Microorganisms in Home and Indoor Work Environments

Diversity, Health Impacts, Investigation and Control

SECOND EDITION

Edited by
Brian Flannigan
Robert A. Samson
J. David Miller

CRC Press
Taylor & Francis Group

Microorganisms in Home and Indoor Work Environments

Diversity, Health Impacts, Investigation and Control

SECOND EDITION

This page intentionally left blank

Microorganisms in Home and Indoor Work Environments

Diversity, Health Impacts, Investigation and Control

SECOND EDITION

Edited by
Brian Flannigan
Robert A. Samson
J. David Miller

CRC Press
Taylor & Francis Group
Boca Raton London New York

CRC Press is an imprint of the
Taylor & Francis Group, an **informa** business

CRC Press
Taylor & Francis Group
6000 Broken Sound Parkway NW, Suite 300
Boca Raton, FL 33487-2742

Version Date: 20111012

International Standard Book Number-13: 978-1-4200-9335-3 (eBook - PDF)

Preface

Since the preface to the first edition was drafted ten years ago there have been major changes in the recognition of the economic and health importance of fungal damage in the built environment. A decade ago, the transition from a focus on outdoor air quality to an understanding that most allergic disease is associated with contaminants found in homes was just under way. We noted then that the National Institute for Occupational Health studied the impact of poor indoor air quality on productivity in the USA. The authors of the study estimated that for around 20% of the US working population a benefit approaching what in 2007 would have been 88 billion US dollars might be realized (*American Journal of Public Health*, 2002, 92,1430-1440). In 2007, estimates of the costs of disease associated with mould and dampness were developed which took into consideration the entire population and residential environments. Again in the US, the direct costs were estimated to be in the 4 billion dollar range (*Environmental Health Perspectives*, 2007, 115, 971-975*; Indoor Air*, 2007, 17, 226-235). The indirect costs of lost work and school days are much larger. The Institute of Medicine of the US National Academy of Sciences produced two expert reports that deal with the effects of mould and dampness on asthma in 2000 and on mould and dampness and health in 2004, as did Health Canada (2004), and in 2004, and again in 2007, the World Health Organization also indicated the importance of mould and dampness to public health. Most recently, an expert panel commissioned by the US Centers for Disease Control found that the elimination of moisture and mouldy materials in homes resulted in improved health [D.E. Jacobs *et al*. (2010) *Journal of Public Health Management and Practice* **16**: S5-S10].

These are only some of the landscape changes that have occurred in this field in less than a decade. The first edition contained information that served as a benchmark for defining future progress. Although much was known in the scientific community about the growth of mould and dampness on building materials and contents, and of epidemiological evidence that such growth represented a public health hazard, there were major scientific challenges. We noted that "Fully elucidating their effects on human health has however been bedevilled by problems of accurate assessment of exposure to microorganisms and precise identification of those present in the environment." This remains true.

As in the first edition, the first three sections of the book review to date the types of microorganism in outdoor and indoor air, their growth and control in home and work environments, and their role in respiratory disease. An entirely new chapter on pollen in indoor environments and its allergenic effects has been added. The remaining sections of the book are given over to addressing the twin problems of exposure assessment and identification, discussing the methodology for and conduct of investigations of indoor environments. As before, the book includes information on key fungi and actinobacteria that reflects advances in knowledge of their occurrence in buildings in different parts of the world, as well as changes in taxonomic status.

What is entirely new is treatment of issues that were emergent at the time of writing the first edition. Epidemiological studies had demonstrated an association of mould with respiratory disease not associated with allergic mechanisms. In this second edition, there is a chapter on the emerging picture of the mechanistic basis for this phenomenon, i.e. of the effect of toxins and inflammatory agents on lung biology and other systems. Similarly, there is a new chapter on the use of molecular methods for determining microbial contaminants. Much new material is also found on the problems of remediation, control and quality assurance; occupational exposures in a wider range of work environments and among remediation workers; infectious fungi in the built environment; and endotoxin. The nomenclature of some common indoor fungi has been recently changed and these changes have been applied lea-ving the "old " name between brackets.

We think that the availability of information on the microorganisms that grow in the built environment, together with information on the limitations of the methods currently available to measure them, will be useful to researchers, public health officials and industrial hygienists. Together with reviewers, the authors from Canada, Sweden, the Netherlands, the United Kingdom and USA have worked hard to produce material that is accurate and timely. In addition to thanking reviewers and authors for their efforts, we should also like to express our gratitude to Margaret Flannigan for invaluable editorial assistance.

Brian Flannigan (Edinburgh), Robert A. Samson (Utrecht), J. David Miller (Ottawa)

PREFACE TO THE FIRST EDITION

While much of the concern about air pollution in the past has been focused on the outdoor environment, in recent years indoor air quality (IAQ) has moved up the agenda. Over the period between 1987 and 1999, more than $1 billion of federal government money was spent on research into indoor air pollution in USA. In March 2000 the Environmental Protection Agency released a report on "Healthy Buildings, Healthy People: A Vision for the 21st Century", which set the objective of achieving major health gains by improving indoor environments. The National Institute for Occupational Health has studied the impact of poor indoor air quality on productivity. The median estimate of these losses is $100 billion per year. Other countries, including Canada, Denmark, Finland, Netherlands and Sweden, also have substantial programmes on residential housing and health. However, there is wide variation in the research effort and expenditure on measures to improve IAQ, and IAQ in some countries is very much lower on the order of priorities.

A document produced in July 2000 by a WHO European working group has further emphasized the global importance of IAQ as a determinant of population health and well being. This document, *"The Right to Healthy Indoor Air"*, sets out nine principles (derived from the general principles in the International Bill of Human Rights). These are intended to inform all those who have an influence on public health of their obligations to honour the right of every individual to breathe healthy indoor air, and influence those national governments that do not have plans for future action on healthy indoor air to put it on their agenda.

Despite the large amount of money spent on research into pollution of the indoor environment, the US General Accounting Office has confirmed that what has been done has pointed to the complexity of the problem and to major gaps in knowledge. Among these gaps are accurate knowledge of the identities and sources of pollutants and of the effects of prolonged exposure to indoor pollutants on health. This book considers one such group of pollutants, namely microorganisms, and more particularly heterotrophic bacteria and fungi. Advances have certainly been made in our knowledge of microorganisms in the home and indoor work environment as research has accelerated in the last decade. Fully elucidating their effects on human health has however been bedevilled by problems of accurate assessment of exposure to microorganisms and precise identification of those present in the environment. The first three sections of the book review the types of microorganism in outdoor and indoor air, their growth and control in home and work environments, and their role in respiratory disease. The remaining sections of the book are given over to addressing the twin problems of exposure assessment and identification, discussing the methodology for and conduct of investigations of indoor environments and providing keys and colour illustrations to assist in the identification of approaching 100 mould, yeast and actinomycete contaminants.

We think that the availability of information on the microorganisms that grow in the built environment, together with information on the limitations of the methods currently available to measure them, will be useful to researchers, public health officials and industrial hygienists. Together with reviewers, the authors from Canada, Sweden, the Netherlands, the United Kingdom and USA have worked hard to produce material that is accurate and timely. In addition to thanking reviewers and authors for their efforts, we should also like to express our gratitude to Margaret Flannigan for editorial assistance, Karin van den Tweel for helping with the drawings and Ans Spaapen-de Veer for preparing the index. Thanks are given to Dr Brian Crook (HSL, Sheffield, UK) for substantive assistance in the preparation of Chapter 3.2.

We have dedicated this book to the memory of a fellow microbiologist, John Lacey, who was internationally recognized for his unique expertise at the interface of stored product microbiology, aerobiology, occupational hygiene and medicine, and was the author of more than 300 publications. The aerobiological, taxonomic and ecological studies of John and his co-workers not only clarified the role of microorganisms in a number of occupational lung diseases, but have a relevance to home and non-industrial work environments as well as to the storage and processing of the particular agricultural materials with which they were first concerned. In his friendly collaboration and links with many workers in overseas institutes, he was generous in passing on his knowledge and expertise to others, and his work and the influence that he has had are reflected in this book.

