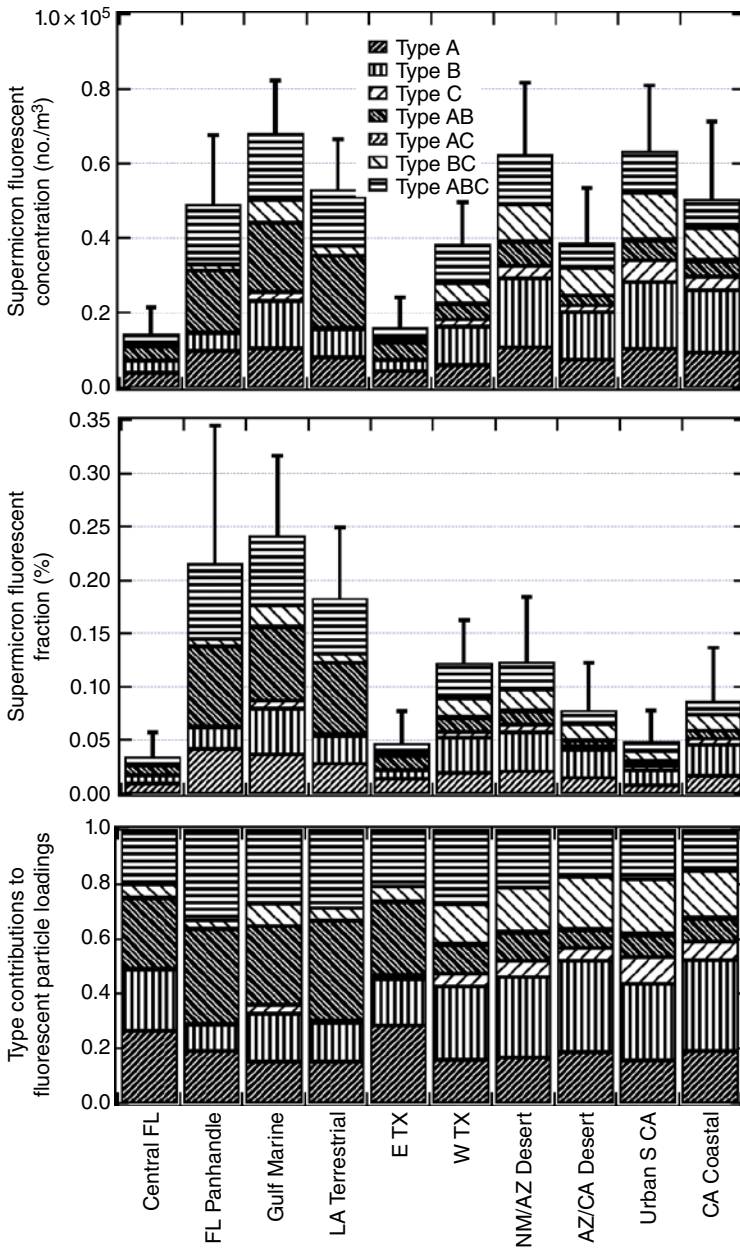
##### Instrumentation

The development of the wideband integrated bioaerosol sensor (WIBS) took place in parallel with that of the other technologies discussed above and was centered in the laboratory of Paul Kaye at the University of Hertfordshire, UK, with sponsorship from the Defence Science and Technology Laboratory of the UK (95–97). The instrument was originally named the wide issue bioaerosol sensor, but given the same acronym. After several generations of modification the WIBS-4 model comprised total aerosol detection using a continuous-wave CW red diode laser and fluorescence detection via pulsed excitation from two xenon flash lamps, optically filtered to provide 280 nm and 370 nm light, respectively. The intensity of fluorescence from the first flash is recorded in two bands: 300–400 nm (FL1 or channel A) and 410–650 nm (FL2 or channel B), whereas fluorescence from the second flash is recorded only in the second channel 410–650 nm (FL3 or channel C) (98). Forward scattering is collected onto a four-quantum detector to provide a rough estimate of particle asymmetry and optical size. Thus, for every particle sampled, five discrete pieces of optical information are recorded: optical size, particle asymmetry, and fluorescence from the FL1, FL2, and FL3 channels. Background fluorescence is recorded by measuring the fluorescent emission when no particles are present, or what is referred to as the “forced trigger” mode. Background values subtracted from the emission intensity of each particle are often determined as the average plus 3 standard deviations of the forced trigger fluorescence intensity in each channel, although other thresholding strategies have been investigated as well (e.g., 99). Perring et al. (100) coined terminology that relates whether an individual particle exhibits fluorescence above a given threshold by listing it by the letters associated with those channels, e.g., A, AB, ABC and so on (Figure 1.4.3).

The instrument is significantly lighter (13.6 kg) and smaller than the commercial 3314 UV-APS, because of its use of xenon flash lamps for fluorescence excitation rather than the Nd:YAG laser used in the UV-APS. The instrument has recently been licensed by Droplet Measurement Technologies, Inc. in Boulder, CO, USA. With relatively small weight and size (30.4 cm width × 38.2 cm length × 17.1 cm height), the WIBS-4A is easy to transport and deploy in most field locations. The WIBS has gone through several



**Figure 1.4.2** Application of the ultraviolet aerodynamic particle sizer (UV-APS) during a field study in remote Amazonia. Size distributions show similarity of results using fluorescent particles from the UV-APS and direct characterization using scanning electron microscopy, elemental analysis, and fungal staining. FBAP, fluorescent biological aerosol particle; OA, organic aerosol; PBAP, primary biological aerosol particle. (Reproduced from Huffman et al. (83).) (See color plate section for the color representation of this figure.)



**Figure 1.4.3** Application of the wideband integrated bioaerosol sensor (WIBS) data summarizing blimp trip across the USA showing particle category breakdowns. AZ, Arizona; CA, California; E, East; FL, Florida; LA, Los Angeles; NM, New Mexico; S, South; TX, Texas; W, West. (Reproduced from Perring et al. (100) with the permission of John Wiley and Sons.) (See color plate section for the color representation of this figure.)

generations of modifications, but the most recent versions offered by Droplet Measurement Technologies, Inc. are the WIBS-4A and the WIBS-NEO.

A recent outgrowth of the WIBS instrument has been the parallel development of two instruments, each with increased spectral resolution from the WIBS-4 and previous models. The first of the two instruments is the multiparameter bioaerosol sensor developed at the University of Hertfordshire. The instrument is conceptually similar to the Hertfordshire WIBS-4, but utilizes an eight-channel PMT for fluorescence detection. The instrument is still in development and has seen limited field use, with no peer-reviewed references to date (<http://www.herts.ac.uk/research/centres-and-groups/cair/particle-instruments-and-diagnostics/other-instruments>). The second parallel development from the WIBS has been the spectral intensity bioaerosol sensor (SIBS), developed and sold by Droplet Measurement Technologies, Inc. The SIBS underwent a number of hardware upgrades from its WIBS-4A predecessor, most importantly the addition of a 16-channel PMT in place of the two parallel single-channel PMTs. The SIBS and WIBS-NEO both originally reported the ability to provide time-resolved information about fluorescence lifetime, but, until further hardware upgrades are designed, the timescale of the lifetime measurement is unlikely to be sufficient for practical application of the feature. The SIBS is currently undergoing laboratory characterization and testing (101).

### Application

The WIBS has found increasing application by aerosol scientists and has been deployed for ambient study in various environments. The first field application of the Hertfordshire WIBS was to study bioaerosol properties in rainforest in Borneo (98,102–104). In the years following, the instrument has been applied in various field environments (38,100,105–114) and for laboratory characterization studies (33,101,115–119). One significant benefit the WIBS instrument has over, for example, the UV-APS is that it records information about each particle sampled. This allows the analysis of very low particle concentrations and at virtually any desired timescale. Recently, the WIBS-4A has flown on several airborne missions to investigate the vertical lofting of bioaerosol particles that might influence ice nucleation and cloud formation (100,120,121).

### 1.4.2.5 Other

Many other instruments utilizing fluorescence spectroscopy for single-particle bioaerosol analysis have been created and used. Some have been applied mostly for laboratory analysis at a given institute, and others have been produced with commercialization and field work in mind.

Starting in the late 1990s many groups around the world put effort into developing bioaerosol detection instruments, often for BWA detection (23,122–132). More recently there has been another wave of technical development. One particularly unique instrument was developed by the Technical University of Tampere in Finland. The excitation source is a pulsed, tunable laser that allows fluorescence excitation in a wide, variable range from 210 to 419 nm (133–135).

Within the last decade several additional bioaerosol fluorescence sensors have become commercially available, many as an outflow from academic research laboratories. The ENVI BIOSCOUT is sold by the Finnish company Environics ([www.environics.fi](http://www.environics.fi)) and uses a 405 nm excitation source and is marketed for the application

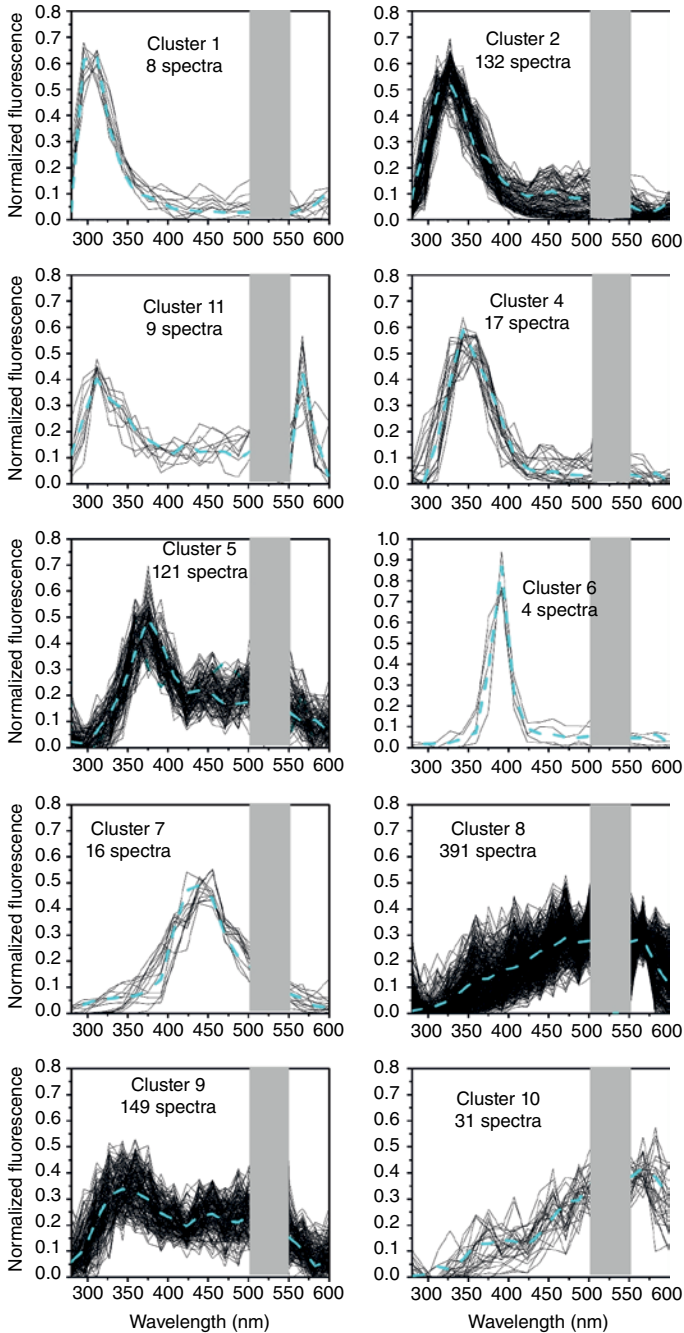
of monitoring harmful airborne biological particles (70,73,136). The instrument also collects air samples for offline analysis. The Biobox is sold by the Swiss company Plair ([www.plair.ch](http://www.plair.ch)) and uses two alternative modes of fluorescence excitation (137,138). Using the one-photon source, a wide band of light from a xenon flash lamp between 245 and 280 nm excites individual particles, whereas a multiphoton source utilizes femtosecond laser pulses at 800 nm. A US company, BioVigilant ([www.biovigilant.com](http://www.biovigilant.com)), sells the instantaneous microbial detection (IMD) device that also utilizes a 405 nm laser for bioaerosol detection and is marketed toward indoor aerosol detection and monitoring of aerosol sources that require clean indoor environments (139). With the recent technical advances in diode laser and UV LEDs, new technologies are being developed for commercial sale and for scientific research applications, and so the list of instruments mentioned here should not be taken as exhaustive (138, 139; e.g., 140).

#### 1.4.2.6 Data analysis strategies

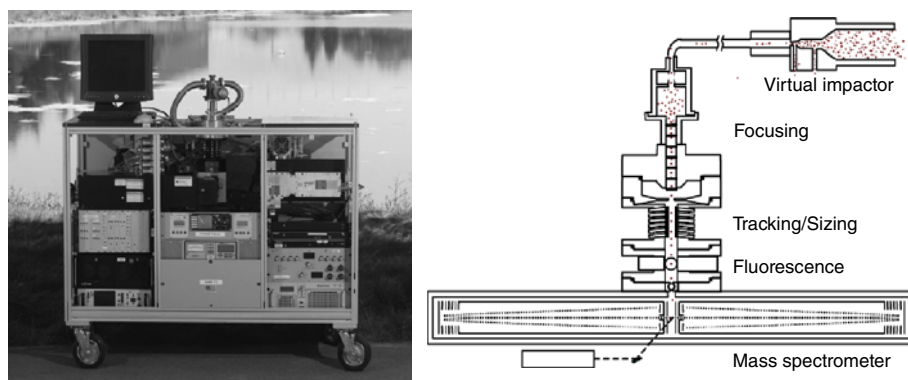
Many of the single-particle fluorescence spectrometers mentioned above have been deployed for either field detection of BWAs or ambient environmental aerosols. The collection of multiple points of information about every individual particle sampled creates huge volumes of data (up to many GB per day in some cases). This challenges standard methods of spectroscopic analysis and requires the application of advanced data analysis strategies. An overview of these strategies is beyond the scope of this chapter, but a few notes are provided here to allow the reader to investigate sources in further detail. One commonly applied method for multivariate analysis is cluster analysis (Figure 1.4.4), which collects fluorescence spectra into common groups that can then be compared selectively with spectra of known materials (82,106,114,141–143). Principal component analysis has also been applied by several groups to organize fluorescence spectra for more detailed interpretation (21,144,145).

### 1.4.3 Bioaerosol mass spectrometry

Mass spectrometry applied to single aerosol particles sampled and analyzed in real time has become an important tool for the investigation of the chemical and physical properties of aerosols in many environments. The full list of mass spectrometer (MS) types used for aerosol science and their applications is well beyond the scope of this chapter, but we will present an overview of instruments that have been widely utilized for the detection of bioaerosols. For a broader survey of aerosol MS work, see various reviews and sources cited here (146–153). Application of aerosol mass spectrometry for real-time bioaerosol detection is challenging, in part because real-time MS instruments typically sample submicron particles, or rarely up to 3  $\mu\text{m}$ . Particle inlets and lenses often do a poor job of collecting particles larger than 1  $\mu\text{m}$  in size, and often only a small fraction of a supermicron particle will be ablated and ionized (154). Most biological particles are larger than this size range (4,155), however, and so important sizes of PBAPs are typically not accessible to MS techniques and quantitative information gained is relatively uncertain.



**Figure 1.4.4** Clusters of individual particles collected in Las Cruces, NM, by the SPFS instrument. (Reproduced from Pinnick et al. (143) with permission from Elsevier.) (See color plate section for the color representation of this figure.)



**Figure 1.4.5** Picture and schematic overview of the BAMS instrument. (Reproduced with permission from Steele et al. (156).) (See color plate section for the color representation of this figure.)

### 1.4.3.1 Bioaerosol mass spectrometry (BAMS)

The bioaerosol mass spectrometry (BAMS) system exists as an internally hyphenated UV laser fluorescence instrument for spectroscopic identification of individual aerosol particles, paired with simultaneous chemical analysis achieved by mass spectrometry (156–161). The instrument utilizes fluorescence excitation from the third and fourth harmonics of a Q-switched Nd:YAG laser at 266 nm and 355 nm, respectively (Figure 1.4.5). Each individual particle is sized, and then desorbed and ionized by a laser pulse before resultant ions are analyzed via time-of-flight (TOF) mass spectrometry simultaneously in both positive and negative ion modes. The tremendous benefit to fluorescence spectroscopy paired with mass spectrometry is the combination of physical and chemical information on a single-particle basis. This allows higher level discrimination between non-biological and biological particles. The instrument was developed by Lawrence Livermore National Laboratory for the detection of biological aerosol threat agents. The BAMS was deployed at San Francisco Airport, CA, for testing (156).

### 1.4.3.2 Aerosol time-of-flight mass spectrometer (ATOFMS)

The aerosol time-of-flight mass spectrometer (ATOFMS) was developed for the chemical analysis of single aerosol particles. The MS operates similarly to the BAMS, in that single aerosol particles are desorbed and ionized by a pulsed laser source and ions are characterized by time-of-flight mass spectrometry. In this system, however, no additional fluorescence excitation is utilized and so analysis of particles is limited to chemical characterization of ions via mass spectrometry. The instrument has been widely used for chemical analysis of ambient aerosols, but it is not specifically designed for application to bioaerosol research. Mass spectrometry results are interpreted through algorithms that search mass spectral libraries for similar ion mixtures. Using key markers such as potassium (K), phosphorus (P), and abundant organic ions (162) the algorithms suggest certain particles to be biological in source. The ATOFMS has thus been utilized to investigate ambient biological particles in a number of field and laboratory experiments over the last decade (162–165).

### 1.4.3.3 Aerosol mass spectrometer (AMS)

Similar to the ATOFMS, the Aerodyne aerosol mass spectrometer (AMS) has been utilized widely for investigation of ambient aerosols (146,166,167); however, it has been only relatively rarely applied to the investigation of biological aerosol particles. The AMS samples individual aerosol particles and focuses them using an aerodynamic lens. Particles are sized by particle time-of-flight between a chopper wheel and the MS detector. Particles undergo flash vaporization on a heated surface before being ionized by electron impact ionization and mass analysis by time-of-flight mass spectrometry. The approach of using thermal vaporization generally limits the AMS to interrogating non-refractory materials, and because whole bioparticles do not vaporize easily, they are not investigated with high efficiency. A number of researchers have used the AMS to look at semi-volatile chemical markers consistent with PBAPs, and thus the instrument can have important application to bioaerosol detection in some environments (84,154,168–170).

### 1.4.3.4 Other

Many other real-time mass spectrometry systems have been developed and applied to ambient detection of PBAPs. A full review of these instrument is beyond the scope of this chapter; however, a few selected publications are given here for reference (129,171–178).

## 1.4.4 Other real-time bioaerosol detection techniques

### 1.4.4.1 Light detection and ranging (LIDAR)

Light detection and ranging (LIDAR) techniques have been utilized frequently for the detection of PBAPs in the atmosphere. They provide data in real time, but integrate the signal over long distances (kilometers) and so do not provide information about individual particles. Fluorescence-based LIDAR systems were developed as early as the 1970s (179). Since that time, a number of LIDAR systems have been developed and deployed (142,179–192).

### 1.4.4.2 Resource Effective Bioidentification System (REBS)

Raman spectroscopy has been utilized to characterize PBAPs (e.g., 191–199), but has not been applied in real time until recently. The Resource Effective Bioidentification System (REBS), developed and sold by Battelle, is a Raman microspectrometer for the characterization of aerosol particles, including airborne microorganisms at the biological species level. The narrow linewidths of Raman spectra allow detailed characterization of aerosol particles of both biological and non-biological origin, while enabling the collection of information at temporal resolutions significantly beyond competing Raman microspectrometers. The instrument represents an important step forward in the real-time detection of biological aerosols, but has not yet found broad application by civilian or academic research groups. The instrument is an autonomous sampling unit that collects airborne particles larger than 300 nm in diameter onto a metalized

tape substrate (200). The tape is then moved forward so that collected particles can be interrogated by a laser diode at 640 nm wavelength, focused to a line so that Raman spectra of a few individual particles can be measured at the same time using an image-preserving spectrograph with a charge coupled device detector. The Raman spectra are measured on a particle-by-particle basis at an approximately 1  $\mu\text{m}$  resolution. Spectra of collected particles are then compared with Battelle-provided spectral databases. In contrast to the current state-of-the-art fluorescence-based instruments, REBS spectra utilize unique combinations of detailed molecular information that claim to deliver biological species-level discrimination for a select set of biothreat agents (200–202).

#### 1.4.4.3 Molecular tracer techniques

A number of molecular tracers have been linked to various classes of PBAPs and are routinely utilized for the investigation of bacteria, fungal spores, and pollen (e.g., 201–206). These techniques typically utilize some form of column chromatographic separation or mass spectrometry of sample suspensions and are thus not easily applied to real-time analysis. Several attempts have been made to perform such chemical tracer analysis in real time, however. Dworzanski et al. (207) built an automated pyrolysis–gas chromatography/ion mobility spectrometer (Py-GC/IMS) for PBAP detection that looked for the presence of picolinic acid and pyridine as biochemical markers in bacterial spores walls. The technique has been steadily refined and frequently used in laboratory and field studies by the developing group (208–212). A group in France has also recently developed a technique to look at anhydrosugars as tracers of fungal spores using an online anion exchange chromatography system (213). They have recently used the system to estimate fungal spore number concentrations in suburban Saclay, France (213).

#### 1.4.4.4 PBAP detection via elemental analysis

Breakdown spectroscopy can be a powerful tool to provide elemental analysis of individual particles, and has been applied to PBAP analysis, although its application in real time has been limited. Laser-induced breakdown spectroscopy (LIBS) and spark-induced breakdown spectroscopy (SIBS) both enable single-particle PBAP analysis (214–218).

#### 1.4.4.5 Automated pollen counting

Motivated by the desire to estimate atmospheric patterns of key allergenic classes of pollen in real time, many attempts are being made to automate pollen counting and characterization. One such approach utilizes the fact that pollen grains of different size and shape scatter light with unique patterns. By comparing simultaneously scattered light by individual particles in the side and forward directions from a 780 nm laser by an algorithm suggests the class of pollen grains sampled and reports an estimated concentration in real time (219).

Many other methods have been investigated for automated palynology to varying degrees of success (220–232), although these are largely not real-time methods and will not be discussed in detail here.

## Acknowledgments

The authors acknowledge assistance from the following colleagues who provided additional material and perspective to the chapter: I. Crawford, K. Froyd, P. Hairston, S. Hill, J. Ho, J. Schneider, V. Sivaprakasam, and L. Williams.

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## **Part II**

### **Sources and Transport of Microbial Aerosols**

## 2.1

### Bioaerosol Sources

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#### 2.1.1 Introduction

The Earth's biosphere provides many diverse and important sources of bioaerosols (Figure 2.1.1). Microorganisms inhabit most plant, soil, and rock surfaces with high concentrations per unit surface area ( $10^4$ – $10^8$  cells  $\text{cm}^{-2}$ ) in various natural environments (1). They can be released from every region of the globe and are ubiquitous in outdoor air. In the near-surface atmosphere, where microbial cell numbers range from  $10^4$  to  $10^6$  cells  $\text{m}^{-3}$  over land (2), microbial cells account for a significant fraction of aerosolized organic carbon. The dominant sources of bacteria in the atmosphere include environments as diverse as soils, waterbodies, leaf surfaces, and animal feces. The relative importance of these sources varies with altitude, season, location, and meteorological factors in general (3–7). In most terrestrial environments the main providers of bacteria in the near-surface atmosphere are plants (i.e., leaf surfaces) and soil (8, 9). The global leaf surface area is estimated to be approximately four times the terrestrial ground surface area ( $\approx 6.4 \times 10^8 \text{ km}^2$  and  $\approx 1.5 \times 10^8 \text{ km}^2$ , respectively).

Human concentrations in big cities, as well as agricultural and industrial activities (livestock, cultivated crops, intensive farming, peat- and wood-processing facilities, waste treatment plants, composting platforms, cooling towers, etc.), constitute important sources of bioaerosols (10). Bowers et al. (3) found that cow fecal material was a dominant source of bacteria in airborne communities in rural areas, together with soils and leaves; in this study, the cow fecal bacteria probably originated from two large cattle feedlots located near the sampling point. Biological aerosol particles originating from animals and humans include debris from skin and hair as well as excrement, brocho-somes, and eggs dispersed into the atmosphere by insects (1). People spend up to 90% of their lifetime indoors, and 50% live in urban areas (11). Indoor air accounts for approximately 0.3 millionth of tropospheric air (12).

In addition to particles coming in from outdoors, important sources of bioaerosols in indoor environments include material resuspended from surfaces as a result of human activities and human fluids emitted via coughing, sneezing, talking, or breathing (13).

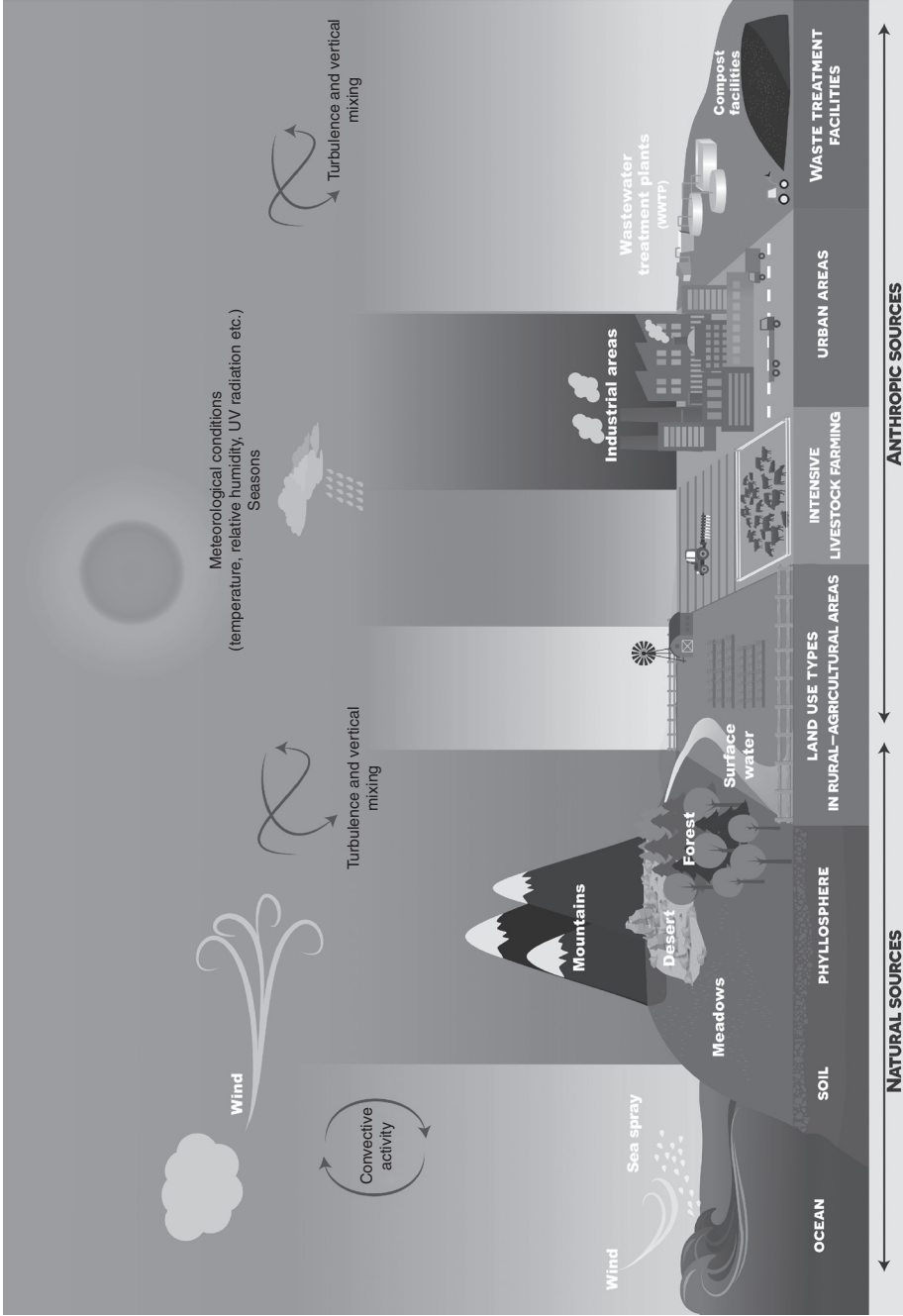


Figure 2.1.1 Outdoor sources of bioaerosols.

Indoor bioaerosols are mainly influenced by the microbial diversity from human sources (12). The direct particle shedding of desquamated skin cells and hair, together with their subsequent resuspension, strongly influences the structure of airborne bacterial populations (13, 14).

The surface of the oceans, which covers 70% of the globe, ejects living and decaying organisms, including archaea, bacteria, fungi, and algae, by bubble-bursting mechanisms. Above biologically active ocean areas (Arctic and Southern), the number of bacteria is still much smaller than the total number of particles, and consists mainly of biogenic organic aggregates and colloids (1). The cryosphere (e.g., Greenland, Antarctica, glaciers), largely formed from precipitation and ice, may contain bacteria that settled through precipitation. Surface snow in wind-blown conditions can also constitute a source of bioaerosols via resuspension. Finally, arid and semi-arid regions of the globe exhibit biological crusts consisting of bacteria, fungi, algae, lichens, and bryophytes in variable proportions, which can be eroded from the surface: small dust particles are produced and subsequently transported over long distances during storms (1).

Microbe aerosolization from these various sources occurs through two main mechanisms that will be described in Section 2.1.2. Aerosolization can be characterized by measuring emission fluxes but this remains a challenging task, as reported in Section 2.1.3. The type of aerosol source plays a role in the various characteristics of the bioaerosols released. In particular, it affects the size of airborne microorganisms and their residence time in the air. According to Burrows et al. (2), the residence time of bacteria may be shorter for bacteria emitted from the ocean than for bacteria emitted from land surfaces, because of more rapid removal by precipitation. Also, consistent with the smaller size of marine bacteria, the median count diameter of particles associated with culturable bacteria has been found to be smaller at coastal sites (around 2  $\mu\text{m}$ ) than at continental sites (about 4  $\mu\text{m}$ ) (15, 16). The impact of the source type on microbial concentration and microbial diversity will be developed in Section 2.1.4.

Studies on outdoor sources of bioaerosols mainly deal with bacteria, pollens, and fungi, whereas data on other primary biological aerosol particles such as archaea, viruses, algae, and cyanobacteria are rather limited. The relative importance of these biological particles can vary drastically with the source type: in pristine tropical rainforest air, for example, fungal spores account for approximately 45% of coarse particulate matter (1). The presence of archaea has mainly been reported in environments rich in biological material: compost piles, biosolids, biogas, or dairy barns (7, 17, 18). The major sources of airborne algae are the aquatic and terrestrial algal populations (19). Algae growing on solid surfaces such as soil, tree bark, rocks, and swamped wall surfaces may indeed be carried away by wind erosion. Little is known about their presence in the atmospheric environment.

## 2.1.2 Emission mechanisms

### 2.1.2.1 Passive and active release

The emission of microorganisms from a source to the air may occur via two processes: active and passive release. Passive bioaerosols are emitted when the material is

disrupted by external forces greater than the bonding forces between the particles and the source surface. Such movement may typically occur as a result of wind or the impact of raindrops (20). Microorganisms can also release biological material by active mechanisms whereby they eject them into the surrounding air. The process of active release is triggered by an internal mechanism that is specific to the biological species, in response to environmental factors such as relative humidity or temperature. Active release is prevalent among some fungi and pollen species. Elbert et al. (21) showed that the active discharge of fungal spores, which is accompanied by the emission of aqueous droplets (*Ascomycota* and *Basidiomycota* in particular), is an important source of primary biogenic aerosols. In this case the presence of liquid water, as either rain or dew, is necessary to stimulate release. Active discharge can also occur upon drying when spores are discharged without accompanying liquids. Active mechanisms of bioaerosol emission include “ejection” by humans or animals through the emission by disease carriers of expelled particles or droplets containing infectious particles ejected by sneezing or coughing in particular (2, 22). By extension, active release can be defined as an emission due to the mechanical disturbance of a microbial source other than agitation resulting from wind or the impact of raindrops (2). For man-made emissions, such as composting, one can distinguish passive emission by wind erosion (without manipulating the composting pile) from active emission when the pile is agitated during mixing, screening, or turning.

Thus, the emission mechanisms from different types of sources depend on forces resulting in two main mechanisms: (i) abrasive dislodgment (suspension of soil, plant, or biological materials by agitation) and (ii) bubble bursting (at the water–air interface). These mechanisms are affected by physical parameters (temperature, air or stream velocity, humidity, radiation on the surface, etc.). Numerous outdoor studies invoke the necessity for a threshold force, commonly due to the mean or instantaneous wind velocity, to remove material from the surface by either blow-off or surface movement (23). At wind speeds greater than  $4 \text{ m s}^{-1}$ , primary marine aerosols are formed via bubble bursting from breaking waves, and material from dry soils with sandy textures can be aerosolized (7, 24). The threshold velocities for plants, which depend on the plant and the material to be released, are smaller than the threshold velocities for soil surfaces.

### 2.1.2.2 Erosion, abrasive dislodgment, and abrasive damage

Wind erosion, wind abrasive dislodgment, or damage are important bioaerosol mechanisms over land, desert, and plants. Over land and desert, wind velocity, soil moisture, and soil texture control soil aerosolization. In agricultural fields, wind aerosolization events may occur many times a year. Wind velocities as low as  $4\text{--}10 \text{ m s}^{-1}$  can aerosolize material from soils with sandy textures and low moisture content (7). Parts of biological soil crusts from deserts and semi-arid regions may be suspended in the air as a result of wind erosion across the continent and lift from local wind (1, 25, 26). In the phyllosphere, the release of biological material from leaf surfaces may be caused by adjacent leaves rubbing each other, by leaves flexing in the wind, by repeated impaction of leaf tips, or by the impaction of wind-borne particles and precipitation onto the leaf surface (27, 28). These processes are presented in greater detail in Chapter 2.3.

### 2.1.2.3 Bubble bursting

Bubble bursting constitutes one of the most important processes for the generation of aerosols from water surfaces (20, 25, 29–31). Löndhal (25) reported three ways of bioaerosol production in open waters: spume drops issued from breaking waves, films drops, and jets drops originating from bubbles. Film drops are produced from the collapse of the thin water film that separates the bubble air from the atmosphere, just before bursting. Jet drops are produced from the jet that rises from the collapsing bubble. The size distribution of film drops ranges from 0.1 to over 10  $\mu\text{m}$  in diameter, whereas the size of jet drops varies by more than a factor of two or three (32). Marine bioaerosols represent a major natural aerosol system via the bubble-bursting process from whitecap generation. Thus, when a wave breaks a large volume of air is entrained in the water and dispersed into a cloud of bubbles that rise to the surface and burst (33). Aerial release can also be achieved during scum and foam formation at the water surface, as well as during their further total or partial disintegration (34). The quantity of biological material carried by the bubbles and then released by their disruption can be enriched by several orders of magnitude, as compared with the concentration in the water from which the aerosol is produced (1, 20). Microorganisms can also be dispersed by bubble bursting when raindrops impact water surfaces or porous soil surfaces (35). When raindrops impact a porous surface, bubbles form at the droplet–surface interface due to the entrapped air. The newly formed bubbles reach the air–water interface and their rupture generates aerosols with tiny water jets, resulting in elements of the porous medium being released into the environment. Thus, there is evidence of aerosol generation from rainfall impaction on the soil, with a strong correlation between aerosol generation, surface wetting properties, and droplet impact velocity (35).

### 2.1.2.4 Emissions from man-made systems

Man-made systems include agricultural, waste-handling processes such as composting and wastewater treatment, but also urban areas. The active production of bioaerosols results from mechanical disturbance. For example, most waste management operations generate episodic or periodic active release during the operational cycle, e.g., unloading, grinding, mixing, turning, screening of compost piles, aeration in wastewater tanks, or land application of biosolids and animal waste. The intensity of the episodic release to the air depends on particle size, surface loading, surface conditions, wind velocity, atmospheric and surface moisture, and the intensity of the mechanical disturbance generating the suspending activities (36). The wide range of processes associated with man-made systems emit concentrated fugitive bioaerosols but also constitute a source of passive bioaerosol emissions (20, 37). For example, in compost facilities, *Aspergillus fumigatus* and actinomycetes concentrations measured during the active turning of green waste compost were typically 3-log higher than previously reported concentrations from static compost windrows (37). Existing estimates of microorganism fluxes to the atmosphere show that these fluxes are significantly lower over undisturbed croplands than over disturbed man-made systems (1). Baertsch et al. (7) showed that, beyond a threshold velocity, wind is able to produce aerosolization and off-site transport of land-applied biosolids (e.g., stabilized sewage sludge) from agricultural fields. The mechanical agitation of the litter material, hay, or silage that is present in animal houses,

or that is moved during feeding operations, is known to be a significant source of bioaerosols (38). In wastewater treatment plants, operations involving the aeration of wastewater by mechanical agitation or air bubbling have also been identified as important bioaerosol sources. Tanks in which oxygen is supplied via mechanical agitation induce a remarkable dispersion of microorganisms. Bubbling aeration systems (air diffusers, surface turbine, horizontal rotors) may have a lesser impact but the choice of the system remains important, as it induces different levels of bioaerosol release (39, 40).

#### 2.1.2.5 Differences in concentration factors between microorganisms: selection during aerosolization

Differences in size, shape, cellular organization, and particle adhesion (to dust, salt, or organic matrices) can lead to differential aerosolization. Concentration or enrichment factors, defined as the ratio of air concentration to the concentration in the bulk source, have been defined to quantify the aerosolization capability of a particular microorganism. Hejkal et al. (41) studied the water-to-air fractionation of different bacterial species and found that the concentration factors of *Serratia marinorubra* and *Micrococcus euryhalis* were generally 10–100 times greater than those of *Escherichia coli*, *Pseudomonas bathycetes*, and spores of *Bacillus subtilis* (41). For aquatic surfaces, differential adherence of bacteria to bubbles during their ascent through the water column, or differences in volatilization during bubble bursting, have been identified as the likely mechanisms responsible for differential aerosolization (42). The morphological and physiological characteristics of microbial cells have an effect on the concentration factor, such as the lipidic composition of the cell or the production of secondary metabolites. Hejkal et al. (41) showed that the concentration factor of *E. coli* increases with cell age, indicating that cell composition may be a factor that influences concentration in jet drops. Prodigiosin, present in some pigmented cells of *Serratia marcescens*, increases aerosolization from water by a factor of 10–80 (43, 44). Pigments help bacteria to better survive the harsh conditions of life in air by trapping the free radicals and oxidants, but they may also affect their ability to be released in the air. Fahlgren et al. (42) analyzed the differential transfer of marine bacteria to aerosols and showed that the selection during aerosolization may explain the elevated proportions of pigmented bacteria frequently observed in atmospheric samples, although the mechanisms remain unexplained. They further found that, although in a majority of cases operational taxonomic units (OTUs) present in the seawater were also present in the aerosols, many OTUs were either selectively enriched in the aerosols or only slightly aerosolized. Some microbes common in the atmosphere use chemical induction of water condensation to increase their dispersal by wind: high dimethylsulfide emission by marine algal blooms may increase dispersal rates by generating a local increase in wind speed favoring the take-off process (45).

Selection during aerosolization was also observed in industrial anaerobic digestors by comparing microbial abundance in the digester and that in the biogas above. Three different patterns of aerosolization behavior were identified: (i) that of non-aerosolized microorganisms, Deltaproteobacteria, Spirochaetes, Thermotogae, Chloroflexi phyla, and sulfate-reducing groups, (ii) that of passively aerosolized microorganisms, including Actinobacteria, Firmicutes, and Bacteroidetes phyla, and (iii) that of preferentially aerosolized microorganisms, including Alpha-, Beta-, and Gammaproteobacteria (46).

Aerosolization of bacteria, archaea, *Synergistes*, *Staphylococcus* spp., and *Propionibacterium acnes* in the anaerobic digestors was investigated *in situ* with quantitative real-time polymerase chain reaction: *Staphylococcus* spp. and *P. acnes* were aerosolized 30 and 220 times more than bacteria, respectively, and archaea and *Synergistes* eight and 20 times less, respectively (18). Opportunistic pathogens may be more susceptible to aerosolization, since they use air as a dissemination vector.

## 2.1.3 Measuring emission fluxes

### 2.1.3.1 Introduction

The emission flux, or source strength, quantifies a number, or a mass, of microorganisms emitted by a given surface area during a given period of time (e.g., number of bacteria emitted per square meter per second). Determining this flux should be very useful for understanding the dynamics of emission and its relation to environmental factors. However, very few studies have provided proper estimates of the emission flux. A vast majority of the observations devoted to atmospheric microorganisms have only been based, up until now, on experimental determinations of the microbial content of air samples, collected in the vicinity of or at some distance from the emitting surfaces. Such measurements can be qualitative (e.g., species identification) or quantitative (air concentration, e.g., number of bacteria per cubic meter of air). They provide a snapshot of the microbial load of the air mass over the sampling duration, but they cannot alone give indications on the intensity and direction of the transport of microorganisms.

One of the main reasons for this lack of results lies in the difficulty of performing adequate flux measurements. Among other problems, the standard method for measuring atmospheric fluxes over large surface areas, the so-called “eddy-covariance method” (see (47)), is not applicable since it requires high-frequency concentration measurements (with sampling intervals typically less than 1 s): such measurements have been repeatedly performed for many gaseous species (e.g., carbon dioxide, water vapor, ozone, nitrogen compounds) but are not currently feasible for bioaerosols owing to the lack of adequate sampling technology. Several other methods are *a priori* possible for measuring emission fluxes, depending on the size of the source under investigation.

### 2.1.3.2 Chamber measurements

The general principle is to enclose for a certain time the emitting surface, or part of it, in a chamber, and count all particles either collected therein (in the case of a “closed chamber”) or carried by a throughflow of air (“open” chamber). This method was used by Jarosz et al. (48) to estimate daily pollen shed in a maize field. Every day during the pollination season they placed polythene bags over the tassels of five plants and left them for a period of 24 h, after which the number of pollen grains collected was estimated with a cell counter. Taha et al. (36) used a portable wind tunnel to estimate the release of fungi and ascomycetes from static compost windrows. Sawyer et al. (49) measured bacterial aerosol emission rates from wastewater aeration tanks. A tower bearing a six-stage viable particle sampler was partly immersed in the aeration basin, so that the sampler was located just above the water surface; the emission flux was determined from the bacteria concentration and the air rise velocity in the tank (49). Note

that the same principle can be used for “virtual” chamber calculations, as Mayol et al. (50) did to estimate the flux of microbes associated with seawater spray. Altogether, such measurements can only be used over small surfaces, and suffer from the fact that any enclosure is prone to modifying the local conditions possibly acting on the emission (wind velocity, turbulence intensity, radiation, air temperature and humidity, etc.).