List of Contributors

Dr Denis Corr
Corr Associates
7 Spruceside Ave, Hamilton L8P 3Y2, Ontario
Canada

Dr Brian Crook
Health Improvement Group, Health and Safety Laboratory
Buxton, SK17 9JN
UK

Prof. James H. Day, MD
Division of Allergy and Immunology
Department of Medicine, Queen's University
Kingston K7L 3N6
and
Kingston General Hospital
76 Stuart St,
Kingston K7L 2V7, Ontario
Canada

Prof. Anne K. Ellis, MD
Division of Allergy and Immunology
Department of Medicine, Queen's University
Kingston, K7L 3N6
and
Kingston General Hospital
76 Stuart St,
Kingston K7L 2V7, Ontario
Canada

Dr Brian Flannigan
Scottish Centre for Pollen Studies
Napier University
10 Colinton Road, Edinburgh EH10 5DT
UK

Dr Jonathan M. Gawn
Health Improvement Group,
Health and Safety Laboratory
Buxton, SK17 9JN
UK
Present address:
Biological Agents Unit
Health and Safety Executive
Redgrave Court
Bootle
Merseyside L20 7HS
UK

Dr Brett J. Green
Sporometrics, Inc.
219 Dufferin Street, Suite 20C
Toronto, Ontario M6K 1Y9
Canada
Present address: Allergy and Clinical Immunology Branch,
Health Effects Laboratory Division
National Institute for Occupational Safety and Health
Centers for Disease Control and Prevention
1095 Willowdale Road MS L-4020
Morgantown, WV 26505
USA

Dr Michael J. Hodgson, MD, MPH
Office of Public Health and Environmental Hazards
Veterans Health Administration
810 Vermont Avenue, NW
Washington, DC 20420
USA

Ing. Jos Houbraken
CBS-KNAW Fungal Biodiversity Centre
P.O. Box 85167, 3508 AD Utrecht
The Netherlands

Dr Timo Hugg
South Karelia Allergy and Environment Institute
Joutseno
and
Institute of Health Sciences
University of Oulu, Oulu
Finland

Prof. J. David Miller
Department of Chemistry
Carleton University,
Ottawa K1S 5B6, Ontario
Canada

Dr Philip R. Morey
ENVIRON International Corporation
2235 Baltimore Pike
Gettysburg, PA 17325
USA

Dr John Mullins
The Centre
33 The Parade, Rooth, Cardiff CF24 3AD
UK

Prof. Thomas G. Rand
Department of Biology
Saint Mary's University
923 Robie Street
Halifax B3H 3C3, Nova Scotia
Canada

Dr Auli Rantio-Lehtimäki
Aerobiology Unit,
Department of Biology
University of Turku, FIN-20014 Turku
Finland

Prof. Timothy J. Ryan
School of Health Sciences
E 344 Grover Center
Ohio University
Athens, OH 45701-2979
USA

Prof. Dr Dr h.c. Robert A. Samson
CBS-KNAW Fungal Biodiversity Centre
P.O. Box 85167, 3508 AD Utrecht
The Netherlands

Dr James A. Scott
Dalla Lana School of Public Health
University of Toronto
223 College Street, Toronto M5T 1R4
and
Sporometrics, Inc.
219 Dufferin Street, Suite 20C
Toronto, Ontario M6K 1Y9
Canada

Dr Richard C. Summerbell
Dalla Lana School of Public Health
University of Toronto
223 College Street
Toronto M5T 1R4
and
Sporometrics, Inc.
219 Dufferin Street, Suite 20C
Toronto, Ontario M6K 1Y9
Canada

Dr Jillian R.M. Swan
Health Improvement Group,
Health and Safety Laboratory
Buxton, SK17 9JN
UK

Dr Eija Yli-Panula
Aerobiology Unit
Department of Biology
University of Turku, FIN-20014 Turku
Finland

Contents

This page intentionally left blank

Chapter 1. Microorganisms in air

This page intentionally left blank

Chapter 1.1

MICROORGANISMS IN OUTDOOR AIR

John Mullins[1] and Brian Flannigan[2]

[1] *The Centre, Rooth, Cardiff, UK;*
[2] *Scottish Centre for Pollen Studies, Napier University, Edinburgh, UK.*

EARLY STUDIES OF OUTDOOR AIR

It had long been believed that the air could bring disease to humans and crops, but it was not until the invention of the microscope in the 17th century that it was possible to observe the array of particles that are carried in the air. With his lens, Antonie van Leeuwenhoek (Dobell 1932) was just able to observe bacteria. It gradually became recognized that the air carried bacteria, yeasts, fungal spores, spores of mosses and ferns, algae, pollen grains and even protozoa. Initial studies were concerned with the controversy surrounding spontaneous generation of organisms and it was Pasteur (1861) who, by drawing air through gun cotton and then dissolving the gun cotton and examining the deposit under a microscope, discovered that the air contained a variety of different particles. However, he did not pursue these studies and the realisation that the air contained a variety of microbes resulted in a concerted effort by medical men to discover the microbes that caused disease (Bulloch 1938). The original work of Miquel (1899) in Paris into airborne bacteria stands as one of the most sustained series of volumetric measurements of the microbial population of the air ever attempted. Samples were collected over a 16-year period in plugs of gun cotton, and after this was dissolved the filtrate was cultured in flasks of filtered saline beef extract. From his studies he discovered that in a park 5 km from the centre of Paris bacteria were nearly three times as numerous in summer as in winter; in the centre of Paris counts were twice those in the park, but with a similar seasonal fluctuation. He also sampled a narrow unhygienic street and the main sewer of Paris, in which the air proved to be no more contaminated than in the streets outside. On average, in the park there were 290 bacteria m^{-3} air, in the centre of Paris 7480 m^{-3}, in the unhygienic street 5550 m^{-3} and in the sewer 3835 m^{-3}. He also noted a steady annual decline, which he attributed to improved street cleaning and washing to lay dust. Miquel came to the conclusion that the source of most outdoor airborne bacteria is the surface of the ground. He also studied the variations during the day, and attributed increases during the course of the day to mechanical causes such as road sweeping and traffic. Miquel lost interest in fungal spores however, and developed media that selectively discouraged mould growth. Fungal spores in the air were then largely ignored until investigated by Cadham (1924), who confirmed spores of cereal rust fungi as a cause of asthma and rekindled an interest in airborne fungal spores driven by allergists.

FUNGAL SPORES IN OUTDOOR AIR

Types and sources of fungal spores

Fungal spores are present in outside air throughout the year (the air spora) with scarcely any exceptions, and in the senior author's experience concentrations in the centre of the British city Cardiff have reached as high a 24-h mean as nearly 85000 m^{-3} air. It is virtually impossible to take a breath without inhaling a quantity of fungal spores. Estimates of the volume of air inspired at rest suggest a value of 10 l min^{-1}, the rate of sampling adopted for Hirst spore traps (Hirst 1952). At this rate, 1 m^3 air would be inspired in 100 min, but any increase in activity would dramatically increase the volume of air inspired, resulting in a greater intake of fungal spores.

The presence of so many fungal spores in the air is a consequence of the mechanism possessed by many fungi as a means of dispersal, viz. the production and release into the air of enormous numbers of spores. The spores of some fungi are dispersed in water and may only become airborne in spray thrown up by wave action. Insects also are responsible for the dispersal of some fungal spores, but the majority of fungi release their spores directly into the air.

Fungi produce both sexual and asexual spores: some produce only asexual spores, some produce only sexual spores, and some produce both. Asexual spore types include sporangiospores, conidiospores (conidia), pycnidiospores, teliospores of the cereal pathogens in the phylum Basidiomycota (basidiomycetes) that are known as smut fungi, and the teliospores, uredospores and aeciospores produced at different stages of the life cycle of the other basidiomycete plant pathogens referred to as rust fungi. The sexual spores include those of the Zygomycota (zygospores, which are mostly sessile), the Ascomycota (ascospores) and the Basidiomycota (basidiospores).

Release and aerosolization of spores

As fungi are relatively small, certain barriers to spore dispersal exist which have to be overcome. There is a static layer of air known as the laminar boundary layer varying in thickness from 1 m to 10 cm from the ground; it is thicker in still conditions and becomes thinner with increased wind speed. If spores are released into this layer they will not be dispersed. Accordingly, mechanisms have evolved in the fungi that ensure that their spores are released into the turbulent layer, which extends above the laminar layer.

Extensive work was carried out by Ingold and his co-workers on spore release mechanisms, to which reference should be made for a more exhaustive treatment of the subject (Ingold 1971). Most of the asexual spores are released relatively passively and rely on wind currents and turbulence to carry them away. Among these, sporangiospores are produced within a sporangium, which is raised up on a sporangiophore, exposing spores to the scouring effects of air currents as the sporangial wall bursts on maturity, exposing the spores for dispersal. In some species of *Mucor* the sporangial wall appears to dissolve leaving a mass of spores in a sporangial drop exposed to drying air currents. In the majority of sporangial fungi the sporangial wall ruptures as the sporangiospores, initially packed into the sporangium in polyhedral shapes, round off as they mature and increase the pressure on the sporangial wall. Although this method of spore release is found in *Mucor* and other fungi in the order Mucorales within the Zygomycota (see Chapter 1.2), despite the abundance of this group in nature relatively few of their spores are isolated from outside air.

The asexual aeciospores of rusts are released by a similar method of rounding off from a polyhedral shape as they mature, exposing them to erosion by air currents. However, by their situation as biotrophic parasites on the leaves of plants, rusts and their spores are effectively raised above the laminar layer, facilitating spore release.

Perhaps the most common asexual spore (or mitospore) produced by fungi is the conidiospore or conidium. These conidia are produced externally, rather than within a sporangium, and the spores are individually budded off or may be formed in chains but raised up on conidiophores, allowing for erosion of the spores by wind currents. This latter mechanism is found in *Cladosporium*, the genus that contributes most to the air spora in temperate countries, including UK (Harvey 1967, 1970). *Cladosporium* and a number of other common airborne fungi, such as *Alternaria*, *Botrytis* and *Epicoccum*, colonize the surface of leaves (the phylloplane), stems and other plant organs, particularly as they senesce, so that these fungi are often referred to as phylloplane fungi. In contrast to these aerially dispersed dry-spored fungi, in those such as *Fusarium*, *Gliocladium* and *Trichoderma* the conidia occur in minute droplets of aqueous slime and are not directly detached by wind currents. Dispersal in these wet-spored fungi is by water — rain-splash and surface water — and in some cases by insects and other arthropods.