### 2.1.3.3 Flux–gradient relationships

Alternate methods to the “eddy-covariance” one were of standard use for gas and energy flux measurements in the field before the former became commonly used. These “classical” methods are based on the fact that in the atmospheric surface boundary layer (say, the lowest 1–100 m of the atmosphere) the vertical profiles of wind, temperature, and scalar variables are linked to the corresponding fluxes (momentum, sensible heat, scalar fluxes, respectively) through similarity relationships (see (51)). Measuring vertical concentration gradients of a given scalar (pollen, spore, or bacteria concentration, for example) can therefore allow the vertical flux of this scalar to be estimated, provided that the conditions of applicability of the similarity laws are fulfilled. It must be noted that this method, which requires sampling at two fixed heights at least, provides a net vertical flux corresponding to the emission flux minus the deposition flux. The latter must therefore be estimated for the emission flux to be derived (see Chapter 2.2).

The first attempt to measure microbial fluxes using flux–gradient relationships was made by Lindeman et al. (52) with the “aerodynamic method.” By measuring horizontal wind speed and bacteria concentration at two different levels above various surfaces (bare soil, maize, wheat, bean, and alfalfa crops), at unspecified times of day, these authors measured upward vertical bacterial fluxes, which varied between 57 and 543 colony-forming units (CFU)  $\text{m}^{-2} \text{s}^{-1}$ . In a later study Lindeman and Upper (53) reported upward fluxes of bacteria over beans during the warmest part of sunny days with an average value of approximately 10 CFU  $\text{m}^{-2} \text{s}^{-1}$ . In this method the bacterial flux,  $F_b$ , is proportional to the differences in horizontal wind speed,  $\Delta U$ , and bacterial concentration,  $\Delta C_b$ , between the two levels, with a proportionality factor that depends on the measurement levels, and on other factors that were neglected in these studies (vertical temperature gradients, or sensible heat flux, in particular).

Later, Lighthart and Shaffer (54) used a modified “Bowen ratio” method to measure the bacterial flux at a desert site displaying a mixture of sagebrush, grass, and bare soil. With this method the bacterial flux was estimated as

$$F_b = F_T \times \frac{\Delta C_b}{\Delta T}$$

where  $F_T$  is the kinematic heat flux, measured independently, and  $\Delta T$  is the temperature difference between the same two levels where  $C_b$  was measured. The authors gathered a set of hourly vertical fluxes over several days. Although these fluxes exhibited very high variability, with alternating positive and negative values, the authors showed a tendency for the mean flux to be positive (i.e., directed upward) during daylight hours, with a maximum value of about 5 CFU  $\text{m}^{-2} \text{s}^{-1}$  in the middle of the day. Hardly any further study was conducted on this topic, apart from Chen et al. (55), who performed flux measurements of bacteria and fungi with the same method, at 30 and

50 m height at an urban site. At 30 m for instance, their bacterial flux oscillated between  $-25$  and  $+40$  CFU  $m^{-2}s^{-1}$ , and the fungi flux was close to 0 for most of the day, except for a peak at about 250 CFU  $m^{-2}s^{-1}$  in the middle of the day.

Using these profile methods requires stringent conditions to be fulfilled. In particular they can only be applied over flat, homogeneous surfaces, of sufficient horizontal extent for the investigated atmospheric layer to be in equilibrium with the surface. As a rule of thumb there is a factor of 100 between the windward distance to a change in surface properties (the “fetch”) and the height of the internal boundary layer in which profile measurements can be safely performed (51). A long enough fetch is therefore required between the edge of the field and the position of the mast bearing the sensors. Given the fact that the separation distance between the two measurement levels must be large enough for the measured concentration difference to be significant, the profile method can only be used over very large fields.

#### 2.1.3.4 A novel method for measuring vertical atmospheric fluxes?

An alternate method for measuring scalar fluxes has been developed, which is less demanding in terms of site characteristics than the profile method and which does not need fast-response sensors, as the eddy-covariance method does. This “relaxed eddy accumulation” (REA) method was introduced by Businger and Oncley (56), and has been used for a wide variety of scalars: aerosols, sulfur and nitrogen compounds, volatile organic compounds, stable isotopes, mercury, herbicides, etc. It is based on fast measurements of wind velocity and slow measurements of concentration. Its principle is to sample the considered scalar conditionally, according to the sign of the instantaneous vertical wind velocity,  $w$  : when  $w > 0$  the air is sent at constant rate to a reservoir, and when  $w < 0$  it is sent to another one. It can be shown that at the end of the integration period (30 min, typically) the flux  $F$  is proportional to the concentration difference between the two reservoirs

$$\Delta C = C_+ - C_-$$

where the subscripts + and – refer to the reservoirs with  $w > 0$  and  $w < 0$ , respectively:

$$\Delta C = \beta \times \sigma_w \times \Delta C$$

Here  $\sigma_w$  is the standard deviation of vertical velocity and  $\beta$  is a parameter depending on the conditions of acquisition (57). Work is currently performed for adapting the REA method to flux measurements of biotic aerosols (58). It seems that its accuracy is acceptable provided adequate operating precautions are taken, allowing in particular  $\Delta C$  to be maximized. Several problems still have to be solved: quality of the sampling, automation, limitation of contaminations, possibility of keeping the microorganisms viable, etc.

#### 2.1.3.5 Downwind dispersion modelling

The aerosols emitted by a surface are submitted to turbulent dispersal in the vertical and horizontal directions. At a given height, the aerosol concentration decreases along the mean wind direction at a rate that depends on environmental characteristics (wind

speed and turbulence intensity, buoyancy forces, surface roughness, etc.). Provided that the source can be clearly identified, this downwind decrease can be modelled in various ways (see Chapter 2.2), using for instance, in the simplest cases, Gaussian plume dispersion equations. The combination of such a model with measurements of the downwind variation in concentration allows the emission flux to be estimated as the source strength, providing the downwind concentration profile that fits best with the measured values.

The success of this back-calculation approach depends on the validity of the model used in the situation considered (site topography, presence of heterogeneities, climatic conditions, etc.). It has been used for estimating microbial emission fluxes from sewage sludge piles (59), green waste compost facilities (37), and manure application sites (60), as well as for estimating the source strength of pathogens at field scale (61) and landscape scale (62). Note that this general principle has also been used at the global scale to derive bacterial emission rates representative of each ecosystem, by matching literature estimates of bacteria concentrations to concentrations simulated by a general circulation model including a module for bacterial emission, transport, and deposition (63). The mean global emission rate of bacteria was found to be in the range  $50 - 220 \text{ m}^{-2} \text{ s}^{-1}$ .

#### 2.1.3.6 Conclusion

As can be seen from this short review, there are a variety of methods that are potentially appropriate to measure fluxes of microorganisms emitted by terrestrial surfaces, and it may be surprising that so few results have been published so far. These results only provide a rough estimate of vertical fluxes (of the order of  $2 - 500 \text{ CFU m}^{-2} \text{ s}^{-1}$  over surfaces undisturbed by human activities; much higher otherwise), with a tendency to be maximum in the middle of the day. However, these results show very large hourly variability, with alternating positive and negative values. Atmospheric microbiology has clearly put much more effort into identifying microorganisms present in the atmosphere and measuring their concentration than into trying to estimate the intensity of their emission. One major reason for this is the difficulty of obtaining accurate flux measurements: measuring a precise concentration requires very careful procedures (see Part 1), so that estimating a concentration *difference* represents a real challenge.

### 2.1.4 Impact of aerosol sources on the concentration and diversity of airborne microbial communities in the near-surface atmosphere

#### 2.1.4.1 Effect of source type on microbial loads

The comparison of bacterial concentration between urban and other environments was historically based on data obtained on culture media, and comparison of Gram-positive and Gram-negative bacteria. Contradictory results were obtained both on bacterial loads and on the predominance of Gram-positive or Gram-negative counts. Culture techniques found more Gram-positive bacteria in urban air, whereas culture-independent techniques found more Gram-negative bacteria. This discrepancy was probably due

to the fact that only a small fraction of airborne bacteria are culturable (64). Most studies found higher microbial concentrations in urban than in rural environments (15, 65), but an opposite effect of urbanization has also been reported (66). The absence of a common trend is probably due to intrasource variability: bacterial concentrations in cities exhibit especially high spatial variation because bacteria are released from strong point sources, in contrast to the more spatially homogeneous release from agricultural fields, for example (1). Furthermore, cities do vary in their bacterial abundance and composition (6). Bowers et al. (4) sampled the near-surface atmosphere above three distinct land-use types (agricultural fields, suburban areas, and forests,  $n = 15$ ), and found no significant difference in mean bacterial abundance, which ranged from  $1 \times 10^5$  to  $3 \times 10^6$  cells  $m^{-3}$ . In comparison with bacterial concentrations in urban and rural environments, CFU counts seem to be in general lower at coastal sites than at inland sites (15). Although oceans constitute both a source and final destination of a large fraction of airborne microorganisms, there is almost no information about the abundance of microbes over them. The study by Mayol et al. (50) estimated the concentration in the atmospheric boundary layer of the North Atlantic Ocean to be between  $6 \times 10^4$  and  $1.6 \times 10^7$  microbes per  $m^2$  of ocean, with millions of microbes leaving and entering the ocean per  $m^2$  every day.

#### 2.1.4.2 Effect of source type on microbial diversity

The airborne microbial communities are dominated by bacteria and fungi. Other microbial groups such as archaea, algae, viruses, or pollen are also present but less abundant. The main bacterial phyla found in the atmosphere are Actinobacteria, Firmicutes (Bacillales, Clostridiales, Lactobacillales), Bacteroidetes (Bacteroidales, Sphingobacteriales), Alpha- (Rhizobiales, Rhodospirales), Beta- (Burkholderiales), and Gammaproteobacteria (Pseudomonadales, Sphingomonadales). The main fungal phyla are Ascomycota (including the genera *Aspergillus*, *Penicillium*, and *Cladosporium*) and Basidiomycota. Recent studies using next-generation sequencing (NGS) have shown that airborne bacterial communities are mainly composed of non-abundant or rare taxons with few genera dominating. This is also the case for fungi, as the high number of fungal species detected only once in culture-independent studies indicates low coverage of species diversity (9). Only a few bacterial and fungal genera are ubiquitous and found commonly in the air. For example, in the study by Barberán et al. (8), who sampled settled dust from 1200 locations across the continental USA, approximately 90% of the bacterial and fungal phylotypes were detected in only 10% of the samples, and 0.003% and 0.02% of the bacterial and fungal phylotypes were found in more than 90% of the samples, respectively. These ubiquitous microorganisms comprise *Sphingomonas* and *Hymenobacter* for bacteria and *Cladosporium*, *Toxicocladosporium*, and *Alternaria* for fungi. In rural air Firmicutes and Actinobacteria are the prevalent groups of Gram-positive bacteria, whereas the dominant Gram-negative bacteria belong to the Proteobacteria (1). Bacteria at coastal and marine sites primarily belong to the phyla of the Alpha-, Beta-, and Gammaproteobacteria, Firmicutes and Bacteroidetes (67). Within the Firmicutes, *Bacillus* seems to be prevalent (15).

In the study of Bowers et al. (4), who sampled the near-surface atmosphere above three distinct land-use types, no effect of land-use type was found on the phylotype

richness or on the overall phylogenetic structure. However, shifts in bacterial community composition were visible in the relative abundance of specific bacterial taxa: Actinobacteria were more abundant at the agricultural and suburban locations, Bacteroidetes in agricultural fields and forests, and Rhizobiales in forests. In their study across the continental USA, Barberán et al. (8) showed that airborne microbial communities exhibit non-random geographic patterns. The airborne microbes found in urban and more rural areas were not distinct in composition but the urbanization seemed to lead to homogenization of the airborne microbiota, as demonstrated by lesser spatial variability at the continental scale. The microorganisms found indoors differ from outdoor air microbes, and indoor microbial communities do not represent a subset of outdoor microbes. Bacterial communities in indoor environments contain many taxa that are absent or rare outdoors. They are dominated by a small number of bacterial taxa commonly associated with humans as commensals or pathogens. The factors affecting the diversity and composition of bacterial communities in buildings are the ventilation source, the airflow rates, relative humidity, and temperature (68).

#### 2.1.4.3 Impact of meteorological factors on source contribution

Meteorological factors strongly shape the concentration and composition of the airborne microflora in a continuous way (temperature, solar radiation), as well as through specific events (rain), whether frequent or rare. Warmer temperatures increase desiccation of soil- or plant-based bacteria, leading to spore dispersal and aerosolization (6). Using multivariate regression techniques, Brodie et al. (6) demonstrated that temporal and meteorological influences can be factors stronger than location in shaping the biological composition of urban air. However, non-significant relationships between meteorological variables and bacterial abundance were found in some studies. In that of Matthias-Maser et al. (69) on the remote continental region of Lake Baikal in Siberia, significant shifts in abundance with changes in atmospheric conditions appeared for large bioparticles (fungal spores, pollen, plant debris) but not for smaller particles (bacteria). Recent studies observed that seasonal shifts in the relative importance of the environmental contribution of different sources impact more the airborne communities than shifts in individual meteorological parameters. Bowers et al. (3) reported a fairly predictable variability in composition with seasonality, which appeared to drive gradual shifts in the relative abundance of individual taxa over time. Leaf sources dominated in the spring and early summer months and dropped in importance in winter and autumn after plant senescence. A seasonal pattern in soil-derived bacteria followed the temporal pattern in crustal element sources, peaking in abundance during late summer and fall when dust loads are probably highest due to low soil moisture levels and increased temperature. The seasonality in airborne communities can also mirror the seasonality in agricultural activities (ploughing, crop cultivation, livestock operations). Finally, it is important to remember here that, if general trends can be found at high taxonomic level, some taxa may display opposing patterns: in the study of Bowers et al. (3), Actinobacteria reached maximum abundance in the late summer whereas Pseudomonadales reached highest levels during mid-spring.

## 2.1.5 Identifying predictors of bioaerosol emission and airborne community composition

### 2.1.5.1 Predictors of airborne community composition

It remains difficult to identify the main factors shaping airborne microbial diversity, since several parameters can be correlated. To analyze the main source contribution to airborne communities and the factors shaping the diversity, one also has to take into account the time scale. Maron et al. (70) suggested that the daily and weekly variability of bacterial communities in cities is mainly influenced by anthropogenic sources, whereas seasonal variations are triggered by climate and atmospheric changes. Factors shaping microbial composition at the source also impact the airborne microflora. For example, Barberán et al. (8), who sampled settled dust in 1200 locations in the USA, found a structuration by soil pH of patterns of airborne *Cellulomonas* (dominant among Actinobacteria) and *Terroglabus* (abundant Acidobacteria). It is well known that soil bacterial communities are strongly structured by pH, Acidobacteria dominating in low-pH soils and Actinobacteria being more dominant in high-pH soils such as those found in deserts. According to these authors, the best predictors of bacterial and fungal community composition in the terrestrial near-surface atmosphere are climatic and soil variables: soil pH, mean annual precipitation, net primary productivity, mean annual temperature, and the diversity of vascular plants. In the end, a complex set of environmental factors, including changes in atmospheric conditions and shifts in the relative importance of available microbial sources, acts to control the composition of microbial bioaerosols in rural and urban environments (3).

### 2.1.5.2 Indicators for monitoring bioaerosol emission

So-called “indicator-taxa” or “source-tracking” approaches consist in identifying taxa indicative of likely microbial source habitats and then determining their relative contributions to the bacterial communities in the near-surface atmosphere (3, 4). This approach is similar to the DNA-based microbial source tracking (MST) analyses used to track the origin of fecal contamination of waterbodies, which is based on the identification and quantification through molecular tools of microbial taxa indicative of one type of animal feces (pig, cow, bird, etc.). To gain quantitative insights into the origin of the detected microbes, the habitat of origin is analyzed by searching for the best matches in public databases. Potential indicators identified by several authors using this approach are gathered in Table 2.1.1. Indicators of bioaerosol emission can help to track the impact of human activities that may lead to sanitary problems for exposed human populations. DNA-based MST methods were used to track specifically and sensitively the aerosolization of land-applied anaerobically digested sewage sludge (biosolids) during high-wind events (7). This approach confirmed that wind is a possible mechanism for the aerosolization and off-site transport of land-applied biosolids. Several microbial indicators are also available for monitoring bioaerosols from composting facilities, based on MST (71). This approach may be generalized to other bioaerosol sources for monitoring biological contamination events such as livestock industry, which currently monitors a number of non-biological indicators such as ammonia, volatile organic

**Table 2.1.1** Microbial taxa that can be used as specific indicators of bioaerosol sources (3, 5–8, 71).

Source environment	Microbial indicators	
Plant	Chloroplasts	
Soil	Solibacteraceae, Chloracidobacteria, Koribacteraceae, Acidobacteria, Acidobacteriaceae, <i>Rhizobium</i> , <i>Bradyrhizobium</i> , <i>Mesorhizobium</i> , Rhodoplanes, <i>Chitinophaga</i> , <i>Solirubrobacter</i> , <i>Opitutus</i>	
Sea	Pelagibacteraceae, <i>Prochlorococcus</i> , <i>Synechococcus</i> , <i>Polaribacter</i> , <i>Pseudoalteromonas</i> , <i>Marinobacter</i> , <i>Nitrosopumilus</i>	
Insect	<i>Wolbachia</i> , <i>Buchnera</i> , <i>Rickettsiella</i> , <i>Cardinium</i> , <i>Rickettsia</i> , <i>Rhabdochlamydia</i> , <i>Fritschea</i> , <i>Regiella</i> , <i>Hamiltonella</i> , <i>Blochmannia</i> , <i>Portiera</i> , <i>Tremblaya</i> , Endomicrobia, Entomoplasmatales, Bartonellaceae, <i>Blattabacterium</i> , <i>Sulcia</i> , <i>Thorsellia</i> , <i>Baumannia</i>	
Skin	<i>Propionibacterium</i> , <i>Staphylococcus</i> , <i>Corynebacterium</i> , <i>Streptococcus</i> , <i>Rothia</i> , <i>Micrococcus</i> , <i>Anaerococcus</i> , <i>Brevibacterium</i>	
Stool	<i>Bacteroides</i> , <i>Faecalibacterium</i> , <i>Lachnospira</i> , <i>Oscillospira</i> , <i>Roseburia</i> , <i>Coprococcus</i> , <i>Ruminococcus</i> , <i>Parabacteroides</i> , <i>Phascolarctobacterium</i> , <i>Sutterella</i> , <i>Blautia</i>	
Anthropic activities	Composting	<i>Saccharopolyspora rectivirgula</i> , <i>Aspergillus fumigatus</i> , <i>Thermoactinomyces vulgaris</i>
	Wastewater treatment plant (human fecal sources)	<i>Arcobacter</i> , <i>Helicobacter</i>
	Land-applied biosolids	<i>Clostridium bifermentans</i> , uncultured bacterium of the class Chloroflexi

compounds, or particulate mass concentrations. As mentioned in Chapter 2.2, atmospheric transport models used in back-trajectory mode can also help to identify the source location of specific microbial communities found in the air at a given place. Substantial progress in identifying bacterial sources is to be expected from the use of such models in conjunction with molecular surveys of airborne communities.

### 2.1.6 Conclusion

Barberán et al. (8) made the following statement: “Given that many bacteria and fungi are capable of long-distance dispersal through the atmosphere, one might expect greater homogeneity in the composition of the bacterial and fungal communities found in outdoor air. This is clearly not the case” (8). Air microbial content and diversity indeed constitute patchworks built from different aerosol sources (ocean, agricultural areas, forests, cities, etc.), shaped by meteorological and physico-chemical factors, and

exhibiting high temporal variability. Human activities introduce new bioaerosol sources with potential impact on health and the environment and on the ecology of some microbial species. Nowadays, NGS techniques give us access to the enormous bacterial and fungal diversity (several thousands of phylotypes), whereas culture-based surveys detect only a few dominant taxa. Although research in this field is still in its infancy, these molecular techniques help us to address some challenging questions: How do changes in land use or land cover influence the diversity and composition of airborne microbial communities? To what extent do airborne bacterial communities overlap with those found in the source environments? What are the factors shaping airborne microbial diversity?

Further prospects for the future include understanding the success of particular taxa at being transferred to aerosols and surviving in the atmosphere (cell size and density, pigments, composition of the cell membrane), as this success expresses a potential to “invade” new environments. A better characterization of the specific features of microorganisms adapted to the atmospheric environment, and the identification of specific indicators, would also be particularly welcome. The use of molecular techniques (metagenomics, NGS, etc.) should be of great help for this. Further, online analyses of microbial loads for environmental monitoring (as already exists for the monitoring of chemical compounds), combined with microbial indicators specific for the different sources, and possibly inverse-transport modelling, should allow us to (i) understand the factors that shape the microbial content and the microbial diversity in outdoor air and (ii) facilitate prevention from health and environmental impacts. One final prospect is to quantify the impact of human activities and climate changes on the sources and associated airborne microflora.

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## 2.2

### Short-Scale Transport of Bioaerosols

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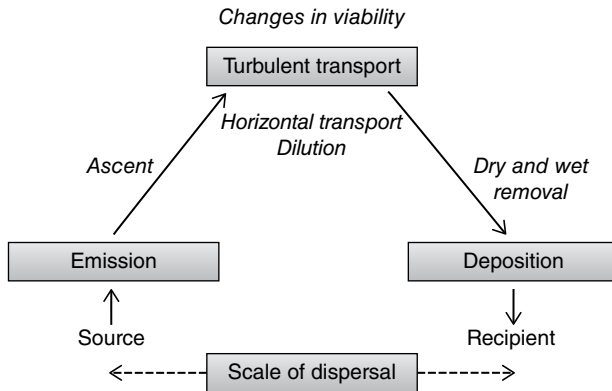
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#### 2.2.1 Introduction

The transport of bioaerosols in the atmosphere, from very local scales to much larger ones, has profound implications for the spread of infectious diseases that may affect humans, domestic animals, and plants. It also contributes to the biogeochemical cycles, and to the maintenance of biodiversity at the Earth's surface (1). It is of importance in meteorology since various classes of microbes are known to play a significant role in the formation of clouds and snow (2). A number of ecological and environmental studies have been devoted to estimating the amount of microbes that can be transported at various distances from a source, the rate of decrease in air concentration and deposition downwind from a source, the spread of specific microorganisms in the atmosphere, as well as the potential for gene dispersal from cultivated species. Examples of sources of interest in industrial and agricultural studies are, respectively: sewage treatment plants, compost facilities, or cooling towers at electricity-generating plants; crop fields with transgenic plants, plants infected with pathogens, manure spreading, or sewage irrigation. In cities, multiple sources of microorganisms usually coexist (3).

Particle dispersal in the atmosphere occurs throughout a range of length scales from millimeters to thousands of kilometers. The definitions of short- and long-scale transport depend very much on the field of investigation. For researchers working on seed dispersal, for instance (e.g., (4, 5)), a horizontal scale of 10 to 100 m may represent a long distance for propagation between the source and the receptor. For others analyzing the transport of lighter particles, such distances are very common and "long-range," or long-distance, dispersal rather refers to horizontal scales of hundreds of kilometers (e.g., allergenic pollen (6) and rust pathogens (7)), or even to the global scale (bacterial cells transported by desert dust (8)). In this chapter, which deals only with outdoor transport, we have adopted a pragmatic approach whereby we mainly focus on the so-called atmospheric microscale (up to a few kilometers of spatial extent) and mesoscale or subregional scale (up to 100 km or so) as an upper bound, following the definitions of (9). Larger scales (e.g., from regional to continental and global) will be dealt with in Chapter 2.3.



**Figure 2.2.1** A general scheme for the transport of microbial particles.

Most studies involving the transport of bioaerosols have been performed on the dissemination of pollen, as well as fungal spores, across a continuous range of scales. Bacteria have received growing attention over the past 10–15 years, with studies focusing on two main scales: very short scales (dispersal in the lee of industrial or agricultural sources) and very long scales (continental and intercontinental transport). Only a limited number of studies have dealt with airborne viruses and microalgae (for the latter, see (10)). As many examples will be given from pollen and fungi studies, it should be pointed out that airborne pollen and spores can be useful markers of potential dispersal distances for other types of microbes of similar sizes.

This chapter on outdoor transport at the short scale will be mostly devoted to the passive transport of biological particles by wind; the reader should note that there are other means of dispersal in the atmosphere (via insects and birds, and also splash droplets), but they will not be considered here.

Atmospheric dispersal of biological particles can be seen as involving three main stages (Figure 2.2.1): (i) emission from a source, (ii) transport in the atmosphere, which includes escape from the canopy air space in the case of emission by vegetated surfaces, ascent into the atmospheric boundary layer or above, and horizontal transport over a range of scales, and (iii) deposition onto various types of surfaces (plants, soil, water, built surfaces, etc.). One additional process to consider when dealing with bioaerosols, as compared with abiotic particles, is the alteration of their biological properties as they travel: microorganisms tend to lose viability under the effect of ultraviolet (UV) radiation or desiccation, for example, at a rate that depends on many factors. Emission processes have been considered in Chapter 2.1. Here, particle deposition will be described first, before we move on to the mechanics of dispersal and the changes in viability during transport. We will then focus on particle transport modeling and give examples of dispersal patterns. Perspectives will be drawn in the concluding section.

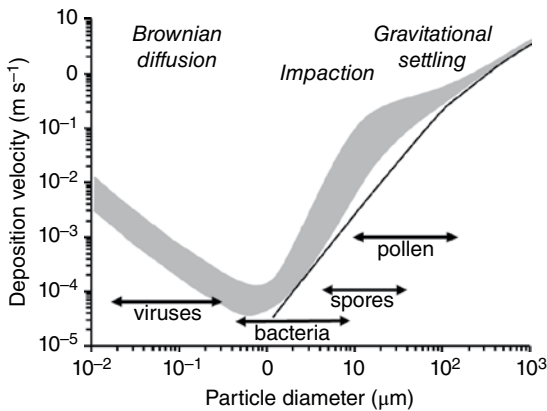
## 2.2.2 Particle dynamics and deposition processes

The term “bioaerosols” covers a wide variety of biological particles that are transported by the atmospheric flow. These particles display a wide range of sizes, from

approximately 10 nm for some viruses to more than 100  $\mu\text{m}$  for some pollen grains. They have different shapes, densities, and surface properties. Despite this variety, the motion of all airborne particles is determined by a set of forces including, principally, aerodynamic drag, aerodynamic lift, buoyancy, and gravity forces; other forces may also be operating, such as the Magnus force due to particle rotation and electrostatic forces (11). These forces mostly depend on key variables such as mean wind velocity, turbulent velocity fluctuations, temperature gradients, and particle characteristics (diameter, density, surface area, drag coefficient (a dimensionless quantity used to quantify the drag of an object in a fluid)). It is the balance of these forces in the transporting fluid that determines the trajectory of the particle and its ability to travel over short or long distances. Showing and discussing each of these forces as well as the equations of motion for microbial aerosols is beyond the scope of this chapter. All relevant information can be found in textbooks such as (11). We will focus here on deposition processes, a key issue to consider.

Particles are removed from the air by two processes: wet and dry deposition. Wet deposition is the removal by precipitation, or washout. Microbial aerosols can be scavenged below clouds if they come into contact with raindrops and are captured. So-called “dust rain,” or rain occurring in an atmosphere loaded with desert dust (12), is particularly effective at removing microbes from the atmosphere. Microorganisms can also be incorporated into cloud droplets by nucleation processes (13), where they act as cloud condensation nuclei or ice nuclei. These processes are described in Chapter 3.1.

Dry deposition is a continuous process whereby particles settle or collide and stick onto surfaces. It is usually quantified through a deposition velocity,  $V_d$ , expressed as the ratio of the downward particle flux,  $F_p$ , to the ambient particle concentration,  $C_p$ . Dry deposition of particles on terrestrial surfaces involves three main processes acting in parallel: gravitational settling, inertial impaction on individual elements, and Brownian diffusion through the boundary layers attached to each element. Each of these processes is a strong function of the particle aerodynamic diameter (the diameter of a spherical particle of unit density with the same gravitational settling velocity as the particle under consideration): the dominant mechanisms are Brownian diffusion for submicrometer particles and gravitational settling for large particles, typically above 100  $\mu\text{m}$ . Impaction dominates in an intermediate size range centered at around 10  $\mu\text{m}$ . The combination of these processes results in a “V-shaped” curve exhibiting a minimum at around 1  $\mu\text{m}$ , where none of these processes is effective (Figure 2.2.2 (14)). It is interesting to note that the deposition of bioaerosols therefore depends to a large extent on their type: viruses are in the submicrometer range where diffusion is the main deposition mechanism, whereas spores and pollen are in the ascending branch and are strongly subjected to gravity forces. Most bacteria are in the diameter range corresponding to the minimum of the curve and therefore have a particularly small dry deposition velocity, giving them the potential to be dispersed by wind over very large distances (up to the global scale); washout (wet deposition) is the main mechanism by which they are removed from the atmosphere. It has indeed been shown that bacteria can remain aloft for between 5 and 10 days typically, in cloud-free conditions, but for a much shorter time when condensed water is present (15, 16). However, this applies to single bacterial cells, but in reality bacteria are often aggregated, or attached to dust particles or small vegetation fragments, so that their mean aerodynamic diameter is larger (e.g., 4  $\mu\text{m}$ , as found at various continental sites (17)), and deposition velocity is



**Figure 2.2.2** Deposition velocity and particle diameter, after the single-layer surface model of Raupach et al. (14). The case represented here is for a vegetation of height 0.06 m and leaf area index 1. The shaded area shows the range of deposition velocities calculated for a range of wind speeds giving friction velocities between 0.35 and 1.40  $\text{m s}^{-1}$ . The solid line is the predicted terminal velocity. Diameter ranges for four classes of microbial particles are shown.

instead at the start of the ascending branch on the right-hand side of the curve. The dependency of deposition velocity on particle size also has consequences for particle segregation during transport. It has been demonstrated that after emission the droplet size tends to decrease with distance from the source, because of preferential deposition of the largest droplets close to the emission site, under the effect of gravity. Shrinking, caused by the evaporation to which the aerosols are submitted during their journey, has also been invoked (e.g., (18)).

The deposition velocity is often expressed as the inverse of a sum of resistances, using an electrical analogy. Its parameterization can be based on surface models of varying complexity. For instance, Raupach et al. (14) used a single-layer model for vegetated surfaces (Figure 2.2.2), and Petroff et al. (19) elaborated a model for  $V_d$  over plant canopies with significant vertical extent, derived from a detailed analysis of canopy flow and deposition mechanisms. The terminal velocity, i.e., the settling velocity under the sole action of gravity in still air, can be approximated by Stokes law, which implies a linear variation with the square of the diameter. In Figure 2.2.2 it appears as an asymptote to the ascending branch on the right-hand side.

### 2.2.3 Transport processes and dispersal scales

From an experimental point of view, the dispersal of particles in the atmosphere has been studied using air concentration measurements at single sites or at various distances downwind from sources. Measurements have been taken near the ground or at human respiratory height (20, 21); they have also been taken at various heights in the atmosphere using sensors set up on a mast (22), or onboard light aircraft (23–25) or unmanned aerial vehicles (26, 27). Downwind variations in particle deposition flux have also been measured (22), and occasionally the investigations have been performed not

on the particle flux itself but on its consequences, such as cross-fertilization by pollen (28) or infection by bacteria and spores (29). Cases of release–recapture field experiments have been reported, using small-scale or field-scale sources of known inoculum (30, 31).

Biological particles emitted at the surface are released into the surface boundary layer (SBL), which extends over the first 10–100 m or so, depending on the time of day, wind and temperature conditions, terrain type, etc. In this region the vertical gradients in all turbulent variables are usually strong, because of the vicinity of the surface. The atmospheric flow is highly turbulent, owing to the high shear exerted by surface elements, and turbulent eddies contribute to a large extent to the spread and diffusion of particles. In the presence of a plant canopy with significant vertical extension, or in an urban context, a specific roughness sublayer forms at the bottom of the SBL, which displays the properties of a plane mixing layer rather than those of a boundary layer (32); in particular, this sublayer exhibits even larger gradients, and turbulence is dominated by intermittent eddies at the canopy scale. The SBL forms the lowest part of the planetary (or atmospheric) boundary layer (PBL), which increases every day under the influence of surface heating, up to typical heights from 500 m to 2 km, before it collapses at night (see (33)). It is well mixed because of the presence of large-scale convective motions. The PBL is usually capped by an inversion layer that limits further ascent of particles.

This vertical structure has strong implications for the distance over which a particle can travel after it has been released. Particles remaining in the SBL, which is often the case for heavy particles such as pollen grains of many cultivated species, are prone to deposition within a short distance from the source. For example, massive deposition of maize pollen grains (diameter  $\approx 100 \mu\text{m}$ ) occurs at plant and soil surfaces in the source field itself or within the first meter or so downwind from it, where air concentration and deposition fluxes quickly decrease with distance (see (34) for a review). Similar behavior, but with a slower decrease, has been observed with lighter particles such as spores: for example, Pruissin et al. (31) showed a logistic-type decrease in the deposition of *Fusarium graminearum* spores ( $\approx 20 \mu\text{m}$  diameter) over the first few hundreds of meters from the center of the source. In fact, even with large particles the dispersal kernel (the curve giving the probability density function of the distance over which a particle travels before it lands) often has a fat tail formed by rare, long-distance dispersal events (35). For oilseed rape pollen, for example, it was found that classical exponential kernels underestimated the probability of pollen deposition at long distances, and that a fat-tailed power-law function provided the best fit to experimental field data (28). It is important to have an accurate estimate of the kernel tails because predicted spread rates closely depend on the probability of long-distance dispersal events. Such behavior may partly result from the possibility that particles will be entrained into the PBL, depending on their weight and density, as well as on the intensity of convective motions. Comparing instantaneous vertical wind velocities in updrafts during convective conditions (which can easily be larger than  $1\text{--}2 \text{ m s}^{-1}$ ) with the settling velocity of particles (of the order of  $3 \times 10^{-1}$ ,  $3 \times 10^{-3}$ , and  $3 \times 10^{-5} \text{ m s}^{-1}$  for particles of  $100 \mu\text{m}$ ,  $10 \mu\text{m}$ , and  $1 \mu\text{m}$  diameter, respectively, in the Stokes regime), it is clear that even heavy particles such as large pollen grains can be lifted to heights well above the SBL. The presence of numerous biological aerosols in the PBL has indeed been demonstrated using light aircraft (23–25) as well as unmanned aerial

vehicles (27). Given the large-scale mixing motions occurring in the PBL they can remain aloft for a major part of the day, and before landing they can therefore drift away from their emission source over large horizontal distances, of several kilometers to hundreds of kilometers (25, 36). Aerial measurements performed by Lin et al. (37) on fungi in the genus *Fusarium* have been used to deduce distances to the potential inoculum sources ( $\approx 1\text{--}4$  km), as well as their seasonal variations. As PBL height plays an essential role in the dilution and dispersal of particles, models including a parameterization, or a full set of equations for the PBL, are therefore better suited than surface layer models for simulating particle dispersal at scales larger than a few tens or hundreds of meters.

In particular conditions, a fraction of the particles may even be entrained above the PBL, through the inversion layer. This is particularly true for light particles with a small deposition velocity such as bacteria: for instance, Mandrioli et al. (24) and Fulton (38) found from aircraft measurements that the decrease in bacterial concentrations above the PBL (at about 6000 and 3000 m, respectively) was much less than that for pollen and spores. The daily peaks in microbial concentration observed at such altitudes were shown to correspond to the peaks in convective activity. Once in the free troposphere, the microbial aerosols are no longer submitted to the intense mixing of the PBL and its daily behavior, so that light enough particles then have the potential to travel over very large distances, at continental or trans-oceanic scales, under the action of trade winds for instance (39) or dust storms (40). A recent modeling study (41) indicates that such potential should become increasingly unlikely for particles greater than 20  $\mu\text{m}$  in diameter, and virtually impossible for particles larger than 60  $\mu\text{m}$ . Bacteria and fungi, shown by Yamaguchi et al. (8) and Prospero et al. (39) to have traveled over very long distances, were indeed smaller than 20  $\mu\text{m}$ ; however, pollen grains between 20 and 80  $\mu\text{m}$  were found in Greenland, with an estimated average transport duration of 5 days (42). More examples will be given in Chapter 2.3.

## 2.2.4 Survival of microorganisms during transport

In the lower atmosphere airborne microorganisms have to cope with adverse conditions including desiccation, as caused by meteorological conditions (air temperature and humidity, radiation, wind velocity), deprivation of nutrients, presence of oxidants (OH, O<sub>3</sub>, H<sub>2</sub>O<sub>2</sub>), and damaging solar radiation (e.g., (43, 44)). The deleterious effect of UV radiation has been mostly considered for conditions representative of higher altitudes, but it has been reported that on sunny summer days at midlatitudes UV radiation greater than about 290 nm can reach the ground in sufficient amounts to kill sensitive spores in a few hours (36).

The capacity to survive in the atmosphere differs significantly from one microorganism to another. Microorganisms described as ubiquitous in the air (*Aspergillus*, *Penicillium*, *Bacillus*, *Clostridium*, *Alternaria*, etc.) have developed mechanisms to cope with the different stresses that allow them to survive for long periods in the atmosphere. Pigmentation, sporulation, and attachment to particles have been assumed by several authors to be important protection mechanisms against solar radiation for the outdoor atmospheric bacteria (3, 44). When associated with particles, bacteria are also better protected against desiccation (3). In contrast, it is conceivable that a large part of

the microbial diversity emitted from the sources will not be able to survive a long time and will only be found locally, close to the emission point. For instance, it has been shown that the diversity of airborne high-alpine bacteria is reduced in comparison with urban and rural sites (45).

The atmosphere therefore constitutes a strong environmental filter: the mechanisms of survival, along with the capacity to disperse, which also depends on the microbial species (size, shape, etc.), do select for some taxa. Bacteria consistently detected in the air comprise several groups of spore formers such as the endospore-forming bacilli and clostridia and the exospore-forming actinomycetes. Cyanobacteria (*Plectonema*) and plant chloroplasts (presumably from pollen) are also often represented (3). On the basis of the existing evidence, some bacterial and fungal species particularly adapted to life in the air may constitute a “background” microflora, often masked in the near-surface atmosphere by the inputs from various aerosol sources.

## 2.2.5 Modeling tools for the transport of microbial aerosols

Simulation of atmospheric transport is a cornerstone for the prediction of particle dispersal across a range of scales. A wide variety of models have been developed in atmospheric sciences to simulate wind flow, as well as energy and mass transport in gaseous or particulate forms, at various scales from the leaf scale to the global scale. The bioaerosol literature reflects this variety: there has been wide use of models to simulate the dilution of particles in the atmosphere after emission, and predict, for instance, air concentration at various locations downwind from sources, or deposition fluxes at remote places. The main types of modeling approaches have been used for this purpose, such as Gaussian plume models, trajectory (Lagrangian) models, Eulerian and large-eddy simulation models.

### 2.2.5.1 Gaussian approaches

The most common model used for bioaerosol dispersal on the short scale is the Gaussian plume model, which provides a simple statistical prediction of the downwind, lateral, and vertical concentration of particles found in the plume, once they have been released from the source. This model is a theoretical solution to the advection–diffusion equation for a passive scalar, emitted from a continuous point source, in steady-state flows with uniform wind field and constant turbulent diffusivity, over horizontally homogeneous surfaces. Its success is to a large extent due to its simplicity, since it has modest input requirements and provides analytical solutions that do not require lengthy numerical integration. Although the above-mentioned conditions are not always fulfilled in atmospheric boundary layers, characterized by vertical gradients in wind velocity and diffusivity, this model has been shown in many cases to provide a reasonable approximation to the actual concentration field, at least for elevated point sources (e.g., several tens of meters, as in (46)).

For sources closer to the surface, where vertical gradients are stronger and turbulence is inhomogeneous, the conventional Gaussian model is not always applicable. For instance, exponential rather than Gaussian vertical scalar distributions have been repeatedly observed. A number of improvements have therefore been instigated to

correct the flaws in the standard model, and in particular to make the representation of vertical diffusion more physically realistic, while trying to keep the model as simple as possible: (i) mean horizontal wind velocity and vertical diffusivity are allowed to vary with height; (ii) stability forces are included through empirical corrections to account for the influence of vertical temperature gradients in the atmosphere—several stability classes are usually considered; (iii) a settling velocity is added to take into account the effect of gravity; (iv) dry deposition is allowed at the lower boundary; and (v) terrain roughness is included, because of its effect on the spreading of the plume through increased turbulence. Many such hybrid or “quasi-Gaussian” models have been developed (e.g., (14, 47–50)). Their domain of validity is typically within a few hundred meters, although some of them have been used at much larger scales.

Beyond simulating dispersion from a continuous point source, Gaussian models have been adapted to line and area sources, and to finite-time release of particles (Gaussian puff models). Some of the models consider a range of droplet sizes, so that the overall solution can be calculated from the superimposition of several elementary Gaussian plumes, e.g., one for each droplet-size category (46). Gaussian models have been integrated into modeling systems taking into account the whole cycle (emission, transport, deposition), and interfaced with geographical information systems. One of these systems is AERMOD, a steady-state gas and particle dispersion model for research and regulatory applications, originally developed by the US Environmental Protection Agency (51) and subsequently used by Jahne et al. (52) to assess microbial risk associated with bioaerosols from a manure application site. This model takes into account the whole convective boundary layer and is therefore able to allow dispersion estimates over several kilometers.

Despite all the improvements brought to Gaussian models, this modeling approach may still be inadequate in various cases such as flow in plant canopies or over heterogeneous terrain, intermittent emission, presence of very light wind, nocturnal dispersion, or large-scale dispersion. More sophisticated models have therefore been developed to address more complex problems.

### 2.2.5.2 Modeling dispersal in plant canopies

In the case of emission within a plant canopy it is necessary to model the motion of particles in the canopy air space and calculate the “escape” flux, i.e., the upward flux of particles crossing the horizontal plane at the canopy top, and subsequently available for atmospheric transport. Turbulent dispersal within plant canopies is substantially different from that in the atmospheric boundary layer: turbulence is dominated by large coherent flow structures that manifest themselves through cycles of fast downward wind gusts and slower updrafts (32). Alternate methods to Gaussian dispersion models have been developed to face this challenge.

One popular category is Lagrangian stochastic models where vast numbers of particles are tracked individually by random walks, accounting for turbulence effects, until convergence of the flow statistics. Among others, this method has been used to model fungal spore trajectories within plant canopies (53, 54), and maize pollen dispersal at field scale (55, 56) (two-dimensional and three-dimensional dispersal, respectively). Lighthart and Kim (18) used it to simulate the trajectory of bacteria-containing droplets

sprayed over a potato field. One interesting property of Lagrangian models is their ability to simulate droplet evaporation and microbial death, since all particles are tracked individually over time.

A drawback of these models is that prescription of an adequate wind field is required, which may pose a problem in the presence of surface heterogeneities or in any case requiring determination of flow properties. Advection–diffusion Eulerian models have therefore been used for this purpose since they can solve coupled systems for velocity and concentration (22). In this category, large-eddy simulation models have proven very efficient at modeling turbulent flow and dispersal in plant canopies. They consist in solving time- and space-averaged flow equations, down to small enough scales for the essential features of turbulence to be explicitly modeled (57). A feature of great interest is that they simulate the direct action of a whole range of turbulent eddies, thereby allowing occasional events of importance for dispersal to be accounted for. Only one application of this promising method to the dispersal of biotic particles has been published so far (58).

### 2.2.5.3 Toward larger scales

Lagrangian principles have been used at larger scales, for example by Pfender et al. (59), who modeled dispersion and deposition of urediniospores at the landscape scale (several kilometers). The authors used the CALPUFF model, originally developed for air pollution studies (60), and interfaced with the meteorological models MM5 and CALMET for prediction of the wind fields at the right scale. Although these models have been developed for mesoscale studies, they can also be used at smaller scales. One popular application of the Lagrangian approach is its ability to help back-calculate trajectories, which allows the sources of observed concentrations to be identified. The most widely used tool for this purpose in aerobiology is HYSPLIT, a code from the US National Oceanic and Atmospheric Administration (61). It has been used repeatedly in pollen studies, either for biodiversity purposes (for example Belmonte et al. (62) found that beech pollen found in Catalonia, Spain, partly originates from an area extending over northern Italy and central Germany), or for health issues due to allergenic pollen (Sikoparija et al. (63) described how ragweed pollen is able to travel from the Pannonian plain to Scandinavia). HYSPLIT has also been used to study the transport of pathogens and bacteria. Skjøth et al. (64) showed for instance that, although most airborne *Alternaria* spores found in Copenhagen, Denmark, have a local or regional origin, some of them come from harvesting operations in central Europe. Also, Amato et al. (65) used back-trajectory analysis to interpret ice nucleation measurements on bacterial strains found in cloudwater. These approaches, mostly used for long-distance transport, will be discussed further in Chapter 2.3.

At similar scales, Eulerian principles were used by several researchers for different particles: Schueler and Schlünzen (66) studied oak pollen dispersal at the landscape level, using the mesoscale model METRAS; Brunet et al. (25) simulated maize pollen dispersal at the subregional scale with the mesoscale model MESO-NH; birch pollen dispersal was simulated by Vogel et al. (67) over Switzerland, using COSMO-ART, and by Sofiev et al. (68) across Europe, using SILAM. Table 2.2.1 provides a summary of modeling exercises performed at the submesoscale and beyond.

**Table 2.2.1** Examples of Eulerian and Lagrangian atmospheric models for the transport of biological particles at different scales.

Model name	Model type	Spatial scale of interest	Particle type	Reference
SMOP	Lagrangian	Field (100 m)	Maize pollen	55
AQUILON	Eulerian	Field (100 m)	Maize pollen	22
MM5-CALMET-CALPUFF	Eulerian–Lagrangian	Landscape (eastern USA, 5 km)	<i>Puccinia graminis</i> , Urediniospores	59
METRAS	Eulerian	Regional (Germany, 200 km)	Oak pollen	66
MESO-NH	Eulerian	Regional (France, 200 km)	Maize pollen	25
KAMM/DRAIS	Eulerian	Regional (Germany, 250 km)	Alder pollen	69
MM5-HYSPLIT_4	Eulerian–Lagrangian	Regional (central USA, 500 km)	Oak pollen	70
WRF-MEGAN-CMAQ	Eulerian	Regional (western USA, 500 km)	Various pollen species	71
MM5-CMAQ-HYSPLIT	Eulerian	Continental (eastern USA, 2000 km)	Birch and ragweed pollen	6
COSMO-ART	Eulerian	Regional (Switzerland, 400 km)	Birch pollen	67
		Continental (central Europe, 2000 km)	Ragweed pollen	72
		Continental (Europe, 3–4000 km)	Fungal spores	73
SILAM	Lagrangian	Continental (northern Europe, 2000 km)	Birch pollen	68
		Continental (Europe, 4000 km)	Ragweed pollen	74

#### 2.2.5.4 Modeling the survival of airborne microorganisms

Whichever model is used, one important requirement when simulating the transport of biological aerosols is our ability to predict the survival of airborne microorganisms during their journey in the atmosphere. In a certain number of cases it is the total number of particles found in the lower atmosphere that matters: for instance, Wéry (75) underlined the effects of endotoxins and glucans present in cell walls on human health. But in other cases it is the quantity of viable microorganisms only, i.e., microorganisms that can ensure biological functions (fecundation for pollen, infection for plant pathogens, etc.). Survival models are usually elaborated from laboratory experiments where the microorganisms are submitted to a range of climatic factors such as air temperature, relative humidity, wind velocity, exposure to UV radiation, etc. Examples of such models can be found for pollen (25, 76), spores (77), bacteria (78), and viruses (79).

## 2.2.6 Dispersal patterns

### 2.2.6.1 Release conditions

The conditions of release of the particles affect their subsequent transport, depending in particular on whether the initial emission velocity allows the particles to escape from the plant canopy, or the emitting surface in general. This may differ considerably between active and passive release (see Chapter 2.1), the latter requiring wind gusts of high enough intensity. The time of emission during the day also matters: in early morning or night-time conditions the levels of turbulence are usually low, with little mixing, which drastically limits the possibility of long-distance dispersal. Maximum atmospheric concentrations of microbial aerosols have been repeatedly observed in daylight hours, with a trend to increase as wind speed and temperature increase (see (80) for a review). However, in the absence of proper measurements of emission fluxes (see Chapter 2.1), it is difficult to disentangle the effects of the source strength from those of horizontal atmospheric transport. In many reported studies the observed correlations between aerial microbial concentration and meteorological factors (temperature and wind velocity in particular) may well only reflect the influence of the latter on the emission processes.

### 2.2.6.2 Concentration variations downwind from sources

In the atmosphere microbial aerosols behave like all particles of similar sizes and shapes. Their dispersal results, in general, in a steady decrease in air concentration and deposition flux with distance downwind from the source. Note that, if the observation point and the source are at different heights, the observed concentration may first increase with distance until a peak is reached, and then decrease, due to the shape of the diffusing plume (see, for instance, (20)).

These downwind variations have been studied in many experimental situations and on all types of microbial aerosols. They are of importance in several fields of research such as genetics (dissemination of genes in the environment), plant diseases (spread of pathogens), and human health (risk assessment for exposure to airborne infectious agents). In the last case, for instance, the typical question asked is: How many viable microorganisms per cubic meter of air are there at breathing height, at a given distance downwind from a continuous point source, and for a given source strength? To answer this question a number of field campaigns have been performed in the lee of concentrated bacterial sources such as manure application sites, wastewater treatment facilities, or composting platforms (for the last, see the review by Wéry (75)). In most cases a very rapid decrease in bacterial concentration has been observed, with for example a drop by two to three orders of magnitude in the first 100 m, and four orders at 1000 m (52, 81). For composting facilities one key issue regarding risk assessment is the prediction of the distance at which air concentration returns to its background value, for comparison with regulatory threshold distances (250 m for instance in the UK). Disparate results have been found regarding this distance: for some authors little impact of the source could be observed beyond about 100 m, but for others it took considerable distances for air concentration to return to background levels (between about 500 m and 1400 m in the review of Wéry (75); between about 2 km and more than 5.4 km in the

work of Galès et al. (82)). Transport models fitted against experimental data are usually used for this purpose (83), as well as for the estimation of the source strength (see Section 2.1.3). When combined with dose–response relationships they can provide predictions of exposure risk at various distances from the facility (52, 84).

For practical purposes, easy-to-use dispersal functions have been derived from observed rates of decrease. Although many types have been tested (see (28)), exponential (i.e.,  $y = a \times e^{-bx}$ ) and power (i.e.,  $y = c \times x^{-d}$ ) laws have been most successful in providing best fits with experimental data. Their relative merits have been discussed at length (30, 85). It has been shown that the exponential law is more adapted when the tendency for particles to deposit on underlying surfaces is much larger than the tendency for them to be transported by turbulence; when turbulence levels are higher, dilution due to turbulence dominates transport and a power law becomes more appropriate (30). At short distance it is difficult to discriminate between these two functions, but at larger distances the power law seems to better represent the occurrence of long-distance dispersal events.

The observed rate of decrease depends on several factors of variation such as source size and height, particle size, wind speed, and stability forces. Chalvatzaki et al. (20) showed that at large enough distance from open storage piles (125 m) particle concentration tends to increase as pile height increases (a higher pile results in increase exposed surface and source height). At shorter distances the maximum concentration is observed at the same height as the pile. Regarding particle size, Peterson and Lighthart (46) showed that, downwind from an elevated point source (a cooling tower), the larger the droplet the closer to the source the concentration peak, and the higher its magnitude. Strong winds and turbulence tend to increase streamwise transport and dilution in the atmosphere: for a given emission flux they yield a lower concentration close to the source, but a larger concentration further away from it. Regarding the effect of source size, larger sources usually provide larger concentrations at short distance; but at larger scale Sundberg (86) observed that the exponent  $d$  of the power law becomes smaller than within short distances from small patch sources, because of the presence of numerous scattered sources at such scales.

### 2.2.6.3 Landscape-scale patterns

Landscape heterogeneities may modify the dispersal patterns. For instance the presence of tall obstacles in the landscape, such as rows of trees or forest plots, has a known effect: particles lifted above tree height by the wind are likely to travel over longer distances before they land (86, 87). However, the effect of landscape fragmentation is complex and depends to a large extent on patch shape and connectivity, as well as on the direction of mean wind speed relative to that of corridors (88). Most of the above-mentioned studies have focused on the impact of a well-defined source at a given location. The question of the impact of multiple sources in a given landscape is also of great interest. In a recent study where they used back-trajectory analyses, Sadyset al. (89) were able to show that a significant amount of airborne fungal spores (*Ganoderma* sp.) are exported from forests to agricultural and urban areas in England. Most of the sources were found within 200 km range from the sites considered, so that landscape-scale and mesoscale transport had more significant impact than long-distance transport (i.e., from continental Europe).

## 2.2.7 Conclusion

The atmosphere has an essential place in the life cycle of many microbes, and atmospheric transport processes are of paramount importance in microbial ecology. They determine the area of influence of all sources, depending on their location, their spatial extension, and source strength.

Transport models have proven very useful to many applications in simulating the atmospheric dispersal of several types of biotic particles over a whole range of scales, from the plant to the globe. The different modeling approaches considered here appear to be well adapted to specific problems, and are complementary. As considered above, microbial aerosols travel over a continuum of scales (35, 86), which the current models have not taken into account well. One exception was the work of Aylor (53), who merged three types of physical models to simulate dispersal over a continuum of scales from the plant scale to the regional scale: a Lagrangian stochastic model for in-canopy and in-field dispersal, a three-dimensional advection–diffusion model for dispersal at the landscape scale, and a Gaussian puff model for longer distance transport. Current modeling systems can now incorporate this range of scales naturally, by solving the flow equations within a series of nested domains. At the smallest scales the large-eddy simulation technique has shown its potential in several fields of research, and should be developed for the transport of biological particles.

One point of concern is the current lack of knowledge about bacterial emission fluxes (see Chapter 2.1). Many concentration measurements are available (see the reviews 74, 90, 91), but only very few direct measurements of source strength have been performed so far. Back-trajectory calculations have been used for this purpose, but with no possibility for evaluating the quality of the prediction. Efforts should be made toward the development of reliable flux measurement techniques, as has been advocated in Chapter 2.1, in relation to model estimates. In the absence of such data we still know little about the aerosolization of bacteria, quantitatively and qualitatively. Also, a reliable knowledge of emission fluxes and short-scale transport patterns is of major importance for larger scale models since they provide the necessary inputs for long-distance dispersal, cloud feeding, etc. Finally, progress should also be made on the time variation in viability of microbial aerosols as they travel in the atmosphere, and on the way it can be represented in models.

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## 2.3

### Global-Scale Atmospheric Dispersion of Microorganisms

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#### 2.3.1 Historical context

The field of aerobiology was born during the heated controversy over “spontaneous generation” in the mid-19th century. Louis Pasteur’s research put an end to that fallacy but not without heated opposition (1). Later in the early 20th century, Lourens Baas Becking coined the phrase “Everything is everywhere, but, the environment selects” to characterize his perception of the magnitude and implications of microbial atmospheric dispersion (2). More recently, there was skepticism that long-range atmospheric transport of diverse microbial communities occurred at any significant scale due to the lethality of prolonged ultraviolet (UV) light exposure and other stressors such as desiccation and lack of obvious substrates (3). However, research by a number of global, independent aerobiology teams has demonstrated that the long-range transport of microorganisms is ubiquitous, that it is common to find viable microorganisms even at extreme altitudes in Earth’s atmosphere, and that microbial cells and spores serve as ice nuclei and thus may play a significant role in influencing weather and climate (4–16).

Generally, the diversity and concentration of microorganisms in Earth’s atmosphere decrease as altitude increases (17–19). However, severely limited access to air samples above the boundary layer (>1–2 km above sea level) has prevented systematic studies across time and space (20). Some of the most basic aerobiology questions in global-scale aerobiology remain wide open. What are the primary sources and sinks of microorganisms in the atmosphere? How long can cells remain aloft before returning to the surface? How does the atmospheric environment influence the survival of transported microorganisms and the ecology of downwind habitats? New experimental approaches and sampling platforms—both airborne and ground based—may provide an improved understanding of distantly transported microbes in Earth’s atmosphere. This chapter reviews the primary sources and sinks (natural and anthropogenic) of atmospheric bioaerosols, innovative approaches for determining transport history, methods for

assessing survivability, efforts to establish the high-altitude biosphere boundary, and overlapping areas between aerobiology and astrobiology.

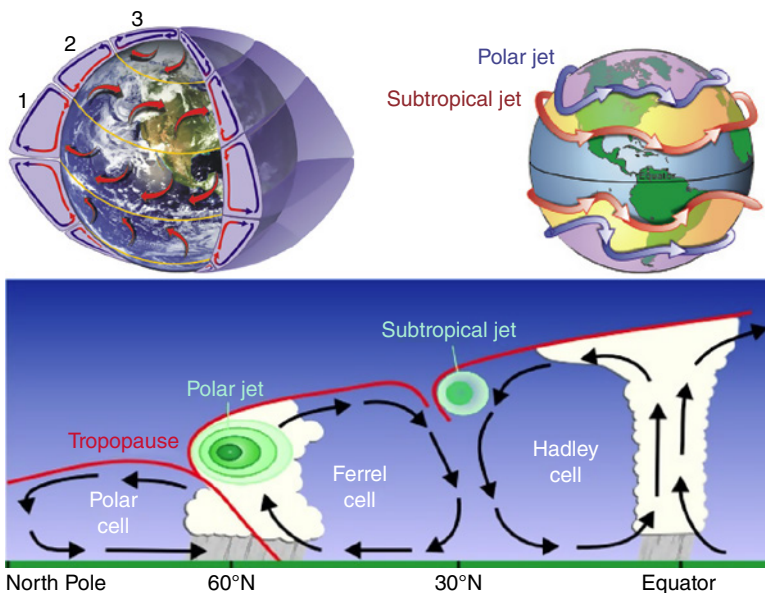
## 2.3.2 Mechanisms of dispersion

### 2.3.2.1 Natural sources

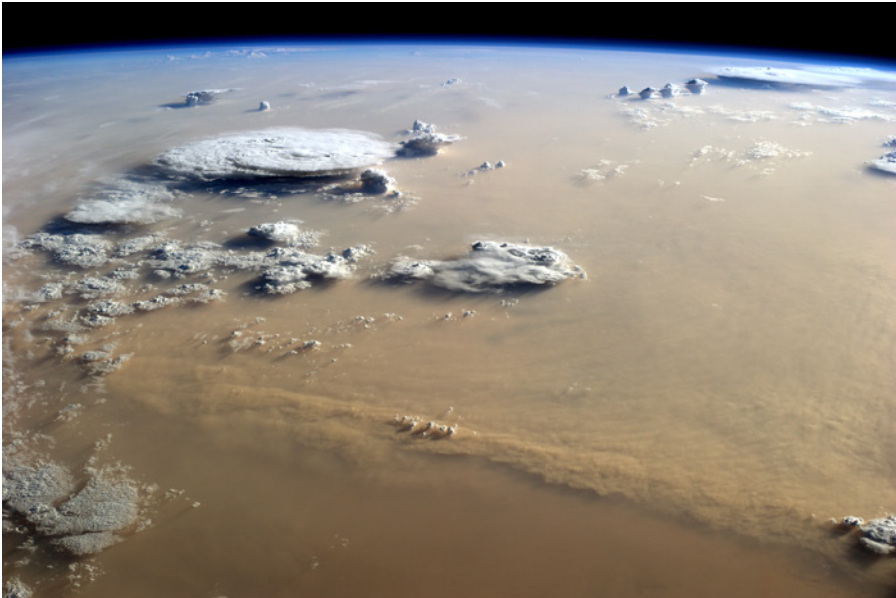
#### Wind

Global wind patterns (Figure 2.3.1) and storms are the primary drivers of long-range dispersion of microorganisms in Earth's atmosphere on intra- and intercontinental scales (4, 21–23). Storms provide the necessary energy required to loft soil and aquatic aerosols high into our atmosphere and transport them vast distances. Dust storm transport is one of the most prevalent and well documented, and these atmospheric events are capable of global dispersion (Figure 2.3.2) (12, 24, 25).

Wind speeds along storm fronts over terrestrial environments as low as  $6 \text{ m s}^{-1}$  can cause deflation and at  $3 \text{ m s}^{-1}$  can cause erosion (25). Normal low-level wind speeds in the vicinity of  $0.5 \text{ m s}^{-1}$  are known to disperse fungal spores from the surfaces of leaves (26). Storm activity over aquatic environments that generate splash and spray may transport planktonic microorganisms into the atmosphere via bubble bursting (27). Aerosols released from bubble bursting in the size range of  $2\text{--}20 \text{ }\mu\text{m}$  carry elementary charges in the range of 200–5000 units and on a global scale “may be a source as well as a sink for the charge that maintains the Earth's positive electric-field” (28). Electric fields



**Figure 2.3.1** Global wind circulation patterns. 1, Hadley cell; 2, Ferrel cell; 3, Polar cell. (Figure courtesy of NOAA's National Weather Service, Southern Region Headquarters, Fort Worth, Texas. Figures located at <http://www.srh.noaa.gov/jetstream/global/circ.htm> and <http://www.srh.noaa.gov/jetstream/global/jet.htm>) (See color plate section for the color representation of this figure.)



**Figure 2.3.2** Desert dust lofted into the atmosphere over the Sahara by storm activity. (Photo courtesy of NASA's Earth Observatory (<http://earthobservatory.nasa.gov/IOTD/view.php?id=84400>.) (See color plate section for the color representation of this figure.)

created by atmospheric processes can enhance vertical aerosol transport by an order of magnitude (29). Like the smoke plume injected into the atmosphere from a 19th century steam locomotive as it moves across a landscape, large-scale cyclones such as hurricanes are capable of driving vertical transport of aerosols into the atmosphere as they traverse oceans (at altitudes that may reach into the lower stratosphere) (30). This mechanism of upper atmospheric loading may explain similarities of algal flora in tropical Atlantic and Pacific waters such as those separated by narrow landmasses in Central America (31). Smaller scale cyclones such as dust devils (Figure 2.3.3) and water spouts can also produce aerosols that may be dispersed on a long-range scale (32).

#### **Biomass burning, earthquakes and volcanic aerosols**

The generation and long-range transport of aerosols from fires is well documented, such as those impacting air quality in North America from biomass burning in Siberia (33, 34). Microorganisms in soils and on the surfaces of plant life can be swept into the atmosphere as fire fronts consume vegetated regions producing smoke and black carbon. This vector played a significant role in the long-range dispersion of associated microorganisms as observed in a study that documented viable fungal spore transport from fire smoke generated in the Yucatán Peninsula that crossed the Gulf of Mexico and impacted air quality in the State of Texas (35). Other potential loading sources of particulate matter to the atmosphere that may result in the long-range atmospheric transport of microorganisms include earthquakes and volcanoes. Outbreaks of disease such as coccidioidomycosis and pneumococcal pneumonia have been demonstrated following exposure to aerosols generated by earthquakes (36, 37). The annual estimates



**Figure 2.3.3** Dust devils lifting exposed agricultural soils into the atmosphere southwest of Ritzville in Adams County, WA, USA (27 September 2015). (Photo credit: D.W. Griffin.) (See color plate section for the color representation of this figure.)

for atmospheric dust loading from volcanic eruptions ranges from 4 to  $25 \times 10^6$  tons and the particle loads from large eruptions are known to force climate change through the resulting effect on planetary solar irradiance (38, 39). It is interesting to note, given the known ability of volcanoes to load Earth's atmosphere with soil and ash, that aerobiology studies of this material are infrequently pursued (40). *Bacillus luciferensis*, which was first identified in volcanic soils (Lucifer Hill on Candlemas Island, South Sandwich Islands), was identified from a sample collected at an altitude of 20 km using culture, as have various species of viable bacteria and fungi in a number of similar studies (6, 41, 42). Volcanoes certainly have the capability to load the troposphere and stratosphere with microorganisms and affect long-range transport rates on a global scale (40).

#### **Desert dust source region: Africa**

Once aerosolized, microorganisms are dispersed by regional and global wind circulation patterns. Two of the dominant circulation patterns for microbial transport between continents are the clockwise patterns over the North Atlantic and North Pacific that facilitate transport of aerosols off the coasts of North Africa and Asia to the Americas. Desert dust is a substantial vector of microorganisms through these atmospheric corridors (4, 43). Dust and other aerosols emanating off the northwest coast of North Africa can be transported across the Atlantic to the Americas by the Atlantic trade winds year round. The Sahara and Sahel regions of North Africa account for approximately 50–75% of the annual total atmospheric dust load (44–47). Annual variation in

transmission load is influenced by large-scale pressure systems such as the North Atlantic Oscillation and events such as El Niño that influence annual and long-term regional precipitation rates (44, 46, 48). Quantities of dust moving across the Atlantic and falling out of the atmosphere into the Amazon Basin were recently estimated at  $2.8 \times 10^7$  metric tons per year (49). A large dust event that occurred in 1992 reached ~30% of the contiguous USA. As these clouds of dust traverse the Atlantic they extend from sea level to an average upper altitude of ~5.1 km near the coast of Africa in the summer and ~3.7 km in the winter (50). African dust transport north and northeast over the Mediterranean Sea can extend up to altitudes of ~8 km (51). Transport times across the Atlantic typically range from 3 to 5 days. During the northern hemisphere's summer, the transport route is to the North Caribbean and the southeast USA and North Caribbean and South America during the winter. Aerosols from North Africa also routinely impact air quality in Europe and the Middle East and transmission of African dust to Asia and across the North Pacific to western North America has been documented (52).

#### **Desert dust source region: Asia**

Asian desert dust and other aerosols typically move across the North Pacific dispersing in Arctic and North American air masses. Transmission is seasonal and occurs primarily from February through June. Ocean–atmosphere patterns such as the Pacific Decadal Oscillation (PDO), El Niño, and La Niña influence precipitation patterns in Asia and the Americas (53). PDO-positive phases result in less dust transport to North America and negative phases result in drought in the southwest USA (53). Transport of dust across the North Pacific occurs at ~45°N during El Niño years and at ~40°N during La Niña years (54, 55). Transport to North America typically occurs over a 5–9 day period, although more rapid transport is possible. For instance, a 2 day transport rate of aerosols from China to just west of San Francisco, CA, via vertical cumulus updrafts in the source region and entrainment into the upper tropospheric jet was reported by Kritz et al. (56). A large Asian dust storm in 1990 moved across the Pacific, North America, the North Atlantic and deposited particulate matter in the French Alps (57). In 1998, another large Asian dust storm impacted air quality in North America and left a chemical fingerprint in the State of Minnesota (58). A recent study reported that an estimated 64 million tons of Asian dust and other aerosol particulate matter impacts North America annually, and that this represents approximately 60–70% of North American foreign dust load (with the remaining 30–40% coming from Africa and the Middle East) (59). Asian desert dust has accumulated at a rate of ~4–15 mg cm<sup>-2</sup> kyr<sup>-1</sup> over the last ~1.9 Myr on the Ontong Java Plateau in respect to total mass accumulation rates due primarily to desert dust and volcanic sources of ~34–90 mg cm<sup>-2</sup> kyr<sup>-1</sup> (60).

### **2.3.2.2 Anthropogenic sources**

#### **Agricultural activity**

Agriculture activity such as tilling and livestock grazing can produce long-term negative consequences for regional surface soils through the breakup of stabilized surface layers and exposure of barren soil constituents to wind (61). A marked increase in dust emission out of the Sahel in the mid-19th century coincided with the beginning of commercial agriculture as observed in marine sediments off the northwest coast of

Africa (62). The authors concluded that “human-induced dust emissions from the Sahel region have contributed to the atmospheric dust load for about the past 200 years” (62). The desertification rate in China between 1975 and 1987 was reported at  $\sim 2100 \text{ km}^2 \text{ yr}^{-1}$  and was attributed to a combination of climate change and harmful agricultural practices (63, 64). Similarly, the “American dust bowl” was the result of a 10 year drought and agricultural plowing that contributed to ecological disaster that drove one-fourth of the human population from the region. A dust storm during that era impacted Washington, DC, aiding in passage of the Soil Conservation Act of 1935 (65). The purposes of the Act were to ban what were recognized as agricultural practices that exacerbated soil erosion and created frequent giant dust storms nicknamed “black dusters” (66, 67).

Diversion of source water for agricultural use also contributes significantly to the atmospheric dust load due to the lowering of lake levels that results in the exposure of fine-grained and dried sediments to surface winds. Lake Owens, CA, was utilized for irrigation and as a drinking water source and was nearly drained dry in the early 20th century. It is now the primary source of atmospheric dust in the contiguous USA (68, 69). In North Africa the current long-term drought and diversion of source water from Lake Chad for agricultural purposes decreased its surface area size by over 94% between 1963 and 1997 and is recognized as a significant source of aerosolized dust (70). Similarly, exposed sediments of the Aral Sea, which lost  $\sim 50\%$  of its surface area between 1960 and 1992 due to source water diversion for irrigation, are a significant source of aerosolized dust (71). Globally it is believed that currently unsustainable agricultural practices, which may contribute to the annual global dust load, result in the loss of  $\sim 10$  million acres of farmland per year (72).

#### **Traffic aerosols**

Vehicle traffic on unpaved roads on arid terrain is a prominent source of aerosolized dust. Out of an annual total of  $25 \times 10^6$  tons  $\text{yr}^{-1}$  of atmospheric particulate matter in the USA, traffic-produced dust accounts for 40%, or roughly  $10 \times 10^6$  tons  $\text{yr}^{-1}$ . (wind erosion generates  $\sim 17\%$ , construction  $\sim 13\%$ , paved roads  $\sim 8\%$ , miscellaneous  $\sim 19\%$ , and other  $\sim 3\%$ ) (73). Smaller areas that have more unpaved roads for a given equivalent area such as South Africa generate an estimated 4.0 million tons  $\text{yr}^{-1}$  (73). “Toyota-ization” was a term used by Dr. Andrew Goudie of the University of Oxford, UK, to describe the widespread use of Toyota 4x4s in the desert and arid regions of North Africa, which along with other military and civilian off-road traffic contribute to the destabilization of these soils (74). It has been suggested that this type of traffic contributed significantly to a 10-fold increase in dust emissions emanating from the Sahara over the last 60 years or more (74).

#### **Flight: aircraft and rockets**

Other factors that may disperse microorganisms high into the atmosphere include aircraft flights, high-altitude rocket launches, and weapons testing (75). Microorganisms can contaminate vehicle surfaces (fuselage, wings, fins, landing gear, and wheels), cracks and crevices of aircraft and rockets during assembly, storage (inside or outside), or while awaiting launch/takeoff on a pad/runway. There are an estimated 100 000 commercial aircraft flights occurring every day (76), injecting unknown quantities of microorganisms into the upper troposphere and lower stratosphere as airflow and turbulence

dislodge contaminants attached to exterior portions of the vehicle. In addition, higher altitudes are frequently visited by “dirty” vehicles used for scientific and military purposes (73, 77, 78). Irregular locations on the vehicle such as air intakes, doors, windows, and hatches have been identified as key transport areas for microorganisms (79). Exterior surface areas of aircraft, which are protected from the airstream during flight, have been shown to move microorganisms (fungi and bacteria) between the airplane’s point of origin to its destination (79). Between 1950 and 2015 there have been ~5500 successful orbital launches (80). Microorganisms can contaminate rockets during assembly, roll-out, and time spent sitting on the launch pad. With coastal launch locations such as the Kennedy Space Center, FL, contamination from sea spray as well as local terrestrial flora may provide substantial surface loading. Liberated ice condensates and turbulence from vehicle stage separations would presumably release microbial contaminants throughout atmospheric ascent, but this effect has not been directly measured to date.

## 2.3.3 Microorganisms associated with long-range dispersion

### 2.3.3.1 Ubiquity

The number of prokaryotes in top soils ranges from  $\sim 10^3$  to  $\sim 10^9$  cells  $\text{g}^{-1}$  and these concentrations may vary annually depending on nutrient and moisture flux (81). The concentration of fungi in 1 g of topsoil is typically in the range of  $\sim 10^6$  (82). Viral concentrations in soil studies have ranged from  $\sim 10^3$  to  $\sim 10^8$  virus-like particles per gram (83–85). In a group of desert soil samples collected in North America, Africa, and the Middle East, bacterial and viral concentrations were observed to range from  $5.5 \times 10^3$  to  $1.5 \times 10^7$  cells and from  $2.2 \times 10^3$  to  $1.1 \times 10^7$  virus-like particles per gram (85). Bacterial concentrations in marine water typically range from  $\sim 10^5$  (nutrient limited waters) to  $\sim 10^9$  (nutrient rich waters) cells per milliliter, and viral concentrations are within the same range or 10–100 times higher (86–89). Marine aerosols are enriched an order of magnitude (on average) by the concentration of bacteria and viruses just below the ocean surface due to aerosol generation as bubbles pass through the sea-surface microlayer (a thin biofilm-like layer located at the surface water–atmosphere interface) (90). In the phyllosphere, concentrations of bacteria on plant leaf surfaces averaged  $\sim 10^6$ – $10^7$  cells  $\text{cm}^{-2}$  of leaf surface area (91). In regard to fungi, mean per leaf 12 h immigrant rates of  $3.0 \times 10^2$  to  $2.9 \times 10^4$  have been reported (92). What these data illustrate is that both terrestrial and aquatic aerosols may be microbially rich, contributing to an already diverse aerosol community (e.g., a passing dust storm). Not surprisingly, it has been estimated that approximately 25% of the atmospheric particles in the size range of 0.2–50  $\mu\text{m}$ , globally, are biological in nature (93).

Numerous fungi and bacteria were recently characterized from archived dust samples (over 160 years old) that were collected by Charles Darwin and others over the Atlantic in the early to mid-19th century (94, 95). Following in Darwin’s footsteps, long-range transport research was pioneered by United States Department of Agriculture scientist Fred C. Meier in the early to mid-20th century. With a focus of transoceanic transport of plant pathogens and the aid of famed aviators such as Charles Lindbergh and Amelia Earhart, Meier identified viable microorganisms using aircraft for collection from the

atmospheres over the Arctic, North America, and the Caribbean (96–98). Research on the North American “*Puccinia* pathway” in 1938 reported source concentrations of an uredospore cloud in the atmosphere over Dallas, TX, at  $\sim 129\,000\text{ ft}^{-2}$  and impacting atmospheres at distances of 300 km (Oklahoma), 560 km (Falls City, NE), 840 km (Beatrice, NE), and 970 km (Madison, WI) at concentrations of 584, 713, 183, and  $18\text{ m}^{-2}$  respectively (99). This study along with others that occur on a global scale for pollen and plant rusts (fungi) were first reviewed by Gregory in Chapter 14 of his 1961 book *The Microbiology of the Atmosphere* (75). Knowledge that certain microorganisms were capable of surviving long-range dispersion in Earth’s atmosphere has driven the synthesis of hypotheses on the intra- and intercontinental dispersion of plant rusts based on outbreak and wind patterns (e.g., the outbreak of a rust in a crop on the north-west coast of Africa followed by an outbreak by the same rust in crops in the Caribbean with the link being trade wind transport) (100). Likewise, transpacific atmospheric transport of Asian influenza viruses into North America has been hypothesized based on historical outbreak timing, spread patterns, and infectious dose data (101). Interestingly, 21 years later, the elevated presence of influenza viruses in the atmosphere of Taiwan was reported during an Asian desert dust event (102). More recently, global wind patterns have driven hypotheses on intercontinental atmospheric dispersion of: (i) plant rusts and pollen on a global scale (100, 103), (ii) influenza between Asia and North America (101), and (iii) microbiota to Antarctica (104). Because of its remoteness, Antarctica has been identified as an ideal location to study the global dispersion of microorganisms (13).

#### **Survival of microorganisms in the atmosphere**

In the lower atmosphere, it has been estimated that approximately  $10^{19}$  microorganisms exist within clouds and that clouds may function as “atmospheric oases” at the microbial scale, providing refuge to the atmosphere environment rich in UVA (315–400 nm) and UVB (280–315 nm) while moving microbes vast distances (105, 106). Microbes are not necessarily passive passengers. In fact, bioaerosols can serve as ice nuclei and influence cloud formation and precipitation (107). Updrafts created by fast moving storm clouds are believed to be a source of microbial cloud loading and that once within the cloud “easily degradable organic compounds suffice to support bacteria growth (Temkiv et al., 2012)” (108–111). The isolation of methanotrophic bacteria from the atmosphere that were able to deplete methane typically found at atmospheric concentrations in laboratory simulations suggested the ability of these types of organisms to exist and function while suspended in the atmosphere (112). The sheer prevalence of microorganisms in the atmosphere has driven exploration into the possibility that their presence is more than just a transient state and that some species may have evolved over time to exist within what may be classified as a unique ecosystem (20). Compared with the relatively mesophilic cloud environment, bioaerosols residing in the Earth’s upper troposphere and lower stratosphere where air is thin ( $\sim 5\text{--}50\text{ mbar}$ ), cold (temperatures from about  $0^\circ\text{C}$  to  $-100^\circ\text{C}$ ), and dry (low water availability at  $<\sim 20\%$  relative humidity) (20) must endure more biocidal conditions. Extremely dry air, oxidizing chemical species (e.g.,  $\text{O}_3$  and free radicals) and higher radiation dosages (both ionizing and non-ionizing), including biocidal UVC wavelengths (200–280 nm), can all inactivate microbes at high altitudes in the atmosphere (113–116). Previous reports of viable species collected and cultivated from the upper atmosphere (see Smith (20) and references

therein) support the notion that microorganisms can withstand this harsh environment, but a fundamental understanding of survival mechanisms and conditions aloft is incomplete. Cell pigmentation, DNA repair, the ability to form spores, and growth in hypobaric conditions are just a few of the known adaptations potentially permitting terrestrial microbes to persist above the boundary layer (4, 6, 117, 118); however, more experimental studies are needed. One critical distinction to be made is that the detection of cells—most of which are dormant or dead according to seasonal studies (5)—should not be confused with signs of active atmospheric regions, but rather limits of persistence. Microbiological measurements at extreme heights can be done through *in situ* exposures and simulated conditions in the laboratory. Recent experimental designs have been developed to explicitly measure microbial activity in cloud chambers. Amato et al. (105) simulated the cloud environment and observed that metabolic functions can be performed at water, temperature, and nutrient levels typical in clouds. Yet, considering the lower limits of bacterial growth and metabolic activity around  $-35^{\circ}\text{C}$  (119–123) and desiccation parameters for life (124–126), the window of potential atmospheric microbial metabolism—where the atmosphere could actually be considered an ecosystem in its own right (127)—is likely capped around 5 km above sea level (a.s.l.) in the free troposphere. Any higher, bioaerosols are subjected to intense desiccation and exposure to UVC wavelengths, surely inhibiting activity and probably limiting persistence to short periods.

Most survival simulations measuring microbial viability (not activity) indicate that sun-illuminated bioaerosols are quickly inactivated in the upper atmosphere through irradiation, desiccation, freeze–thaw, free radicals, or a combination of the aforementioned factors (105, 118, 128–134). For instance, Smith et al. (118) showed that 99.9% of monolayered *Bacillus subtilis* endospores exposed to simulated stratospheric conditions (at 20 km ASL) were killed within 6 h using the following experimental parameters:  $-70^{\circ}\text{C}$ , 56 mb, 10–12% relative humidity, and 0.00421, 5.11, and 54.64  $\text{W m}^{-2}$  of UVC (200–280 nm), UVB (280–315 nm), UVA (315–400 nm), respectively. Results revealed that direct exposure to UV limited bacterial survival while simulated stratospheric air pressure, temperature, and desiccation had no measurable effects on viability. In contrast, other experiments mimicking lower altitude cloud environments showed a similarly low probability of relatively stable *Dioszegia hungarica*, *Sphingomonas* sp., *Pseudomonas syringae* 32b-74, *Pseudomonas syringae* 13b-2, and *Arthrobacter* sp. populations exposed to ambient conditions, daytime light flux, freeze–thaw cycles, osmotic shock, and  $\text{H}_2\text{O}_2$ . Joly et al. (132) concluded that freeze–thaw was primarily responsible for viability reductions in the multifactorial, multispecies experiment. Similarly, Amato et al. (105) simulated cloud conditions expected to be present at lower altitudes and measured an exponential decrease in the viability of *P. syringae* and *Pseudomonas fluorescens* with a half-life time of about 3.5–4.5 h. The study also found that ice nucleation improved survivability outcomes. It is also expected that the absence or attenuation of UV light, perhaps from the coaggregation with dust or adjacent dead cells, can lead to greater endurance to the upper atmosphere environment (118). Solar simulations are performed differently at every laboratory, making comparisons across study difficult. Notably, no artificial light source can simulate the full spectrum of radiation wavelengths expected in the atmosphere. It is also not feasible to simulate dynamic illumination inside environmental chambers (including diurnal cycles and scattering effects). Instead, most simulations expose microorganisms continuously to

UV irradiation at dosages mimicking a fixed solar zenith, altitude, and place (118, 133, 134). Light does not behave this way in nature but the approach simplifies procedures. Accurately mimicking UV dosages is fundamental to producing robust, meaningful data for studies simulating atmospheric conditions in the lower or upper atmosphere.

As a result of known limitations associated with ground simulation experiments, exposing terrestrial and marine taxa to regions above the boundary layer by intentionally transporting microorganisms in payloads onboard scientific aircraft and balloons is an alternative approach to measuring atmospheric survival outcomes. To be successful, environmental influences from preflight, ascent, descent, and landing must be minimized. An ideal exposure experiment occurs at targeted altitudes, alone. Second, aseptic conditions within a closed payload system have to be maintained and monitored to verify the sample integrity of microorganisms transported to the upper atmosphere. Smith et al. (135) demonstrated a new hardware system capable of delivering microorganisms to the stratosphere for controlled exposure experiments on large scientific helium balloons capable of carrying instruments and experiments on a gondola later returned to the surface on a parachute. On two previous flights launched from New Mexico the Exposing Microorganisms in the Stratosphere (E-MIST) payload carried known quantities of *Bacillus pumilus* SAFR-032 to approximately 36 km above sea level (135). During the test flights, which ranged from 5 to 25 h in duration, the hardware system's sample holders contained bacterial spores deposited on the surface, rotating open at hourly time intervals and exposing the specimens to the stratosphere. Bacteria used for experiments were metabolically dormant; hence, the stratosphere exposure caused cumulative damage to internal cellular components which could be measured against known starting populations. During ascent and descent, samples remained enclosed within dark cylinders at ~25°C. Payload components included a UV radiometer (400 to 230 nm), humidity and temperatures sensors, a flight computer, and a camera system. The payload returned to the ground on a parachute with environmental data stored on its flight computer, and then samples were transported back to the laboratory for biological assays. A negative control coupon was located on the payload and used for the monitoring of possible contamination. Experiments of this sort can be useful for testing a variety of microorganisms at various altitudes and locations across the globe to better measure and model the expected delivery of viable microorganisms in long-range transported air masses.

In summary, ground- and flight-based experiments have revealed that the survivability of microbes in the upper atmosphere depends on (i) atmospheric residence time, (ii) the atmospheric conditions to which microbes are exposed, and (iii) genomes and phenotypes of microbial populations. These aspects will vary as a function of microbial source region, injection mechanisms, and factors influencing deposition. More fully integrated experimental approaches (i.e., modeling transport pathways, identifying cotransported aerosols and trace gases, and intercomparing these with the results of microbial metagenomics) will be necessary to generate testable hypotheses about environmental conditions experienced aloft and key ecological implications when microorganisms return to the surface. Collectively, aerobiology survival experiments conducted to date indicate microorganisms cannot linger above the boundary layer perpetually. Most bacteria, even hardy endospore-forming species evaluated in previous ground studies, were inactivated by direct exposure to sunlight within hours. Hence, the detection of viable microbes in the upper atmosphere, repeatedly, from a variety of research teams across the planet remains

a compelling paradox for the field of aerobiology. Three general areas should be examined in the years ahead to better explain why viable microbes continue to be identified in samples collected from extreme altitudes, as follows.

- i) *Stowaway hypothesis*: To what extent does cotransportation and shielding provided by dust particles and/or dead biomass permit the survival of certain microorganisms?
- ii) *Sunscreen hypothesis*: Are previously unmeasured, unique atmospheric phenotypes, including resistance to UV, responsible for the survival of airborne microbes?
- iii) *Contamination hypothesis*: Are false positives (stowaway microbes on hardware) responsible for life detected at extreme altitudes? (A possibility to be discussed in Section 2.3.5.1.)