Sexual spores are produced by fruiting bodies, or sporocarps, of the Ascomycota and Basidiomycota. The members of these two phyla are frequently referred to as ascomycetes and basidiomycetes. In the Ascomycota, members of the large subphylum Pezizomycotina (the cup fungi) produce ascospores in groups of eight within a sac known as an ascus. Asci may be produced within, or over the surface of the sporocarp, the ascocarp (or ascoma). In response to the appropriate stimuli the ascus releases its spores explosively into the air. The stimulus may be a change in relative humidity (RH) or even a response to light (Walkey and Harvey 1966 1968a). In the Basidiomycota, members of the subphylum Agaricomycotina (which includes mushrooms, toadstools and bracket fungi), the whole of the sporocarp, the basidiocarp,

may be raised above the ground on a thick stalk or it may grow out from decaying wood, as in the bracket fungi. The basidiospores develop externally, usually as a group of four, each on a separate sterigma, on a basidium. Depending on the type of basidiomycete, the basidia cover the surface of sheet-like gills or surround pores in the basidiocarp. They are forcibly ejected by a mechanism that is not yet fully understood into the space between the gills or into pores, and thereafter fall by gravity into the turbulent airflow below the cap. In puffballs and the earth star fungi a different mechanism has evolved in which the spores are produced inside a thin papery capsule with an apical opening, the ostiole. The dry spores are either forced out of the capsule by the impact of raindrops on the surface of the capsule or are drawn out as the air passes across the ostiole.

Rain affects the numbers of not only puffball and earth star spores in the atmosphere. For instance, over a period of five days in central London Battarbee *et al.* (1997) recorded the size of airborne particles impacting on the sticky tape of a Burkard automatic volumetric spore trap (see below under Sampling the Air Spora). As well as pollen grains and occasionally diatoms, conidia of *Cladosporium, Alternaria* and *Epicoccum*, ascospores of *Leptosphaeria* and lichens, and basidiospores were among the more common recognizable spores on the tape. On two consecutive dry summer days 1-4% of particulate matter of an aerodynamic diameter up to 10 µm (PM10) consisted of such spores. Light rain on the day after and heavy rain the day after that raised the level to 3-6%. On the day following the heavy rain, 23-27% of the PM10 comprised fungal spores, this increase being attributed to initiation of spore release by the rain. Collectively then, the various types of fungal spore may form a sizeable proportion of particulate matter in outdoor air. Based on a Canadian study of glycerophospholipids in airborne particles <2.5 µm in size (PM2.5), it has been suggested that at three sites in the Toronto area fungal spores and pollen grains between them accounted for 12-22% of the organic carbon fraction in the outdoor air, or 4-11% of the total mass on a fresh weight basis (Womiloju *et al*. 2003).

The length of time spores remain in the air will depend in part on the size of the spore, as large spores will naturally tend to be deposited from the air more rapidly than small spores. The rate of fall of a spherical spore in still air is given by Stoke's Law:

$$V = \frac{\sigma - \rho}{\mu} g r^2$$

where

V is terminal velocity in cm sec^{-1}
σ is the density of the spore
ρ is the density of air
g is acceleration due to gravity (981 cm sec^{-1})
μ is the viscosity of air (1.8×10^{-4} g cm^{-1} sec^{-1} at 18°C)
r is the radius of the spore.

Since the density of most spores is 1.0 and the density of air is so small that it can be ignored, this can be simplified to:

$$V = \frac{2gr^2}{9\mu}$$

The rate of fall of a spore is then proportional to the square of the radius, giving rates of fall of 2.0-2.8 cm sec^{-1} for the very large spores of the microfungus *Cochliobolus sativus* (*Helminthosporium sativum*), which are 80 x 15 µm, compared with 0.05 cm sec^{-1} for the spores of the puffball *Lycoperdon pyriforme* (4 µm diameter).

Long distance transport of spores

Fungal spore distribution from a point source has always been of interest to plant pathologists who wish to predict the distance which spores will travel, and to workers attempting to predict concentrations of smoke screens, gas clouds, radioactive particles or pollen released from genetically modified crops (Emberlin *et al*. 1999).

Gregory (1973) described the extensive work done in this field, both in his own studies at Rothamsted and by others. It is apparent that producing a mathematical model to predict the distance to which a spore will travel is extremely difficult. Unlike gas clouds, spores are eroded from the cloud by deposition, but Chamberlain (1966) considered that for grass pollen (with grains around 20 µm in diameter) and other pollen released at about knee height the evidence suggests ranges in the order of 1 km, and for pollen released at tree top level distances of travel in the order of tens of kilometres. Raynor *et al.* (1970) found that 1% of ragweed pollen released from a point source remained airborne at a distance of 1 km from the point of release. As agents by which plant disease may spread, spores and the distances they may travel have also

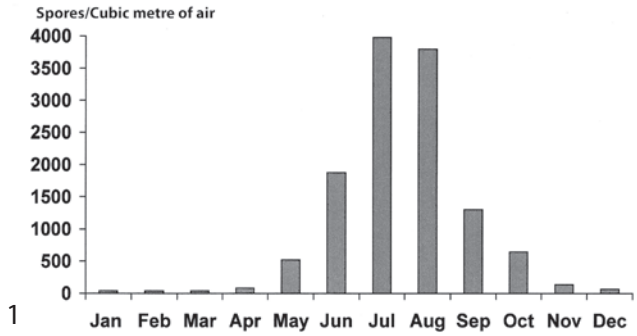
long been of economic interest. Stakman and Hamilton (1939) reported uredospores of stem rust of wheat (*Puccinia graminis tritici*) at distances of some 970 km from the source, which was a vast area of winter wheat in the southern USA. Hirst *et al.* (1967) had the opportunity to sample airborne particles from an aircraft travelling over the North Sea, and some 400-500 km from the English coast recorded unexpected clouds of fungal spores. These corresponded to spores, which had been released the previous day over the land and had been carried eastward by the prevailing winds. There was a further increase between 500 and 600 km from the coast of spores characteristic of those released at night, again which had been released over land, but the previous night. As Gregory (1973) noted, the extent of spore dispersal is sufficient to spread plant diseases between countries and across continents.

Sampling the air spora

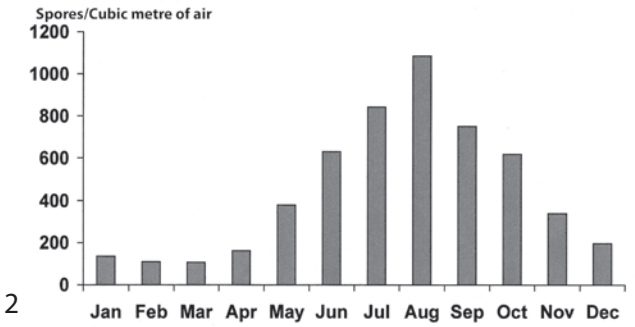
Estimates of spore concentrations in the air are obtained by air sampling. Two methods are widely used to carry out a census of the atmosphere. The first of these involves collecting spores onto culture plates, counting the colonies which develop from these and identifying the fungi from the characteristics of the colonies. Although spores may comprise the bulk of airborne fungal material, there can also be pieces of the mycelium, hyphal fragments, which may also give rise to colonies (see below). Aggregated clumps of spores, not just individual spores (or in the case of yeast, not only individual cells but clumps), may also be trapped on culture plates. To allow the spores simply to settle on the plate is to invite sampling errors because of the different rates of fall of different spores. Consequently, an efficient sampling device such as the Andersen sampler (Andersen 1958), based on the cascade impactor devised by May (1945), is required to ensure volumetric sampling. Because of the danger of overloading the culture plates, the time for which the plates can be exposed for sampling purposes is limited. Therefore, sampling has to be restricted to "spot" or "grab" samples or a series of samples taken at intervals throughout the day and night, with the attendant problems of incubating and subsequently examining a large number of culture plates. There is also the problem that different culture media tend to be somewhat selective in the fungi which will grow on them, and many fungal spores will not germinate on culture plates, including most of the sexual spores which may form a significant part of the air spore. As one cannot be certain as to what exactly colonies have arisen from – individual spores, spore clumps or hyphal fragments – counts on culture plates are expressed volumetrically as propagules or colony forming units (CFU) m^{-3} air.

The alternative is to collect the spores on a glass microscope slide using a volumetric trap such as the Hirst trap (Hirst 1952) or the Burkard automatic volumetric spore trap (AVST) into which it evolved. Irrespective of whether the spores collected for visual identification under the microscope are viable (culturable) or non-viable, identification is carried out and is dependent on the morphological characteristics of the spores. Counts are expressed as spores m^{-3} air, and not as CFU m^{-3} as in culture-based methods. Some spores are easy to identify but others are very difficult to distinguish, either because of their small size or their lack of distinguishing features. In consequence, many spores tend to be counted according to categories such as colour and shape. This is particularly true of the ascospores and basidiospores, which frequently occur in the air in high numbers and are likely to have originated from many different species. It may therefore be necessary to use a culture method when it is the only way of confidently estimating the concentration of airborne spores of a particular species of fungus, particularly when visual identification is not possible (Mullins *et al.* 1976).

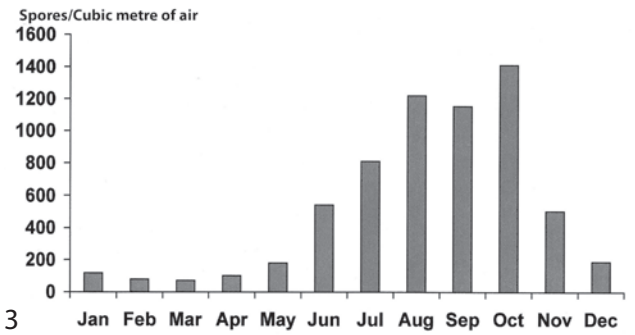
With the advent of PCR (polymerase chain reaction) and other modern molecular methods, advances have been made in identifying specific organisms, such as those that only occur in the air in small numbers, e.g. the human pathogen *Pneumocystis carinii* (Wakefield *et al*. 1998), so that such techniques are proving to have more widespread applications. For example, PCR-based techniques have also been used for prediction of crop disease epidemics by monitoring the pathogenic fungi in the ambient air (see West *et al.* 2008). A very recent demonstration of the use of modern methods is a study carried out in Mainz, Germany, by Fröhlich-Nowoisky *et al*. (2009), who used a high-volume dichotomous sampler to separate airborne particles of aerodynamic diameter >3 µm from those <3 µm in samples of approx. 3000 m^3 of the ambient air, which is representative of a mixture of urban and rural continental boundary layer air encountered in central Europe. On analysis, DNA extracted and amplified from the two fractions suggested to the authors that >1000 fungal species were present in the sampled air, although around 70% of these were found only once. More plant pathogens were found in the coarser material (>3 µm) and more



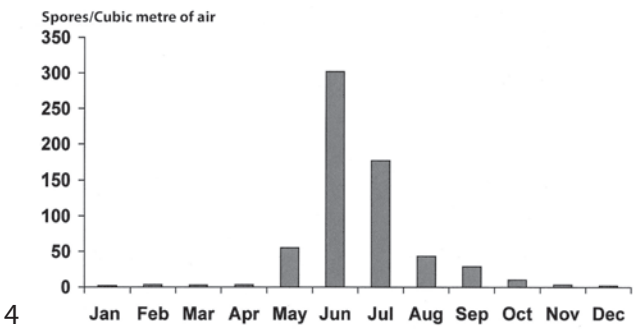
1



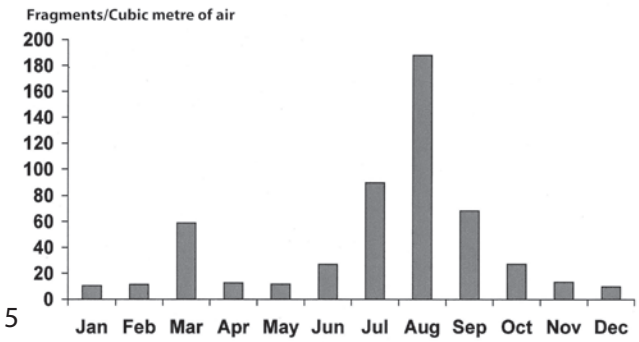
2



3



4



5

fungi allergenic and pathogenic for humans in the finer material. Basidiomycetes accounted for 64% of all fungi identified and ascomycetes (including mitosporic species) only 34%, leading the authors to conclude that in earlier studies, particularly those using culture-based methods, the presence of basidiomycetes may have been underestimated.

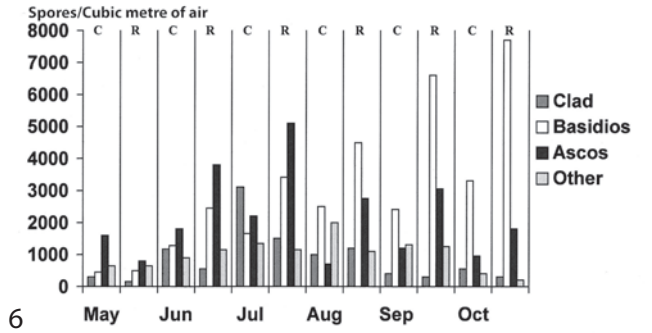
Factors affecting the composition of the air spora

The variety and concentration of spores in outdoor air are subject to continuous diurnal and seasonal variation. Contributory factors include availability of substrate, activities such as mowing grass and harvesting grain, and climatic factors, particularly temperature and rainfall (see, for example, Rodriguez-Rajo *et al*. 2005, Stepalska and Wolek 2005), which have a direct effect on the release of spores into the air. Rain and warmth also promote the development of vegetation on which parasitic and saprophytic fungi subsequently develop. Broad correlations between the composition of the air spora and climatic factors enable predictions to be made that certain spore types will be more abundant in warmer, drier summers, whereas others will be more abundant during damp weather.

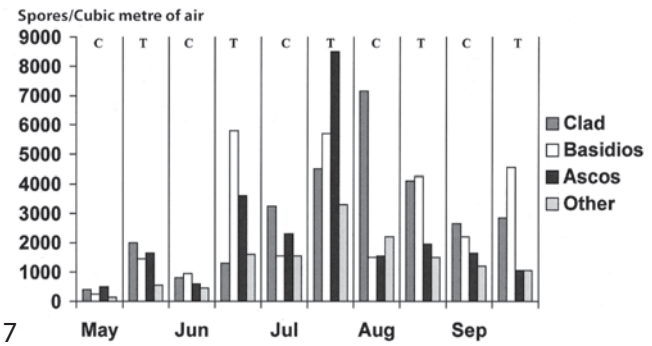
The outdoor air spora is largely derived from spores produced by moulds and other fungi growing on natural and cultivated vegetation and on surface vegetable debris. Not all fungi on leaves have spores that are easily aerosolized, e.g. yeasts and *Phoma*, which are dispersed by rain-splash. However, the colonization of leaf surfaces by those with dry spores that are readily dispersed into the air, such as *Cladosporium* and *Alternaria*, can make a major contribution to the fungal burden in the air (Levetin and Dorsey 2006). In a review on fungal endophytes, i.e. fungi that grow intercellularly or invade single cells in leaves and other plant organs, Schulz and Boyle (2005) noted that the majority in temperate habitats belonged to more or less ubiquitous genera. These include species in genera that are known to colonize the phylloplane and are commonly isolated from air, not only

Fig. 1. Incidence of airborne *Cladosporium* spores trapped by an AVST at roof top level in Cardiff.
Fig. 2. Incidence of airborne ascospores trapped by an AVST at roof top level in Cardiff.
Fig. 3. Incidence of airborne basidiospores trapped by an AVST at roof top level in Cardiff.
Fig. 4. Incidence of airborne rust and smut spores trapped by an AVST at roof top level in Cardiff.
Fig. 5. Incidence of airborne hyphal fragments trapped by an AVST at roof top level in Cardiff.

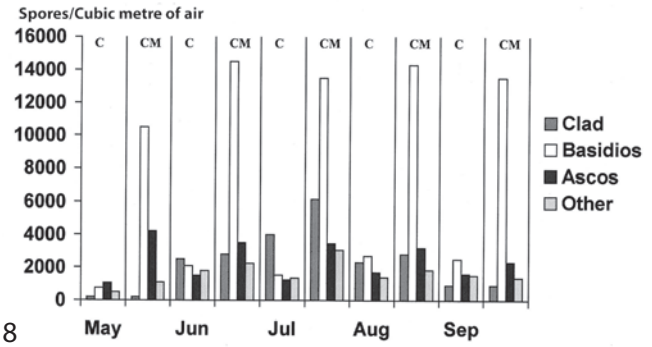
Cladosporium and *Alternaria* but also *Acremonium*, *Coniothyrium*, *Epicoccum*, *Fusarium*, *Phoma*, *Pleospora* and others. Unterseher and Schnittler (2009) recently reported that the endophytes that they most frequently isolated from healthy beech leaves were unspecified ascomycete cup-fungi, and species of *Phomopsis*, the pink yeast *Rhodotorula*, *Paecilomyces*, and *Nodulisporium*. *Alternaria alternata*, *Aureobasidium pullulans, Cladosporium cladosporioides*, *C. herbarum*, *Chrysosporium* sp. and *Epicoccum nigrum*. While *Alternaria* and *Cladosporium* are well-known as being allergenic, it should be mentioned that the anamorphic ascomycete *Nodulisporium* (teleomorph *Xylaria*) has been noted as causing allergic fungal sinusitis (Cox *et al*. 1994).



6



7



8

Fig. 6. Comparison between airborne fungal spores at a city centre site in Cardiff and at a coniferous woodland site at Resolven.
Fig. 7. Comparison between airborne fungal spores at a city centre site in Cardiff and at a mixed deciduous/coniferous woodland site at Tintern.
Fig. 8. Comparison between airborne fungal spores at a city centre site in Cardiff and at a mixed deciduous woodland site at Cefn Mably.

Surveys of the air spora at any site tend to be dominated by spores of local origin, with others of more distant origin forming only a smaller part of the total census. Some species are practically ubiquitous, whereas others are more or less confined to certain localities. It is therefore not surprising that the air spora of towns and cities will tend to be less abundant than that of the surrounding countryside, where such agricultural activities as mowing and grain harvesting result in the aerosolization of large numbers of spores and hyphal fragments.