### 2.3.3.3 Long-range transport studies by method type

#### Culture-based methods

A primary limitation of culture-based approaches for bacterial analyses is that less than 1% of the existing community that reside in most aquatic and terrestrial environments are culturable (136). Other factors that can negatively influence culture data for aerobiology studies are the state of cell stress from desiccation, humidity extremes, atmospheric and incubation temperatures, and UV exposure that affect the ability of a typically culturable microorganism to grow within classical incubation periods (4, 42). Another critical factor needed to maximize recoveries of culturable microorganisms from atmospheric samples is low-nutrient medium that limits medium shock and gives cells the time to repair damaged components and grow at a normal rate (42, 137, 138). Regardless of their recognized limitations, culture-based assays have proven valuable in demonstrating survival and viability of community members in any number of real or simulated extreme environments (6, 41, 42, 139, 140). Concentration data in colony-forming units (CFU) from a number of long-range transport studies have ranged in distance from source to sample site from 400 to 9200 km (5, 6, 141, 142). Of those studies reporting bacterial or fungal CFU m<sup>-3</sup>, for source distances ≤ 1100 km, concentrations ranged from 0 to 995 and from 0 to 703 respectively (7, 143–147). In contrast, data from air samples collected from the atmosphere over the Canary Islands (Tenerife, ~500 km off the northwest coast of Africa) over a 3 year period demonstrated a combined average count of  $6.27 \times 10^5$  CFU m<sup>-3</sup> for both bacteria and fungi (148). For source distance ranges of >1100 to 2600 km, counts ranged from 0 to 15 700 bacterial and from 0 to 6992 fungal CFU m<sup>-3</sup> (11, 22, 149–153). Bacterial and fungal concentrations in CFU m<sup>-3</sup> for distances >2600 km ranged from 0 to 353 and from 0 to 90, respectively (3, 5, 6, 87, 95, 154–156). Two Taiwanese studies noted spore counts at concentration ranges of 6078 and 29 038 spores m<sup>-3</sup> during dust events (151, 152). In contrast to these studies, the percent culturability is reflected in the two Taiwanese studies that utilized fungal culture to study aerobiology during Asian dust events and reported lower ranges of 915 (11) and 3664 spores m<sup>-3</sup> (149). It should be noted that few of these studies utilized the same approach for collection and culture, leading to a highly variable range of observations. Data from many culture-based, long-range transport studies published prior to 2007 demonstrate a high degree of airborne bacterial and fungal diversity on a planet-wide scale (4, 75). A study conducted in the atmosphere over the mid-Atlantic

ridge at  $\sim 23^\circ\text{N}$  identified 13 bacterial and 14 fungal genera that were primarily recovered when African dust was impacting the research site (156). Similarly at a coastal tower research site in Erdemli, Turkey, eight bacterial and seven fungal genera were identified primarily during dust event periods (146). It is interesting that, at a site closer to the source region in Mali, Africa, more bacteria genera (20) were identified than fungal (3) (the ratio of bacteria to fungi goes down as transport distances increase), which demonstrates the lethality of atmospheric transport for many bacterial types (150). Research conducted at Mount Bachelor Observatory, a North American site  $\sim 9600$  km from aerosol source regions in Asia, identified 18 species from six bacterial genera (all Gram-positive bacteria capable of forming endospores) and 31 species from 26 fungal genera with reproductive spores (5).

### Molecular-based methods

Nishimura et al. (145) reported concentrations of bacteria impacting Beijing, China ( $\sim 1000$  km from the dust storm source region), while utilizing quantitative PCR (qPCR) at a range of  $1 \times 10^6$  to  $3.5 \times 10^9$  cells  $\text{g}^{-1}$ . In a later, related project conducted within the same city, similar concentrations of bacteria were noted when dust was present that ranged from  $1 \times 10^8$  to  $2 \times 10^9$  cells  $\text{g}^{-1}$  (7). Approximately 1600 km downwind from this same source region in Osaka, Japan, qPCR concentrations during dust events ranged from  $5 \times 10^3$  to  $2 \times 10^5$  cells  $\text{m}^{-3}$  (22). In 2010, Chen et al. (102) reported elevated (versus clear atmospheric conditions) dust-associated qPCR influenza A concentrations that ranged from non-detects to 25 copies  $\text{m}^{-3}$ . In a 4 year study conducted on Tenerife in the Canary Islands, enteric viruses were routinely detected in air samples collected during both normal and dust days (157). At the Mount Bachelor Observatory in Oregon, USA, Smith et al. (5) reported average DNA concentrations in the range of  $4.9 \times 10^{-5}$  to  $4.8$  ng  $\text{m}^{-3}$  for transpacific bacteria and fungi, respectively, via qPCR. In this study bacterial isolates primarily belonged to the family Bacillaceae and fungal isolates represented over 20 different genera (5).

The following is a summary of a number of the long-range desert dust research projects that have been published since 2007 using culture assays, molecular assays, or a combination of both methods. In 2008, De Deckker et al. (158) reported identifying six phyla from fallen dust samples collected in Canberra, Australia ( $\sim 1100$  km from the source region, using 16S rRNA PCR sequencing). In that same year, Polymenakou et al. (159) collected Saharan dust samples in Heraklion, Crete ( $\sim 700$  km from the source region, using 16S rRNA amplicon sequencing), and reported the presence of eight phyla. In 2009 and 2010, several Asian desert dust studies that utilized amplicon sequencing reported the identification of various genera to include *Bacillus*, *Propionibacterium*, *Ralstonia*, *Serratia*, and *Aquabacterium* sp. in samples collected in Beijing, China, Seoul, Korea, and Suzu, Japan (145, 153, 160). Studies conducted in the Canary Islands using culture and molecular assays identified 58 bacterial and 37 fungal genera dominated by *Bacillus*, *Arthrobacter*, and *Staphylococcus*, and *Penicillium*, *Cladosporium*, and *Alternaria*, respectively (148). Smith et al. in 2013 (142) identified thousands of distinct bacterial taxa using microarray analyses of Asian dust samples collected at the Mount Bachelor Observatory. Studies that have been reported since 2013 employing a variety of metagenomic techniques illustrate an even greater depth of bacterial and fungal diversity in samples collected at ranges  $\geq \sim 1000$  km from suspected source regions (7, 10, 142, 161–167). Bacteria and fungi, both CFU and sequences, have

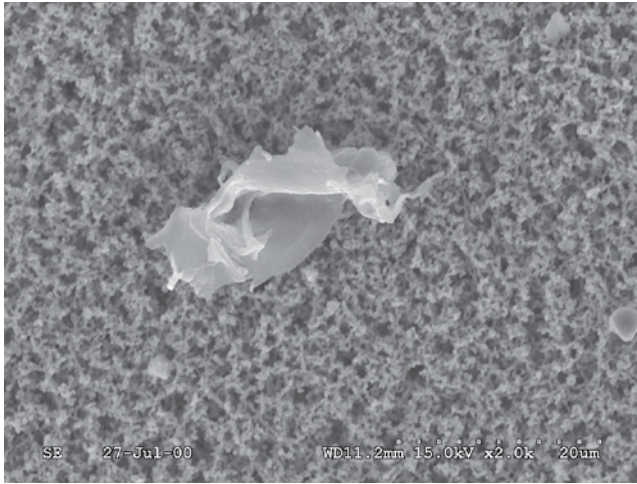
been identified in air masses classified as “clear” or “dust associated,” regardless of whether samples were collected at mid-oceanic locations or at alpine observatories (5, 87, 142, 156).

## 2.3.4 Residence time, transport history, and emission models

### 2.3.4.1 General principles

Residence time—defined generically here as the total period that microorganisms remain aloft in the atmosphere—can vary from minutes to months depending on interrelated physical and biological factors. The beginning and end of the process is associated with distinct sources and sinks (25, 168). Bioaerosols emanating from a wide array of terrestrial and marine environments on the surface (discussed previously) reach altitudes in the atmosphere dependent on wind conditions, the size of the particles, and fallout factors including precipitation (17, 25, 169, 170). Most airborne microorganisms return to the surface prior to exiting the boundary layer due to wet or dry deposition (171). Large-diameter bioaerosols of the PM<sub>10</sub> fraction such as fungal hyphae, spores, pollen, bacteria, and archaea attached to larger substrates generally have shorter residence times in the troposphere owing to higher rates of sedimentation (172). However, storm intensity and high-speed winds may greatly enhance dispersion with large inorganic soil particles, as “giant-sized” quartz particles (>62.5 μm) were observed in the Pacific up to 10 000 km from their source (173, 174). Fungal spores typically range in size from 2 to 40 μm with various morphologies to include globose, elliptical, curved, rod-like, and asymmetric (175). Estimated settling rates from an altitude of 3000 m is ~3.5 days for *Alternaria* sp., ~116 days for *Aspergillus fumigatus*, and ~1157 days for *Coccidioides immitis*. Bacteria, which typically range from 0.5 to 1.5 μm have a settling rate (using an average of 1.0 μm) that is equal to that estimated for *C. immitis* at 0.003 cm s<sup>-1</sup> or a settling rate from 3000 m of 1157 days (176). Non-aggregated bioaerosols the size of the PM<sub>2.5</sub> fraction (≤2.5 μm) might have residence times in the upper troposphere of the order of weeks or even months (17, 77). Viruses are typically much smaller than 1 μm (Enteroviruses ~0.04 μm; Ebola, which is considered a large virus, ~0.97 μm) and, because of their smaller size, may remain airborne indefinitely (177). Whereas fungal spores emanate from parent organisms as an adaptive dispersal strategy, bacteria and viruses are typically cotransported with organic detritus and soil particles from surface environments (178–182). Figure 2.3.4 is a scanning electron microscope image of a piece of asymmetric organic detritus that is approximately 20 μm in length (collected during an African dust event in the Northern Caribbean). Organic detritus such as this could act as an atmospheric “sail” moving rafted microorganisms beyond typical dispersion ranges. Particle transport can also provide UV shielding for microbes embedded within cracks and other surface irregularities.

Overall, the tropopause (ranging from 9 to 17 km above sea level) is likely a natural barrier for bioaerosols in the atmosphere, representing a major bottleneck in the free exchange of PM<sub>2.5</sub> fraction bioaerosols with the surface–atmosphere system. While the troposphere is turbulent and well mixed, physical properties of the stratosphere limit convection. Still, some of the previously discussed uplift mechanisms, including



**Figure 2.3.4** Scanning electron microscope image of organic detritus collected by membrane filtration during an African dust event that impacted the northern Caribbean in the summer of 2000. Light asymmetric particles like this can serve as atmospheric rafts and prolong the atmospheric suspension of associated microorganisms. (Photo credit: D.W. Griffin.)

convective overshooting and strong updrafting forces generated by processes associated with thunderstorms, hurricanes, typhoons, monsoons, electrostatic levitation, gravitophotophoresis effects, volcanic eruptions, and anthropogenic activities, could all contribute to bioaerosols being swept into the stratosphere (20, 183–188). Altogether, the primary portion of the aerial microbiome that directly interfaces and exchanges with Earth’s surface biomes is probably restricted to altitudes below the tropopause (with results from exceptional high-altitude case studies, to be discussed in Section 2.3.5).

### 2.3.4.2 Global and regional models including biological aerosols

A number of global and mesoscale models (Table 2.3.1) have been used since 2005 for the Eulerian calculation of the emission, dispersion, and atmospheric fate of various biological particles. In Eulerian models, the prognostic equations for the concentrations of tracers (here, the biological aerosol particles) are solved at fixed gridpoints. The prognostic equation has the following form (without turbulence effects):

$$\frac{\partial c}{\partial t} = -(\vec{v} \cdot \nabla)c - \nabla \cdot \overline{F_{\text{turb}}} - \frac{\partial}{\partial z} F_{\text{sed}} - \lambda c + Q$$

Here,  $c$  is the (number or mass) mixing ratio,  $\vec{v}$  is the advection velocity,  $\overline{F_{\text{turb}}}$  is the turbulent diffusion flux,  $F_{\text{sed}}$  is the sedimentation flux,  $\lambda$  is the washout coefficient, and  $Q$  is a source term. The source term needs to be parameterized, usually in the form of a surface emission flux  $F_{e,s}$ , yielding  $Q$  from the following equation:

$$Q = -\frac{\partial}{\partial z} F_{e,s} \approx \frac{F_{e,s}}{\Delta z_s}$$

Here,  $\Delta z_s$  is the vertical extent of the surface layer.

**Table 2.3.1** Summary of recent global and mesoscale model studies of bioaerosol emission and dispersion.

	<b>Independent variables in emission parameterization</b>	<b>Model domain and resolution</b>	<b>Global/regional source strength</b>	<b>Global/regional burden</b>	<b>Reference</b>
Bacteria	Fractional coverage of 10 ecosystem types	Global (1.8°)	0.74 (0.4-1.8) Tg yr <sup>-1</sup>	8.7 Gg	17
	Fractional coverage of 10 ecosystem types	Global (2.8°)	0.75 Tg yr <sup>-1</sup>	4.3 Gg	195
	TKE, vegetation fraction, soil class, month of year	Global (4° × 5°)	28.1 Tg yr <sup>-1</sup>	N/A (310 Gg for bacteria, spores, and pollen together)	255
	Fractional coverage of five ecosystem types	Global (2.8°)	2.58 Tg yr <sup>-1</sup>	28 Gg	199
	Fractional coverage of seven ecosystem types	Global (2.8°)	N/A	11 Gg	193
Fungal spores	LAI, water vapor mixing ratio	Global (2.5°)	28 Tg yr <sup>-1</sup>	180 Gg	191
	TKE, LAI, relative humidity, vegetation fraction, soil class, month of year	Global (4° × 5°)	186 Tg yr <sup>-1</sup>	N/A (310 Gg for bacteria, spores, and pollen together)	255
	LAI, specific humidity	Global (2.8°)	31 Tg yr <sup>-1</sup>	94 Gg	195
	Fractional coverage of five ecosystem types	Global (2.8°)	3.97 Tg yr <sup>-1</sup>	1 Gg	200
	LAI, water vapor mixing ratio	Global (2.8°)	N/A	150 Gg	193
	LAI, specific humidity, month of the year	Global (1.9° × 2.5°)	60 Tg yr <sup>-1</sup>	N/A	256
FBAP (assumed to be dominated by fungal spores)	Specific humidity, temperature, LAI	Europe (14 km)	N/A	N/A	192
Marine organic aerosols (partly primary biogenic material)	Sea salt emissions, 10 m wind, chlorophyll <i>a</i>	Global	15.9–18.7 Tg C yr <sup>-1</sup> (2.8–5.6 Tg C yr <sup>-1</sup> submicron)	N/A	257

(Continued)

**Table 2.3.1** (Continued)

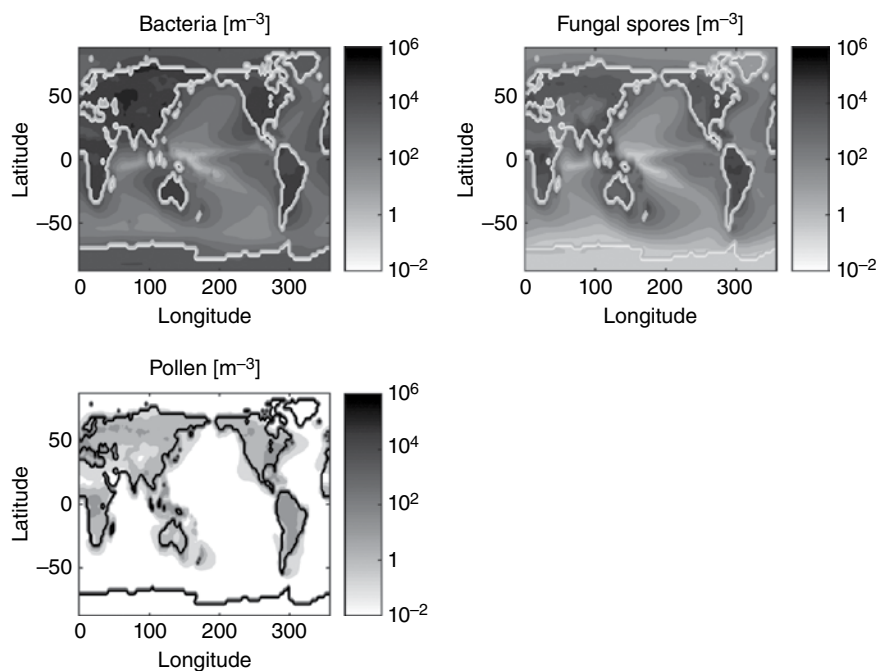
	Independent variables in emission parameterization	Model domain and resolution	Global/regional source strength	Global/regional burden	Reference
Pollen	Sea salt emissions, MOA fraction TKE, LAI, vegetation fraction, soil class, month of year, hour of the day	Global Global (4° × 5°)	32.6 Tg yr <sup>-1</sup> (8.24 Tg C yr <sup>-1</sup> submicron) 84 Tg yr <sup>-1</sup>	N/A N/A (310 Gg for bacteria, spores, and pollen together)	258 255
Birch pollen	LAI, month of the year Number of pollen grains produced during one season, likelihood to bloom, friction velocity	Global (2.8°) Switzerland (7 km)	47 Tg yr <sup>-1</sup> N/A	22 Gg N/A	195 194
Ragweed pollen	Number of pollen grains produced during one season, likelihood to bloom, friction velocity	Europe (7 km)	N/A	N/A	259
Birch pollen	Maximal daily amount of pollen, vegetation fraction per species, course of the pollen season, precipitation, TKE, temperature, RH, LAI	Europe (7 km)	N/A	N/A	189
Birch and ragweed pollen	Friction velocity, LAI, humidity, wind speed, precipitation, birch/ragweed plant density	Northeast USA (12 km)	N/A	N/A	260
Birch, grass, olive, and ragweed pollen	Temperature sum, precipitation, humidity, wind speed at 10 m, convective velocity scale	Europe (0.2° or 0.25°)	N/A	N/A	190, 261, 262
<i>Betula</i> , <i>Bromus</i> , <i>Juglans</i> , <i>Morus</i> , <i>Olea</i> , <i>Platanus</i> , and <i>Quercus</i> pollen	Onset and duration of the pollen season (depending on temperature and precipitation), potential amount of pollen released per day, canopy height, friction velocity	California (12 km), southern California (4 km)	N/A	N/A	263
Ragweed pollen	Friction velocity, LAI, humidity, wind speed, precipitation	Europe (50 km)	N/A	N/A	198
Primary biogenic aerosols <10 μm	Annually averaged above-ground biomass, constant year-round	Global (1.8°)	186 Tg yr <sup>-1</sup> (0.164 Tg P yr <sup>-1</sup> )	N/A	197

FBAP, fluorescent biological aerosol particle; LAI, leaf area index; MOA, marine organic aerosol; N/A, not applicable; RH, relative humidity; TKE, turbulent kinetic energy.

At present, most global and mesoscale models subdivide biological particles into bacteria, spores, pollen, and marine organic aerosols and either treat these categories with different prognostic variables (for number and/or mass of the particles, sometimes subdivided into different size bins) or they treat only a subset of them, depending on the application of the model. Table 2.3.1 lists relevant model studies that provide estimates of the emission and dispersal of biological particles on large scales. The level of detail in the emission parameterization varies substantially between particle types and between different models. In general, bacteria are treated with the simplest emission functions (fixed emission fluxes for different ecosystems (17, 168)), while pollen emissions are treated with detailed physical and biological dependencies. Pollen grains are also the only biological particles which are usually subdivided into species. The reason for this high level of detail is threefold. First, the sources are unambiguously related to the occurrence of specific plants. Second, pollen grains of different species are relatively easy to identify by classic microscopic pollen count methods. Third, their individual occurrence by species is of interest for health effects, such that pollen forecasts with Eulerian dispersion models have already been implemented by operational weather services (e.g., (189, 190)). For fungal spores, emission functions have been developed based on observed correlations between concentrations and meteorological variables (191, 192). Marine organic aerosols (which are partly of primary biogenic origin) are scaled to sea salt emission fluxes.

In general, the spread between different global emission estimates for a certain type of biological particle is very large (one to two orders of magnitude), even when the emission parameterizations follow similar forms. The reasons for these discrepancies are differences in the considered size classes, the definition of the considered particle type, and the lack of suitable data to evaluate the models. In comparison with *in situ* measurements, the simulated concentrations in global models often deviate significantly (e.g., (193)), but mesoscale models—which are to some extent tuned to local observations—have a higher skill in capturing short-term variability (192, 194). The global distribution of biogenic particles (Figure 2.3.5) features generally higher concentrations over land and lower concentrations over oceans, with maxima over the African and South American tropical forests and boreal regions in central Asia (17, 193, 195).

Relative to other coarse-mode particles such as sea salt and mineral dust (e.g., (196)), the estimates of the global emissions and burden of biological particles are smaller by at least two orders of magnitude. Therefore, the interest to include them into aerosol models stems less from their role for aerosol microphysics or direct radiative effects, but is motivated by special properties such as the phosphorus content (197), their role as allergens (e.g., (198)), and their ice nucleation ability and potential impact on clouds. With respect to the last point, all available global model studies agree that the low concentrations of biological particles limit their potential impact as ice nuclei on global average cloud properties (193, 195, 199, 200). However, Sesartic et al. (199, 200) and Spracklen and Heald (193) point out that some impact on the liquid and ice water paths is found in boreal regions, where bacteria and fungal spore concentrations are relatively high. The impact is expected to be limited to the mid- and lower troposphere (400–600 hPa), where temperatures are often too warm for inorganic ice-nucleating particles to be active (193). Global model studies of marine organic aerosols suggest that these may be important as a source of ice-nucleating particles in the remote oceans, such as the Southern Ocean, where mineral dust is scarce (201, 202).



**Figure 2.3.5** Annual mean near-surface number concentrations of bacteria, fungal spores, and pollen, simulated with a global aerosol model (195). (Reproduced with permission from IOP Publishing.)

### 2.3.4.3 Determining transport history with proxy aerosols

Injection and deposition mechanisms are only part of the full picture required for describing atmospheric residence time. Travel patterns and environmental stressors above the boundary layer are critical to addressing the ecological consequences of aerosolized microorganisms. Since bioaerosols are dilutely distributed in the upper atmosphere, remote measurements from the ground or space satellites are not yet feasible. However, proxy aerosols in more easily detectable concentrations, with identifiable source regions and unique mixing ratios (molecular “clocks”), can be used to establish most likely pathways for long-range bioaerosol transport. To enable such observations, a number of physical and chemical aerosol measurements, along with satellite data and meteorological models, must be considered as supplementary information to microbiological characterization. Basic aerosol properties including wavelength-dependent scattering and absorption coefficients can be derived from a variety of instruments such as X-ray fluorimeters in order to provide information on key elements (such as Na, Mg, Al, Si, S, Ca, and Fe) within bioaerosol aggregations to help establish aerosol type and candidate source regions.

Many of the aerosol species listed in Table 2.3.2 are commonly detected in atmospheric samples containing microorganisms and are in sufficiently dense levels to be monitored remotely through existing satellites and modeling methods. Cotransportation with bioaerosols allows these proxy aerosols to stand in for an otherwise cryptic aerobiological transport history. Combining aerosol identification, model analysis, and

**Table 2.3.2** Tools for characterizing aerosol transport history.

Sensor/instrument	Aerosol types or products	Reference
<i>Ground-based</i>		
AERONET	Mineral dust, urban dust, smoke and soot, marine aerosols	264
Micro Pulse Lidar Network (MPLNet)	Vertical profile of aerosol backscatter and depolarization	265
Rosenstiel School of Marine and Atmospheric Science (RSMAS) network	Non-sea-salt sulfate, dust, methanesulfonic acid	266
Interagency Monitoring of PROtected Visual Environments (IMPROVE)	Ions, metals, metalloids, organic carbon, elemental carbon, fine soil	267
<i>Airborne</i>		
High Resolution–Time of Flight Airborne Mass Spectrometer (HR-ToF AMS)	Urban/industrial organic aerosols, biomass burning	268
High Spectral Resolution Lidar (HSRL)	Smoke, mineral and urban dust, pollution	269
<i>Satellite</i>		
Moderate-resolution Imaging Spectroradiometer (MODIS)/Terra and Aqua	Smoke, mineral and urban dust over land and ocean	270
Multangle Imaging Spectroradiometer (MISR)	Marine aerosols and dust	271
Cloud-Aerosol Lidar with Orthogonal Polarization (CALIOP)	Smoke, mineral and urban dust	272
Ozone Monitoring Instrument (OMI)/Aqua	Aerosol index for dust/smoke from pollution and sea salt	273
Atmosphere Infrared Sounder (AIRS)/Aqua	Dust, CO, relative humidity and temperature profiles	274
Ozone Mapper and Profiler Suite (OMPS)/NPP	Aerosol index for O <sub>3</sub> , dust/smoke	275

metagenomic information is required to decipher dynamic transport histories. Using microbial biogeography and the detection of isolates from unique ecosystems is, alone, rarely sufficient to pin distant source regions responsible for emitting the bioaerosol collected downwind. Coupling atmospheric chemistry data, meteorological models, and metagenomic results was demonstrated by an alpine study monitoring the transpacific movement of microorganisms in the free troposphere at the Mount Bachelor Observatory in Oregon, USA (5, 142). Convergence of results from independent, interdisciplinary methods all pointed to the presence of Asian long-range transport. During sampling periods, meteorological and atmospheric chemistry data were collected for aerosol elemental composition (i.e., ammonium sulfate ((NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>), soil, and trace metals), carbon monoxide (CO), ozone (O<sub>3</sub>), water (H<sub>2</sub>O) vapor, total gaseous mercury (THg), temperature, atmospheric pressure, wind speed, and direction (203). Air mass trajectories were calculated with the Hybrid Single-Particle Lagrangian Integrated Trajectory (HYSPLIT) using a time resolution of 3 h, a spatial resolution of 1° latitude

by 1° longitude, and a vertical resolution of 23 pressure surfaces between 1000 Pa and 20 hPa (204). Trajectories were run at multiple heights (1200, 1500, and 1800 m above ground level) surrounding the sampling station. Transoceanic aerosol plumes were then modeled with the Navy Research Laboratory Aerosol Analysis and Prediction System (NAAPS) to compare them with HYSPLIT long-range transport patterns depicting the transpacific migration of airborne sulfate, dust, smoke, and sea salt at a total aerosol optical depth of 0.1–0.2 (205). The NAAPS model produced a total aerosol forecast at an optical depth of 550 nm that included sulfate, smoke, dust, and sea salt mass concentrations. Low humidity and high O<sub>3</sub> sampled at the same time as bioaerosols indicate that the air plume likely mixed with regions from the upper troposphere/lower stratosphere during transport (5). Enhancement between the chemical species THg and CO were used as cotransportation tracers, and, similarly, soil and (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> concentrations were correlated, with homogenization indicative of atmospheric residence time of the order of 10 days (206).

## 2.3.5 Implications for planetary exploration

### 2.3.5.1 Aerobiology informs astrobiology

Earth's atmosphere provides a thin barrier to the severe conditions of space. Globally, terrestrial biology from our planet's surface moves through and interacts with the blanketing atmosphere, analogous to how marine life drifts through oceans. A century of exploration has allowed oceanographers to characterize marine life at every depth. Such knowledge was enabled by routine access—specifically, submersible vehicles with sample return systems. The outer reaches of Earth's atmosphere probably contain trace signatures of life swept up from the surface but high-altitude exploration has been severely constrained by a shortage of reliable, affordable sample acquisition systems. Where does the natural boundary of the biosphere end in the atmosphere? Where does meaningful activity stop? Is it possible to fly clean enough instrumentation to collect bioaerosols in low concentrations? Answers to these fundamental questions are directly relevant to the discipline of astrobiology searching for signs of life in the universe (20, 207). For example, the stratosphere is a natural laboratory for examining habitable microbial environments on the surface of Mars, which possesses a similar combination of conditions (high levels of UV light and low extremes of temperature, pressure, and relative humidity). Moreover, low biomass detection instrumentation developed exploring Earth's upper atmosphere may contribute to strategies for detecting biology in atmospheres of other worlds.

#### Natural boundary of the biosphere

At some yet-to-be-determined point, traveling vertically up through a column of Earth's atmosphere, signatures of bioaerosols will likely be absent. The altitude range where this occurs, referred to as the upper boundary of the terrestrial biosphere (208), has not been firmly established by aerobiology studies. Conclusions based on observations of microbial growth and DNA signatures from samples collected in the upper atmosphere have resulted in extraordinary claims for the tenacity of life (6, 22, 23, 41, 42, 118, 130, 131, 135, 208–221). While pioneering, many of the previous studies have been

qualitative in nature and lacking rigorous control measurements to verify the authenticity of the results obtained (20, 222). Based on contamination control issues and the absence of reproducible data, results from all upper atmosphere missions must be conservatively interpreted. Viable microbes recovered from 77 km a.s.l., the highest biosphere boundary commonly cited in literature, were collected by a single rocket flight to the mesosphere by Imshenetsky et al. (208). However, the authors did not describe in any detail how their system was sterilized or prevented contamination beyond the untested, unmeasured assertion that heat of friction during ascent should have sterilized portions of the nose cone. A subsequent mission was never flown, and the simplest explanation for the spores collected in the mesosphere is that the microbes originated from the rocket itself or from soil contamination after the system impacted the desert landing site.

Strides to establish the upper limits of the biosphere boundary have been constrained by inadequate sampling systems capable of collecting bioaerosols in rarefied air. Smith (20) reviewed the current collection methods used by research teams flying balloons, aircraft, and sounding rockets and protocol improvements for low concentration DNA-based bioaerosol collectors was recently reviewed by Luhung et al. (223). More sensitive molecular methods (reviewed by Yooseph et al. (224) and Prussin et al. (225)), paired with microfluidics and miniaturized, autonomous instruments, might also create new *in situ* collection and analysis opportunities (226–229). Additionally, the commercialization of space with numerous companies now offering suborbital flights may provide the field of aerobiology with unprecedented missions of opportunity in the coming decades (230, 231).

#### **Reducing false positives and improving contamination control**

Similar to aerobiology, astrobiological questions guiding international space missions require a comprehensive understanding of potential contamination sources. Detecting low biomass at the upper reaches of Earth's atmosphere shares many similarities with the effort to measure biology in pristine extraterrestrial environments. Preventing forward contamination—the transport of viable terrestrial microorganisms to other planets—is required by international policy agreements (232). Despite comprehensive protocols to reduce bioburden, it is now commonly understood, as revealed by sensitive high-throughput methods, that spacecraft leaving Earth inadvertently carry microorganisms onboard embedded within surfaces, wiring, electronics, and other inaccessible areas that cannot be sterilized (233, 234). Spacecraft assembled in cleanrooms are not actually clean, despite best efforts (234–238). Ultimately, engineers and technicians operating around the flight hardware are the primary source of contamination, and, ironically, attempts to clean hardware with H<sub>2</sub>O<sub>2</sub>, alcohol, heat treatments, and irradiation sources might not have a desirable effect, potentially killing off the weaker contaminants and leaving hardy survivors preadapted to harsh conditions behind (238–240). Considering this body of knowledge from international space programs, Earth's upper atmosphere environment is clearly susceptible to contamination delivered by flight hardware systems in the pursuit of aerobiological exploration (222). Consequently, every high-altitude aerobiology study must convincingly demonstrate that microorganisms sampled from the environment are native cells (as opposed to false positives).

Bryan et al. (221) published methods describing a stratospheric balloon hardware system featuring, arguably, the strictest contamination control measures implemented

to date in the field of aerobiology. Life's Atmospheric Microbial Boundary (LAMB) is a balloon payload that passively samples aerosols at an ascent rate of  $\sim 305 \text{ m min}^{-1}$  into the upper stratosphere ( $\sim 38 \text{ km a.s.l.}$ ). Payload doors open and close at preprogrammed altitudes during ascent and allow sampling of the air using a solid impactor rod collection method (40 rods per chamber). Each rod samples  $\sim 15 \text{ L min}^{-1}$  over any altitude range. Rods can be decontaminated by exposure to germicidal, 254 nm ultraviolet radiation for 20 min, soaking in sodium hypochlorite (2000 parts per million (p.p.m.)) for 20 min, rinsing with 70% (v/v) ethanol, and then dried in a laminar flow hood. After applying a thin layer of silicone grease for sample collection, the rods are sterilized one last time through ethylene oxide exposure. For each flight, a dedicated LAMB sample chamber flies unopened, serving as a procedural control for all measurements. A cut mechanism allows the payload string to be separated from the balloon at any desired altitude, returning the payloads safely to the ground on a parachute. Preliminary results showed a collection efficiency of about 100 cells per rod on a typical ascent through the stratosphere (221).

Potential false positives associated with upper atmosphere aerobiology studies will linger behind every publication unless special operating considerations and controls are documented, repeated, and verified. All collection sites and platforms require stringent experimental approaches. At a minimum, content to include within manuscripts or supplementary information files should address:

- description and verification of all sterilization methods
- description and verification of nucleic acid removal methods
- ground control results (samples and sampling units transported to field site)
- hardware control results (samples and sampling units transported to field site, installed in equipment, but not activated)
- assay controls (handling and laboratory-processing quality controls)
- any contamination results from the aforementioned assays to assess signal-to-noise ratio of collection system and assays.

### Life in other atmospheres

Widespread distribution of airborne microbes in Earth's atmosphere demonstrates a need to examine other planetary atmospheres with future astrobiology exploration missions. Arguably, habitable skies (regions where liquid water is stable and available) could be more common than habitable surfaces throughout the universe. More than 3000 exoplanets have been detected to date, the vast majority have atmospheres (due, in part, to biases associated with transit-based detection strategies), and around 100 billion exoplanets are thought to be present in our galaxy (241). It is not unreasonable to consider whether extraterrestrial life could thrive in exoplanet atmospheres. Here in our solar system, there may be habitable regions within the atmospheres of planets (e.g., Venus) and moons (e.g., Titan) (242). Even if life emerged on Earth, alone, viable terrestrial microorganisms still may have reached other habitable atmospheres over billions of years by meteoritic exchange (243) and spacecraft entries. Aerobiology in the atmospheres of gas giants (including Jupiter and Saturn) seems less likely due to extreme convection and radiation conditions.

In the Venus cloud layer roughly 48–65 km above the surface, temperatures and pressures are well within the known tolerances of terrestrial life (244, 245). The

atmosphere consists mainly of CO<sub>2</sub> (96%) and N<sub>2</sub> (3%) but there are other trace gases including water vapor and a variety of ingredients for theoretically powering redox reactions (242, 246, 247). Irwin and Schulze-Makuch (242) argue that some terrestrial acidophilic microbes might be able to persist in these harsh conditions if they are capable of outcompeting the natural absorption of water by sulfuric acid haze. Water may be only several hundred p.p.m. in the lower cloud layers and the droplets would be at a pH of 0 but some acidophiles (e.g., bacterium *Ferroplasma acidarmanus* or archaean Picrophilaceae) can tolerate this acidity level. Schulze-Makuch and Irwin (247) hypothesized that acidophilic microbes in Venusian clouds could theoretically make a living from either phototrophic or chemotrophic metabolic reactions. Sulfur-utilizing chemotrophic microbes on Earth, both within the bacterial and the archaeal domain of life, are examples for such a lifestyle. For instance, *Thiobacillus* is an extremely acidophilic bacterial species that obtains energy by reducing SO<sub>2</sub> to either H<sub>2</sub>S or COS, and the H<sub>2</sub> necessary for driving this reaction should be available from the surface of Venus. Regarding phototrophic possibilities, green sulfur bacteria, purple sulfur bacteria, and some cyanobacterial species on Earth use H<sub>2</sub>S as their electron source, depositing sulfur outside the cell. While none of these microbial groups are tolerant to extreme acidity (pH < ~4.5), selective pressures at Venus as the planet descended into a runaway greenhouse might have driven persisting cells toward adaptations not present on Earth. To cope with UV in the upper atmosphere of Venus, photoprotective pigments or sulfur allotropes might serve as a non-reactive UV absorber. However, ionizing radiation would be another biologically challenging constraint (248).

Interestingly, an unknown UV absorber has been observed in the upper cloud layer of Venus; although biosignatures were not detected during a spacecraft flyby (249), Schulze-Makuch and Irwin (247) argued that elemental sulfur incorporated by hypothetical aeroplankton in the atmosphere would mask hydrocarbon signatures. The borderline habitable environment in the upper atmosphere of Venus and unresolved observations make a case for returning to Earth's nearest neighbor. Since Venus is closer to Earth, it is substantially cheaper to reach, and an atmosphere analysis spacecraft would not have to softly land a spacecraft or protect it from the harsh surface conditions, further reducing mission costs. Since the beginning of the NASA Discovery Program in 1992, at least 24 proposals for Venus missions have been submitted but none selected for launch. Parachute drop and balloon flotation missions introduced by Schulze-Makuch and Irwin (247) and Crisp et al. (250) have considered mission concepts, and others have discussed potential life detection instrumentation (247, 250, 251). In the future, a simple descent probe (with a parachute opening at 70 km) with a fast working microfluidics payload (e.g., (228, 229)) might be capable of detecting potential Venusian cloud life. Such a probe might only have an entry mass of 100 kg, possibly a piggyback craft heading toward the outer planets using a Venus–Earth–Venus flight trajectory boost. Recently, NASA has selected five Discovery-class science investigations for refinement and potential flight opportunities as early as 2020 (252). One of the planetary missions down-selected (Deep Atmosphere Venus Investigation of Noble gases, Chemistry, and Imaging (DAVINCI), led by Lori Glaze of NASA's Goddard Space Flight Center in Greenbelt, MD) aims to explore the chemical composition of Venus's atmosphere during a 63 min descent. It would examine whether volcanoes are active on Venus and how the surface interacts with the atmosphere of the planet. While not biological in nature, such a mission could set the stage for future

aerobiology/astrobiology investigations seeking biosignatures of sulfur-based, phototrophic acidophiles.

Outside the solar system, exoplanet surveys have already detected hundreds of planets with dense atmospheres, and the discovery of terrestrial-sized planets with substantial atmospheres is expected to follow as Earth- and orbit-based telescope capabilities improve. In astrobiology, it has been proposed that biological byproducts, such as O<sub>2</sub>-derived molecules or CH<sub>4</sub>, could indirectly reveal the presence of life on exoplanets (see (253) and references therein). Direct detection of life on an exoplanet surface will require more sophisticated instrumentation and modeling (254). Today, in special cases using currently available methods, transit measurements allow atmospheric spectral data to be obtained as host starlight passes through exoplanet atmospheres. It is therefore conceivable, with airborne biology in a sufficiently dense concentration, that biosignatures within thick exoplanet cloud ecosystems could be detected. This is a far-fetched notion, admittedly, but so was the thought of detecting exoplanets not more than two decades ago. Such observations would permanently link aerobiology and astrobiology, generating one of the most profound discoveries in the history of science.

## Acknowledgments

The authors would like to thank the U.S. Geological Survey (USGS) Environmental Health Toxic Substances Hydrology Program for support. We would also like to thank Eugene Shinn and Jason Westrich for reviews and constructive comments. This content has been peer and administratively reviewed and has been approved for publication by the USGS. Note that approval does not signify that the contents necessarily reflect the views of the USGS, but rather the authors. Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the U.S. Government.

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## **Part III**

### **Impacts of Microbial Aerosols on Atmospheric Processes**

## 3.1

### Impacts of Bioaerosols on Atmospheric Ice Nucleation Processes

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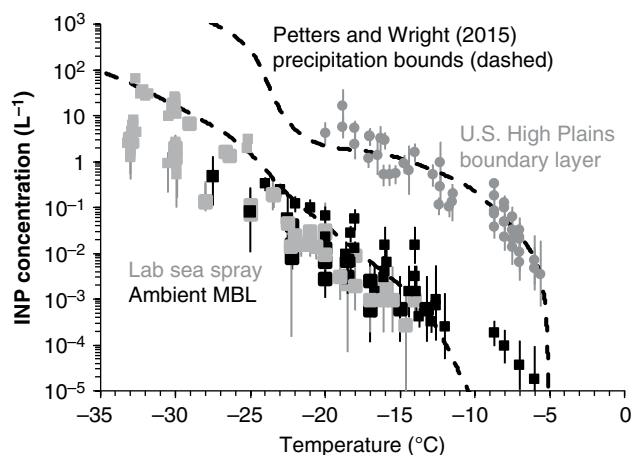
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#### 3.1.1 Introduction

In the absence of particles or other entities (e.g., soluble molecules or monolayers) able to nucleate ice, cloud droplets will supercool in the atmosphere until about  $-38^{\circ}\text{C}$ . Ice nucleation in cloud droplets occurs either via the freezing of water already condensed around a particle, by water vapor condensing and simultaneously freezing onto a particle, or by a particle contacting a supercooled droplet (1). Although there are a variety of materials with surface structures that can assist ice nucleation in the atmosphere, it is commonly stated that mineral dusts are the major source of ice-nucleating particles (INPs, using the terminology of Vali et al. (1)) at temperatures colder than about  $-20^{\circ}\text{C}$ , whereas particles of biological origin may dominate atmospheric INPs above about  $-15^{\circ}\text{C}$  (2–5). However, this commonly accepted assumption can be difficult to prove given the extremely low number concentrations of INPs at warmer temperatures, which range from as low as 0.00001 per liter of air at  $-5^{\circ}\text{C}$  in some air masses (6, 7) to usually not more than 10 per liter of air at  $-20^{\circ}\text{C}$  (6, 8, 9) (Figure 3.1.1).

Existing methods have been improved and new methods developed in recent years to directly measure the INP concentration in the ambient atmosphere (e.g., 8, 10–17) or to measure the INP concentration in substrate-collected or water suspension samples of atmospheric aerosols (e.g., 18–21). These measurements confirm a naturally wide range of atmospheric INP concentrations, the result of variations in total INP concentrations and of variations in their characteristic ice-nucleating activities at different temperatures. Both are greatly affected by the source(s) of INPs, such as whether they are dominated by mineral dusts (22–24) or by particles of biological origin (4, 25–29) (see Section 2.1). Many laboratory experiments have identified these bioaerosols that induce ice formation at modest supercoolings (degrees below  $0^{\circ}\text{C}$ ) at  $-5$  to  $-10^{\circ}\text{C}$  (see Section 3.1.3). However, the concentration and distribution of these

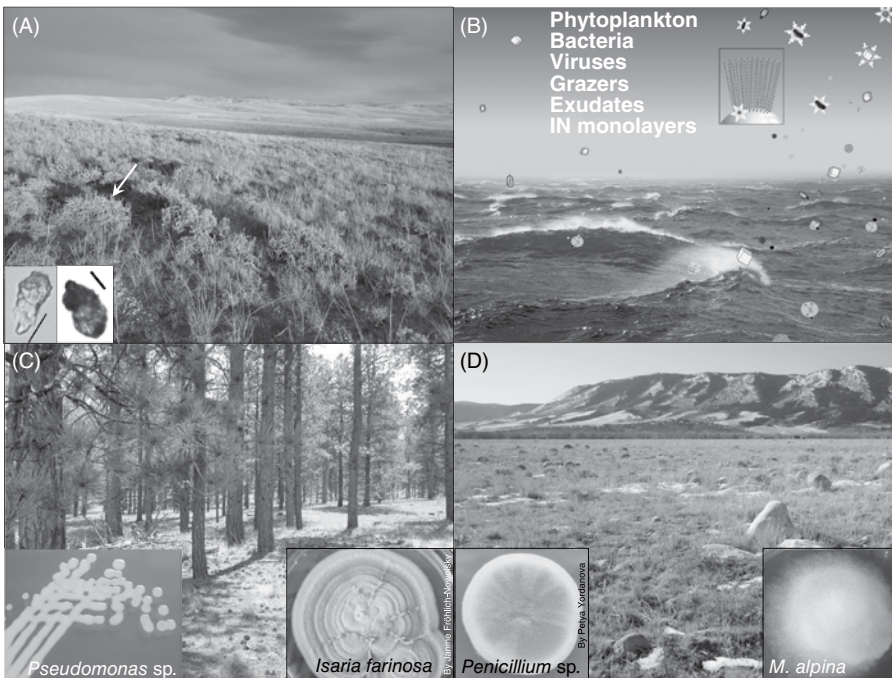


**Figure 3.1.1** Measurements of bounding curves for precipitation sampling of ice-nucleating particles (INPs) (adapted from Petters and Wright (7) overlain on aerosol measurements of INPs from DeMott et al. (6)). The agreement of bounding curves with land-sourced particle data on the upper end and sea spray aerosol INPs on the lower end is remarkable and potentially revealing of a specific impact of regional INPs on precipitation. The warm temperature “bubble” of INPs over land is often seen and is undoubtedly of biological origin. Sea spray aerosol INPs are also implicated as biogenic, and yet these are clearly different in their behaviors. This warns against describing all biological INPs as a single type or as always being of superior efficiency at modest supercooling.

species in the atmosphere and their contribution to total INP abundance remains unclear.

Biological INPs, and their component subsets, can be measured with a variety of approaches (4, 30). Indeed, significant progress has been achieved in recent years in investigating the compositions or active surface structures of atmospheric INPs, including the use of indirect methods such as thermal, chemical, or enzymatic destruction of certain components prior to INP reanalysis (31–34) or the direct analysis of atmospheric ice particle residuals with aerosol mass spectrometry (35–38) or electron microscopy (39, 40). The use of this suite of new tools in a variety of field and laboratory studies is expanding our understanding of the contributors, reservoirs, and emissions of INPs with biological origins (Figure 3.1.2), and, consequently, the potential role of these INPs in cold cloud processes (i.e., in most clouds, in which precipitation forms via freezing of cloud droplets). Initially, the focus was on several species of ice-nucleating (IN) bacteria. Now, it has broadened to include cell-free fungal proteins (41), carbohydrates released by pollen grains (42), components of the soil organic matter (34), and even phytoplankton exudates presumed to be emitted from the sea surface microlayer (43, 44).

This chapter includes an introduction to and summary of instruments and methods being applied to identify biogenic INPs in atmospheric aerosols and study them in isolation, recent findings from laboratory experiments and field studies that are shaping our understanding of the role of bioaerosols as INPs, and the atmospheric implications of these diverse studies. Together, results from recent studies provide a foundation for the development of parameterizations for predicting the atmospheric INP abundance in cloud, weather, and climate models (see Section 2.3.4).



**Figure 3.1.2** Recently discovered ice-nucleating (IN) entities. (A) IN particles directly isolated from soil beneath this sagebrush shrubland (34); both particles inset in the main micrograph nucleated at  $-7^{\circ}\text{C}$  (bar lengths are  $10\ \mu\text{m}$ ). Sagebrush (arrowed) tissue also ice nucleates at  $-12^{\circ}\text{C}$  (34). (B) IN entities potentially contributing to the pool of bio-INPs emitted from oceans, especially so during the decay phase of phytoplankton blooms. (C) IN bacterium *Pseudomonas putida/fluorescens* isolate BF81Fb (19) (reproduced with the permission of the American Society for Microbiology) and *Isaria farinosa* (one of three IN fungi) isolated from the air at this ponderosa pine forest (117). (D) IN fungi isolated from the air above and soil of this native grassland. Shown are an IN *Penicillium* isolate (157), grown from boundary layer air, and *Mortierella alpina*, which can release copious numbers of cell-free IN proteins active between  $-5^{\circ}\text{C}$  and  $-6^{\circ}\text{C}$ , isolated from the soil (41).

### 3.1.2 Measurements of ice-nucleating particles

In this section, we will briefly describe the suite of field and laboratory methods used to measure the concentrations of INPs and to identify their nature and origin. We will distinguish between directly measuring the INP concentration via instruments sampling atmospheric air and methods for collecting aerosol particles onto substrates (e.g., filters) or into water for later offline analysis in the laboratory.

#### 3.1.2.1 Online and offline measurements of single ice-nucleating particles using diffusion chambers

Ice-thermal diffusion chambers operate on the principle of creating gradients of temperature and water vapor between surfaces (usually ice coated) held at different temperatures, which exposes particles to a chosen temperature and varying values of supersaturation of water vapor. A variety of instruments expose collections of particles

on horizontally oriented hydrophobic substrates to controlled temperature and supersaturation conditions (20, 45, 46). This permits assessment of INP activation properties at relative humidities below water saturation. Water vapor supersaturation is achieved in some cases, and, if this is done at near 0°C prior to continued cooling, the immersion freezing process can also be used on such cold plate devices (47, 48). Compositional information has been gathered from some of these types of instruments through the use of integrated environmental scanning electron microscopy or Raman spectroscopy (49), and this may be their special utility.

Continuous flow diffusion chambers (CFDCs) add the element of real-time detection (50, 51). Ice activation times are limited, and sample volume flow rates are typically only a few liters per minute, which together affect the CFDC's detection limit. Geometry and orientation are variable among the present generation of devices, with both vertical and horizontal airflows between either flat or cylindrical concentric plates (15, 50, 52–54). Continuous flow instruments are ideally suited to collect ice crystal residuals, which will contain the INP, for single-particle analyses of compositions via collection onto substrates for electron microscopy, Raman spectroscopy, and other methods. Alternatively, ice crystals can be sent for analysis of their residuals directly to online devices such as single particle mass spectrometers (55). Their ability to process free-flowing aerosols above water saturation (where condensation to droplets will occur) for comparison with immersion freezing methods is attractive.

### 3.1.2.2 Offline ice-nucleating particle measurements using bulk aerosol and precipitation samples

A range of methods are used to collect aerosols for INP analyses. The most common is the use of filters because of their overall simplicity and because they facilitate long-period collections; such bulk collections are necessary when INPs active at modest supercoolings are of interest since these are typically both rare and probably biogenic. Sampling large volumes is essential when INP concentrations are low, such as in polar regions and over oceans (to quantify an average concentration of 1 INP m<sup>-3</sup> with a precision better than ±50%, one needs to sample at least 4 m<sup>3</sup> of air).

Bigg (e.g., 56, 57) used cellulose ester filters to quantify INPs in his still unparalleled broadscale studies across Australia and over the Southern Ocean. Now, polycarbonate Nuclepore membrane filters are often used, since they have accurate pore sizes and are inert (19). On the other hand, quartz fiber filters can be operated at much higher flow rates and are already in use for gravimetric determinations of particulate matter concentrations in air (e.g., aerosols <10 μm in diameter, PM10). Further, they have no lower cut-off size, so they efficiently collect even small IN macromolecules (58). Small circles can be cut out from quartz filters, immersed in an aliquot of water, and tested directly to -15°C in a freezing assay (18).

Alternatively, particles can be collected onto impervious, clean substrates. This includes the collection of aerosols onto hydrophobic glass coverslips in a multistage cascade impactor designed to separate INPs into bins ranging from 0.1 to 10 μm (47, 48) or electrostatic precipitation onto silicon wafers (20, 59). Particles can also be collected directly into water using impingers (e.g., the SKC BioSampler collects ≥80% of particles ≥0.3 μm). Thus far, no studies have compared filters with liquid samplers for collection efficiency and sample preservation.

The INP concentration in precipitating clouds can be indirectly estimated from the INP concentration of rain, hail, or snow (7) (see Table 3.1.1 and Figure 3.1.1). Conversion to INPs per cubic meter of air at cloud level is done by assuming that clouds have an average condensed water content, typically  $0.4 \text{ g m}^{-3}$  (7). Many factors can alter the INP concentration of the cloudwater in the process of precipitation formation and descent, but the limited available evidence suggests it results in only a modest net increase, primarily from particle scavenging and droplet evaporation as it falls (7).

A common means for assessing the number concentration of INPs or ice-nucleating entities is to disperse them, if not already in water, into a few milliliters of water. Then, many equal volume aliquots (picoliters to hundreds of microliters) are cooled in an offline device, and observed as they freeze. A common offline immersion freezing method is to array droplets of a suspension onto a hydrophobic surface placed on a cooled stage, either by nebulization, in an emulsion (picoliter to microliter droplets), or by aliquoting (microliter droplets), and then recording the freezing events, often under a microscope, as the temperature is lowered (see the summaries in Hiranuma et al. (60)). To screen larger volumes (5–400  $\mu\text{L}$  aliquots) polymerase chain reaction (PCR) tubes or 96-well PCR plates can be used, which are then cooled in a bath or block (34, 61). Other methods aim for greater realism by observing the freezing of droplets (<1 nL to 5  $\mu\text{L}$ ) suspended in cooled airstreams. This can be achieved using a vertical wind tunnel (62), by “floating” a droplet on a standing ultrasonic wave (63) or by using an electrodynamic balance to levitate charged water droplets (64). Estimation of INPs starts by assuming their random distribution among the aliquots. Since each aliquot is only able to register the presence of its warmest INP, a simple statistical formula (equation 13 in Vali (65)) is used to estimate the total number of INPs from the fraction of all aliquots frozen.

Repeated freezing and melting of the same array of droplets generally produces very similar results (66). However, there are limits to the stability of biological INPs once immersed in water. For example, in precipitation samples stored at  $4^\circ\text{C}$  INPs active around  $-5^\circ\text{C}$  increased over the course of days (61). Hence, while there is evidence that INPs from diverse sample types collected onto substrates are stable when stored at ambient conditions (20), it is typical to immediately process or store samples frozen.

### 3.1.2.3 Cloud simulation laboratories

Cloud chambers are capable of storing and investigating aerosols over periods of hours to days under well-controlled environmental conditions, including cloud formation cycles, by simulating the expansion cooling of air parcels as they ascend. By that means, cloud formation and cloud internal processes can be investigated as a function of temperature, pressure- and cooling rate. In the Aerosol Interaction and Dynamics in the Atmosphere (AIDA) cloud chamber, expansion cooling is controlled in a cylindrical aluminum chamber with a volume of  $84 \text{ m}^3$  (67). The whole chamber volume is well mixed, so that various instruments measuring *in situ* or sampling from the chamber at different locations can all record and investigate the temporal evolution of a similar cloud parcel. A typical immersion freezing cloud experiment is started at a constant temperature of between  $-5^\circ\text{C}$  and  $-30^\circ\text{C}$ , and at a relative humidity with respect to ice of 90–95%. Aerosols of interest are added in the required amounts to act as both cloud condensation nuclei and INPs during each cloud expansion simulation. Time series of

number concentrations and size distributions are recorded for aerosols, cloud droplets, and ice crystals, and are analyzed for the ice nucleation efficiency per aerosol surface area, the so-called ice nucleation active site density (30, 68). Cloud droplets and ice crystal residuals can be selectively sampled and residuals analyzed with a variety of methods like aerosol mass spectrometry (69, 70).

In recent years, a variety of heterogeneous ice nucleation experiments have been conducted using AIDA for various aerosol types, including mineral dust (71), bacteria (72), and cellulose (73). Since aerosols have long residence times within the chamber, long-duration experiments have been conducted, such as on the viability and ice nucleation activity of IN bacterial cells under well-defined environmental conditions (74).

A disadvantage of the AIDA chamber is the relatively long turnover time of up to 4 h between cloud expansion runs for cleaning and refilling the chamber system. The Dynamically Controlled Expansion Cloud simulation Chamber (DCECC), operated by the Meteorological Research Institute in Tsukuba, Japan (75), has a smaller volume of about 1.4 m<sup>3</sup> and therefore has shorter turnover times. Another advantage with DCECC is that the cloud chamber walls are actively cooled during the expansion experiments, creating isothermal conditions between the chamber volume and the walls, and allowing expansion experiments over a much wider range of supercooling with respect to the start temperature (75).

Cloud condensation and ice formation conditions can also be simulated with the Leipzig Aerosol Cloud Interaction Simulator (LACIS (76, 77)), a vertically oriented laminar flow device with independent control of temperature, relative humidity, and supersaturation achieved by mixing airflows of different temperature and moisture content. The system can be operated at temperatures between 25°C and -50°C for detailed studies of cloud condensation and ice nucleation properties of various aerosols.

#### 3.1.2.4 Contact freezing measurements

Experiments in recent years have confirmed that, when an INP collides with a supercooled cloud or rain droplet, freezing may occur at a warmer temperature than would otherwise occur if the INP was already immersed within the droplet. The actual mechanism of contact freezing that leads to this enhanced freezing efficiency is unresolved. The process also depends critically on particles remaining interstitial (i.e., not scrubbed by nucleation or previously scavenged by cloud droplets) in clouds when they reach supercooled temperatures, and on subsequent collision rates and collection efficiencies. Hence, the contribution of contact freezing to cloud ice activation is uncertain, and is most often considered as a potential factor for long-lived stratiform clouds.

Recent progress in quantifying contact freezing rates has been achieved with a new experimental set-up which directs a flow of size-selected aerosol particles toward a supercooled droplet levitated in an electrodynamic balance trap (78). In this experiment, both the number of collisions between aerosol particles and the supercooled droplet and the subsequent freezing time can be accurately measured and analyzed for the efficiency of contact freezing compared with immersion freezing of the same aerosol particles at the same temperature. Further laboratory experiments have been developed in recent years to quantify the contact nucleation efficiency of aerosols (79) and IN and non-IN bacteria (80), or measure the relative importance of contact versus immersion freezing processes (81, 82).

### 3.1.2.5 Compositional analyses of ice-nucleating particles

#### Analyses of ensembles of particles

The vast majority of particles in a collected ensemble will not be ice nucleation active, especially at the relatively warm temperatures where biological INPs may be important. For example, INPs active at  $-8^{\circ}\text{C}$  at a high-altitude mountain station accounted for only one particle in 10 million (for particles  $>0.5\ \mu\text{m}$  (83)). While it is clearly not valid to make inferences about INPs from the characteristics of the bulk aerosol, a particle ensemble can be treated to inactivate a class of INP before retesting. With such challenge tests one can gauge the abundance of a range of INP types. For example, if the removal of organic matter with  $\text{H}_2\text{O}_2$  results in a substantial reduction in INPs, one can conclude that the majority were organic (31, 32, 34). Similarly, heat can be used to denature and deactivate relatively labile biological INPs. Incubation at  $60^{\circ}\text{C}$  will lower the onset activity of IN bacteria from above  $-3^{\circ}\text{C}$  to below  $-8^{\circ}\text{C}$  (19, 84), and denature some fungal INPs (85) but not others (41, 86), which require temperatures approaching  $100^{\circ}\text{C}$  for deactivation. A temperature range of  $95\text{--}105^{\circ}\text{C}$  is the most common heat applied to test for labile biological INPs. Pollen INPs, however, are still active after being boiled (42). More focused tests can be used to deactivate specific classes of INPs, such as digestion with lysozyme to lyse bacteria (34, 87), or use of papain to digest IN proteins produced by *Mortierella alpina* (34, 41). Size separation through filters (88) or by size exclusion chromatography allows differentiation into cells, larger fragments, and cell-free IN proteins (41, 89).

Next-generation sequencing is now an inexpensive tool for comprehensive profiling of the bacteria and eukaryotes (e.g., fungi, plant tissues, pollen) collected in an aerosol sample (90) (Figure 3.1.3), while quantitative PCR (qPCR) can be used to directly count the number of IN bacteria with high sensitivity (19). Nucleic acid-based methods work only with INPs containing DNA and cannot detect, for example, free IN molecules released by fungi or pollen grains. For more information on these methods, see Section 1.3.3.

#### Analyses of individual ice-nucleating particles

Various approaches can be used to isolate and identify individual INPs for analysis, but all exploit the ability of INPs to freeze water, either by capturing ice crystals in real time (33, 36, 39, 91) or after collection on substrates or in liquid (34, 92). Their morphology may then be studied by light or scanning electron microscopy (environmental or standard SEM), their elemental composition by combining SEM with energy-dispersive X-ray microanalysis (EDX) (33, 36, 91, 92), and the analysis of their major molecular groups using Raman spectroscopy (13). It is important to note that the composition of the INP may differ entirely from the ice-nucleating entity to which it is attached (see Section 3.1.3).

### 3.1.3 Findings from laboratory experiments, field collections, and field studies

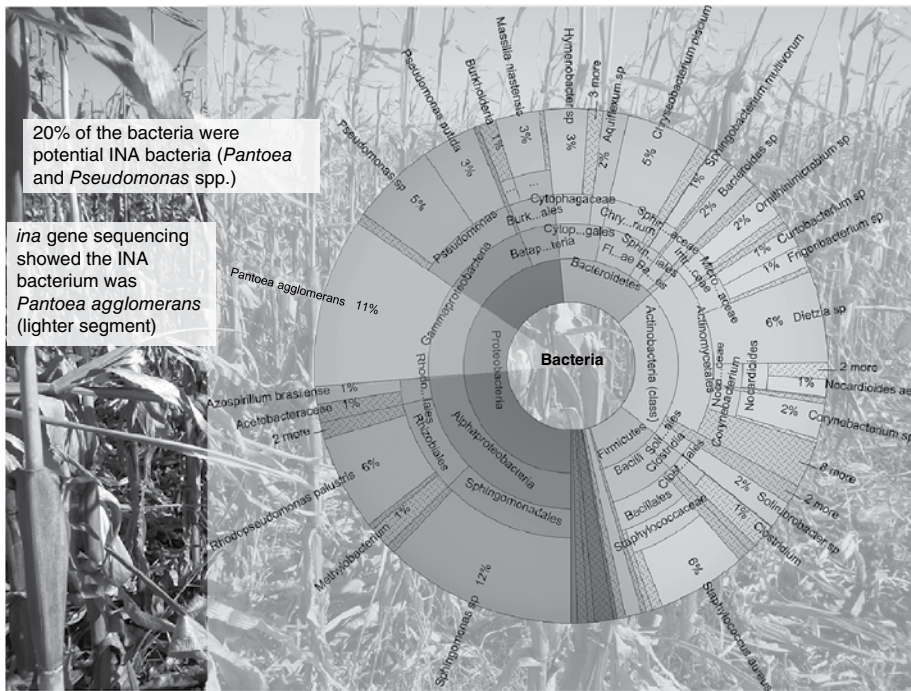
In this section, we will briefly describe laboratory experiments and field studies that have investigated the IN properties of atmospheric aerosol particles, with special emphasis on particles of biological origin.

Research into the possibility that particles of biological origin may significantly influence ice nucleation in clouds, with the attendant potential for them to modify cloud radiative properties, lifetime, and the dynamics of precipitation, has evolved through several stages. Because of their early discovery, much work focused on one group: the IN or ice nucleation active bacteria. These are spread among three genera, *Pseudomonas*, *Pantoea*, and *Xanthomonas*, but one species, *Pseudomonas syringae*, has received most attention (25, 93–95). All of them produce variants of the same IN protein, the core of which contains mostly hydrophilic amino acids that arrange water molecules in their vicinity to spawn an ice embryo (96). Aggregates of the protein can trigger nucleation at up to  $-1^{\circ}\text{C}$  (97), although typically several degrees colder (95). Only a small proportion of IN bacterial cells express the protein under normal culturing conditions. Accordingly, 0.07–0.5% of IN bacterial cells sprayed into the AIDA cloud chamber acted as INPs in the temperature range  $-8^{\circ}\text{C}$  to  $-10^{\circ}\text{C}$  (72). Pioneering work identified *P. syringae* as the agent responsible for high-temperature nucleation of alder leaf litter (98–100), and the same species inspired the bioprecipitation hypothesis (see Section 3.1.4).

However, while the known IN bacteria are enriched in rainfall compared with cloud-water (95, 101–105), and released by the action of rainfall (106) and harvesting (9) (Figure 3.1.3), in the absence of a significant physical disturbance their abundances seem to account for only a minor fraction of the biological INPs in the air above crops (9). The same was found in fresh snow (19), but they may play a more significant role in the formation of thunderstorm hail (19, 107). The concentration of IN bacteria in air at cloud height can be estimated from their abundance in precipitation by assuming, as mentioned above, that  $1\text{ m}^3$  of cloud contains 0.4 g of condensed water. Applying this conversion to snow samples, values of  $\leq 0.0002$  IN bacteria per liter of air were found using qPCR of the *ina* gene (19), while means of approximately 0.001–0.002 IN *P. syringae* per liter of air were obtained from culturing (95, 108). For rain, values ranged from 0.0001 to approximately 0.002 IN *P. syringae* or IN bacteria per liter of air (95, 108, 109).

While other, still-to-be identified IN bacteria may also be present (87), the bulk of the biological INPs could well be contributed by eukaryotes; for example, ice nucleation activity at warm temperatures (approximately  $-5^{\circ}\text{C}$ ) has been found in lichens (110, 111), plant tissues (34, 112–114), and especially in an ever-increasing diversity of fungi (41, 85, 86, 115–118) (Figure 3.1.2). Pollen and the pollen rinse water of many common species nucleates at colder but still atmospherically relevant temperatures (42, 85). For example, recent studies using LACIS, the vertically oriented laminar flow device, showed steeply increasing ice nucleation activity of birch pollen below about  $-15^{\circ}\text{C}$  (119). All of these sources could contribute to the prodigious reservoirs of organic INPs present in most topsoils (31, 32, 34). Using laboratory-generated soil dusts tested in the AIDA cloud chamber, Steinke et al. (120) have shown that ice nucleation efficiencies of three agricultural soil dusts are 10 times higher than desert dusts at  $-19^{\circ}\text{C}$ , although the difference narrowed below  $-26^{\circ}\text{C}$ . By contrast, using laboratory-generated arable soil dusts tested with the CFDC, Tobo et al. (33) found that unidentified organic INPs predominated over mineral INPs across almost the entire range over which heterogeneous freezing operates.

Of likely profound significance are recent laboratory studies showing that IN fungi release copious numbers of small (e.g.,  $<15\text{ nm}$ ), cell-free INPs (41, 85, 89) and that pollen of widespread species such as silver birch release IN carbohydrate macromolecules



**Figure 3.1.3** Use of next-generation sequencing of the 16S rRNA gene to profile the bacteria present in aerosol downwind of corn harvesting in Nebraska (see sampling details in Garcia et al. (9)). Twenty percent of bacteria were potential ice-nucleating (IN) species, but sequencing of *ina* genes, which code for the active protein, revealed that *Pantoea agglomerans*, which accounted for 11% of all bacterial sequences, was the primary IN species.

when immersed in water (42, 85, 119). Further, other INPs active at colder temperatures may exist as a result of the serendipitous attribute of a molecular class. If so, then these additional organic INPs may be produced by many organisms (e.g., particles of cellulose or other macromolecules associated with them (73)). Indeed, recent cloud simulation experiments with DCECC have demonstrated the relatively high ice nucleation activity of cellulose particles (73). Macromolecular INPs are readily adsorbed onto clays, conferring on them their ability to catalyze ice nucleation at relatively warm temperatures (121–123). Sorption studies have shown that only 3 mg of organic carbon is required to cover the surface area of a gram of pure clay minerals (124). In other words, organic entities that constitute only 0.3% of a particle's mass may define the properties of its surface. Their presence may thus go undetected by SEM/EDX and be “difficult or even not possible” to unambiguously discern even with single-particle mass spectrometry (122). If this is the case, then the ice nucleation activity of apparently purely mineral particles will sometimes actually have a biological root. Interestingly, while Pratt et al. (36) found that 50% of ice crystal residues in a wave cloud were mineral, most of these also contained humic and/or biological material (see also Creamean et al. (37) and Tobo et al. (33)). These findings suggest that biogenic sources could contribute much more than the very low global average ice nucleation rate (<0.6%) estimated by Hoose et al. (125), which included only IN bacteria, fungal spores, and individual pollen particles.

A range of methods have provided direct and indirect (i.e., via correlations) measurements or assessments indicating the significant contributions of biogenic INPs in various ambient aerosols, especially at higher than  $-12^{\circ}\text{C}$  in precipitation samples, but also at colder temperatures in both agricultural and forest ecotypes (Table 3.1.1). In addition, the influence of biological INPs can be inferred from sudden increases in concentrations of warm-temperature-active INPs, such as after a downpour (126) or after the passage of marine air over vegetated land (127). Stopelli et al. (128) used multiple linear regression, calibrated and validated on about 100 precipitation samples, to develop a model that explained  $>75\%$  of the variability of INPs active at  $-8^{\circ}\text{C}$  in precipitation; a high abundance of such effective INPs, which are likely biological, occurs when high winds coincide with air masses having experienced little or no precipitation prior to sampling. Put another way, conditions in which such INPs are sufficiently abundant to initiate the ice phase in clouds may frequently coincide with meteorology that favors precipitation, such as frontal passages. The further development of these methods, and the reconciliation between field and laboratory studies, will lead to the full understanding of their abundance and role.

Above oceans, INP concentrations are relatively low, typically  $<0.02\text{ L}^{-1}$  at  $-15^{\circ}\text{C}$  (6, and references therein). Recent investigations focusing on identifying marine INP sources and emissions have variously suggested that candidate INPs are small ( $<0.2\text{ }\mu\text{m}$ ),

**Table 3.1.1** Direct and indirect measurements of the relative contribution of biogenic ice-nucleating particles (INPs) to ambient aerosols.

Method	Finding	Reference
Use of heat ( $\sim 100^{\circ}\text{C}$ ) to denature biological INPs suspended in water	<b>Air</b>	
	INPs active at higher than $-18^{\circ}\text{C}$ in the air above fields were predominantly heat sensitive	9
	<b>Cloudwater</b>	
	65–100% of cloudwater INPs active at $-10^{\circ}\text{C}$ were heat sensitive	104
Use of heat ( $\sim 300^{\circ}\text{C}$ ) in real time to denature biological INPs	<b>Precipitation</b>	
	100% of INPs active at $-9^{\circ}\text{C}$ or higher were heat denatured in $\geq 70\%$ of snow and rain samples	87
	Heat-sensitive INPs predominated in snow to approximately $-12^{\circ}\text{C}$	
	Submicron, heat-sensitive INPs in rain ( $482\text{ L}^{-1}$ ) and snow ( $199\text{ L}^{-1}$ )	19 109
Use of lysozyme to lyse IN bacteria	<b>Air</b>	
	INPs released by harvesting (e.g., sorghum and wheat) showed strong reductions after passage through a heating tube upstream of the CFDC	129
Use of lysozyme to lyse IN bacteria	<b>Precipitation</b>	
	Digestion eliminated the majority of INPs active at $-9^{\circ}\text{C}$ or higher in 64%, 50%, and 25% of precipitation samples from France, Montana, and Louisiana, respectively	87

Table 3.1.1 (Continued)

Method	Finding	Reference
Correlations of INPs in real time with PBAPs <sup>a)</sup>	<b>Air</b>	
	Biological INPs predicted to dominate at higher than $-25^{\circ}\text{C}$ in rainforest	39
	INPs active from $-15^{\circ}\text{C}$ to $-25^{\circ}\text{C}$ highly correlated with FBAPs <sup>a)</sup> during high humidity and rain events in forest	117, 130–132
	INPs strongly correlated with terrestrial FBAPs between $-15^{\circ}\text{C}$ and $-25^{\circ}\text{C}$ at a coastal site. Averaged over various locations, 79% of INPs active at $-20^{\circ}\text{C}$ had diameters $>1\ \mu\text{m}$	48
Correlations of INPs in real time with mass spectrometer particle signatures <sup>b)</sup>	<b>Cloud ice particle residues</b>	
	33% of ice crystal residues sampled at $-31$ to $-34^{\circ}\text{C}$ designated as biological	133
	Higher levels of INPs coincided with aerosol residuals enriched in dust, dust/biological, and pollution (soot and biomass burning) in mid-level cloud ice layers	37
Electron microscopic analysis <sup>c)</sup> of ice crystal residuals	<b>Air</b>	
	Carbonaceous INPs active at $-30^{\circ}\text{C}$ dominant in 4/7 samples in rainforest	39

- a) Primary biological aerosol particles (PBAPs) (and fluorescent biological aerosol particles (FBAPs)) measured using fluorescence with an ultraviolet aerodynamic particle sizer or a wideband integrated bioaerosol sensor (WIBS-4A), and aerodynamic particle sizes measured with the micro-orifice uniform-deposit impactor (MOUDI) droplet freezing technique.
- b) Using aerosol time-of-flight mass spectrometry (A-ATOFMS).
- c) Scanning electron microscopy with energy-dispersive X-ray spectroscopy (SEM/EDX). CFDC, continuous flow diffusion chamber; IN, ice nucleating.

heat-sensitive organic entities associated with phytoplankton cell exudates (44), or that they are both microbes and much smaller particles occurring under different circumstances, such as during the decay phase of phytoplankton blooms (134–136). “Nanoscale” INPs are not unique to seawater: they are often common in terrestrial precipitation (88, 137), and may be biological (109).

### 3.1.4 Atmospheric implications

#### 3.1.4.1 Ecological advantages of ice nucleation and the bioprecipitation hypothesis

There are several explanations as to how ice nucleation may constitute a selective advantage for organisms that have evolved the capability. For example, a frozen dew

drop on a leaf will potentially penetrate the underlying tissue, releasing nutrients and providing access for the microorganisms to the host plant (138). Furthermore, Morris et al. (139) show that this trait is correlated with the level of pathogenicity, increasing the likelihood of the strain becoming a fitter pathogen on a wider plant host range. Since water vapor pressure above ice is lower than that above liquid water, early-forming ice crystals will grow at the expense of nearby supercooled droplets. In arid environments this mechanism could be utilized by dew-covered lichens to gather water (110). Further, microorganisms and plants might benefit from inducing freezing of topsoils to cleave soil or mineral aggregates to gain access to otherwise occluded organic matter or phosphorus (41).

In the atmosphere, ice nucleation may provide two benefits. First, a species exploiting aerial dispersion must limit its time aloft, since desiccation and ultraviolet irradiation will cause an exponential decline in survival with time (74, 140). Nucleating an ice crystal, which then precipitates out, is akin to manufacturing your own parachute to return to Earth (see 128, 141). Interestingly, however, in the AIDA chamber it was found that while viability of cells decreased over several hours the amount of ice formation remained unchanged, indicating that the ice nucleation efficiency can be retained in non-viable cells (74). Second, precipitation deposits microorganisms in conditions fitting for both them and their host plants, thereby “procuring a gain in fitness that leads to positive selection of ice nucleation activity” (5, and references therein). The watered plants would support the growth and subsequent release of more IN microorganisms (94, 142), thereby closing the feedback loop. A positive feedback could occur even if the majority of biogenic atmospheric INPs are sourced from the soil, and whether or not they are produced for a specific purpose by IN fungi or from the serendipitous characteristic of a polymer such as cellulose.

This concept, termed bioprecipitation, was first proposed by David Sands and co-workers (143), following his observation of the sudden and simultaneous appearance of a disease caused by *Pseudomonas syringae* across a large wheat field, with circumstantial evidence pointing to rainfall as the likely source. The three prerequisites for bioprecipitation to operate are that: (i) a mechanism must exist by which bacteria or other biological INPs can be readily taken up into the atmosphere, (ii) biological INPs are present in sufficient numbers, and (iii) ice nucleation is the limiting factor for rainfall initiation (143). Morris et al. (5) judged that the crux of the debate lies in the second requirement. In Section 3.1.4.3 we discuss ways that the third requirement represents a significant challenge for future research.

#### **3.1.4.2 Correlation with precipitation cycles (stimulation of ice-nucleating particle release by rainfall?)**

Prefrontal humidity and rainfall stimulate both immediate and delayed releases of high-temperature-active biogenic INPs (117, 130, 131, 144, 145). Bigg et al. (142) and Morris et al. (146) investigated the potential sensitivity of precipitation to these emissions of biological INPs by mapping the long-term intensity and patterns of rainfall feedback in southeastern and southwestern Australia, and at 1250 sites in the western USA. In the latter study, orographic precipitation was the clearest factor associated with a positive feedback, especially along the California coast west of the Sierra Nevada mountain range. This makes sense, since orographic clouds are those most likely to be influenced

by a flush of INPs stimulated by local rainfall. However, synoptic weather patterns may also have influenced this apparent feedback, such as the arrival and persistence for several days of so-called atmospheric river-induced rainfall, when tropical moisture flows impinge on the topography of the US west coast (147).

### 3.1.4.3 A special role for bioaerosols in secondary ice generation and precipitation formation?

A case exists for the additional special atmospheric role for biological INPs, commonly mentioned in association with the bioprecipitation hypothesis, because of the fact that in certain clouds (the proportion of which is undetermined) a secondary ice formation process is triggered by the ice that first forms at temperatures where biological INPs would appear to be the only significant source for the primary ice nucleation. Even in clouds with tops not colder than  $-10^{\circ}\text{C}$ , where past measurements of INP concentrations range from  $\ll 0.001$  to  $1\text{ L}^{-1}$ , the number of ice particles can suddenly surge by three or more orders of magnitude, which in some cases (the proportion and requirements for which are also undetermined) stimulates rain formation. For example, Hobbs and Rangno (148) observed a maritime cumulus go from no detectable ice to  $>350$  crystals  $\text{L}^{-1}$  in 9 min even though no colder than  $-8^{\circ}\text{C}$ . One explanatory process was first reproduced in the laboratory, and conditions for its occurrence circumscribed, by Hallett and Mossop (149), who found that the filigree-like riming of an ice surface (as occurs on a graupel particle) can lead to copious ice splinter production. This secondary ice production process is only active in the  $-3^{\circ}\text{C}$  to  $-8^{\circ}\text{C}$  range and requires a mix of both large ( $>24\text{ }\mu\text{m}$ ) and smaller cloud droplets. In the absence of ice crystals sedimenting from higher altitudes to serve as the rimer particles, this Hallett–Mossop rime-splintering process likely involves production, via collision and coalescence, of supercooled raindrops which then freeze via included INPs and immediately begin to rime to create splinters. These ice splinters grow by vapor deposition, which may lead to subsequent precipitation (150–152). Moderate updrafts help by keeping the riming particles suspended within the zone of maximum aerodynamic collection of smaller droplets and splinter production (153). Other secondary ice formation processes have been documented, although none of these is operative in the temperature regime warmer than  $-8^{\circ}\text{C}$  (154).

While evidence suggests that biogenic INPs nearly exclusively catalyze ice formation between  $-3^{\circ}\text{C}$  and  $-8^{\circ}\text{C}$ , a number of factors highlighted in the recent review of Field et al. (154) indicate that proof of a special influence of primary biological INPs upon secondary ice and precipitation formation does not yet exist. Quantitative support for the Hallett–Mossop process in clouds is not comprehensive, and all the processes leading to secondary ice formation may not be known (151, 155). The frequent association of secondary ice formation with the presence of millimeter-sized drops occurring at modest supercooling (154) favors secondary ice generation in clouds with deep cloud regimes warmer than  $0^{\circ}\text{C}$  and lower cloud condensation nuclei number concentrations. This situation is most common in maritime clouds, which are typically farthest from the strongest emission sources of known biogenic INPs. These same conditions also favor warm rain production of precipitation that may compete with (just as it stimulates) cold-phase processes. The minimum concentrations of INPs needed for activation of secondary processes also remains poorly studied. One study (156) suggested a

direct relation between ice crystal enhancement via the Hallett–Mossop process and INP concentrations in the cloud case they modeled. Finally, modeling studies regarding the overall quantitative role of secondary ice formation in production of precipitation in specific clouds types, or globally, produce inconsistent results (154).

Cloud systems involve an array of complex processes within varied meteorological contexts. Vertical cloud structure, mixing and shear, cloud particle interactions, hydro-meteor trajectories, scavenging removal of particles prior to ascension to supercooled levels, and a host of other processes and feedbacks occur. This complexity means it is currently premature to offer any definitive conclusions with regard to the specific or vital role of biological INPs in cloud evolution and precipitation. The complex nature of cloud microphysical and dynamic processes will require robust parameterizations to be developed to represent various INP classes, including those of biogenic origin, for implementation in simulations from cloud to regional to global scales. As indicated in Chapter 2.3, such investigations are in their early stages.

### 3.1.5 Conclusion and future needs

In this section, current information on the properties of biogenic INPs has been reviewed, including an introduction to the methods being used to assess their abundance and behaviors, and the potential role of these INPs in cloud and precipitation processes. Reflecting recent findings, the point has been made that biological INPs emitted from land and ocean are not only discrete entities, but also that they may occur within or on inorganic and other organic particles as the by-products of biological action. This makes the task of identifying the role of biogenic particles on atmospheric processes challenging, but also exciting.

Research into biogenic ice nucleation processes and impacts is rapidly progressing. A key objective is the measurement and prediction of the full spectrum of biogenic INP sources and of their abundances. These measurements are critical to evaluating hypotheses regarding the role of biogenic INPs in ice formation in clouds, and subsequent impacts on precipitation, as well as the more elaborate, holistic bioprecipitation hypothesis. The path to evaluation includes an expansion of specialized measurements and their implementation, at a range of scales, in numerical models of clouds and precipitation. Current evidence also suggests that the focus should be on comparing and contrasting specific cloud types in varied locales, rather than at a global scale.

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## 3.2

### Impacts on Cloud Chemistry

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#### 3.2.1 Introduction

Clouds play a key role in the atmosphere due to their effects on the Earth's radiative budget, on the hydrological cycle, and on tropospheric chemical compositions. Clouds interact with incoming solar radiation and long-wave (infrared) radiation emitted from the ground, and consequently perturb atmospheric photochemistry features and the global radiative budget. Clouds also vertically redistribute emitted trace compounds from the boundary layer to the free troposphere and remove them from the air through washout processes (precipitation). Cloud lifetime is driven by atmospheric dynamics at the synoptic scale and by microphysical processes on a smaller scale. These processes transfer chemical compounds throughout various reservoirs: gaseous, particulate, liquid, and ice phases.

The chemical composition of the cloud aqueous phase is formed through the uptake of gases, through the dissolution of cloud condensation nuclei (CCN) constituents, and through chemical reactions. Cloud chemical compositions are highly variable in time and space and in terms of droplet size. Understanding their various compositions is crucial, as clouds drive important atmospheric processes: the formation and transformation of chemical species (1), the production and consumption of oxidants such as hydrogen peroxide and free radicals (2, 3), droplet activation and growth (4), and finally the transport and deposition of pollutants (5, 6).

Cloud chemistry processes can lead to the formation of new low-volatile compounds that modify aerosol particles in terms of their (micro)physical and chemical properties (particle size, chemical composition, and morphology). These changes affect the effective radiative forcing from aerosol–radiation interactions (ERFari) and from aerosol–cloud interactions (ERFaci) (7), i.e., they affect the interactions of aerosol particles with incoming radiation by scattering/absorbing, thus affecting future cloud and fog cycles. Particles modified over a cloud's lifetime generally present a larger soluble fraction after evaporation. These particles form cloud droplets at lower levels of supersaturation, leading to the formation of clouds composed of smaller droplets. The cloud/particle lifetime

is consequently increased, and cloud precipitation occurrence levels are affected. Cloud chemistry features consequently affect the climate system. Sulfates are produced as a result of cloud reactivity, while secondary organic aerosol (SOA) mass may form as a result of chemical reactions in cloud droplets and aerosol water (8), which are referred to as “cloud aqSOA” and “aerosol aqSOA,” respectively (9, 10).

In this context, microorganisms (bacteria, yeasts, and fungi) have been discovered as new potential actors of cloud chemistry. Microbial concentrations in clouds reach values of roughly  $10^5$  bacteria per milliliter and  $10^4$  fungi and yeasts per milliliter in cloudwater. The main genera of cultivable microorganisms encountered in cloudwater include *Pseudomonas*, *Sphingomonas*, *Streptomyces*, *Rhodococcus*, and *Bacillus* bacteria and *Dioszegia*, *Udeniomyces*, and *Cryptococcus* yeasts. A number of recent studies based on molecular biology confirm the presence of Proteobacteria, Actinobacteria, and Firmicutes (11–13). However, these authors also found new elements such as Cyanobacteria and other bacterial families such as Methylobacteriaceae and Oxalobacteraceae. Recent studies show that microorganisms can resist and survive harsh conditions encountered in clouds. Joly et al. (14) measured the survival rates of microorganisms isolated from clouds and exposed to various atmospheric stresses: exposure to  $H_2O_2$  and ultraviolet light (oxidative stress), evaporation–condensation cycles (osmotic shock), and freeze–thaw cycles (multiple stresses: cold shock, oxidative stress, and osmotic stress). These authors show that freeze–thaw cycles are the most difficult form of stress to endure, that rates of survival are strain dependent, that the opposite is the case for oxidant exposure, and that light has almost no negative effect on any strain. Microorganisms have developed various means of enduring atmospheric stress: (i) the presence of pigments—roughly 50% of strains isolated from clouds are pigmented (15), protecting microorganisms from extreme and cold temperatures; (ii) exopolymeric substance (EPS) and biosurfactant (16) synthesis allows for the formation of aggregates and biofilms (17); and (iii) some microorganisms form spores (*Bacillus*, yeasts, and fungi).

Microorganism capacities to survive in clouds render them metabolically active. Sattler et al. (18) were the first to show that bacteria can survive and grow in supercooled cloud droplets. Amato et al. (19) showed that microorganisms isolated from clouds can grow in cloudwater incubated in a laboratory. This metabolic activity was largely confirmed by assaying the adenosine 5′-triphosphate (ATP) content found in cloudwater samples (20). A long-term survey of these *in situ* measurements conducted at the puy de Dôme station (France) shows that ATP content remains rather stable independent of the season or of the air mass geographical origins involved (15). Hill et al. (21) also showed that 76% of all bacteria remain alive in cloudwater, as they are able to take up 5-cyano-2,3-ditolyl tetrazolium chloride dye. The presence of metabolic activity in clouds implies an uptake of molecular compounds by cells as nutrients, thus proving their contributions to cloud chemistry. Indeed, microorganisms can be considered biological catalysts that may compete with chemical reactions and with photochemistry features in cloudwaters in particular.

### 3.2.2 Chemical composition of clouds

The cloud aqueous phase is composed of a multitude of organic and inorganic species; their variety and quantity heavily dependent on the history of the air mass involved. The

organic fraction includes oxygenated volatile organic compounds, such as alcohols, carboxylic acids, aldehydes, and ketones. A large fraction of the organic compounds dissolved in clouds has not been characterized or quantified (22). *In situ* measurements of cloudwater generally quantify dissolved organic carbon (DOC), which corresponds to the total concentration of organic compounds in the aqueous phase. For heavily polluted clouds, DOC values can reach  $200 \text{ mgC L}^{-1}$ , as measured at Mount Taishan in China (23). Currently, DOC values range from 5 to  $20 \text{ mgC L}^{-1}$  on average for continental clouds (24, 25) and fall below  $5 \text{ mgC L}^{-1}$  for clouds of marine origin (26).