It appears that most hyphal fragments have cross walls, or septa, and can be simple or branched. Pady and Kramer (1960) reported that they can vary considerably in size, with most 5-15 µm in length, but occasional fragments can be up to 100 µm in length. In their study in Kansas, these authors found that the majority of fragments were dark-coloured (mostly brown) and thick-walled, but hyaline fragments were also present. The fragments were frequently the terminal portions of conidiophores, sometime comprising only a single cell and often with an immature spore attached. They were noted throughout the year, but were more numerous in summer (175-1800 m^{-3} air) than in winter (35-210 m^{-3}). When collected on water agar in a slit-sampler, 29-82% of such fragments germinated and gave rise to colonies of *Alternaria*, *Cladosporium* and *Penicillium*, leading Pady and Kramer (1960) to consider that they would be an important means of asexual reproduction.

With the advent of scanning electron microscopy (SEM), it became possible to detect extremely small aerosolized particles of hyphae and spores. These particles are <1 µm in size and are referred to as submicron particles or fragments, and will be discussed in relation to the indoor environment in Chapter 1.2.

AVST surveys of fungi in outdoor air

Local variation in the temperate air spora

To illustrate the main characteristics of the outdoor air spora and some factors, which have a bearing on it, data gathered by the senior author from air sampling in and around Cardiff in UK will be examined. Cardiff, the capital city of Wales, lies in the southern part and is a large seaport on the north side of the Bristol Channel, approximately 220 km west of Lon-

don. Air in Cardiff has been sampled continuously since 1954 using an AVST at roof top level in the centre of the city.

The data obtained can be regarded as being typical for a north temperate climate and the incidence of different spores throughout the year in the UK and comparable climatic areas of mainland Europe are broadly similar.

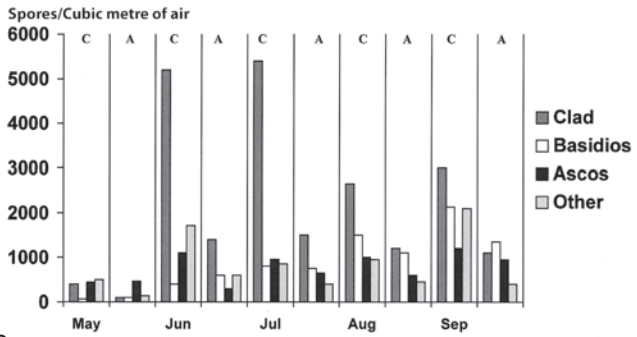
City centre site

In Figs 1-5, the incidence in the air of *Cladosporium*, ascospores, basidiospores, rust and smut spores, and particles of hyphae throughout the year is shown. It will be noticed that *Cladosporium* reaches its peak in July and August (Fig. 1), hyphal fragments reach their highest levels in August (Fig. 5), and rusts and smuts are most numerous in June when smut fungi infect the flowers of grasses (Fig. 4). Ascospores also reach their highest levels in August (Fig. 2), but basidiospores continue at high levels through the months of September and October (Fig. 3). Relative to *Cladosporium* conidia, ascospores and basidiospores (Figs 1-3), hyphal fragments (Fig. 5) form a minor component of the airborne fungal burden. Other investigations have also shown that hyphal fragments account for only a few per cent of fungal particles in outdoor air, e.g. those of Li and Kendrick (1995) and Delfino *et al.* (1997). Despite this, they can be a significant factor in asthma symptom severity (Delfino *et al.* 1997).

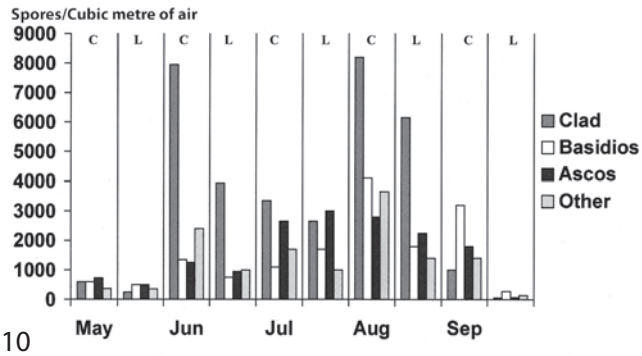
Comparison of the data obtained in urban Cardiff with counts obtained during the summer months at various outstations at woodland, coastal, upland valley and coastal plain sites shows the influence of local factors on spore concentrations. Since only one site distant from the city centre site was sampled in any particular year, it is not however possible to make direct comparisons between the outstations except as they contrast with Cardiff.

At Resolven (R), where the trap was positioned on the edge of a field of rough grass surrounded by plantations of Sitka spruce, Douglas fir and Japanese larch, spore concentrations were consistently higher than at Cardiff (C) after May (Fig. 6), because of the release of large numbers of ascospores and basidiospores in the plantations. Overall, the basidiospore totals were 217% and ascospore totals 206% of those at Cardiff. *Cladosporium* totals at Resolven were 61% of those at Cardiff and the other categories of fungi, including other imperfect fungi and rusts and smuts, were 83% of those recorded at Cardiff. This was due to relative paucity of local substrates for growth of these fungi within the plantations.

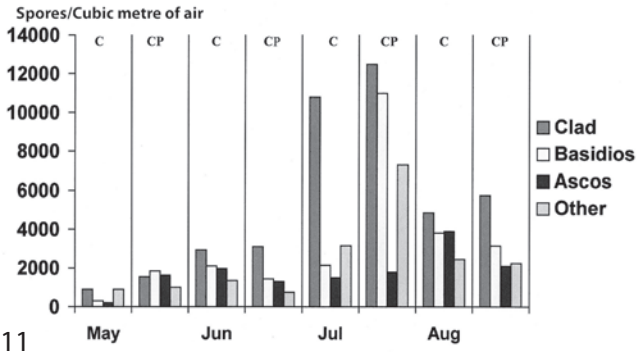
At Tintern, alongside a stream in a field surrounded by mixed deciduous and coniferous woodland with intervening fields and small orchards, the total spore concentrations (Fig. 7) were greater than at the city centre site each month with the exception of August. During that month there was a very high urban concentration of *Cladosporium*, which was 175% of that at Tintern, with an average daily concentration of 7000 spores m^{-3} air. Over the survey period basidiospore totals were 337% and ascospore totals 254% of those in Cardiff.



9



10



11

Fig. 9. Comparison between airborne fungal spores at a city centre site in Cardiff and at a seafront site at Aberystwyth.
Fig. 10. Comparison between airborne fungal spores at a city centre site in Cardiff and at an upland valley site at Llwynypia.
Fig. 11. Comparison between airborne fungal spores at a city centre site in Cardiff and at a farmland site at Cleppa Park.

Table 1. Mean concentrations of airborne fungal spores collected outdoors by Kramer-Collins drum sampler in and around Manhattan, Kansas, over a 10-day sampling period in 1979 (Kramer and Eversmeyer 1984).

Mean concentration (spores m^{-3} air)				
Location	*Cladosporium*	*Alternaria*	Ascospores	Basidiospores
Prairie	3852a	307ab	509ab	426a
Experimental farm	995b	183b	94b	121b
City park	2750a	452ab	894a	468a
New suburbs	4751a	561a	689ab	503a
Old suburbs	2872ab	403ab	688ab	465a
City centre	4063a	608a	685ab	555a

Mean values in the same column followed by the same letter are not significantly different from each other.

A trap at Cefn Mably (CM) was surrounded for a distance of 500-900 m by mixed deciduous woodland, parts of which had been neglected for some time and included large quantities of decaying timber. Total spore concentrations at this site were >300% greater than at Cardiff (Fig. 8). Basidiospore totals were 755% greater than at Cardiff and ascospores 375% greater. *Cladosporium* totals were also greater at this woodland site, averaging 130% of those at Cardiff. Owing to the proximity of many basidiocarps associated with decaying timber, the usual annual periodicity seen in Cardiff over a period of years was not replicated at Cefn Mably, where basidiospore levels were consistently high throughout the summer months.

These woodland surveys indicate that woodlands are, as might be expected, a prolific source of ascospores and basidiospores, the unmanaged mixed woodland (Fig. 8) being a greater source of spores than the managed coniferous or managed mixed coniferous and deciduous woodland. Herxheimer *et al.* (1969) demonstrated that basidiospores and ascospores can cause allergy and induce asthma attacks. Many cases of late summer asthma can be attributed to the inhalation of spores originating in woodlands.

Coastal site

Spore concentrations over the sea are generally much lower than those over the land, particularly at some distance from the land, owing to deposition of the spores on the sea without any compensating addition of spores (Hirst *et al*. 1967), so that traditionally a sea cruise was considered an effective way of avoiding seasonal allergens.

As the prevailing wind direction in UK is from the west, a west-facing coast should benefit from onshore air currents comparatively free of spores, unlike the east coast, which will receive the spore load picked up as the air currents cross the land. Fig. 9 shows a comparison between Cardiff city centre and a west coast site on Cardigan Bay.

This roof top site was at Aberystwyth (A), 120 km NW of Cardiff, and faced west near the seafront, from which town streets containing relatively few trees and gardens extended 600-900 m to the east, with agricultural land lying beyond that. Spore concentrations were consistently lower over the period of the survey, with total concentrations being only 44% of those in Cardiff. While both ascospore and basidiospore concentrations in Aberystwyth were around 80% of those in Cardiff, *Cladosporium* totals were only 32% of the corresponding Cardiff totals. The Aberystwyth counts confirm the trend to lower spore counts near a west-facing coast, particularly so in the case of *Cladosporium*.