Among the most studied organic compounds, the most common organic acids found in cloudwater include acetic ( $\text{CH}_3\text{CO}(\text{OH})$ ), formic ( $\text{HCO}(\text{OH})$ ), succinic ( $\text{CO}(\text{OH})\text{CH}_2\text{CH}_2\text{CO}(\text{OH})$ ), malonic ( $\text{CO}(\text{OH})\text{CH}_2\text{CO}(\text{OH})$ ), and oxalic ( $\text{CO}(\text{OH})\text{CO}(\text{OH})$ ) acids (27–32). These compounds represent approximately 10% of the DOC and derive from several origins: they can be dissolved from the gas phase to the aqueous phase (main source of acetic and formic acids); they can also be transferred into the aqueous phase through the dissolution of the soluble fraction of particles that act as CCN (main source of dicarboxylic acids such as succinic, malonic, and oxalic acids) (33, 34); finally, aqueous phase reactivity will lead to the formation of carboxylic acids (35). Free radicals such as hydroxyl radicals ( $\text{HO}^\bullet$ ) are produced in the aqueous phase (see Section 3.2.3) and lead to the oxidation of organic matter that produces carboxylic acids (1). Carboxylic acids detected in cloudwater present variable molecular masses ranging from one carbon atom to 30 carbon atoms (36). Their size vary from nanometers to several hundreds of micrometers (31, 32, 37–39).

Water-soluble organic compounds (WSOCs) have also been measured in cloudwater; they are produced in the gaseous phase and dissolve in the aqueous phase depending on their Henry's law constants (40). In wet aerosol, due to much higher salt concentrations, Setschenow constants should be preferred (41). The concentrations of WSOCs scale inversely with the volume of the particles, resulting in typical organic compound concentrations in approximately the molar and micromolar range in wet aerosol and cloud droplets, respectively (42).

In the aqueous phase, the oxidation of carbonyl compounds produces carboxylic acids but can also lead to the formation of oligomers (43–47) (see Section 3.2.6). Concentrations of formaldehyde, acetaldehyde, glyoxal, and methylglyoxal have been measured in cloudwater (31, 48–51). Among carbonyls, formaldehyde is efficiently produced in the gas phase through the photochemical oxidation of methane and non-methane hydrocarbons or is directly emitted through biomass burning and fossil fuel combustion. This compound is consequently transferred into the aqueous phase owing to its high solubility, explaining its high concentrations in cloudwater.

Undetermined organic matter in cloudwater still represents a large fraction of the DOC. Humic-like substances have been identified (52–54) and correspond to large multifunctional compounds (LMCs). For instance, molecules such as proteins, cellulose, dicarboxylic acids, polyols, amino acids, fatty acids, sugars, polysaccharides, and aliphatic and aromatic hydrocarbons have recently been detected in cloudwater (55, 56). For instance, total amino acids have been found to account for 13% of DOC in fog waters and approximately 10% of WSOCs in  $\text{PM}_{2.5}$  (57). These compounds are emitted into the atmosphere as particulates from natural and anthropogenic sources and dissolve into the aqueous phase. Aqueous chemistry features may also be responsible for the formation of these compounds and/or may transform them (58). A recent

study (59) has revealed the presence of organosulfates and oligomers in cloudwater that may be related to aqueous aerosol-phase chemistry or cloud-processing processes. SOA formation via aqueous processing, especially through the oxidation of volatile organic compounds of biogenic origin, is hypothesized to contribute significantly to the global aerosol burden (9). Aromatic compounds (60), polycyclic aromatic hydrocarbons (61), and pesticides (62) may also affect the DOC. High-resolution mass spectrometry (HRMS) is employed to identify aerosol particles and organic compounds present in atmospheric waters (56, 63, 64). HRMS constitutes a very valuable tool for identifying chemical species, though quantifications can be extremely difficult to perform (65).

Cloudwater is also composed of inorganic compounds that control the acidity of the aqueous phase and its oxidative capacity. Among these compounds, we find sulfate ( $\text{SO}_4^{2-}$ ), nitrate ( $\text{NO}_3^-$ ), chloride ( $\text{Cl}^-$ ), and ammonium ( $\text{NH}_4^+$ ) as well as alkaline Earth metal cations ( $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Mg}^{2+}$ ,  $\text{Ca}^{2+}$ ) of ammonium. These chemical compounds originate from various sources. For instance, nitrate and ammonium can enter cloudwater as constituents of condensation nuclei as well as through the gas to liquid scavenging of gaseous  $\text{HNO}_3$  and  $\text{NH}_3$ ; potassium, magnesium, and calcium ions are mainly developed from the mineral fraction of aerosol particles in soil; sulfate derives from the oxidation of gaseous precursors that are dissolved in cloud droplets such as  $\text{SO}_2$ . Recent studies have shown that additional sulfate mass is produced within clouds, potentially as a result of chemical production or the uptake of gaseous  $\text{H}_2\text{SO}_4$  (66, 67). Over polluted regions, the oxidation of  $\text{NO}_2^-$  and  $\text{SO}_2$  in the gas phase is a major source of sulfuric and nitric acids that control cloudwater acidity. In coastal regions and over marine surfaces, sodium chloride ( $\text{NaCl}$ ) constitutes a main component of inorganic compounds. The acidity of cloudwater depends heavily on air mass history characteristics and on strong inorganic acid levels. Field measurements show pH values ranging from 2.2 to 7 (21, 68–70). For example, for polluted clouds, the pH level at the puy de Dôme station (France) is the most acidic due to higher amounts of nitrate and sulfate found here (pH of approximately 4) (26).

The atmospheric aqueous phase also presents significant concentrations of oxidants. Cloudwater is an oxidizing environment with a redox potential of up to 200 mV owing to the presence of radicals ( $\text{HO}^\bullet$  and  $\text{HO}_2^\bullet/\text{O}_2^{\bullet-}$ ) and their precursors ( $\text{H}_2\text{O}_2$  and metals) (26). Measured  $\text{H}_2\text{O}_2$  concentrations vary depending on air mass history features. These aqueous concentrations are a product of aqueous chemical reactions and photochemistry features and of phase transfer exchanges between the gas and aqueous phase (71). For instance, values ranging from 0 to  $3.2 \mu\text{m}$  at Kleiner Feldberg (Germany) (72) and from 0.1 to  $57.7 \mu\text{m}$  at the puy de Dôme (France) with an average of  $7.8 \mu\text{m}$  have been recorded (26). Some field campaigns have revealed extremely high concentrations (up to  $247 \mu\text{m}$ ) (e.g., values reported from Whitetop Mountain, USA) (73). Transition metal ions (TMIs) including iron (Fe), copper (Cu), and manganese (Mn) are also present in the cloud aqueous phase. Metals are emitted into the atmosphere in particulate form and are transferred into the aqueous phase through dissolution processes (74–76). The most common metal found in cloudwater is iron, which reaches concentrations of several micromolar in polluted clouds (77).

Numerous researchers have characterized the chemical composition of clouds and inorganic fractions developed, as clouds are responsible for the transformation of sulfur and nitrogen compounds (78–80). Most of these studies are based on field

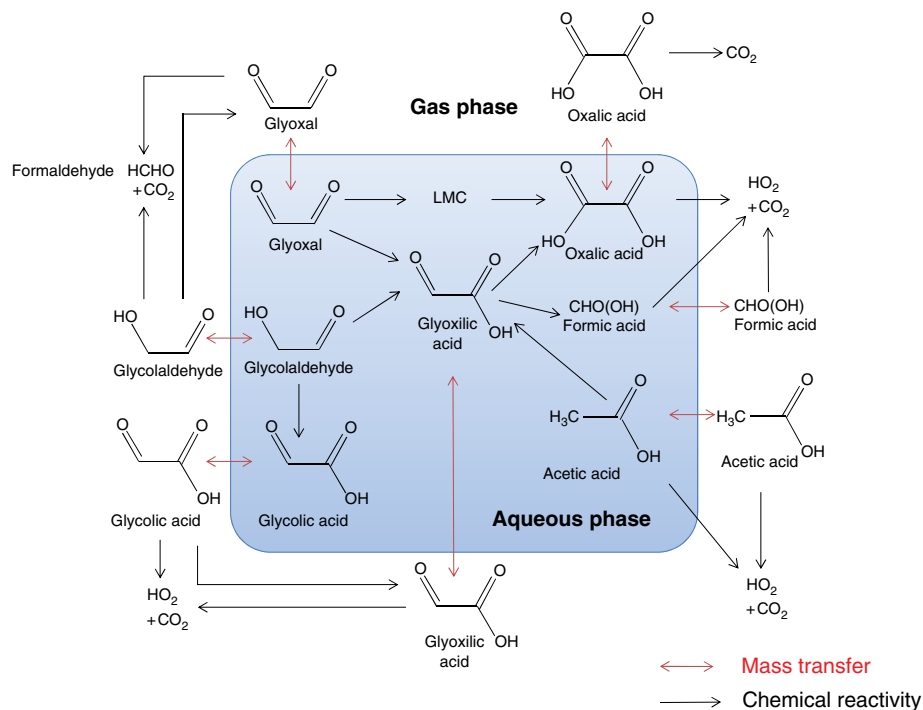
campaigns and specifically examine the effects of pollutant sources, of liquid water content levels, of photochemistry features, etc. (25, 28, 81–83). Recently, several international campaigns have been organized (e.g., the Field Investigations of Budgets and Conversion of Particle Phase Organics in Tropospheric Cloud Processes (FEBUKO) campaign (31, 84) and the Hill Cap Cloud Thuringia (HCCT) campaign (3, 70, 85, 86)) to characterize chemical transformations, and organic compounds and particles found during a cloud cycle in particular. Measurements show strong variations in the compositions and concentrations of the cloud aqueous phase that are controlled by gaseous compounds emitted during air mass transport via cloud microphysics (dilution/concentration) and aqueous biological and chemical transformations. Some researchers have attempted to describe variations in cloud chemical compositions based on several cloud events, though they mainly examined the composition of inorganic compounds (82, 87, 88). Variations in the chemical compositions of clouds depending on environmental conditions (air mass origins, daytime/night-time conditions, liquid water content, etc.) are essential. For this reason, statistical studies on numerous cloud samples that relate chemical compositions to environmental conditions must be conducted. However, it is not trivial to obtain a large number of cloud samples of sufficient water volume to conduct a full characterization (chemistry, biology, and microphysics).

### 3.2.3 Clouds as oxidative reactors

Most atmospheric sulfate forms in cloud droplets, as the oxidation of sulfur(IV) by hydrogen peroxide and ozone, occur much faster than oxidation by the  $\text{HO}^\bullet$  radical in the gas phase. The formation of inorganic acids in the atmosphere has been studied extensively, as it is related to the acidification of rainwater. Recently, it has been proposed that SOA mass may form as a result of chemical reactions occurring in cloud droplets and aerosol water. This may serve as an explanation for the presence of small dicarboxylic and keto-acids and high-molecular-weight compounds in aerosol particles, cloudwater, and rainwater (45).

Organic compounds are transformed into the aqueous phase through oxidation processes due to the presence of free radicals ( $\text{HO}^\bullet$ ,  $\text{NO}_3^{\bullet-}$ , etc.). In illustrating this complex process, Figure 3.2.1 depicts the oxidation of organic compounds with two carbon atoms resulting from the oxidation of a biogenic compound: isoprene (89–91). In the gaseous phase, isoprene induces the formation of glycolic acid, glyoxylic acid, acetic acid, glycolaldehyde, and glyoxal. These compounds are highly soluble and dissolve in the aqueous phase. They then oxidize, leading to the production of oxalic, acetic, and formic acids. Aqueous reactivity then results in the creation of shorter (fragmentation process) and more heavily oxygenated molecules (functionalization). Accretion processes can also occur in cloudwater, leading to the creation of organic compounds of higher molecular mass than their precursors. LMCs can enter the aqueous phase (mainly for wet aerosols, in which concentrations of organic compounds are higher) as a result of organic precursors such as carbonyls (glyoxal, etc.) and carboxylic acids (acetic acids, etc.) (9, 41).

In the following paragraph, we briefly recall the sources of radicals and oxidants of the atmospheric liquid phase and the oxidation of organic matter.



**Figure 3.2.1** Oxidation processes of organic compounds with two carbon atoms in the gas and aqueous phases and exchanges of these chemical compounds between the two phases (“mass transfer”). LMC, large multifunctional compound. (Modified from Ervens et al. (91). Reproduced with the permission of John Wiley and Sons.) (See color plate section for the color representation of this figure.)

$\text{H}_2\text{O}_2$  and metals control the oxidative capacity of the aqueous phase (77). They serve as a significant source of the hydroxyl radical  $\text{HO}^\bullet$ , which is responsible for the oxidation of organic matter. A significant *in situ* source of this radical in the aqueous phase is the so-called “Fenton” reaction between  $\text{H}_2\text{O}_2$  and iron(II). This reaction produces hydroxyl radicals and iron(III)-hydroxy complexes that are efficiently photolyzed (mainly the  $(\text{Fe}(\text{OH}))^{2+}$  aqua-complex, which dominates the relevant atmospheric pH levels of between 3 and 5), thus accelerating the formation of  $\text{HO}^\bullet$  in the aqueous phase. Aqueous concentration levels of  $\text{HO}^\bullet$  are also driven by the gaseous phase, as this radical is taken up from the gas phase (92). Finally, the photolysis of aqueous  $\text{H}_2\text{O}_2$ ,  $\text{NO}_3^{\bullet-}$ , and  $\text{NO}_2^{\bullet-}$  serves as an effective source of  $\text{HO}^\bullet$  in the aqueous phase (93–95). The relative importance of different hydroxyl radical sources depends on chemical compositions of the aqueous phase, which are highly variable. Hydroxyl radicals are also scavenged in the aqueous phase, and primarily by DOC; evaluations of this sink are difficult to conduct, as dissolved organic matter is diverse, complex, and poorly characterized (96). Moreover, organic matter can also contain complex metals, though such complexations are not well characterized in natural cloudwater; the photochemistry of these metal–organic compounds is also not thoroughly understood. Currently, cloud chemistry models only consider the complexation of iron by oxalate (97). The simulated production rates of  $\text{HO}^\bullet$  may be overestimated by existing models (41, 98).

Under night-time conditions, the  $\text{HO}^\bullet$  is produced less, and oxidation processes by the nitrate radical ( $\text{NO}_3^{\bullet-}$ ) become significant (99). This radical's main source in the aqueous phase is its uptake from the gaseous phase. The oxidation of organic matter by ozone and hydrogen peroxide can also occur in the aqueous phase, though in comparison with radical reaction rate constants such values are roughly 10 orders of magnitude smaller (100). Additionally, reactions of oxidized TMIs with  $\text{H}_2\text{O}_2$  and the photolysis of metal–organic acid complexes such as iron(III)–oxalate complexes can act as sources of  $\text{HO}_2^\bullet/\text{O}_2^{\bullet-}$  (101).  $\text{HO}_2^\bullet/\text{O}_2^{\bullet-}$  reacts with iron(II)/iron(III) and copper(I)/copper(II) to form  $\text{H}_2\text{O}_2$  (102).

$\text{HO}^\bullet$  radicals are reactive electrophilic species but are not very selective in nature compared with other atmospheric free radicals. The two main mechanisms of  $\text{HO}^\bullet$  radicals with organic compounds in aqueous solution are H-abstraction reactions with saturated compounds and addition reactions (e.g., C=C double bonds in unsaturated compounds). The probability of electron transfer processes of  $\text{HO}^\bullet$  radicals depends on reduction potentials and on reactant structures. For instance,  $\text{HO}^\bullet$  can undergo an electron transfer in the presence of anions (and especially in the presence of carboxylate compounds). The alkyl radical  $\text{R}^\bullet$  produced directly reacts with oxygen to form a peroxy radical  $\text{RO}_2^\bullet$ . It is assumed that a peroxy radical reacts with itself or with another peroxy radical to form a tetroxide that quickly decomposes. The decomposition of tetroxide can occur through various pathways depending on the nature of the initial peroxy radical. When a hydroxyl moiety occupies an alpha position of the peroxy function, the peroxy radical can undergo  $\text{HO}_2^\bullet$  elimination. To roughly summarize, the transformation of organic compounds via radical chemistry occurs when alcohols in the aqueous phase are oxidized into carbonyls that form carboxylic acids (103).

The kinetic dataset on  $\text{HO}^\bullet$  radical reactions in aqueous solutions is rather important. For instance, the reactivity of alcohols, carboxylic acids, carbonyls, certain aromatic compounds, and certain halogenated alkanes in aqueous solutions has mainly been investigated in laboratory settings because these materials have been examined in cloudwater. However, because of the numerous organic compounds found in cloudwater (Section 3.2.5), not all oxidation pathways have been fully documented or studied. To compensate for this lack of information, structure–activity relationships as well as reactivity correlations are now available in the literature for estimating missing parameters such as kinetic constants, branching ratios, hydration constants, acidity constants, etc. (93, 104–106). However, reaction pathways for aromatic compounds, sugars, organo-nitrates and sulfates, and other functionalized aliphatic reactants are not yet well understood, and products and branching ratios must be identified and quantified. Moreover, some uncertainties also remain in regards to the reactivity, formation, and distribution of transient species and stable reaction products. Furthermore, temperature and pH levels can influence reaction rates, although these effects are not typically measured in laboratory settings.

### 3.2.4 Clouds as spaces of biodegradation

As microorganisms are alive and metabolically active in clouds, they are expected to interact with the cloud medium, and thus microbial metabolism may serve as an

alternative route to pure abiotic processes involved in cloud chemistry. To test this hypothesis, two strategies were employed:

- i) Design model microcosms where a pure microbial strain is incubated in a phosphate buffer solution containing a single substrate or in artificial cloudwater mimicking cloud compositions.
- ii) Directly study real cloud samples of biodiversity and complex chemical mixtures.

The results show that cloud microorganisms degrade organic compounds present in cloudwaters and can interact with  $\text{H}_2\text{O}_2$  and iron. Sections 3.2.4 and 3.2.5 provide an overview of these results.

### 3.2.4.1 Biotransformation of carboxylic acids, methanol, and formaldehyde

Short-chain mono- and dicarboxylic acids as well as C1 compounds (methanol and formaldehyde) are formed during successive oxidation processes in cloudwaters, and they are found at rather high concentrations (in the range of micromolars) and can be used as substrates by cloud microorganisms. Indeed, most can enter into the central metabolisms of several microorganisms, and these metabolites can be used to maintain energy levels through the production of ATP to synthesize larger molecules and produce biomass (proteins, nucleic acids, membranes, etc.). All known metabolic pathways can be found from the Kyoto Encyclopedia of Genes and Genomes data available online (<http://www.genome.jp/kegg/pathway.html>).

#### Biodegradation of short-chain carboxylic acids

One of the main pathways involved in the biodegradation of carboxylic acids (e.g., acetic, citric, succinic, fumaric, and malic acids) is the Krebs tricarboxylic acid (TCA) cycle. Microbial metabolism is extremely complex and involves hundreds of interconnected reactions. For instance, the TCA cycle is related to pyruvic acid metabolism, glyoxylic and dicarboxylic acid metabolism, glycolysis, amino acid metabolism, etc. Further information on these pathways can be found online at <http://www.genome.jp/kegg/pathway.html>.

In regards to dicarboxylic acids, Ariya et al. (107) and Côté et al. (108) found that malonic, succinic, glutaric, adipic, pimelic, and pinic acids can be efficiently degraded by airborne fungi. Using solid-state  $^{13}\text{C}$  nuclear magnetic resonance (NMR), they showed that [ $^{13}\text{C}$ ]-succinate can be integrated into *Geotrichum* sp. macromolecules. By contrast, oxalic acid is typically not biotransformed and, if it is, it occurs slowly. Our research team also confirmed that cloud microorganisms cannot degrade oxalate while active in succinic acid. This was proven using single strains isolated at the puy de Dôme station (France) and incubated in phosphate buffer (109, 110) or in artificial cloud medium that mimics polluted or unpolluted clouds (111). The same result was observed when real cloud samples issued from three different air mass origins and containing the entire uncultivated microbial population were used (112). DeLeon-Rodriguez et al. (11) described the presence of oxalate-degrading bacteria (Oxalobacteraceae) in samples collected at high altitudes over the Atlantic Ocean using DNA-based methods; these results are in contradiction with the laboratory experimental results described above.

This may be attributable to the fact that this genus is not active under real atmospheric conditions or to the fact that its presence depends on the sampling site used.

In regards to monocarboxylic acids, Herlihy et al. (113) were the first to study the degradation of formic and acetic acids by bacteria in rainwater. Amato et al. (20, 109) showed that 60 microbial strains present in cloudwater are able to transform formic, acetic, and lactic acids. This study was performed at 27°C using pure cultures with one single substrate in phosphate buffer. Various metabolic intermediates such as pyruvate and fumarate were detected by *in situ* <sup>1</sup>H-NMR, demonstrating that microbial metabolism can serve as a sink but also as a source of organic compounds for atmospheric chemistry. Further experiments were performed at temperatures closer to those encountered in clouds for microcosms containing single strains or real cloudwaters, and all of these experiments confirmed that acetic and formic acids are easily degraded (110–112). In these latter studies, the biodegradation rates of acetic and formic acids were not heavily affected by the chemical composition of the cloud medium (natural or artificial) (and in particular by changes in pH and sulfate and nitrate concentrations, which are markers of cloud pollution). This was also observed in the case of succinic acid.

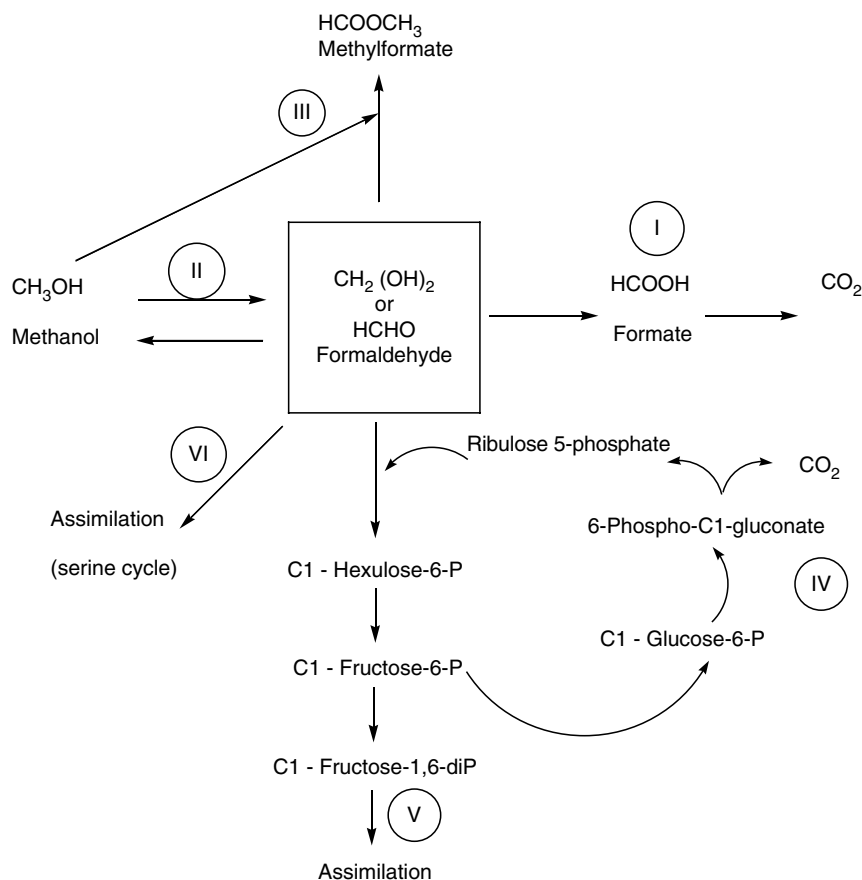
The degradation of carboxylic acids has also been found in microorganisms isolated in other atmospheric environments, including hailstones and Arctic snow (109, 114), Krumins et al. (115) have presented evidence that a *Sphingomonas aerolata* strain aerosolized in a rotating gas-phase bioreactor can increase its cellular 16S rRNA content when acetic acid is added to it, exhibiting its metabolization. This work is particularly interesting, as it demonstrates the potential directly degradation of volatile organic compounds (VOCs) in the air in addition to cloudwater.

#### Biodegradation of methanol and formaldehyde

Some microbial strains can transform methanol and formaldehyde (C1 compounds) using various metabolic routes (Figure 3.2.2) (see also <http://www.genome.jp/kegg/pathway.html>).

First, formaldehyde can be transformed into final products such as methanol (II), methylformate (III), or formate and CO<sub>2</sub> (I), and methanol can be converted into formaldehyde by oxidation (I). These dissimilation routes are typically used by microorganisms to discard formaldehyde, which is considered a toxic compound. It should be noted that routes I and II lead to the production of intermediates that are very similar to those of radical chemistry (116); however, the main difference here rests in the fact that, in radical chemistry, only oxidation occurs, while in metabolic pathways formaldehyde can be reduced to methanol. In addition to these dissimilation routes, formaldehyde can be integrated into the central metabolisms of microorganisms and can be used as a source of carbon and energy for producing sugars (IV and V) and amino acids (VI).

These metabolic pathways are present in microorganisms referred to as “methylotrophs” (facultative or obligatory methylotrophs) and can be found among Alpha-, Beta-, and Gammaproteobacteria and Gram-positive bacteria, which are found in cloud droplets (15). For instance, Šantl-Temkiv et al. (114) found *Methylobacterium* strains (Alphaproteobacteria) in a storm cloud. These strains are known to serve as facultative methylotrophs. Amato et al. (109) screened numerous bacteria and yeasts isolated from clouds at the puy de Dôme station (France) for their ability to transform methanol and formaldehyde. In these experiments, 60 isolated strains were incubated in phosphate buffer and the transformation of formaldehyde and methanol was monitored via *in situ*



**Figure 3.2.2** Main metabolic routes for the biotransformation of formaldehyde.

H-NMR. All of the bacterial and yeast strains examined actively degraded formaldehyde, while different behaviors were observed for the transformation of methanol: Gram-positive bacteria mainly degraded methanol, Gram-negative bacteria degraded but mainly produced methanol, and yeasts formed and degraded methanol equally. A specific strain of *Bacillus* sp. 3B6 (Gram positive) was studied at length by Husarova et al. (117) using  $^{13}\text{C}$ -formaldehyde. *In vivo*  $^{13}\text{C}$ -NMR spectra showed that this strain uses different pathways including routes I and II in addition to the serine pathway (route VI). Finally, Vaitilingom et al. (112) showed that the microbial community present in real cloud samples can fully degrade formaldehyde. Interestingly, it was shown that decreasing the temperature of incubation from  $17^\circ\text{C}$  to  $5^\circ\text{C}$  has little impact on the biodegradation rates of four different strains isolated from clouds (two *Pseudomonas* sp., *Bacillus* sp., and *Frigoribacterium* sp.) (117).

### 3.2.4.2 Comparison between biodegradation and radical chemistry

The studies cited above clearly show that cloud microorganisms are fully equipped to biocatalyze the transformation of di- and monocarboxylic acids, methanol, and

formaldehyde. It has been shown for simplified incubation media with single substrates and pure strains and for highly complex natural samples that the vast majority of cloud microorganisms can perform these reactions.

The main remaining scientific question concerns evaluating the relative contributions of these biological processes in relation to abiotic processes in determining the real effects of microorganisms on cloud chemistry. It is thus necessary to compare the rates of transformation for organic compounds via biodegradation versus radical chemistry. For this purpose, two main strategies have been employed:

- The first involves comparing the measured biodegradation rates with the abiotic rates of transformation determined based on the reactivity constants of  $\text{HO}^\bullet$ ,  $\text{NO}_3^{\bullet-}$ , and  $\text{HO}_2^\bullet$ , and of  $\text{O}_3$ .
- The second strategy involves designing experiments where both biotic and abiotic degradation rates can be measured experimentally. It is also possible to combine photo- and biodegradation using specifically designed photobioreactors.

Ariya et al. (107) measured the biodegradation rates of dicarboxylic acids (malonate, succinate, adipate, pimelate, and pinate) by *Geotrichum* sp. isolated from air and determined their lifetimes, which fell within a range of 1.5–10 days. These lifetimes fell within the same range as those calculated from the reactivity of  $\text{HO}^\bullet$  (0.03–24 days) but were much shorter than those calculated for  $\text{O}_3$  (>771 days) and  $\text{HO}_2^\bullet$  (>2000 days).

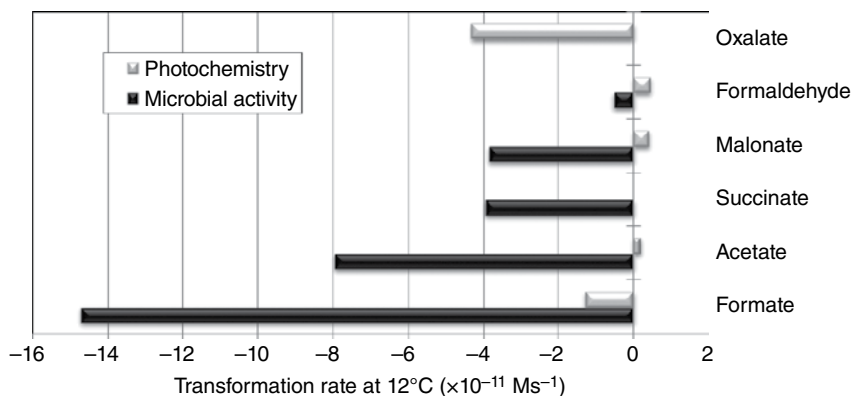
Husárová (118) measured the biodegradation rates of methanol and formaldehyde using four strains (two *Pseudomonas* strains, one *Bacillus* and one *Frigoribacterium* strain) isolated from cloud samples at the puy de Dôme station (France). These rates were compared with those resulting from reactions between methanol and formaldehyde species and free radicals  $\text{HO}^\bullet$  and  $\text{NO}_3^{\bullet-}$ . They showed that microorganism contributions to cloud chemistry are most prominent at night when only  $\text{NO}_3^{\bullet-}$  are present. During the day,  $\text{HO}^\bullet$  reactivity is dominant. Microorganism contributions were found to be more significant in polluted clouds where  $\text{NO}_3^{\bullet-}$  concentrations are higher (due to higher  $\text{NO}_x$  concentrations in urban cases) relative to those of unpolluted clouds.

Using the same approach, Vaïtilingom et al. (111) carried out an extensive study on 17 strains isolated at the puy de Dôme station (France), covering a wide variety of bacterial strains belonging to *Sphingomonas*, *Pseudomonas*, *Bacillus*, *Arthrobacter*, *Clavibacter*, *Frigoribacterium*, and *Rhodococcus* genera. The researchers measured the biodegradation rates of acetate, succinate, and formate, and average values calculated for the 17 strains were compared with those obtained based on the reactivity of  $\text{HO}^\bullet$  and  $\text{NO}_3^{\bullet-}$ . Two extreme cases were considered: one involving a high radical concentration ( $10^{-12}$  M  $\text{HO}^\bullet$  and  $10^{-13}$  M  $\text{NO}_3^{\bullet-}$ ), the other involving a moderate concentration ( $10^{-14}$  M  $\text{HO}^\bullet$  and  $10^{-14}$  M  $\text{NO}_3^{\bullet-}$ ). The results clearly show that bacterial contributions are dominant at night in both cases.  $\text{HO}^\bullet$  reactivity is more pronounced during the day when the highest concentrations are considered, but it is relatively equivalent to microbial activity in moderate cases. The main problem associated with using this theoretical approach is that no real measurements of  $\text{HO}^\bullet$  and  $\text{NO}_3^{\bullet-}$  concentrations in cloudwaters are available. Values are derived from modeling work where cloud chemistry models are used to evaluate concentrations that vary by a few orders of magnitude (from  $10^{-12}$  to  $10^{-16}$  M for  $\text{HO}^\bullet$ ). For this reason, it is more appropriate to directly measure degradation rates using microcosms specifically designed for use in photo- and biodegradation experiments.

In the same study (111), photodegradation experiments were conducted in a photoreactor mimicking solar light in parallel with biodegradation experiments. The photodegradation rates measured gave very similar results to those found using moderate radical concentrations ( $10^{-14}$  M  $\text{HO}^\bullet$  and  $10^{-14}$  M  $\text{NO}_3^{\bullet-}$ ). Under these more realistic experimental conditions, biodegradation and photodegradation rates of formate, succinate, and acetate were well within the same range during the day, with bacteria being dominant actors at night.

All the experiments described above were conducted using pure strains in artificial cloudwater, and an even more realistic experiment was designed using three real cloudwater samples collected from the puy de Dôme station (112). Each sample was divided into two components. One was filtered until it was sterilized and was then exposed to light, and the other was unfiltered and contained microorganisms left in the dark. Figure 3.2.3 shows the relative contributions of microbial activity versus photochemistry to the transformation of acetate, succinate, malonate, formate, oxalate, and formaldehyde.

With the exception of oxalate, which is only photodegraded, all of the other compounds were biodegraded efficiently. Microbial activity is the major actor (compared with photochemistry) that degrades formate, acetate, succinate, formaldehyde, and malonate. Because of the complex chemical compositions of natural samples, formaldehyde, malonate, and acetate are photoproducts and are not photodegraded. This experiment suggests that global microbial activity may have strong impacts on the carbon budget of the aqueous phase of clouds.



**Figure 3.2.3** Transformation rates of organic compounds present in a real cloud sample collected at the Puy de Dôme (France) station resulting from microbial activity (black) or radical chemistry (white).

## 3.2.5 Interactions with cloud oxidants

### 3.2.5.1 Interactions with reactive oxidant species

As shown in Section 3.2.4, cloud microorganisms may transform organic matter as an alternative route to radical chemistry reactions involving  $\text{H}_2\text{O}_2$ ,  $\text{HO}_2^\bullet/\text{O}_2^{\bullet-}$ , and  $\text{HO}^\bullet$ ,

which are considered catalysts of atmospheric chemistry. However, cloud microorganisms can interact directly with these extracellular reactive oxidant species (ROS), which can diffuse into cells across the cytoplasmic membrane. These extracellular ROS are mainly produced through the direct photolysis of  $\text{H}_2\text{O}_2$  or through photo-Fenton reactions ( $\text{Fe} + \text{H}_2\text{O}_2$ ). Aerobic microorganisms are equipped to face such ROS, as they naturally produce the same molecules intracellularly during respiration when  $\text{O}_2$  diffuses inside the cell. This protective mechanism against ROS, which can be deleterious to cells by damaging major cellular components (proteins, DNA, lipids, etc.), is referred to as “oxidative stress metabolism” (119). First, microorganisms can produce antioxidant molecules that serve as radical scavengers; these include vitamins (ascorbic acid,  $\alpha$ -tocopherol, etc.), glutathione, and pigments (carotenoids) (120, 121). As noted above, most atmospheric microorganisms are pigmented: 50% in clouds and 80% in the air (15, 20, 122, 123). A second mechanism of protection is based on the activity of specific enzymes that can transform ROS:  $\text{O}_2^{\bullet-}$  is biotransformed efficiently in  $\text{H}_2\text{O}_2$  by super oxide dismutase, and then  $\text{H}_2\text{O}_2$  is transformed by catalases into  $\text{O}_2$  and  $\text{H}_2\text{O}$ , which are no longer toxic to cells (124, 125). These catalases are thus suspected to act directly on  $\text{H}_2\text{O}_2$ , which is transferred into the cell from an extracellular medium (cloudwater). Owing to their oxidative stress metabolism, cloud microorganisms can thus modify the oxidant capacities of clouds. To test this hypothesis, we designed a specific experiment that allowed us to evaluate cloud microorganism interaction with  $\text{H}_2\text{O}_2$  present in natural cloudwater or radicals induced by photochemical reactions (112). Cloud samples were collected from the puy de Dôme station (France) and were divided into two parts, as follows.

One part was filtered on a  $0.22 \mu\text{m}$  filter to eliminate microbial strains; it was thus considered sterile. This sterile sample was divided again into two parts: one was exposed to artificial solar light and the other was stored in the dark. These two experiments allowed us to study non-photochemical radical reactions (dark) and photochemical reactions (light).

The other part was unsterilized and contained all of the microbial strains; it was also divided into two parts that were either exposed or not exposed to solar light. Reactions that occurred in the dark revealed the contributions of microbial activities to the degradation of  $\text{H}_2\text{O}_2$ . The experiments involving light exposure allowed us to evaluate microorganism interactions with  $\text{HO}^\bullet$  radicals.

Dark and light conditions were also used to mimic night-time and daytime conditions. The relative contribution of microbial activity during the day is equal to 20% and is dominant at night (76%). Finally, it was shown that the metabolic activity of cells measured by the ATP/ADP ratio does not change under dark or light conditions, indicating that microorganisms are not impacted by the presence of  $\text{HO}^\bullet$  radicals. They are instead able to protect themselves against ROS.

### 3.2.5.2 Interactions with iron

As shown in Section 3.2.3, iron plays a key role in cloud chemistry, as it affects the concentration and cycling of radicals ( $\text{HO}^\bullet$  and  $\text{HO}_2^\bullet$ ) and oxidants such as  $\text{H}_2\text{O}_2$ . One major concern is related to the organic speciation of Fe(III) in clouds, which remains unknown. Recent studies conducted by Bianco et al. (98) show that the production of  $\text{HO}^\bullet$  in cloudwater cannot be explained by iron–oxalate complexes only. Our hypothesis

is that Fe(III) can be chelated by other organic ligands of biological origin (1, 22), thus modifying the availability of iron as a source of radicals.

Siderophores are produced by microorganisms for the uptake of iron, which is required to support their metabolism, particularly because iron is part of the active site of metalloenzymes such as mono- and dioxygenases and is involved in electron transfers during aerobic respiration. These very efficient complexing systems are excreted in extracellular environments to chelate Fe(III), and then the Fe(III)–siderophore complex is transported to cells by microorganisms. Some microorganisms are not able to synthesize siderophores but can take up siderophores synthesized by other microorganisms (126–128).

Siderophores are complex molecules with a large variety of chemical structures, though they share functions found in chelate Fe(III): catechols, hydroxamates, and carboxylates. Some siderophores bear specific chelating functions while others can combine two or three of them in one molecule. The existence of siderophores such as Fe(III) organic ligands in the ocean has been known for a long time (126, 128). These biological molecules have a high affinity for iron (complexing constants  $K > 10^{20}$ ). For rainwater, Cheize et al. (129) also found organic Fe-complexing ligands with very high stability constants (log  $K$  equal to 21–22), which is consistent with the presence of siderophores. As rainwater results from the collision/coalescence of cloud droplets, iron may also be complexed by siderophores in cloudwater. To test our hypothesis on the presence of siderophores in cloudwater, we screened 450 bacteria and yeasts isolated from 37 cloud events at the puy de Dôme station for their capacities to produce siderophores using a Chrome Azurol S method (130). We found 42% of them to be siderophore producers, and bacteria from the *Pseudomonas* genus were found to be the most active and abundant (they represent approximately 50% of all active strains). Siderophore production trends were also studied by siderophore type; among the active strains examined, 38% produced catechol, 18% produced hydroxamate, and 44% produced mixed-function siderophores. In addition, *Pseudomonas* strains predominate in the production of mixed functions; this is consistent with the fact that they can produce pyoverdines. Interestingly, we found that the number of strains that produce or do not produce siderophores and siderophore types correlated with the air mass origins of clouds.

Pyoverdines are of special interest as they bear a chromophore and are thus photoactive molecules that could be easily phototransformed into the aqueous cloud phase, thus directly affecting cloud chemistry. In a recent work, Passananti et al. (131) studied the photochemical behaviors of a Fe(III)–pyoverdine complex. They showed that quantum yields of formation for Fe(II) and HO• are lower (factor 10) than values reported for iron–oxalate complexes. As the complexation constant of oxalate for Fe(III) is much lower than that for pyoverdines, pyoverdines may compete with oxalate to chelate iron and may ultimately produce fewer radicals than expected from actual models of atmospheric chemistry; the presence of pyoverdines in clouds may impact iron contributions to the oxidative capacities of the cloud aqueous phase.

In conclusion, the organic speciation of iron in cloudwater remains largely misunderstood, and our results suggest that siderophores may exist as Fe(III) complexes. Although their actual presence must be demonstrated, siderophore photochemistry may change the redox cycling of iron and the production of ROS in clouds. Siderophores could be introduced into new atmospheric models in addition to iron–oxalate complexes.

## 3.2.6 Clouds as spaces of organic compound functionalization

In this section, we compare the formation of high molecular weight compounds (HMWCs) via microbial activity versus radical chemistry.

### 3.2.6.1 Formation of high molecular weight compounds *via* chemical reactions

Fragmentation in the aqueous phase by radical processes constitutes a significant path toward organic compound transformation in clouds; however, for concentrated solutions, compounds of higher molecular mass (LMCs) may be formed (132, 133). This reactivity may especially take place in deliquescent aerosols and during the evaporation of cloud droplets (134). It appears however that the formation of LMCs, which are generally polar and highly soluble, may also occur in cloud droplets, i.e., to weaker concentrations (91). These compounds must then take part in the formation of SOA mass referred to as “cloud aqSOA” and “aerosol aqSOA.” The most likely precursors of aqSOA are small carbonyls ( $\leq C5$ ), as these compounds are soluble, are highly reactive in the aqueous phase, and are formed quickly, primarily from VOCs emitted in the gas phase (9). Therefore, several studies describe the formation of LMCs from precursors such as glyoxal (135, 136), methylglyoxal (52), and pyruvic acid (89). AqSOA formation from a given precursor will be significant when simultaneous gas-phase reactions are not the main loss processes involved. Only very few carbonyl compounds present aqueous-phase processes in competition with their gas-phase sinks (e.g., pyruvic acid) (137).

Experiments have also been carried out on isoprene oxidation products such as methacrolein (MACR) and methyl vinyl ketone (MVK); these compounds, which are oxidized by a radical hydroxyl in the liquid phase, lead to the formation of LMCs as an intermediate chemical product (43). Two different mechanisms have been proposed to explain LMC formation in the aqueous phase: accretion by acid catalysis and radical mechanisms (134).

Currently, most groups agree that radical mechanisms prevail in oligomer formation from atmospheric carbonyl compounds such as methylglyoxal, glycolaldehyde, MVK, and MACR (43, 44, 46, 47). Renard et al. (138) showed, by means of the ion-mobility spectrometry–mass spectrometry technique, that mixtures of unsaturated conjugated precursors had the ability to co-oligomerize, i.e., forming only one complex oligomer system bearing monomers of different structures (138). This capacity allows for oligomer formation from a lower individual concentration of the precursor. Griffith et al. (139) demonstrated that photolysis of aqueous pyruvic acid also produces oligomers. Pyruvic acid is currently used as a proxy for  $\alpha$ -dicarbonyls in atmospheric models and is abundant in both the gas phase and the aqueous phase. Rossignol et al. (140) highlighted a third pathway for oligomer formation. Products bearing an imidazole ring, such as imidazole-2-carboxaldehyde, can act as a photosensitizer, initiating a radical chemistry in the aerosol phase under realistic irradiation conditions; and thus an aerosol growth in the presence of gaseous volatile organic compounds.