Another example of this phenomenon has been recorded by Rodriguez-Rajo *et al*. (2005), who observed that a rooftop AVST in the west-coast city of Vigo, in the province of Galicia, NW Spain, gave lower spore counts than at two inland Galician locations. The total number of spores trapped in Vigo was less than 60% of the counts at the other two sites. At all three locations, *C. herbarum* predominated over the year, accounting for 57-68% of the annual total, with *C. cladosporioides* comprising 30-44% and *Alternaria* spp. no more than 1%. *Cladosporium* spore counts were again particularly low relative to the inland sites. As will be discussed later, another instance illustrating the difference between the air at coastal and at inland sites was noted by Prospero *et al*. (2005) on the Caribbean island of Barbados.

Upland valley site

At Llwynypia Hospital, in the Rhondda Valley 25 km northwest of Cardiff, a sampler was sited on the roof top of a building on a west facing slope covered for the most part by rough pasture and scrub vegetation with some hedgerows. On the opposite side of

the valley was an extensive conifer plantation, as well as more pasture and scrub vegetation. The valley, like all South Wales valleys, runs roughly in a north-south direction, and Davies (1969a,b) suggested that such a valley might mimic the situation of upland valleys in Switzerland. The relatively poor vegetation plus wind flows across the Swiss valleys produce environments with low spore concentrations, which have favoured the construction of sanitoria in these areas for the relief of asthma.

During the period from May to September (Fig. 10), overall spore counts (L) at Llwynypia were found to be 57% of those recorded in Cardiff, with basidiospores being 49%, ascospores 73% and *Cladosporium* 65% of the corresponding Cardiff totals. When Williams and Higgins (1959) carried out a survey of the incidence of asthma in the Rhondda Valley they did in fact observe a lower incidence of asthma compared with Cardiff, and lower concentrations of grass pollen, another cause of asthma, were also noted in the survey at Llwynypia mentioned above.

Coastal plain site

South of the valleys in South Wales is a coastal plain leading to the Bristol Channel. A trap was situated at Cleppa Park (CP), which is a rural site 14 km east of Cardiff. The trap was surrounded by fields used for cereal and vegetable production and for pasture, with small woodlands of mixed conifer and broad-leaved trees some 550-1190 m away.

The incidence of spores (Fig. 11) was higher than at Cardiff overall, with counts of basidiospores being 209%, ascospores 90% and *Cladosporium* 118% of those in Cardiff. However, in June and August, ascospore and basidiospore concentrations at Cardiff exceeded those at Cleppa Park. Surprisingly for a rural site, rust and smut spores in total were marginally lower than in the city, but together rusts and smuts accounted for only some 2-3% of the total air spora recorded at both sites. During July, the spore concentration at Cleppa Park was particularly high in relation to Cardiff, being some 186% of the city concentration.

The comparisons between the air spora at Cardiff and at the different outstations around the city illustrate, within one geographical area, the importance of local sources of spores in the concentration and make-up of the air spora. When Kramer and Eversmeyer (1984) sampled the air at four sites within the city of Manhattan, Kansas, and two sites outside the city, all within a radius of 10 km of the city, they found no significant difference between mean spore concentrations in major spore types at the different sites when tested with Duncan's multiple range test (Table 1). However, the authors noted that variations in concentration of the air spora between different sites were primarily due to differences in environmental conditions which influence the numbers and kinds of fungi that develop, sporulate and release spores, although the site descriptions do not indicate large vegetational differences between the environs of the different sites. However, the differences noted by Kramer and Eversmeyer (1984) were most pronounced in periods during which rain was sufficient to allow for abundant growth and sporulation of fungi locally. Conversely, during dry periods development of fungi in the area is slowed severely, if it occurs at all, so that overall the greatest percentage of the air spora at all these sites is composed primarily of spores transported through the atmosphere from remote sources. Unfortunately, the time of year during which the concentrations shown in Table 1 were recorded is not given, so comparisons cannot by made with data from other sites. The high numbers of *Alternaria* spores in the air suggest that the prairie is the likely source of spores found in the city.

Another instance in which high numbers of *Alternaria* in an urban environment were attributed to the growth of cereals in surrounding areas occurred when Corden *et al.* (2003) compared daily *Alternaria* records in the coastal city of Cardiff with those in urban Derby for the period 1970-1996. They noted that there had been a marked upward trend in the seasonal total for Derby, while the trend in Cardiff had been downwards. It was suggested that the difference could have been due to increased cereal production, together with higher midsummer temperatures, in the area around Derby and the smaller amount of arable production round Cardiff. Latterly, *Alternaria* counts had exceeded 10^3 spores m^{-3} air on some days in Derby, possibly triggering asthma attacks in *Alternaria*-sensitive patients, as they are known to provoke asthma attacks. Despite such differences, however, an investigation in Madrid, which has a continental Mediterranean climate, illustrates the broad similarity between the air spora in Cardiff and that in other European urban environments. In Madrid, Herrero *et al*. (2006) used a rooftop AVST and collected 70 different spore types, but three-quarters of the annual spore total was accounted for by four categories of spore. These were conidia of *Cladosporium* (41.1%); teliospores of the rust *Tilletia* (0.1%) and the smut fungus *Ustilago* (17.6%); basidiospores of *Coprinus* (8.7%) and other members of the Basidiomycota (5.9%); and ascospores of *Pleospora* (1.5%), *Leptosphaeria* (1.5%)

Table 2. Dominant fungal spores during 1992 from outdoor air of Waterloo, Ontario, expressed as mean concentration and percentage of total airspora (after Li and Kendrick, 1995).

Type	Whole year		May-October		November-April	
	Spores m^{-3}	% of total	Spores m^{-3}	% of total	Spores m^{-3}	% of total
Alternaria	62	1.8	139	1.9	13	1.3
Aspergillus/Penicillium	130	3.8	139	1.9	125	12.4
Cladosporium	1425	41.0	3018	40.8	421	41.9
Epicoccum	46	1.3	64	0.8	35	3.5
Leptosphaeria	183	5.3	454	6.1	12	1.1
Unidentified ascospores	185	5.3	403	5.5	48	4.7
Coprinaceae	174	5.0	444	6.0	5	0.5
Ganoderma	249	7.2	641	8.7	0.8	0.1
Unidentified basidiospores	422	12.1	992	13.4	62	6.2
Other unidentified spores	294	8.5	563	7.6	125	12.4
Hyphal fragments	171	4.9	258	3.5	116	11.6

and others (1.5%). An eight-year AVST study of airborne ascospores in another city with a true Mediterranean climate, Heraklion on the island of Crete, showed *Leptosphaeria* to be much more prominent in the air spora (Gonianakis *et al*. 2005). It formed 6.5% of the total fungal spora and 47.1% of the ascospore load, which also included spores of *Chaetomium* and, more sporadically, *Didymella*, *Leptosphaerulina* and *Pleospora*. Overall, the mean ascospore concentration was 30 spores m^{-3} air day^{-1}, which approximates to 13.9% of the total airborne mycobiota (Gonianakis *et al.* 2005).

The predominance of *Cladosporium* can again be seen in the southern hemisphere, where in a comparable temperate climate Mitakakis and her colleagues (Mitakakis *et al*. 1997, Mitakakis and Guest 2001) surveyed the air spora in the Australian city of Melbourne. Among the spores of 29 genera and five other groups collected by AVST, *Cladosporium* conidia (41.7% of the total spores), *Leptosphaeria* ascospores (14.9%) and *Coprinus* basidiospores (14.6%) comprised nearly three-quarters. Rust and smut teliospores amounted to 12.0% of the total, ascospores other than *Leptosphaeria* 5.5%, and *Ganoderma* basidiospores 2.1%. None of the other fungi, including *Alternaria*, *Periconia*, *Drechslera* and other phylloplane fungi such as *Epicoccum*, *Fusarium* and *Stemphylium*, amounted to more than 1% of the total. Small hyaline spores, including those of *Aspergillus* and *Penicillium* were not recorded, although it was known from Andersen sampler culture plates that they were present in the air. The total counts showed marked annual variation, with the count one year being nearly three times those of the succeeding two years (Mitakakis *et al*. 1997). In the third year, *Cladosporium* numbers were approximately 1.5 times those in the two earlier years.

Seasonal and diurnal variation in the air spora

Seasonal variations similar to those in Cardiff (Figs 1-5) have been recorded in Waterloo, Ontario (Table 2), where Li and Kendrick (1995) sampled outside air on a balcony over a period of a year using a particle sampler, which took 10-min samples of the air every two hours. Compared with the spore concentrations recorded in Cardiff in 1992, the concentrations of *Cladosporium,* ascospores and basidiospores recorded in Ontario were all higher, with respective average concentrations of 1425, 368 and 845 spores m^{-3} air in Ontario and 871, 161 and 248 m^{-3} in Cardiff, and the percentages of the total air spora respectively 41, 10.6 and 24.1% in Ontario compared with 55, 10.2 and 15.7% in Cardiff. However, concentrations for Cardiff in 1992 differ from the 30-year averages for the city, in which *Cladosporium* accounts for 41% of the total air spora, ascospores for 17.2%, and basidiospores 20.6%.

Trends broadly similar to those shown in Fig. 1 have been noted for *Cladosporium* elsewhere in Europe. Gravesen and Schou (1997) noted that in a Danish study *Cladosporium* spores were most abundant in late July/August (Fig. 12), whilst *Alternaria* peaked in late August, both much later than allergenic tree pollens, which variously peaked between March and May, and grass pollen, which was most abundant in June (Fig. 12). In the Spanish province of Galicia, Rodriguez-Rajo *et al*. (2005) observed that the highest counts of *C. herbarum*, the more abundant of two

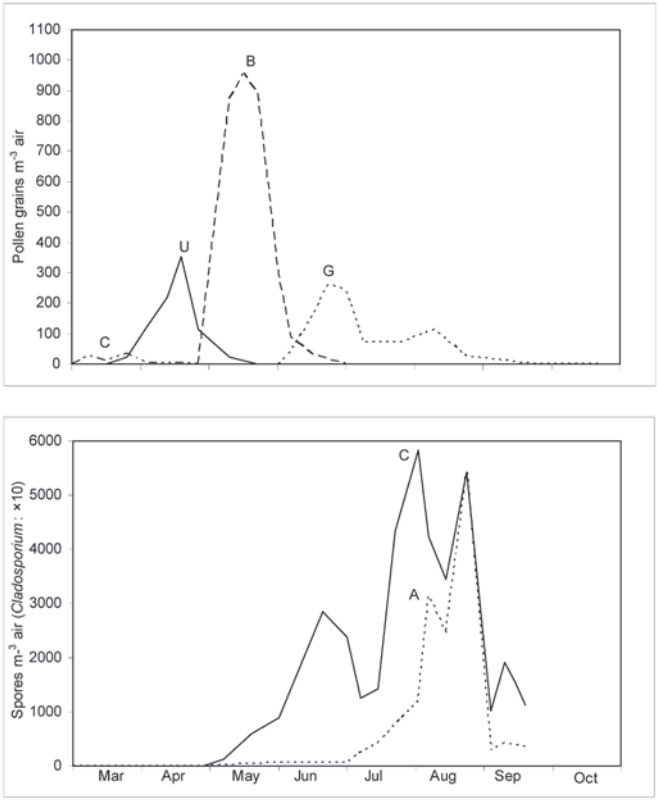


Fig. 12. The seasonal occurrence in outdoor air of pollen and mould spores relevant to allergy (adapted from Gravesen and Schou 1997). Upper panel: B, *Betula* (birch); C, *Corylus* (hazel); G, *Gramineae* (grasses); U, *Ulmus* (elm). Lower panel: A, *Alternaria*; C, *Cladosporium*.

species of *Cladosporium*, occurred in the summer months, particularly June-September, with peak levels in August. On the other hand, *C. cladosporioides* levels did not begin to rise until August and the highest counts were encountered in September-October. For *Alternaria,* the greatest counts were recorded between July and September (Rodriguez-Rajo *et al*. 2005). In contrast, in the city of Leon in the neighbouring province *Cladosporium* counts peaked in October one year, but during the following year maximum levels were reached in July (Fernández *et al*. 1998). *Alternaria* counts were at their greatest in August of the first year and in July of the second year.

That such variation in seasonality between different studies is largely a result of climatic differences can be seen from three European studies previously mentioned. In Madrid (Herrero *et al*. 2006), with its continental Mediterranean climate, the maximum occurrence of the four main spore types differed from those presented in Figs 1-4 for Cardiff. Teliospores peaked earlier (May-June), and ascospores (September/October), *Cladosporium* (October) and basidiospores (October/November) all peaked later than in Cardiff. In the true Mediterranean climate of Heraklion (Gonianakis *et al*. 2005), total ascospore numbers were relatively high in mid-spring and remained so throughout the summer, with *Leptosphaeria* reaching maxima of up to 70 spores m^{-3} air in June. In an AVST survey in the continental climate of the Polish city of Cracow (Stepalska and Wolek 2005) counts of the spores of most of 13 selected fungi peaked in August. These included *Alternaria* and *Epicoccum* conidia and *Ganoderma* basidiospores, but numbers of *Didymella* ascospores were highest in July. In contrast to both Cardiff (Fig. 1) and Madrid, *Cladosporium* numbers peaked during June.

In addition to recording seasonal variation, Li and Kendrick (1995) noted diurnal periodicity in ascospore and basidiospore concentrations, with an increased prevalence during the early hours of the morning, similar to that for the prevalence of *Didymella* ascospores (Allitt 1986) observed in UK (Richardson 1996). Richardson derived his data using an AVST for continuous sampling on a rooftop and found an association between increased spore concentrations and relative humidity during the early hours of the morning. In addition to the diurnal periodicity of ascospores and basidiospores, Li and Kendrick (1995) observed periodicity in the prevalence of conidia of *Cladosporium* and other imperfect fungi, which are released passively. These conidia tended to increase in number during the afternoon, with the highest concentration of *Cladosporium* at 16.00 h and of *Alternaria* at 18.00 h. Three AVST studies in Spain indicate, however, that there can be marked temporal variation in peak concentrations between different locations. In the first of these, *Alternaria* was described by Giner *et al*. (2001) as being a "late afternoon taxon" in SE Spain; its "maximum" numbers in the air were reported to occur between 13.00 and 21.00 h. However, the data appear to show an absolute maximum at around 19.00 h on each of six years. In the northwestern province of Galicia, Rodriguez-Rajo *et al.* (2005) observed the greatest numbers of *C. cladosporioides, C. herbarum* and *Alternaria* spp. between 19.00 and 22.00 h, whereas in the neighbouring province Fernández *et al*. (1998) noted maximum numbers of *Cladosporium* and *Alternaria* conidia between 12.00 and 14.00 h.

Culture plate surveys of fungi in outdoor air

Temperate climates

The limitations of trapping airborne inoculum on agar plates has already been mentioned, but it is worth considering the mistaken impressions of the

air spora which may result from relying on sampling methods based on culture. When comparing AVST and volumetric culture plate sampling by a wind-orientated Andersen sampler, Burge *et al.* (1977) noted that *Cladosporium* spore concentrations assessed by both methods varied directly. However, as AVST spore levels rose the culture plate data progressively underestimated prevailing concentrations, giving low estimates of prevalence (20-40%) at levels below 100 spores m^{-3} and falling to below 5% at levels above 500 spores m^{-3}. Several additional taxa were also substantially understated in abundance and regularity. However, in a recent study in the Greek city of Athens, Pyrri and Kapsanaki-Gotsi (2007) found that for *Cladosporium* a single-plate Burkard sampler gave counts only 5% less than corresponding total counts on a Burkard personal volumetric air sampler, and *Alternaria* 35% less.

Sampling for culturable airborne fungi in Copenhagen using a slit sampler (Table 3) indicated a much higher percentage incidence of *Cladosporium* at 68.9% (Larsen 1981) and 77.8% (Larsen and Gravesen 1991) than was found using the AVST in Cardiff or the particle sampler in Ontario (Li and Kendrick 1995). Whereas it is possible to differentiate a much greater number of imperfect fungi in the air and to record a range of yeasts by culture-based methods, it is important to realise that the large numbers of ascospores and basidiospores in the air are not recorded on culture plates, and so sampling onto agar medium is likely to have biased the values for the percentage incidence of *Cladosporium* recorded by the Danish workers.

Despite their limitations, culture plate methods are frequently used in assessing the indoor air spora and have also been used to effect in surveys of outdoor air. For example, using the Andersen N6 single-plate sampler (Shelton *et al*. 2002) carried out a countrywide survey over three years, sampling inside and outside some 1700 buildings in USA, and taking 12,000 samples in all, 2,400 of which were outdoors. There were considerable differences between regions: the highest levels of culturable fungi outdoors (in summer and autumn) were found in the Southeast, Southwest and Far West regions, and the lowest in the Northwest region. The commonest of the culturable fungi in the outdoor air during all seasons and in all regions were *Cladosporium* (when detected, median concentration approx. 200 CFU m^{-3} air), *Penicillium* (approx. 50 CFU m^{-3}), *Aspergillus* (including *Eurotium* and *Emericella,* approx. 20 CFU m^{-3}) and non-sporulating fungi (approx. 100 CFU m^{-3}). A wide range of other taxa were detected, including the toxigenic genus *Stachybotrys*, which was present in the outdoor air at 1% of the buildings under study. Just how wide the range of culturable fungi in outdoor air can be is evident in a qualitative study in Turin, Italy (Airaudi and Marchisio 1996). In a yearlong survey employing a Surface Air Systems single-plate sampler, these workers isolated 170 different species. However, when Lugauskas *et al*. (2003) used a combination of a liquid impinger, an open-faced filter sampler and settle plates to examine the airborne mycobiota near busy streets in five Lithuanian cities, some 430 species were recorded. Among these, the vast majority (83%) were mitosporic fungi, i.e. those for which no sexual stage has been found. Some 45 species were in the Zygomycota (more than one-half in the family Mucoraceae) and 21 species in the Ascomycota, but no members of the Basidiomycota were mentioned in their paper. Surprisingly, *Aspergillus fumigatus* and *A. niger* were among the species most frequently isolated (detection frequencies approx. 57 and 84%, respectively), together with *Alternaria alternata* (63%), *Aureobasidium pullulans (57%)*, *Botrytis cinerea* (43%), *Cladosporium herbarum* (64%), *C. cladosporioides* (57%), *C. sphaerospermum* (28%), *Geotrichum candidum* (33%), and *Penicillium funiculosum* (46%). The two active sampling methods used revealed that propagule counts were greatest in the industrial areas of Vilnius, and those close to heavily used highways, reaching approx. 6400 CFU m^{-3} air in summer. Corresponding counts for the four other (smaller) cities ranged from approx. 500 to 4500 CFU m^{-3}.