### 3.2.6.2 Formation of high molecular weight compounds via microbial activity

As described in Section 4.2.3, microorganisms can metabolize various carbon compounds present in cloudwaters, and this metabolism can lead to the production of CO<sub>2</sub> but also to the synthesis of molecules of higher molecular mass than the original substrates. These compounds of higher molecular mass can be macromolecules integrated in their own biomass (proteins, DNA, etc.) or they can be excreted in an external medium and thereby considered SOA. This research topic is rather new and has not been widely explored in previous studies.

#### Glycerol derivatives

Husárová et al. (117) studied metabolic pathways involved in the transformation of [<sup>13</sup>C]-formaldehyde by the *Bacillus* sp3B6 strain isolated from clouds. In addition to the formation of methanol, formate, and CO<sub>2</sub>, *in vivo* <sup>13</sup>C-NMR spectra revealed the presence of unexpected glycerol, 1,2-, and 1,3-propanediol. This result shows that these bacteria can synthesize carbon compounds with three carbons from a substrate with one carbon compound. The synthesis of such compounds results from the successive transformation of formaldehyde via the serine cycle, glycerophospholipid metabolism, and glycerolipid metabolism.

#### Oligosaccharides and exopolymeric substances

Various classes of saccharides have been reported as important constituents of ambient atmospheric aerosols, including: sugar alcohols, monosaccharides, disaccharides, trisaccharides, and anhydrosugars; the presence of such saccharides in the atmosphere constitutes a hallmark of biogenic sources of aerosols (17). Because of their high solubility in water, these sugars are suspected to be present in cloudwater. A previous study investigated the transformation of sucrose, a disaccharide which is one of the most abundant sugars in aerosols, by the *Bacillus* sp3B6 strain isolated from clouds (141). Different fractions were obtained after dialysis and separation via size exclusion chromatography of the incubation medium. These fractions contained various fructo-oligosaccharides of levan and inulin with varying degrees of polymerization and levan exopolysaccharide (EPS). In addition, three types of trisaccharides were identified: 1-ketose, 6-ketose, and neo-ketose. The same team (17) performed an extensive study on the transformation of a wide variety of atmospheric sugars by the same strain. Their results show that most these sugars can be metabolized by *Bacillus* sp. 3B6. *In situ* <sup>1</sup>H-NMR has revealed the synthesis of EPSs during incubation with L-arabitol, D-fructose, D-glucose (monosaccharides), sucrose (disaccharide), and cellotetraose (tetrasaccharide). Two-dimensional NMR experiments have found these EPSs to include 1,6- $\alpha$ -galactan and partially acetylated polyethylene glycol.

These results clearly show that cloud microorganisms can synthesize oligosaccharides and EPSs of high molecular mass from monosaccharides to tetrasaccharides. Natural microorganisms are protected when they are embedded in EPSs to form aggregates or to grow within biofilms adhered to solid surfaces (119, 142). EPSs are of special interest to cloud microorganisms, as they can help cloud microorganisms survive atmospheric stress. Monier and Lindow (143) showed that a *Pseudomonas syringae* strain is more likely to survive as an aggregate than a solitary form on a leaf surface

exposed to desiccation. It is worth noting that *P. syringae* is one of the most abundant cultivable species found in clouds (15). Tong and Lighthart (121) also reported that cell aggregation and association with particles increased survival levels during the day due to shielding effects.

Finally, the synthesis of EPSs or oligosaccharides may affect the formation of CCN, as these highly functionalized molecules can easily interact with water. It has been shown that EPSs synthesized by marine phytoplankton serve as a source of CCN (144).

### Biosurfactants

Microorganisms can synthesize biosurfactants (surface active agents), which can remain at the surface or be released into an environmental medium. These molecules are highly complex, presenting considerable levels of structural variability; the same microorganisms can produce one or several biosurfactants depending on the availability of their substrates. Biosurfactants are amphiphilic compounds with hydrophobic (lipids, etc.) and hydrophilic (sugars, amino acids, etc.) moieties, and they can therefore reduce surface and interfacial tension. They are extremely efficient compared with classical surfactants, as they can lower the surface tension ( $\sigma$ ) of water to less than  $30 \text{ mN m}^{-1}$ . Two main classes of biosurfactants can be identified (145, 146): (i) low molecular mass biosurfactants are mainly composed of glycolipids (rhamnolipids, trehalolipids, and sophorolipids) and cyclic lipopeptides (viscosin, surfactin, polymyxin, syringomycin, etc.) but also of fatty acids and phospholipids; (ii) high molecular mass biosurfactants are polymeric structures (approximately 1 MDa) composed of polysaccharides, proteins, lipopolysaccharides, lipoproteins, or complex mixtures of these polymers (alasan, emulsan, etc.).

Research on biosurfactant production in the environment has mainly focused on microorganisms isolated from soils, the rhizosphere and phyllosphere, and the marine environment (147–149). However, few studies have shown that microorganisms isolated from clouds and rainwater can produce biosurfactants. Ahern et al. (150) showed that 70 fluorescent *Pseudomonas* isolates are biosurfactant producers, with 43 constituting high producers. Recently, our group conducted an intensive screening of 480 strains isolated from clouds of approximately 23.3% Gammaproteobacteria (mainly *Pseudomonas*), 19.8% Alphaproteobacteria (mainly *Sphingomonas*), 24.2% Actinobacteria, and 19.6% Basidiomycota, and 41% were considered biosurfactant producers ( $\sigma \leq 55 \text{ mN m}^{-1}$ ). We found that 7% of the active strains can reduce the surface tension of water to under  $30 \text{ mN m}^{-1}$ , and these strains predominantly belong to the *Pseudomonas* genus (151). This work highlights the importance of *Pseudomonas* strains to atmospheric waters as the main group encountered and as the more active group in terms of biosurfactant production. While the presence of biosurfactants in cloudwater has not yet been proven, they have been found in atmospheric aerosols (152–154).

The presence of biosurfactants in the atmosphere could have consequences for atmospheric processes. First, the activation of aerosol particles into cloud droplets is dependent on surface tension levels (155), and so biosurfactants, which are present in aerosols or are associated with microorganism surfaces, are likely to affect Earth's radiation budget (156) and cloud-forming properties by reducing surface tension levels (154). Second, the existence of biosurfactants along the surfaces of cloud droplets may

change the efficiency of mass transfer processes between cloud gas and aqueous phases. While only Henry's constants have been considered, this organic layer could completely change transfers relative to a "simple" water–air interface.

### 3.2.7 Conclusion

The effect of clouds on the climate is still subject to debate and presents uncertainties related to cloud formation and to their microphysical and chemical properties, which are highly variable in time and space (7). One factor contributing to these uncertainties is the current lack of knowledge on multiphase chemistry processes (gas/aqueous phase and aerosol particles) occurring in clouds. Cloud chemistry studies are therefore crucial for scientists to evaluate the effects of clouds on climate change.

Until now, only radical chemistry has been studied to investigate the transformation of organic matter present in the cloud aqueous phase. This chemistry is highly complex and involves the presence of strong oxidants such as  $\text{H}_2\text{O}_2$ , iron complexes, and radicals ( $\text{HO}_2^\bullet/\text{O}_2^{\bullet-}$ ,  $\text{HO}^\bullet$ ) (whether photoinduced or not). Major chemical routes lead to the oxidation of organic matter into aldehydes, carboxylic acids, and finally  $\text{CO}_2$ ; recent studies also report that cloud chemistry may shape the formation of HMWCs such as oligomers. The recent discovery of metabolically active microorganisms in clouds has raised new scientific questions regarding their potential impacts on cloud chemistry. Rather, is microorganism metabolism an alternative route to radical chemistry? Only a limited number of studies have involved the use of model strains isolated from clouds or real cloud samples.

Regarding biodegradation processes, it has been shown that microorganisms may play a dual role in the transformation of carbon compounds.

- First, they can directly degrade carbon compounds such as formaldehyde, methanol, and C1 into C4 organic acids owing to their carbon metabolism.
- Second, they can act indirectly through their interactions with oxidants by modifying concentrations of  $\text{HO}^\bullet$  that can degrade organic matter in clouds. They can transform  $\text{H}_2\text{O}_2$  via their oxidative stress metabolisms, which serve as a major source of radicals.

Alternatively, they can synthesize siderophore-Fe(III) complexes, which are also involved in ROS production.

One comparison between the rates of biotic and abiotic transformations in microcosms mimicking cloud environments showed that biodegradation can be competitive with radical chemistry features. However, such laboratory experiments have been performed under bulk conditions that poorly reflect multiphase (gas/liquid/solid) chemistry processes that occur in clouds. Cloud chemistry is also modified by microphysical and dynamical processes. The next step will thus involve integrating biological reactions within cloud chemistry models.

Regarding the formation of HMWCs, few mechanisms have been presented in the literature, and these mechanisms must be evaluated through cloud chemistry models (41). Research on biological contributions to the production of these compounds is at its infancy. The biotransformation of biogenic substrates should be investigated in greater detail in reference to particular sugars and amino acids. Oligomers produced

may have implications not only for chemistry features but also for cloud microphysics, as they may play a role in cloud condensation or nucleation processes.

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## **Part IV**

### **Impacts of Bioaerosols on Human Health and the Environment**

## 4.1

### Health Impacts of Bioaerosol Exposure

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#### 4.1.1 Introduction

Indoor and outdoor environments can be contaminated with complex bioaerosols that are implicated in a variety of diseases. While bioaerosols from indoor and outdoor environments are associated with several health problems including infectious, toxic, and hypersensitivity diseases, aerosolized biological agents have also been used for malicious purposes. The concentration and the nature of the components of these bioaerosols are important factors affecting their hazardous potential. Yet, the exact role of bioaerosol components and their interplay regarding mechanisms of disease are ill defined. This chapter lists indoor environments contaminated with bioaerosols and pulmonary diseases associated with exposure, as well as the components known to impact on human health. We also cover bioterrorism related to bioaerosols, current strategies to monitor pollens and spores, as well as some exposure limit values, when applicable.

#### 4.1.2 Hazardous potential of bioaerosols

##### 4.1.2.1 Factors affecting the hazardous potential of bioaerosols

Several factors influence the impact of airborne biological particles on human health, including their nature, their immunogenicity, their concentration, their size, and their ensuing penetration in the respiratory tract (1, 2). Host-related factors, such as the genetic background and environmental history, are also to be considered (3). Viable microorganisms and microorganisms compatible with the environmental conditions offered by the human body can cause infection. Moreover, several non-viable components of bioaerosols, which are not infectious, can still cause diseases through toxic or immunopathological mechanisms.

The size of bioaerosol particles is a major determinant of their impact on human respiratory health (4). Indeed, the human respiratory tract is composed of a nasopharyngeal section comprising superior airways (nose, mouth, larynx, and pharynx), a tracheobronchial section composed of inferior airways from the trachea to the terminal bronchioles, and, finally, a pulmonary section, the alveoli, where gas exchange occurs (1, 2). Aerosolized particles are classified in different subcategories according to their aerodynamic diameter and the region of their deposition in the respiratory tract. Bioaerosol particles that possess a 100–200  $\mu\text{m}$  aerodynamic diameter can only deposit in the upper airways. Particles with an aerodynamic diameter ranging from 10 to 100  $\mu\text{m}$  can reach superior airways and are thus considered inhalable. The thoracic fraction gathers particles with an approximate aerodynamic diameter of 10  $\mu\text{m}$  and their deposition occurs within the larynx and the bronchioles, or above in the airways. Finally, the fraction of the particles that can reach the terminal bronchioles and the alveoli display an approximate 3.5  $\mu\text{m}$  aerodynamic diameter and are deemed breathable (5). These particles are considered to be the main threat for human health because they can penetrate deep into the respiratory tract, where there is no mucus surface allowing the disposal of foreign matter (2). Yet, when it comes to hypersensitivity diseases such as asthma, an emerging theory called “united airways” stipulates that a link could be made between allergic diseases of the upper airways, as in the case of rhinitis, and lower airway diseases, as in asthma, under the premise that sensitization occurring in one site could predispose to distal immunopathological responses, or that both diseases are somewhat connected by the individual’s propensity to develop allergic reactions, also called atopy (6, 7). In addition, large biological particles such as pollen grains (20–100  $\mu\text{m}$ ), which stop in the superior airways, can release microparticles or pauciparticles (0.01–0.5  $\mu\text{m}$ ) containing many allergens that can trigger and/or exacerbate several respiratory diseases and allergic symptoms. Thus, non-culturable components of bioaerosols can reach different locations in the respiratory tree depending on their size, where they can either be removed silently or cause a number of non-infectious diseases.

#### 4.1.2.2 Epidemiological data in documented environments

Several environments pose a higher risk for bioaerosol-related diseases owing to the nature of the activities, the degree of confinement, and the nature of agents that are aerosolized. These environments have been well described elsewhere and classified into four categories regarding their level of airborne bacteria and endotoxin contamination (8, 9). Briefly, the highly contaminated environments include very confined locations where biological burden is extremely high, such as farm and agricultural settings, industrial composting facilities, and wastewater treatment plants (8, 9). In these environments, airborne contamination can reach up to  $10^9$  total bacteria per cubic meter and is associated with the development of several diseases such as rhinitis, asthma, chronic bronchitis, organic dust toxic syndrome (ODTS), and hypersensitivity pneumonitis (HP) (10, 11). A higher prevalence of these diseases among workers in such highly contaminated environments was reported (11–13) (Table 4.1.1). Environments with moderate airborne contamination mostly include plant processing facilities and machining industries, where airborne bacteria can reach up to  $10^5$  colony-forming units (CFU)  $\text{m}^{-3}$ . In such facilities, asthma-like syndrome, chronic bronchitis, ODTS, and HP are predominant (10, 12–15) (Table 4.1.1). Finally, weakly contaminated environments are

**Table 4.1.1** Bioaerosol-related diseases and occupational environments where they can occur.

Disease related to bioaerosols	Occupational environment	Reference
HP	Dairy farms, crop workers, livestock farm workers	10, 13
ODTS	Waste and compost industries, livestock farm workers, crop workers	10, 41
Asthma and asthma-like syndrome	Crop workers, livestock farm workers, cotton industry	13, 41
Rhinitis	Livestock and dairy farm workers	10
Sick building syndrome	Office, school, hospital, and care home workers	16
Chronic obstructive pulmonary disease	Landscape or horticultural workers, forestry workers, fishery workers, crop workers, dairy workers	10, 13
Histoplasmosis	Crop workers	13
Pneumonia	Crop workers, forestry workers	13
Influenza	Crop workers, livestock farm workers	13
Tuberculosis	Crop workers, livestock farm workers, forestry workers	13

HP, hypersensitivity pneumonitis; ODTS, organic dust toxic syndrome.

those where the biological burden mostly comes from humans, such as hospitals, schools, offices, and homes. The airborne bacterial counts in these environments are very low, i.e., around  $10^2$ – $10^3$  bacteria  $m^{-3}$ . These environments can be associated with sick building syndrome (16) and retain a significant infectious disease risk (17).

### 4.1.3 Infectious diseases associated with bioaerosols

#### 4.1.3.1 Identification of agents with infectious potential in bioaerosols

Several microorganisms, when aerosolized, have the potential to cause infectious human diseases. Although some airborne bacteria and molds can cause infection, the most susceptible group of microorganisms to induce bioaerosol-related infectious diseases are viruses. These include the influenza virus, severe acute respiratory syndrome (SARS), coronavirus, respiratory syncytial virus, and rhinoviruses (18). A study by Bonifait et al. (19) even suggested an airborne infectious potential of the norovirus. Bacterial infectious bioaerosol-related diseases include, among others, tuberculosis (*Mycobacterium tuberculosis*), plague (*Yersinia pestis*), legionellosis (*Legionella pneumophila*), and pneumonia (*Chlamydia pneumoniae* and *Haemophilus influenzae*) (11). Airborne molds can also cause lung infection, as seen in histoplasmosis (*Histoplasma capsulatum*) and aspergillosis (*Aspergillus*). The sources of these bioaerosol components can vary, but they are mainly produced by infected humans and animals, as well as contaminated water (20). The Canadian Agency for Drugs and Technologies in Health (CADTH) recommends wearing a mask when there is a possibility of exposure to several infectious microorganisms (21).

#### 4.1.3.2 Determinants of maintenance of infectious potential in bioaerosols

Several physical and environmental factors can influence the maintenance of infectious agents in bioaerosols. Indeed, the concentration of these airborne agents is a major factor determining the infectivity of aerosols (4). Moreover, the particle composition, i.e., the density of the particle and its dynamic particle size, affects its aerosolization time, which is a determinant of infectious potential (1, 2). Environmental factors such as relative humidity (22), temperature or ultraviolet exposure can also affect the infectivity of bioaerosol agents, mostly viruses (23). For example, the infectivity of the airborne influenza virus can reach up to 77% in low-humidity environments, i.e., under 23% of relative humidity (22). Moreover, a phage model for influenza virus (24) showed complete loss of infectivity when exposed to 30°C (23). The immunopathological potential of non-infectious agents is presumably little affected by environmental variations observed in indoor environments, unless surface antigens, immunogenic components, or toxic products are altered by passage in the aerosol state, which has not been documented.

#### 4.1.4 Toxic and hypersensitivity disease-associated bioaerosols

##### 4.1.4.1 Balance of biological mechanisms determining toxic reactions and hypersensitivity

The non-culturable components of bioaerosols modulate pulmonary immune responses and induce toxic and hypersensitivity diseases (9). The main sites of bioaerosol exposure, such as the airways, employ a number of mechanisms to insure proper control of the inflammatory response. The absence of immunopathological responses to bioaerosols results from either ignorance or tolerance. In the first case, agents are neutralized and processed at the level of the epithelial barrier and/or ignored by the acquired arm of the immune response. Tolerance, on the other hand, results from a number of active processes, including antigen presentation and generation of a tolerogenic lymphocytic response leading to downregulation of inflammation in response to a specific agent. If lungs are exposed to agents that do not induce ignorance or tolerance, this can lead to inflammation, which can be hazardous to respiratory health. Non-culturable components of bioaerosols can induce immunopathological responses, and this section will focus on the cellular responses that are triggered by classical and novel non-culturable agents.

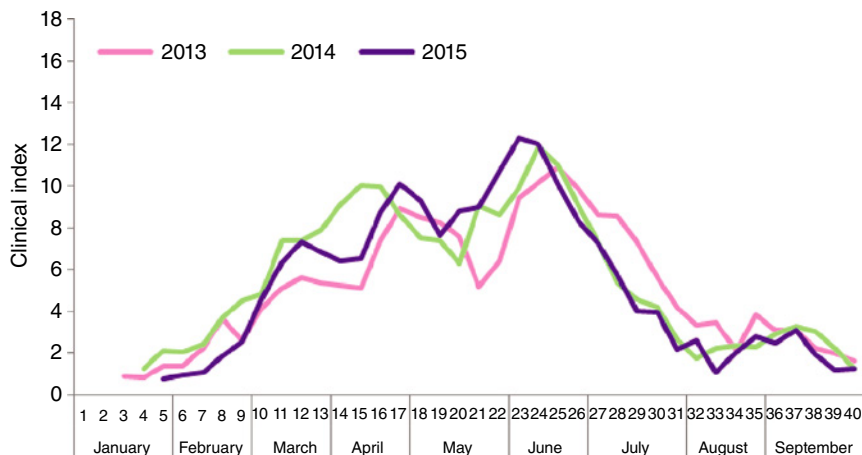
##### 4.1.4.2 Airborne agents responsible for immunogenic responses

The non-culturable fraction of bioaerosols can *per se* induce immunopathology. The most documented non-culturable agents causing an immune response are perhaps endotoxins (25–27), which induce a strong acute inflammatory response or act as adjuvants in the context of hypersensitivity reactions such as allergies. An abundant literature also supports that other subcellular components, including other sugars (10), nucleic acids (28), proteins, and lipids (29), can trigger pulmonary immune responses. Accordingly, these immunogenic factors were termed microbe-associated molecular patterns (MAMPs), which are part of the broad family of “danger signals.” Numerous

MAMP receptors (pattern recognition receptors (PRRs)) have been cloned and characterized (30). Upon MAMP exposure, signaling cascades lead to the secretion of proinflammatory mediators by “sentinel cells,” which trigger the recruitment of leukocytes that cause an immunopathological response involving tissue damage and/or impairment of organ function. It is paramount that the MAMP paradigm does not fulfill the reality of bioaerosol exposure since they encompass non-culturable organic components that are of both microbial and non-microbial origins, with immunogenic mechanisms that are, at least partially, independent of PRR activation. For instance, antigen-enriched proteolytic enzymes are associated with inflammatory/allergic reactions (31, 32). Also, newly discovered airborne non-culturable agents and their immunomodulatory mechanisms have yet to be defined. For example, archaeal species or their subcomponents were shown to modulate mucosal immunity (33). Indeed, purified lipids from archaea species can act as a potent adjuvant for liposome-delivered antigens (34). Importantly, archaeal species sampled in the air of a number of working environments (35–38) induce different patterns of immune responses ranging from asthma-like to hypersensitivity-like pneumonitis, depending on the archaea species or the size of the inoculum (33). Interestingly, differential immunogenic properties of these new agents might rely on their ability to sustain a strong antigen-presenting myeloid dendritic cell response. Moreover, metals found in bioaerosols can trigger toxic cellular responses (39) and compromise pulmonary immune responses and render the airways more susceptible to infections agents (40). Thus, a number of complex or yet to be defined mechanisms are triggered to induce immune responses following bioaerosol exposure, even though these bioaerosols are of a non-culturable nature.

#### 4.1.4.3 Pollen grain and fungal spore surveillance

With real seasonality (spring and summer for pollen and summer and autumn for mold spores), pollen grains and spores measuring from a few micrometers to 100  $\mu\text{m}$  are at very important concentrations in bioaerosols. The Réseau National de Surveillance Aérobiologique (RNSA) at the French level and the European Aerobiology Society at the European level measure the qualitative and quantitative amounts of these airborne particles during the whole season. A very large part of these particles can induce symptoms of allergy such as rhinitis, conjunctivitis, cough, as well as asthma. The main allergenic pollens are Cupressaceae, *Betula*, Poaceae, Oleaceae, and *Ambrosia*. The main allergenic spores are *Alternaria*, *Cladosporium*, and some Aspergillaceae. The analysis of these airborne biological particles is carried out using a Hirst sampler combined with optical microscopy. The sampling and analysis follow the new technical sheet number 16868 of the European Committee for Standardization. Every week, RNSA produces forecasted information relating to the RAEP (allergic risk due to pollen exposure) for the upcoming week. The information about the airborne pollen content, obtained by the analysis of samples collected by pollen traps, is not sufficient to determine alone the allergic risk. RNSA has organized a clinician network (specialists and non-specialists) in the main areas of France. The clinical data allow us to follow the evolution and the intensity of allergic symptoms (conjunctivitis, rhinitis, cough, asthma, cutaneous signs). This information is sent to RNSA through an electronic clinical report, indicating the evolution of symptoms but also the variations in their intensity. By assigning coefficients to each of these symptoms, it is possible to establish a clinical index (Figure 4.1.1) that is correlated



**Figure 4.1.1** Weekly evolution of the clinical index in France in three successive years, on a scale of 0–18. (See color plate section for the color representation of this figure.)

with the evolution of the pollen index. In addition to meteorological and phenological factors, these indices are a precious source of information for the RAEP and show how much clinical data are linked with airborne agents. All these data allow a real forecast to be established of the pollen-related allergic risk. Given the fact that the various pollens and fungal spores present a very unequal allergenic potency, it is not possible to set a universal threshold above which exposure would represent a risk. Such a threshold is specific to each species; for instance, it is very low for *Ambrosia* pollen, intermediate for Poaceae pollen and rather high for *Betula* pollen.

#### 4.1.4.4 Diseases associated with non-infectious culturable and non-culturable fractions

Non-infectious agents are usually associated with toxic or hypersensitivity conditions. They can cause a wide range of diseases, such as asthma, chronic bronchitis, HP, and ODS (Table 4.1.2). The impacts of non-culturable bioaerosol components on respiratory diseases have been reviewed by Blais Lecours et al. (9). This section overviews the major bioaerosol-related diseases. Exhaustive lists of diseases caused by non-infectious bioaerosol agents were reviewed by Douwes et al. (41) and Hauswirth and Sundry (3).

Asthma is a complex syndrome deemed to result from the interplay between genetic predisposition and environmental factors, including early age exposure to respiratory virus. It is generally associated with atopy and is most of the time characterized by a type 2 T-helper ( $T_H2$ ) cell immune response where eosinophils are the main granulocytic cell subset found in the airways. Occupational asthma is considered to be the main contributor to adult-onset asthma (42, 43) and hundreds of agents were hypothesized to cause and/or exacerbate asthma (44). While the archetypal environmental agents associated with work-related asthma are isocyanates (45), emerging literature supports that biological agents *per se* could be sufficient to enhance asthma in working environments. One hypothesis gaining interest is that protease activity appears to be linked to the immunogenicity of a number of bioaerosols including house dust mite (46, 47),

**Table 4.1.2** Etiology, symptoms, and immunopathologies of bioaerosol-related diseases. (Adapted from Blais Lecours (78) and May et al. (10).)

Bioaerosol component	Respiratory disease	Symptoms	Immunopathologies	References
<i>Saccharopolyspora rectivirgula</i>	Farmer's lung (HP)	Fever, malaise, nausea, chest tightness, headache	Lymphocytosis, granuloma, lung fibrosis, high IgG titers	10
Endotoxins, muramic acid, ammonia, hydrogen sulfide, house dust mite	Chronic bronchitis	Sputum-producing cough (for at least 3 months)	Neutrophilia (epithelium surface), tissue monocytes and lymphocytosis	10, 79
Endotoxins, mold spores, mycotoxins	ODTS	Fever, malaise, dyspnea, chest tightness, headache	Neutrophilia, IL-1, IL-6, IL-8, TNF cytokine production	3, 80–82
Allergens, endotoxins, peptidoglycan, bacterial DNA	Asthma, asthma-like syndrome	Cough, chest tightness, dyspnea, wheezing	IgE (allergic asthma), eosinophilia, chronic inflammation, bronchial hyperreactivity, tissue remodeling	10, 83, 84
Allergens, endotoxins, peptidoglycan, bacterial DNA	Allergic rhinitis	Congestion, rhinorrhea, sneeze, pruritus, nasal mucous membrane inflammation	Neutrophilia, IL-8, IL-6	10, 83
Mold spores	Sick building syndrome	Congestion, pruritus, dry throat, fatigue, headache	Unknown	16, 41

HP, hypersensitivity pneumonitis; Ig, immunoglobulin; IL, interleukin; ODTS, organic dust toxic syndrome; TNF, tumor necrosis factor.

cockroach, salmon, and crab (48), to name but a few. A number of mechanisms have been proposed to explain this apparent correlation, including modulation of protease-activated receptors leading to the release of proinflammatory mediators (49–51); disruption of intercellular epithelial junctions leading to altered/enhanced penetration of foreign particles in subepithelial layers where professional antigen-presenting cells (dendritic cells) are located (52), which would facilitate the unfolding of a  $T_H2$ -polarized acquired immune response; and cleavage of immunomodulatory receptors on leukocytes (53). While the presence of adjuvant molecules in bioaerosols in the immediate environment is still considered a main contributor to asthma (54, 55), emerging literature supports that growing up within environments with higher bioaerosol burdens could exert protective effects on the later asthma outcome (56). Thus, while early age exposure to environments rich in bioaerosols would confer protection against asthma, late exposure to environments contaminated with enriched bioaerosol components and with adjuvant molecules would rather enhance its burden.

HP occurs in response to repeated airway exposure to numerous classes of agents, including bacteria, fungi, and proteins derived from animals or insects (57). In its most severe form, as opposed to asthmogenic stimuli, the antigens responsible for HP mainly induce  $T_H1$ - and  $T_H17$ -polarized immune responses. Accordingly, patients with HP present with various degrees of lymphocytic and neutrophilic infiltrates in the lung and in bronchoalveolar lavages. Exposure to these antigens results in disease progression in a fraction of the exposed population and the etiology of this syndrome has been described in detail elsewhere (57, 58). In some patients, the disease is fully reversible upon antigen avoidance, while in others it could progress toward fibrosis and to a less than 5 year survival prognosis. While HP has been associated with farming activities, current trends in lifestyle and use of technology present emerging environments favorable to HP. For example, indoor compost plants show recrudescence of *Saccharopolyspora rectivirgula* (59), the causal agent of “farmer’s lung,” and HP cases have emerged in contemporary settings including in hot-tub users (60) and metal-working plant workers (61). Thus, HP-causing antigens found in bioaerosols are still sampled in numerous and changing environments and remain a significant threat to human health.

A newly described disease entity called mucous membrane irritation syndrome (MMIS) is a non-allergic inflammatory reaction that can be present as cough, phlegm, rhinitis, or conjunctivitis. Symptoms of MMIS can represent asthma, chronic bronchitis, or chronic obstructive pulmonary disease and are induced by exposure to non-infectious bioaerosols (62, 63). There is inherent difficulty in understanding the precise role of each bioaerosol component in the development of these respiratory and systemic problems since their prevalence is relatively low and the bioaerosol content is complex.

## 4.1.5 Biological agents used for bioterrorism

### 4.1.5.1 Bioterrorism

Bioterrorism could be simply defined as the deliberate spread of microorganisms in order to cause diseases or mortality. More broadly, bioterrorism is the use of or threat to use microorganisms such as viruses, bacteria, fungi, or toxins in order to destroy human life, animals, or plants. This type of event can occur insidiously. Although there are

several possibilities in the case of a bioterrorist attack, it is believed that the agents would be preferentially released into the air as bioaerosols, which are invisible, silent, odorless, tasteless, and thus difficult to detect quickly (64). A terrorist attack using aerosolized biological agents could happen without warning, and the first indication of such an attack would be hundreds or even thousands of sick or dying individuals. Advanced biological weapons from recent advances in biotechnology are even more threatening. For example, it is possible to make antibiotic-resistant bacteria or introduce genes of additional virulence (65). Currently, we are rather poorly equipped to rapidly detect such agents potentially modified by genetic engineering bioterrorism.

To detect drastic changes in normal outdoor background microflora, more information on the normal range and variability of the types and concentrations of bacteria is required. Ambient air contains naturally occurring microorganisms including bacteria, molds, fungi, viruses, and eukaryotic cells (animal, vegetal, and unicellular organisms) as well as numerous organic and inorganic particles to which humans are exposed. Other factors such as the presence of various contamination sources and the distance from them, relative humidity, wind, and human activities also play a role in the dispersion of contaminants and may influence the outdoor airborne microbial burden. The normal microbial content of outdoor air also varies in nature and concentration depending on the time of the day, the temperature, and other factors (66). A so-called background range, within which variations in microbial content would be considered normal, may be established in a given geographical area. It is important to be able to detect drastic changes in outdoor bacterial levels with respect to the normal background range and to detect the introduction of new sources of microorganisms, especially pathogens, quickly, efficiently, and in real time in order to react in a timely fashion to a potential threat to human health. The deliberate introduction into the air of pathogens represents a real threat that the modern world is now facing. This reality is at the heart of all the destructive potential that can represent bioterrorism.

#### 4.1.5.2 Classification of bioterrorism agents

More than 250 pathogens have been reported as potential agents for bioterrorism (64–67). In the USA, the Centers for Disease Control and Prevention categorizes bioterrorism agents into three distinct classes A, B, and C. Table 4.1.3 summarizes the main potential agents. Class A includes the most dangerous organisms, easily disseminated or transmitted from person to person, which could result in a very high mortality rate in the exposed population. Class B represents the second priority agents, moderately difficult to spread, resulting in moderate to low mortality. Class C includes the third priority agents and emerging pathogens. One of the most well-known agents but also one of the most feared is *Bacillus anthracis*, the etiological agent of anthrax. Because of its spore formation and resistance to stresses, this agent is at high risk of being spread by the airborne route. The following sections present the three most feared bioterrorism agents: *Bacillus anthracis*, *Francisella tularensis*, and botulinum toxin.

##### ***Bacillus anthracis***

*Bacillus anthracis* has been a potential biological weapon since the end of World War II and was particularly hyped in the wake of the September 2001 attacks in the USA (68). *B. anthracis* is a spore-forming Gram-positive bacterium present among others in

**Table 4.1.3** Most important potential bioterrorism agents.

Class	Disease	Causal biological agent
A	Anthrax	<i>Bacillus anthracis</i>
	Botulism	<i>Clostridium botulinum</i> toxin
	Plague	<i>Yersinia pestis</i>
	Small pox	Variola virus
	Tularemia	<i>Francisella tularensis</i>
	Hemorrhagic fever	Ebola virus
B	Brucellosis	<i>Brucella</i> sp.
	Psittacosis	<i>Chlamydia psittaci</i>
	Q fever	<i>Coxiella burnetii</i>
	Enterotoxin B	<i>Staphylococcus aureus</i>
	Typhus	<i>Rickettsia prowazekii</i>
	Epsilon toxin	<i>Clostridium perfringens</i>
	Ricin toxin	<i>Ricinus communis</i>
	Viral encephalitis	Alphaviruses
	Water safety menace	<i>Vibrio cholerae</i> , <i>Cryptosporidium parvum</i>
	Food safety menace	<i>Salmonella</i> sp., <i>Escherichia coli</i> O157:H7, <i>Shigella</i> sp.
C	Emerging infectious diseases	Nipah virus, hantavirus

the soil. The bacterium is non-motile and facultatively aerobic. *B. anthracis* is genetically very similar to *Bacillus cereus* and *Bacillus thuringiensis*. The phenotypic differences between *B. cereus*, *B. thuringiensis*, and *B. anthracis* are mainly due to the presence of different plasmid genes. *B. anthracis* possesses different virulence factors encoded on two plasmids (pX01, pX02): a capsule of poly-D-glutamic acid allowing it to escape phagocytosis and two toxins (edema factor toxin and lethal toxin).

*B. anthracis* spores are produced when bacteria encounter unfavorable conditions for growth and are a means for bacteria to become dormant until the return of favorable growing conditions. They are highly resistant (drying, heat, disinfectants, gamma rays, ultraviolet rays) and can survive for decades in the soil. The spores do not need a source of C or energy to survive and persist in the environment. Endospores are first formed within vegetative cells (one spore in one vegetative cell); once mature, they are released and called spores.

Anthrax is a zoonotic disease and especially develops in herbivores (e.g., sheep, goats, horses). There are three forms of anthrax: cutaneous, intestinal, and inhalational. Once introduced into the human body by abrasion, inhalation, or ingestion, the *B. anthracis* spores are phagocytized by local macrophages that transport them to the nearest lymph node. Spores germinate into vegetative cells within macrophages; bacteria are released from the macrophages and multiply in the lymphatic system. They can then enter the bloodstream and then sepsis occurs. Cutaneous anthrax is rare in animals, but it is the most common form in humans (95% of infections). Skin infection is due to the entry of *B. anthracis* spores in a wound. *B. anthracis* is usually transmitted through close contact

with an infected animal or carcass. A macula, then ulcerations surrounded by vesicles develop at the site of inoculation and spore germination. The button becomes dry and then covered with a black coal-like crust. Localized disease is curable (less than 1% mortality). However, if it is not recognized, spread through the lymphatic system may cause fatal septicemia. Gastrointestinal anthrax is rare and is caused by the consumption of meat containing *B. anthracis* spores. Herbivorous animals become infected mainly by ingestion. Carnivores are relatively resistant against it as they have a higher gastric acidity. In humans, the digestive gateway only exists in countries with poor hygiene. Gastrointestinal anthrax is associated with the development of an oral or esophageal ulcer and regional lymphadenopathy. If there is infection of the lower digestive system, nausea and vomiting are quickly followed by bloody diarrhea, perforation of the intestine, and septicemia.

Inhalational anthrax is from inhalation of aerosolized spores and causes the highest mortality rates. Inhalation is followed by a few specific flu-like symptoms with fever, muscle aches, headache, and dry cough pain. Severe respiratory failure, acute chest pain, and hypotension are observed after 2–4 days. Hemorrhagic meningitis or septicemia anthrax are possible complications due to the input of spore masses ( $<4\ \mu\text{m}$ ) or single spores (about  $1\ \mu\text{m}$ ) phagocytized by alveolar macrophages in the blood or lymphatic circulation. In humans, pulmonary anthrax represents only 5% of cases. The mortality rate is estimated at 90–100%; however, this decreases to less than 50% in the forms related to bioterrorism. For example, the accidental release of anthrax spores following the explosion of a military complex in the city of Sverdlovsk (Russia) was lethal to 86% of patients (68 out of 79 cases). The cloud of spores contaminated people up to 4 km away. The minimum infectious dose to humans is not well known. It was estimated at 2500–55 000 viable spores, although it can be significantly lower for some people.

*B. anthracis* is sensitive to various antibiotics (e.g., penicillin, macrolides, tetracycline). In Canada, the USA, and most European countries, the use of ciprofloxacin or doxycycline is recommended whenever there is suspicion of anthrax. The strain of *B. anthracis* used in contaminated letter attacks following the September 11, 2001, attack was sensitive to the antibiotics prescribed. Antibiotics are effective only against the vegetative form of *B. anthracis* and, given the possibility of late germination of certain *B. anthracis* spores, antibiotics are taken for 60 days. There is a vaccine but it is not available to the general public (“anthrax vaccine adsorbed”). People most at risk for anthrax are among breeders of affected animals, veterinarians, and persons handling animal products often imported from countries where animal charcoal is widespread (e.g., weavers wool and goat hair).

It was estimated that the release of 50 kg of *B. anthracis* spores on an urban population of 5 million people could infect up to 250 000 people and kill 100 000 (68). *B. anthracis* spores are highly resistant to various environmental stresses. Indeed, spores of *Bacillus globigii* (*B. anthracis* surrogate) aerosolized from a flight from the US Army Base near Johnston Atoll in the South Pacific were found to be viable up to 60 miles from the original place of aerosolization (65). So, once in the atmosphere, the spores are a danger, even remotely, because they can be transported by wind away from their place of aerosolization. During World War II, the British conducted tests on the aerosolization of anthrax spores on the Scottish island of Gruinard (69). Sheep were used as a model to assess the effect of exploding bombs composed of anthrax spores. All sheep on the island died within 3 days. Spores persisted and remained viable for 36 years and the

island was decontaminated with 280 tons of formaldehyde between 1979 and 1987. The island is now considered completely decontaminated. These examples clearly show the pathogenicity, persistence, and spread of aerosolized *B. anthracis* spores.

### Botulism

*Clostridium botulinum* is a Gram-positive rod that is motile, strictly anaerobic, and spore-producing. The bacterium is widespread in soil and sometimes present in the intestines of certain animals (e.g., pig, fish). Other forms are foodborne botulism (ingestion of botulinum toxin, from canned ham, or inadequately sterilized food in which the bacterium has developed and released the toxin), infant botulism (ingestion of *C. botulinum* spores in infants), and skin botulism. Different forms of botulism are associated with the entry of botulinum toxin into the bloodstream from the intestines or injury. *C. botulinum* toxin cannot penetrate intact skin. Skin and infant botulism are due to the growth of *C. botulinum* bacteria in devitalized tissue injury (anaerobic) or in the intestines. Inhalational botulism caused by inhalation of the toxin is primarily a form of botulism created by humans and is manifested following the entry of botulinum toxin into the blood from the lungs. This is the most powerful known poison (40 million times more than cyanide). The toxin is resistant to acids and digestive juices, but sensitive to heat (heat labile). Botulism is not contagious. All forms of botulism are associated with neurological symptoms. Following incubation for 12–72 h a flaccid paralysis emerges that is more or less severe depending on the dose and the nature of the toxin (70). Patients may have blurred vision, swallowing and speech difficulties, and even paralysis of the respiratory system. Death is then avoided by mechanically ventilating patients for weeks and even months. A passive vaccine, an equine antitoxin (antibodies produced in horses and neutralizing toxins A–G), is administered to severely ill patients. The lethal dose of toxin A for a 70 kg human is approximately 0.09–0.15 µg by the intravenous or intramuscular routes or 0.70–0.90 µg by the inhalation and ingestion routes.

### Tularemia

*Francisella tularensis* is responsible for tularemia. Like anthrax, the disease has taken on greater significance since the events of September and October 2001 in the USA. *F. tularensis* is a very small Gram-negative rod (0.3–0.6 µm), which is non-motile and aerobic. The bacterium is virulent when it has a capsule. The lipids of the cell wall and those of the capsule are in unusual proportions (70% and 50%, respectively) for a Gram-negative bacterium. It is resistant to various environmental stresses and survives several weeks at low temperatures in water, moist soil, hay, straw, and animal carcasses. *F. tularensis* is found mainly in lagomorphs (e.g., hares, rabbits) and rodents (voles, squirrels, beaver, muskrat, lemming, coon, mice, guinea pigs) and regularly strikes hunters who catch it through injury when preparing hunted animals. *F. tularensis* can be present in water, soil, and vegetation.

There are various routes of infection: ulceroglandular (the most common type representing 75% of all forms), glandular, oropharyngeal, pneumonic, oculoglandular, and typhoidal (71). Symptoms of tularemia differ depending on the dose and virulence of the agent. A lesion forms at the site of inoculation of the agent (e.g., scratch or an insect bite) and causes ulceration. From the lesion, the bacterium accesses a lymph node and multiplies in it. Pneumonic tularemia is rare but is to be feared in a bioterrorist attack and the symptoms are high fever, asthenia, chills, headache, malaise, dyspnea, and chest pain.

The incubation period is 3–5 days and convalescence stretches over several weeks or even several months. Inhalation of as few as 10–20 bacteria is required to cause the disease in humans. In 1969, an expert committee of the World Health Organization estimated that aerosolization of 50 kg of virulent *F. tularensis* over a densely populated urban area (5 million) could be responsible for 19 000 deaths and 250 000 cases of infection (72).

#### 4.1.5.3 Point detection of biological agents and exposure limit values of bioaerosols

The point measurement and detection of biological aerosols in real time, such as Fluorescence Aerodynamic Particle Sizer (FLAPS) and Light Detection and Ranging (LIDAR) technologies, has been extensively studied by Ho et al. (73–75) and is reviewed in Chapter 1.4. Most of the automated detection technologies were developed to be applied on *B. anthracis* spore detection, because of the high probability of this agent being used as a bioaerosol and the intrinsic fluorescence of the bacterial spores. Aerosolized *B. anthracis* spores are odorless, invisible, and can cross several kilometers before dissipating (76).

Because they are a threat to human health in various occupational settings, bioaerosols, and more specifically several bioaerosol-related agents, need exposure limit values (ELVs). Unfortunately, because, among other things, of the non-specific nature of several bioaerosol-related diseases and the wide variety of etiologic agents, only a few ELVs have been established. Eduard et al. (29) listed those established in the USA and Norway. Walser et al. (77) targeted facilities where ELVs are needed and identified several exposure concentration limits and indicator parameters that could be used for ELV determination.

#### 4.1.6 Conclusion

The impact of agents found in bioaerosols on human health is increasingly recognized. Several agents, when aerosolized, have been designated as hazardous in the bioterrorism issue. Environments highly contaminated with bioaerosols show increased prevalence of diseases that are not infectious in nature. By diverse mechanisms, exposure to several components of bioaerosols causes diseases that relate to hypersensitivity or toxic reactions. Specific agents have been associated with specific health conditions, but it remains difficult to prevent bioaerosol-related illnesses/diseases given our partial knowledge of bioaerosol components and since we do not fully understand how the interplay between these components influences their pathogenic potential. As a result, and because the development of diseases involves a complex relationship between the environment and the host, it is difficult to develop reliable exposure values.

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## 4.2

# Impacts of Microbial Aerosols on Natural and Agro-ecosystems: Immigration, Invasions, and their Consequences

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### 4.2.1 Introduction

The previous chapters and the literature cited therein clearly illustrate that a wide range of microorganisms voyage extensively in the atmosphere. Their deposition with rainfall or settling out in dry air serve as their passage as immigrants to the full range of the habitats—more or less hospitable—on Earth's surface. Although certain publications incite fear because of the presence of animal or plant pathogens from far-off places among the microorganisms transported by the atmosphere, their survival and multiplication upon arrival in new habitats are far from certain.

The invasion of a habitat by a microorganism that has survived long-distance aerial dissemination seems like an oxymoron. First, aerial transport and subsequent deposition can deliver microorganisms to any type of surface that is exposed to the air, from pavement to boiling mud pools to salt licks and cow pies. The rate of death due to incompatibility with the new environment is likely to be high. Second, survival of the environmental conditions that accompany such a voyage often requires a physiological state that is near or *bona fide* dormancy. Capacity for rapid growth in reaction to a sudden increase in the abundance of exploitable resources is generally not a trait associated with such dormancy. Successful invasion of microorganisms immigrating via the atmosphere occurs under rather special conditions characterized by a microbiological void in the new habitat, access to a highly specialized niche, or the capacity of the disseminating particle to avoid the dormant-like state through association with a protective particle during transport. Here we will give examples of cases where microorganisms that have been transported via the atmosphere successfully establish in, and sometimes invade, a new habitat and we will describe the consequences for ecosystem functions and microbial evolution.

## 4.2.2 Colonization of virgin and extreme habitats

### 4.2.2.1 The emergence of terrestrial eukaryotes

In terms of terrestrial habitats, what could be more virgin territory than volcanic rock freshly cooled from a spew of Earth's molten magma or the barren rock that recently pierced into the troposphere from a submarine abyss after the collision of tectonic plates. Such places, for which the word habitat seems rather inappropriate, are usually devoid of soil, and particularly of nitrogen (N) and carbon (C) sources. N and C eventually enter these systems mostly through biological processes (N fixation and photosynthesis) or via wet or dry deposition from the atmosphere (1). Arguably the earliest invasion of such virgin territory by aurally disseminated microorganisms was the colonization of land masses that emerged with the collision of continents and the drop of the sea level that started in the mid- to late Ordovician period (ca. 450 Mya). These events led to the ascent of land plants. But the earliest invaders were hardly plants as we know them today. They were associations of microorganisms where fungi played critical roles as "roots" to conquer—by dissolution—the otherwise inert mineral substrates.

There is evidence that colonization of land by fungi predated that of primitive land plants by about 200 Mya (2). Studies of endolithic microetching in rocks has also revealed the presence of algae in rocks at about the same time as fungi in Earth's evolutionary history and that this was preceded by another 200 Mya by cyanobacteria and archaea in rocks (3). All these organisms have the capacity to dissolve rocks, but fungi are very well characterized for their ability to weather rocks (described in Section 4.2.2.3) via a range of acids they can excrete. This liberates minerals that are a source of essential elements for most organisms. The earliest colonizers of Earth's surface were most likely associations of the phototrophs and fungi adept at eking out a living in this oligotrophic environment (4). Lichens are one evolutionary consequence of these associations. Likewise, it is particularly interesting to note that almost all modern phototrophs adapted to terrestrial ecosystems have mutualistic associations with fungi in the form of either endophytes or as mycorrhizae as a vestige of the ancient origin of this association (5).

The earliest terrestrial colonists probably came from the cyanobacterial mats, stromatolites, and biotic crusts that established in subaerial settings well before the colonization of continents by land plants. This could have occurred as early as 1.43 Gya, a date that corresponds to some of the earliest fossil evidence for the existence of eukaryotic fungal-like organisms (6). But in the Precambrian period, microbial life forms were probably confined to shorelines (7), leaving much of the rest of the emerged rocks barren. Aerial dissemination of bits of these life forms assured further conquest of continental land masses. Subsequent colonization of land by fungi and plants led to the evolution of the multitude of processes (from primary production to degradation) that bacterial–fungal–plant complexes achieve today.

### 4.2.2.2 Modern rebirth of pristine land: colonization in the wake of volcanic eruptions

New pristine land can emerge in modern times from volcanic eruptions in the form of substrates such as lava rock, tephra, and volcanic ash deposits. The colonization of these substrates has been assessed mostly for plants, and in some cases for microorganisms for

sites where volcanic eruption is recurring, such as in Hawaii, USA, and Japan, or where it is a rare occurrence, such as the major eruption in 1980 of Mount St. Helens in the state of Washington in the USA. The new terrestrial substrates resulting from these eruptions are highly oligotrophic with total N and organic C contents each less than about 0.01% (1). These rocks are used as substrates for growth by chemolithotrophs and autotrophs that can grow in the presence of hydrogen and CO<sub>2</sub>.

Ammonia-oxidizing bacteria are among the early microbial colonizers of the volcanic ash deposited in the wake of volcanic eruptions. Their capacity to oxidize ammonia gives them a particular advantage in the absence of nitrate or nitrite that characterizes these habitats and also starts the successional process leading to the formation of soils. In the areas of the Llaima Volcano in Chile, for example, that were recolonized after lava eruptions in 1640, 1751, and 1957, ammonia-oxidizing bacteria and archaea were found in acidic volcanic soils with the archaea being the most abundant (8). The surprising diversity of the bacteria observed at this site indicated that the pioneer species were not clonal. The authors of this work did not investigate the origin of the ammonia oxidizers, but the arrival of immigrants via the atmosphere would be compatible with the non-clonal profile of the invaders. Interestingly, in a study of archaea in the atmosphere, the majority (86%) were from the Thaumarchaeota phylum (9). All organisms of this phylum identified to date are chemolithoautotrophic ammonia oxidizers, suggesting that there is a pool of immigrants in the atmosphere to colonize these oligotrophic environments.

Ash produced in many volcanic eruptions was originally magma and therefore is effectively sterile at the time of its ejection from the volcano. However, some ash arises from abrasion of particles during an explosion and might not have been subjected to heat as intense as in the former case. Nevertheless, during an eruption ash is projected into the atmosphere up to tens of kilometers and can travel hundreds of kilometers before settling (10). It is likely that ash particles scrub the atmosphere of the smaller particles they encounter, including microorganisms. In a study of the microbiology of volcanic ash on Miyake-Jima Island, Japan, it was noted that bacterial population densities of freshly fallen ash from an explosion in 2000 were about 10<sup>7</sup> colony-forming units (CFU) g<sup>-1</sup> dry "soil"—the same density as observed in the soil that was forming from ash from an explosion 22 years earlier (1). Therefore, for new habitats formed by volcanic ash, the early microbial colonizers from atmospheric microflora might be captured by the ash when airborne and as it forms the nascent habitat rather than simply being deposited from the atmosphere independent of the ash. The microbiology of cinder cones that arose in December 1967 in the sunken caldera of Port Foster, Deception Island in Antarctica as the result of volcanic eruptions was studied 1 year after they emerged (11). The authors remarked that the site was not pristine from the point of view of microbiology because of the considerable activity of human and animals close by. They noted that potential sources of microorganisms for colonization of the cinder cones included wreckage from ships, rotting food, bedding, whale skeletons, wood, tar, and coal. Nevertheless, for some samples of "soil" from the cones no culturable microorganisms could be detected. When present, microbial species consisted mainly of corynebacteria, *Bacillus* spp., *Penicillium*, and the algae *Chlorococcum humicola*, illustrating that capacity for invasion of such habitats is likely confined to certain species in spite of the capacity to immigrate to the habitat. The important role of the selective pressure exerted by the habitat on immigrants was also illustrated in a study of lands exposed by retreating glaciers. In a study of such newly exposed soil, the microorganisms arriving via the atmosphere were not part

of the inoculum sources that had the most influence in shaping the microbial populations that installed in these soils. A more influential source was the set of microorganisms associated with the debris deposited by the glacier as it melted (12). Therefore, although transfer of microorganisms via the atmosphere between diverse and distant ecosystems is very plausible (13), the impact of newly arriving strains will depend on their capacity to adapt to and invade their new habitat.

#### 4.2.2.3 The conquest of rocks: weathering and the liberation of mineral nutrients

Lichens can uncontestedly travel long distances via the atmosphere, as illustrated by the lush growth of widely distributed species on isolated islands and archipelagoes as summarized by Printzen (14). Although there is debate about the timescale for long-distance dissemination and the full range of distance at which this can occur (14), the diaspores of lichens have aerodynamic properties that allow them to travel via the air with greater efficiency than propagules of vascular plants. Therefore, they can readily be disseminated to extreme habitats such as the polar regions where they can constitute the pioneer species on rocks.

A key trait in the success of certain fungi, lichens, and other microorganisms in colonizing rocks is their ability to degrade inert minerals through chemical processes. This is referred to as weathering and is the primary source of all essential elements, except for C and N, for the growth of organisms (4). Weathering can also occur from mechanical processes and in particular mechanical disruption due to hyphal penetration, the swelling action of salts from fungal activity, and freeze–thaw cycles of water that seeps into cracks made by this disruption. Lichens, in particular, are known for these effects on rocks via their endolithic—as well as epilithic—phases (15). Weathering by lichens can contribute to karstic features of limestone through the creation of microscopic pits and troughs that become mesoscopic features ((16) and references therein). Such weathering can also lead to flaking of sandstone such as that observed for Beacon sandstone in Antarctica (17) and the exfoliation of South African sandstone by cyanobacteria (18) among many other examples. Traces of the capacity of lichens to degrade minerals are found in their thalli where fragments of minerals, including quartz, feldspar, and mica, can accumulate (15). The degradation of rocks by lichens opens interesting questions about the origin of ice nucleation activity in lichens (19–21) and the selective pressure that maintains this trait in this group of organisms. It has been suggested that this activity provides a fitness advantage by helping the lichens sequester water (21). However, there is no direct proof of this adaptive advantage of ice nucleation activity for lichens. Interestingly, the bacterial microflora of lichens is dominated by Alphaproteobacteria (22), bacteria known for their resistance to severe stress such as desiccation—more so than the Beta- and Gammaproteobacteria that are less tolerant of water stress and more common on higher plants. This suggests that the surface of lichens is an arid microclimate that is not hydrated via ice nucleation. In light of the importance of freeze–thaw cycles in the degradation of rocks, it seems more likely that ice nucleation activity would provide a means of reinforcing this process, thereby enhancing the capacity of lichens to break rocks and give them access to minerals. The accumulation of certain minerals in the lichen thallus, which are themselves ice nucleation active such as feldspar (23), could contribute to the ice nucleation activity.

#### 4.2.2.4 Colonization of sculpted and painted rocks: deterioration of cultural heritage

In addition to weathering of rock surfaces, microorganisms also are responsible for patinas and other types of colorings or “discolorations” of rocks. Cases of stone deterioration due to aerially disseminated microorganisms are critically important as summarized in relatively recent, extensive overviews of this problem (24, 25). Buildings, monuments, temples, tombstones, and works of art are generally made of stone that can be engraved or sculpted by artisans and often is composed of marble, limestone, sandstone, or granite—all of which are known to be substrates for microbial growth. Surfaces of these structures are exposed not only to microorganisms but also to the other types of particles in the atmosphere, including pollutants and organic dusts. These latter can serve as growth substrates or influence the chemistry of the environment being colonized by microorganisms. The initial invaders of the stone surfaces are generally photosynthetic microorganisms (algae and cyanobacteria), but eventually heterotrophic microorganisms can invade the surface. There are also endolithic colonizers of the stone, but they are very distinct from the surface colonizers, indicating that the processes of contamination of the stone and the dynamics of their growth are independent from those of the surface colonizers.

One of the most well-known and hotly debated cases of discoloration of rock surfaces is that of the 18 000-year-old rock paintings in the Lascaux Caves in France. About 20 years after the opening of the caves to the public, the paintings became noticeably degraded by microbial growth, leading to various changes in the dominant colors of the paintings. The microorganisms implicated were first photosynthetic microalgae that produced green biofilms on the rocks. After efforts at disinfection, the algae were replaced by various other microorganisms including *Fusarium* and *Pseudomonas* spp. However, the opening of the cave to visitors was not the source of contaminants that could have arrived via the atmosphere from outside or from the cloud of aerosols carried by the human visitors. Rather, the opening of the caves caused changes to the microclimate (humidity and light, in particular) that favored the development of microorganisms indigenous to the cave. Their spread seems to have been assured by arthropods living in the cave (26). Hence, this famous case of rock-surface deterioration is not strictly linked to atmospheric dispersal, although it illustrates very well the serious deleterious effect that microorganisms can have on seemingly inert surfaces and the difficulty of eliminating these microorganisms.

#### 4.2.2.5 High-altitude/latitude environments

The importance of aerially disseminated particles in the ecology of high mountain lakes has received considerable attention because these lakes are usually oligotrophic and receive few nutrients from their catchment basins that usually are sparsely vegetated and have thin soil covers. Atmospheric dust can be a sort of fertilizer for these lakes as well as a source of microorganisms. Several studies have focused on lakes above 2000 m in the Pyrenees mountains that can receive an input of Saharan dust (27, 28). Overall, the results illustrate that bacteria at the air–water interface in these lakes resemble more the bacteria of the atmosphere above the lakes than the bacteria in the lower layer of the lake, suggesting that the air is a major source of bacteria at this interface.

Furthermore, the capacity of the bacteria to colonize the lakes upon their arrival via aerial dissemination depended on their capacity for rapid growth upon arrival, a trait that typifies the Gammaproteobacteria in particular. The greater capacity of the Gamma- and Betaproteobacteria to rapidly respond to being newly introduced into an aquatic environment—more so than the Alphaproteobacteria—was also illustrated for bacteria arriving with rainfall over a high-altitude lake in Austria (29). The communities that result from these spurts of colonization can be relatively stable at the water–atmosphere interface because there are long periods of calm weather whereas in marine environments the regular wave motion and wind action do not lead to such stability. The authors of this work proposed that changes in the species composition at the air–water interface of these lakes could be indicators of the microbial species entering these high mountain areas with Saharan dust events that could be increasing in frequency with global change (28).

### 4.2.3 Invasion of agriculture

Although microorganisms emitted from cropped regions are sources of microbial aerosols for other types of habitats (13), aerial dissemination of microorganisms from one cropping region to another is frequent and can lead to significant invasions. In agricultural habitats, which are favorable to rich and diverse biological colonization, certain plant pathogens that arrive via the atmosphere can outcompete the other microorganisms that are already inhabiting plants. Higher plants have a system of so-called non-host resistance that keeps most microorganisms at bay (30), allowing for only superficial colonization of outer plant surfaces and/or endophytic populations that do not attain a density sufficient to set off symptom expression. Plant pathogens are variants of certain species of microorganisms (mostly bacteria, fungi, and viruses) that can either physically bore into plant cells (some fungi) or enter via wounds, natural openings, or with plant-feeding insects and then deploy various biochemical means to disarm plant defenses and exploit the nutritional reserves that plant tissues provide. Via these processes they have a marked competitive advantage relative to other plant-inhabiting microorganisms. Although all plants, whether cultivated or wild, can be susceptible to invasion by pathogens, plants in agricultural contexts—and in particular those in monoculture—provide expansive homogeneous swaths of habitat, thereby favoring subsequent lateral expansion of the disease foci that develop from the inoculum deposited from aerial dissemination. Furthermore land use has changed drastically over the past century. By the early part of the 20th century, anthropogenic biomes have expanded to now constitute 50% of terrestrial land cover; with crops, rangelands, and pastures constituting over 80% of anthropogenic land use (31, 32). In light of this extensive transformation of vegetated land, the limited genetic diversity of many of the major crops, and the intensive production of certain crops well outside their natural range of diversification, successful invasions by aerially disseminated plant pathogens could be expected to increase markedly.

Successful invasions have been demonstrated mostly for very host-specific pathogens and for organisms that are obligate pathogens needing specific host plants in order to survive. This does not mean that invasion cannot occur for other types of aerially disseminated plant pathogens. This apparent bias in our knowledge is due to the relative

ease in linking source and sink for host-specific or obligate pathogens compared with pathogens that are widely distributed, making it very difficult to identify source and sink populations. These invasions by plant pathogens have occurred due to long-distance dissemination between continents and across oceans and also within continents. The most remarkable invasions due to transoceanic dissemination are the devastating expansions of various rust diseases caused by *Puccinia* spp., including the spread of stem rust of wheat from Africa to Australia in 1969, of stripe rust of wheat from Australia to New Zealand in 1980, of sugar cane rust from Cameroon in West Africa to the Dominican Republic in 1978, and of coffee rust, caused by *Hemileia vastatrix*, from Angola to Brazil in 1970 (31). The important genetic homogeneity of wheat and the considerable capacity of wheat rust to disseminate via the atmosphere has led researchers to suggest that the most recent newly emerged races of stripe rust of wheat could be the eventual demise of modern lines of wheat (33). Invasion by pathogens after their initial introduction via intracontinental aerial dissemination can also lead to considerable demise of crops. Examples include the spread of mildew of potato (caused by *Phytophthora infestans*) across Europe leading to the Great Irish Famine in 1845, of Sigatoka disease (caused by *Mycosphaerella fijiensis*) across all regions where banana and plantain are grown, and of rusts of wheat from Mexico to Canada with the instauration of the Wheat Belt in North America in the early 1900s and northwestward across China from major wheat-producing regions in Gansu and Yunnan provinces (31, 33).

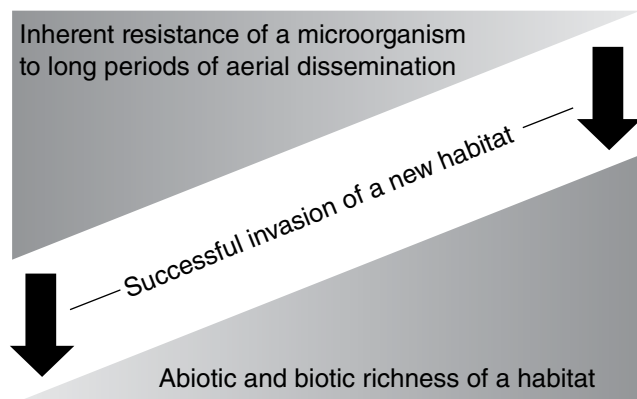
Invasion of the microbial communities associated with plants can have effects that are less drastic than inciting famines or the failure of major cash crops. Aerial dissemination is one of the four processes that contribute to the size and structure of the microbial populations on leaf surfaces. These processes are immigration, multiplication, emigration, and death and they contribute to leaf surface community structures in the same way that they contribute to the colonization of islands (see the theory of island biogeography as applied to leaves (34, 35)). Studies of leaves from the perspective of the theory of island biogeography have led to the realization that population sizes or composition on leaves can be limited by the rate of immigration. This suggests that the immigrants successfully invade upon arrival. For example, citrus is a plant species on which *Pseudomonas syringae* and bacteria in general grow poorly on the leaves. On leaves of navel oranges, epiphytic population sizes of total bacteria and ice-nucleation active bacteria were highest at the upwind edge of orchards bordering other plant species. This was also reflected in the higher rates of deposition of bacteria in these upwind sites and in the higher epiphytic populations on other plant species (36). Likewise, the abundance of *P. syringae* on leaves of various cultivars of snap bean, bred to inherently harbor low population sizes of this bacterium, could be enhanced by growing them adjacent to cultivars of bean that harbored high population densities of this bacterium, thereby serving as sources of aerial inoculum (37).

As observed for the fate of microorganisms immigrating to extreme oligotrophic habitats described above, the survival of microorganisms to the leaf surface is not necessarily assured, even in the absence of competitors. In experiments with apple leaves, the population sizes of fungi on these leaves was lower than what is predicted by immigration as a sole source of individuals for colonization (38). This suggests that death of immigrants (or their emigration) is an important factor in the fungal population dynamics on apple leaves. In particular, even in the absence of competing microorganisms, the fungus *Chaetomium globosum* has much greater difficulty in persisting after arrival by

immigration than does the yeast *Aureobasidium pullulans* (39). Although such dynamics might seem to be only of esoteric or academic importance, in fact the means by which microorganisms can invade leaf surfaces suggests the conditions that must be achieved for their successful use in biological control of plant disease (40).

#### 4.2.4 Opportunities for research

The full extent to which aerially disseminated microorganisms impact natural and agricultural habitats needs to be explored further—for a greater range of microorganism–habitat couples than the high-altitude, oligotrophic, or obligate biotrophic examples described above (Figure 4.2.1). One approach to assessing this impact has been used in studies on the origin of certain rust epidemics. This approach is based on corroborative evidence and involves demonstrating (i) the kinship of the target microorganism in the source and the sink, (ii) the presence of the microorganism in the source prior to that in the sink, and (iii) the existence of air mass movements that would assure the needed trajectory between the source and sink habitats (41). For this, the challenge will be to identify potential sources and sinks that are unique in space or time and to identify the traceable microorganisms associated with these habitats. Knowledge of changes in land use will be critical to developing such hypotheses. Such changes could involve recent



**Figure 4.2.1** The general relationship between the inherent capacity of a microorganism to survive long periods in the atmosphere and the nature of new habitats that it can successfully invade. We propose this relationship based on the diverse range of examples where aerially disseminated microorganisms have successfully invaded the new habitats they attain when deposited from the atmosphere. Survival of long-distance trajectories generally requires persistence in the atmosphere for long periods. Such survival is favored by dormancy and other mechanisms for organisms to be indifferent to various environmental parameters leading the organism to be slow in responding to the environmental parameters in the new habitat. Microorganisms with such traits would not be competitive in new habitats that are rich in microorganisms and would be better adapted to poor, oligotrophic habitats. The competitiveness needed to be invasive in rich habitats depends on rapid growth in response to the resources encountered in the new habitat. Such responsiveness could be unfavorable for survival during aerial dissemination. Hence, successful invaders of rich habitats are likely to travel shorter distances, to travel in routes where the speed of travel is great, or to have means of temporary protection such as associating with other particles.

introductions of crops to new regions, the expansion of roof-top urban agriculture, the building of dams and the associated intensification of irrigation, the intensification of animal farms, etc. The massive continent of plastic in the Pacific Ocean (great Pacific garbage patch) could also be a source of unique organisms whose trajectories to various destinations could be predicted and then validated through studies of microbial community dynamics at the sites of the hypothetical sinks. Climate change that leads to changes in aerial dissemination pathways could also give clues as to where changes in sinks might occur. Another approach would involve more direct experimentation to assess the fate of microorganisms that immigrate into a habitat via aerial dissemination. The study of the fate of human pathogens on plants (42) or of microorganisms with potential for biological control of disease (43) are examples of such fate, but they do not concern the majority of microorganisms naturally present in the atmosphere and they involve the use of inoculum concentrations well above those that would arrive via the atmosphere. Addressing this question for natural aerial dissemination will be a special challenge and in particular for sink habitats that are rich in resources and in microbial diversity, such as agricultural habitats. Studies to address the fate and impact of natural immigrants to rich habitats will involve a combination of innovative experimental design, like that used in the context of island biogeography studies of leaf colonization (34, 35, 44), and metagenomic-like tools that are powerfully quantitative.

As illustrated above, invasion of natural and agricultural habitats by aerially disseminated microorganisms can have numerous practical consequences leading to important economic losses. In habitats at high altitudes or extreme latitudes, changes in the microbiology of pristine exposed surfaces can be an indication of the arrival of plumes of pollution carrying not only microorganisms but also certain nutrient sources that facilitate invasion by the newcomers. Insight into the processes involved in invasion could have practical benefits for managing plant health or for monitoring signals of changes in air mass circulation patterns, for example. Research in microbial ecology will be key to harnessing these benefits.

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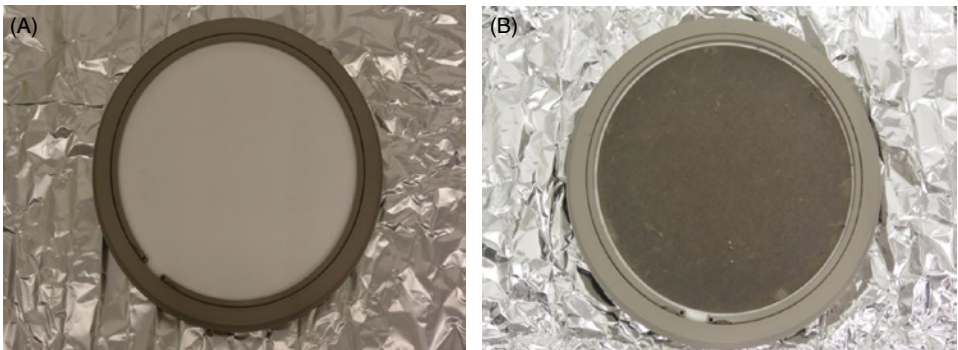
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**Figure 1.2.1** Digitel DHA-80 high-volume filter sampler operating from the roof of a building. (Photos: Anna Kunert, Max Planck Institute for Chemistry, Mainz, Germany.)



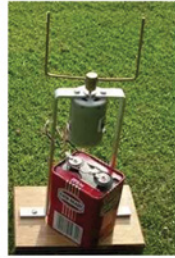
**Figure 1.2.2** Quartz fiber filter in its holder before (A) and after 1 week of aerosol sampling (B) with the Digitel DHA-80 high-volume sampler. (Photos: Anna Kunert, Max Planck Institute for Chemistry, Mainz, Germany).



(A)



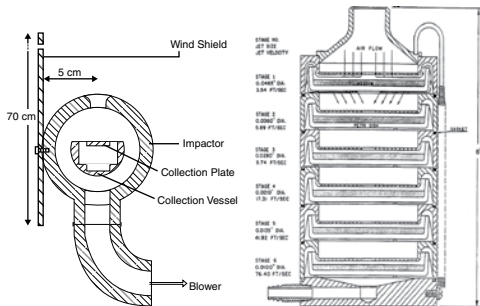
(B)



(C)



(D)



(E)

(F)

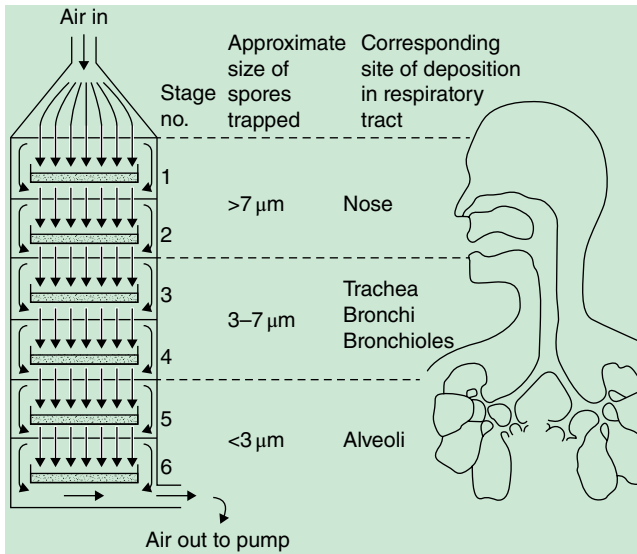


(G)



(H)

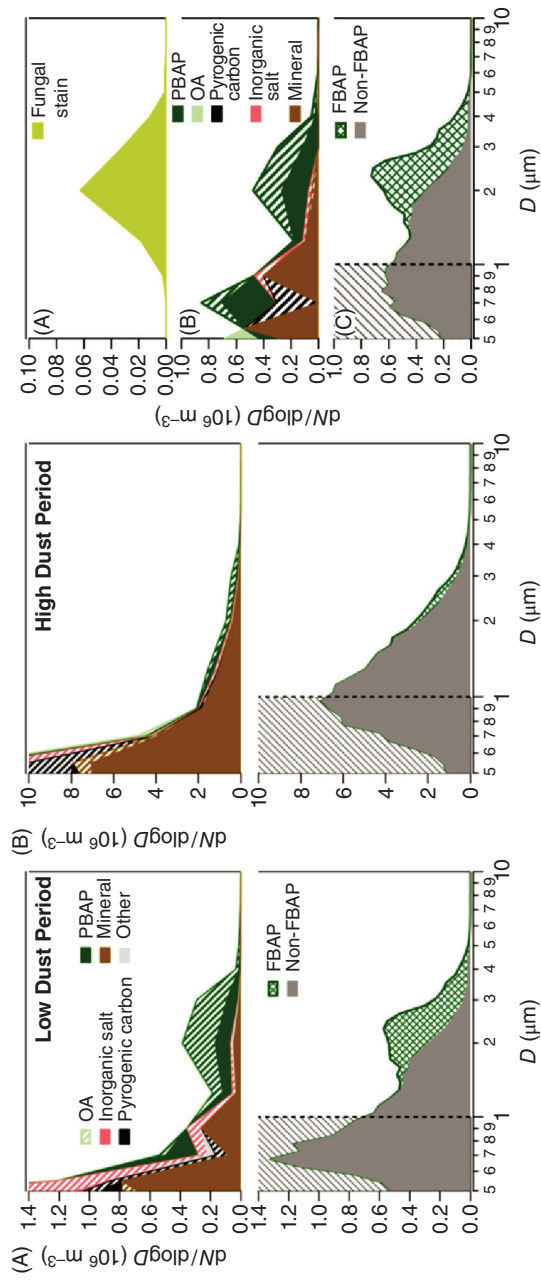
**Figure 1.2.3** Examples of inertia-based samplers used for collecting bioaerosols. A, High-throughput “jet” spore and particle sampler: this sedimentation sampler is used for collecting spores and pollens (Burkard Scientific). (Courtesy of Burkard Scientific.) B, String collector: a passive impactor used for collecting cloudwater droplets (57). (Reproduced with permission from the Royal Society of Chemistry.) C, Rotating arm collector: an active impactor used for collecting pollens and spores. (Courtesy of Jim Deacon, The University of Edinburgh.) D, Seven day volumetric spore trap: an active impactor used for monitoring spores and pollens. (Courtesy of Burkard Scientific.) E, Cloud droplet impactor: an active impactor designed for collecting cloudwater at high wind speeds (58). (Reproduced with the permission of Backhuys Biological Books.) F, Six-stage Andersen sampler: an active cascade impactor with impaction plates consisting of nutritive agar medium (59). (Reproduced with the permission of American Society for Microbiology.) G, Coriolis®  $\mu$ : a centrifugal liquid impactor. (Courtesy of Bertin Technologies.) H, Reuter Cyclone Sampler (RCS®) and agar strips: a centrifugal impactor on agar medium. (Courtesy of Merck-Millipore.) See text for details on the different categories of samplers.



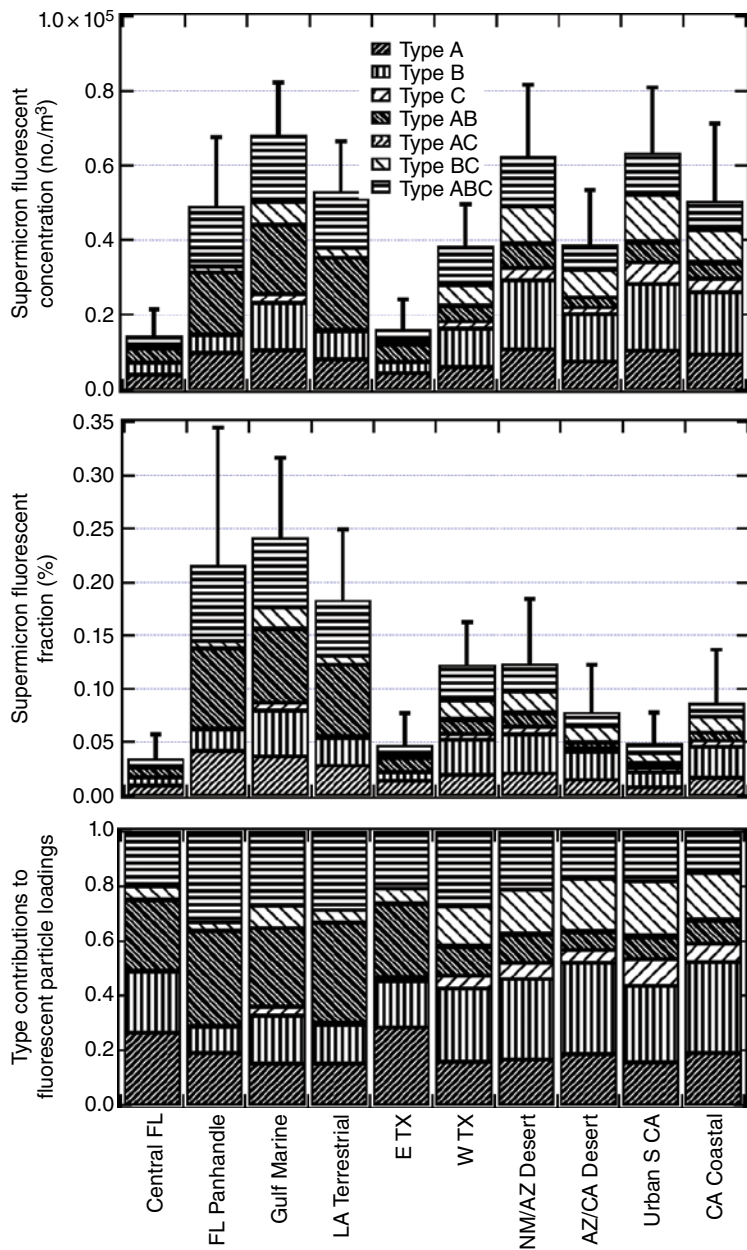
**Figure 1.2.4** Representation of the cut-off diameters of each stage of the cascade Andersen sampler, and corresponding sites of deposition in the human respiratory tract. (Courtesy of Jim Deacon, The University of Edinburgh.)



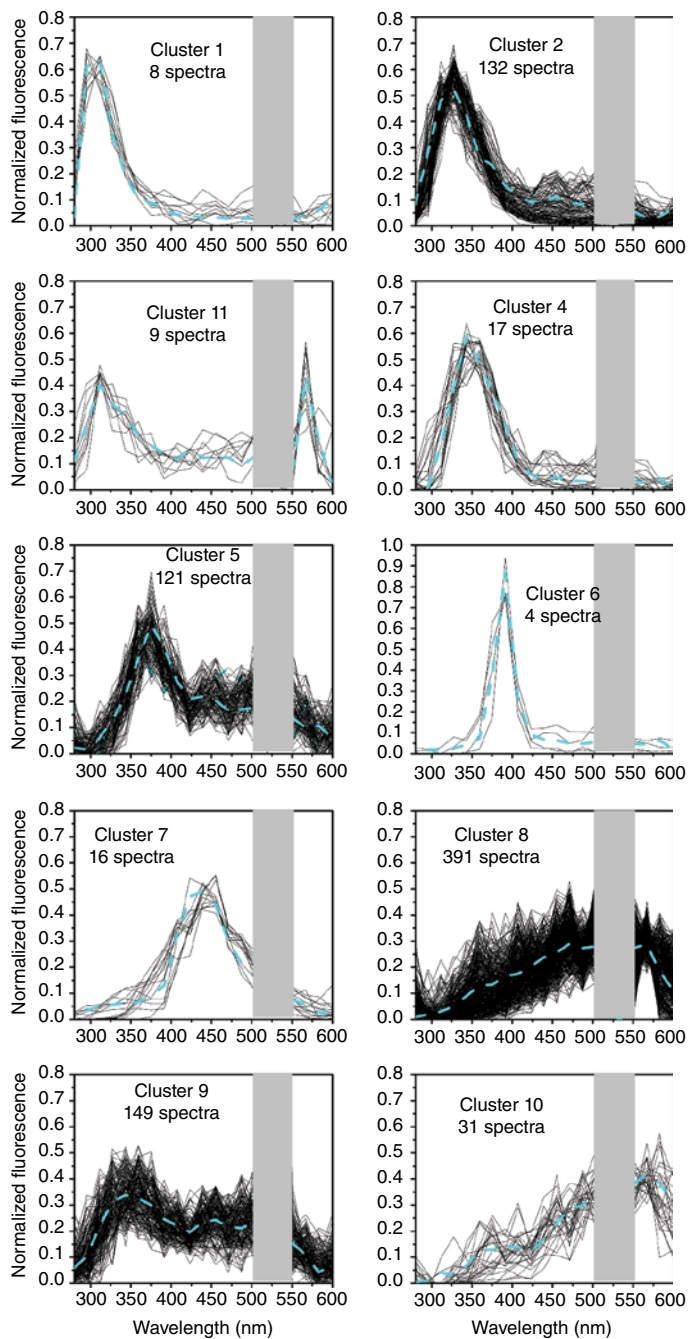
**Figure 1.3.1** Colony-forming units on potato-dextrose-yeast extract agar (PDYA) medium from air sampled with a Casella CEL Airborne Bacteria Sampler (slit sampler). (Photo: T.C.J. Hill, Colorado State University, Colorado, USA.)



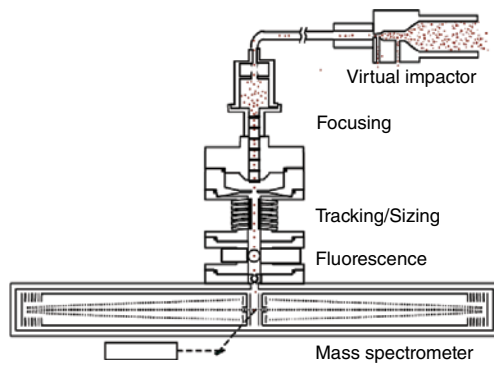
**Figure 1.4.2** Application of the ultraviolet aerodynamic particle sizer (UV-APS) during a field study in remote Amazonia. Size distributions show similarity of results using fluorescent particles from the UV-APS and direct characterization using scanning electron microscopy, elemental analysis, and fungal staining. FBAP, fluorescent biological aerosol particle; OA, organic aerosol; PBAP, primary biological aerosol particle. (Reproduced from Huffman et al. (83).)



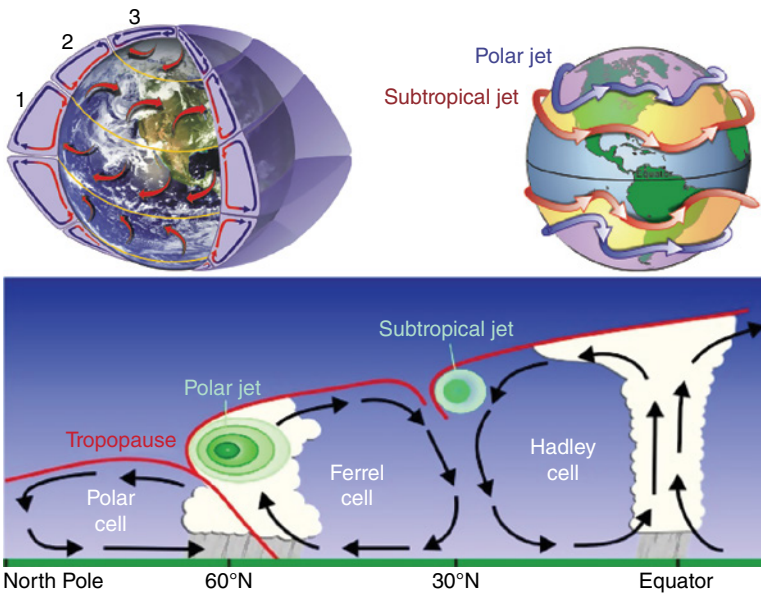
**Figure 1.4.3** Application of the wideband integrated bioaerosol sensor (WIBS) data summarizing blimp trip across the USA showing particle category breakdowns. AZ, Arizona; CA, California; E, East; FL, Florida; LA, Los Angeles; NM, New Mexico; S, South; TX, Texas; W, West. (Reproduced from Perring et al. (100) with the permission of John Wiley and Sons.)



**Figure 1.4.4** Clusters of individual particles collected in Las Cruces, NM, by the SPFS instrument. (Reproduced from Pinnick et al. (143) with permission from Elsevier.)



**Figure 1.4.5** Picture and schematic overview of the BAMS instrument. (Reproduced with permission from Steele et al. (156).)



**Figure 2.3.1** Global wind circulation patterns. 1, Hadley cell; 2, Ferrel cell; 3, Polar cell. (Figure courtesy of NOAA's National Weather Service, Southern Region Headquarters, Fort Worth, Texas. Figures located at <http://www.srh.noaa.gov/jetstream/global/circ.htm> and <http://www.srh.noaa.gov/jetstream/global/jet.htm>)

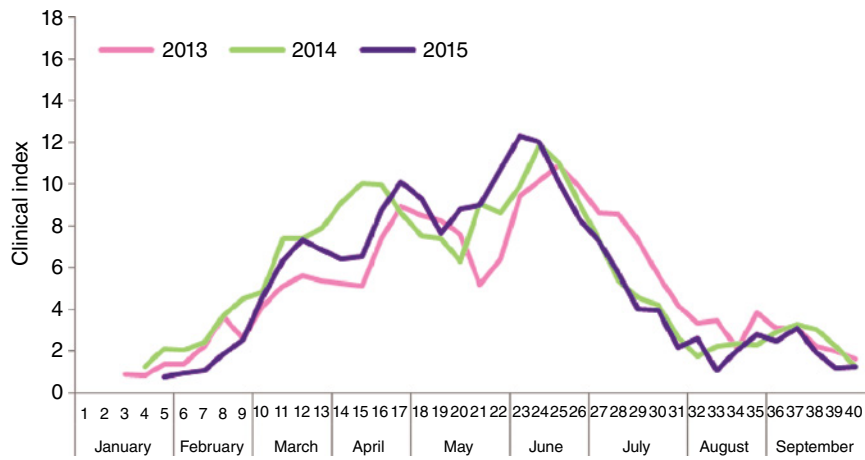


**Figure 2.3.2** Desert dust lofted into the atmosphere over the Sahara by storm activity. (Photo courtesy of NASA's Earth Observatory (<http://earthobservatory.nasa.gov/IOTD/view.php?id=84400>.)



**Figure 2.3.3** Dust devils lifting exposed agricultural soils into the atmosphere southwest of Ritzville in Adams County, WA, USA (27 September 2015). (Photo credit: D.W. Griffin.)





**Figure 4.1.1** Weekly evolution of the clinical index in France in three successive years, on a scale of 0–18.

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