As mentioned above, culture-based sampling methods enable a wider range of yeasts to be identified than can be differentiated by cell morphology after AVST. For example, when Rantio-Lehtimäki (1988) sampled the air specifically for yeasts in southern Finland over a one-year period using an Andersen sampler with yeast-peptone-D-glucose agar culture

Table 3. Principal viable fungi collected by slit sampler from outdoor air in Copenhagen, as percentage of total colonies on slit-sampler collection plates.

Type	1977-79[a]	1978-87[b]
Cladosporium	68.9	77.8
Alternaria	9.4	3.0
Penicillium	6.0	2.5
Aspergillus	2.5	0.8
Non-sporing filamentous fungi	6.3	n.s.
Yeasts	4.1	n.s.

a Larsen (1981); b Larsen and Gravesen (1991).

plates, she found that concentrations of yeasts in the air never exceeded 50 CFU m^{-3} air. The predominant yeast genus isolated on the agar plates was *Sporobolomyces,* followed by *Rhodotorula* and *Cryptococcus,* all of which are basidiomycetous yeasts. Ascomycetous yeasts including *Candida* were caught infrequently.

Employing a slit sampler, Larsen (1981) found that yeasts comprised only 4.1% of the air spora in Copenhagen. The mirror yeasts *Sporobolomyces* and *Tilletiopsis* can be identified on AVST slides or tapes, and Gregory and Sreeramulu (1958) reported *Sporobolomyces* concentrations of up to 10^6 spores m^{-3} air over an estuary in the south of England; and routine monitoring of the air at Cardiff over 30 years showed an average *Sporobolomyces* spore concentration of 167 m^{-3} air, with a maximum of 27297 m^{-3}, and an average for *Tilletiopsis* of 48 m^{-3} air, with a maximum of 7920 m^{-3}. This reinforces the view of Burge *et al.* (1977) that culture plate methods seriously underestimate the spore content of the atmosphere.

Dust events

In the same era as Miquel observed a boost in the air spora with dust-raising activities such as street cleaning, Carnelley *et al*. (1887) recorded that in dry weather the raising of dust by wind increased numbers of microorganisms overall, and greatly increased the ratio of bacteria to fungi in the streets of two large Scottish towns. Over the years since then, aerobiological studies have continued to demonstrate the importance of risen dust as a contributor to both the outdoor and the indoor air spora. Two types of dust event that can result in augmentation of the outdoor air spora are discussed below.

The first type occurs during building construction and destruction, which are significant in altering the nature and magnitude of the air spora locally. Airborne dust and associated spores generated and dispersed externally during constructional work can penetrate into nearby buildings. Such penetration is of particular relevance in hospitals, since it presents a potential health risk to patients. Among the spores associated with dust particles that are generated and dispersed during construction those of thermotolerant species are of particular concern. These fungi, able to grow at body temperature (37°C), include some aspergilli that can behave as opportunistic pathogens and are a particular hazard to immunocompromised patients. *Aspergillus fumigatus* is the most important of these pathogens, but *A. flavus*, *A. niger* and *A. terreus* may also present a risk (Fitzpatrick *et al*. 1999).

Demolition of buildings may have an even greater effect than construction activities in elevating microbial numbers in outside air. In reporting the effect of explosive demolition of a hospital building in Minnesota, Streifel (1983) noted that relative to levels before the explosion samples taken 15 m from the building 2 min after the explosion revealed a 1.8-log increase in thermotolerant fungi to 1.6×10^5 CFU m^3 air. Among these thermotolerant fungi, *A. fumigatus* and *A. niger* showed 3.3- and 1.5-log increases to 8.4×10^2 and 1.4×10^4 CFU m^3, respectively. At a sampling site about 60 m away from the building, the collective counts, and those for *A. fumigatus* and *A. flavus*, remained high for 45 min before declining. In another study of hospital demolition, in Madrid (Bouza *et al*. 2002), concentrations of airborne fungi were found to be much lower than in the Minnesota investigation, however. During the five days before demolition by controlled explosion the thermotolerant average count was 17.6 CFU m^3 air; after the explosion it was 70.2 CFU m^3; and after falling to little over half that level it rose again to 74.5 CFU m^3 four days later during a second, mechanical phase of demolition; not until 11 days after the explosion had counts fallen back to levels roughly the same as before demolition. Although the identity of two-thirds of the isolates was not established by Bouza *et al*. (2002), the principal identified taxon was *A. fumigatus,* and *A. niger, A. flavus, Mucor, Alternaria, Fusarium* and unspecified penicillia were also isolated.

While building and demolition activities have a local effect on the air spora, it has been estimated that dust events of the second type, desert dust storms, result in 2.2×10^9 metric tons of dust becoming airborne and being transported annually across continents and oceans (Goudie and Middleton 2001). Satellite imagery has shown long distance migration of dust clouds from desert areas in Asia, Africa, North America and also Australia and South America. It is those originating in the 9×10^6 km^2 of the Sahara Desert which have the greatest global impact, with the dust reaching Southern, and much less frequently, Northern Europe and the British Isles; the Eastern Mediterranean; Caribbean islands; and North and South America (Griffin et al. 2001b). With the knowledge that spore dispersal can spread plant diseases between countries and across continents (Gregory 1973), it has been a concern that microorganisms in the surface layers of the soil that is aerosolized might have implications for human health and ecosystems downwind of desert storms. It is this concern that has driven research into these dust events, especially so in relation to the deposition of Saharan dust in the

Caribbean islands and south-eastern USA (Griffin *et al*. 2001a, 2003, Prospero *et al*. 2005).

In an investigation on the west coast of St John in the US Virgin Islands airborne microorganisms were collected on membrane filters during dust events, cultured on agar plates and subsequently identified by comparison of 16S and 18S rDNA sequences with those in GenBank (Griffin *et al*. 2001a). Very few airborne fungi were found. *Cladosporium cladosporioides*, *Coccodinium bartschii*, *Gibberella pulicaris* and *Pleospora rudis* were isolated on a clear day, but in a dust event one week later only the first two species were detected. Three days after that, during a second dust event, *C. bartschii*, *Cochliobolus sativus* and *P. rudis* were the only fungi isolated. However, using traditional culture-based identification methods Prospero *et al*. (2005) isolated a much wider range of species on the east coast of Barbados. Although nearly 60% of the isolates were not identified, *Arthrinium* and *Periconium* together comprised three-quarters of those that were, with (in descending order) *Penicillium*, *Curvularia*, *Cladosporium*, aspergilli (principally *Aspergillus niger*, but also *A. fumigatus*, *A. clavatus*, *A. terreus* and *A. flavus*), *Neurospora* (*Chrysonilia*) and *Alternaria* being minor components. Inland, numbers were much greater and the composition was rather different, with *Cladosporium* amounting to one-third, and in descending order *Aspergillus* (*A. niger*, *A. flavus*), *Bipolaris*, *Curvularia* and *Penicillium* collectively comprising one-half of the total. In a follow-up to Griffin *et al*. (2001a), airborne culturable fungi at a site on the south coast of the neighbouring island of St Thomas were collected during an African dust event (Griffin *et al*. 2003). The fungi isolated belonged mainly to the genera *Cladosporium*, *Aspergillus* and *Penicillium*. Smaller numbers of *Microsporium*, *Bipolaris*, *Paecilomyces*, *Acremonium* and *Nigrospora* were encountered. In none of these investigations is there clearcut evidence that the source of the fungi detected was Africa; the evidence from Prospero *et al*. (2005) suggests that the composition of the air spora at the sampling sites on St John and St Thomas (Griffin *et al*. 2001a, 2003) could have been influenced by *Cladosporium* and other phylloplane fungi from local sources being entrained by the trade winds crossing the islands from the east.

Nevertheless, Griffin *et al*. (2006) found that the airborne mycobiota at a mid-Atlantic research site in the transatlantic pathway from West Africa included common penicillia and phylloplane fungi such as *Alternaria* spp., *Aureobasidium pullulans* and *Phoma herbarum* (Table 6). The most frequently isolated species were the *Cladosporium*-like ascomycete, *Lojkania enalia*, and another ascomycete, *Neotestudina rosatii*, which is common in tropical soils and is a cause of mycetoma in humans. There was a statistically significant relationship between the number of culturable microorganisms recovered and atmospheric dust levels. However, the concentration of culturable fungi in the air was only around 1% of that recorded during desert storms in Mali, West Africa (Kellogg *et al*. 2004), where *Alternaria* sp., *Cladosporium cladosporioides, Aspergillus niger* and *A. versicolor* were identified by their micro-morphology. This could be expected because of the extended period of exposure to UV light, desiccation and other stresses during long-distance transport, and would suggest that an even smaller proportion of microorganisms reaching the Americas would be culturable.

Table 4. Percentage frequency of viable airborne fungi collected during 1988 on settle plates exposed to outdoor-air in the city of Natal in tropical Brazil (after de Oliviera *et al.* 1993).

Type	Dry season	Rainy season
Aspergillus	77.7	55.3
Penicillium	62.9	40.0
Fusarium	16.6	44.6
Cladosporium	25.9	10.7
Curvularia	11.1	20.0
Rhizopus	11.i	16.9
Rhodotorula	12.9	9.2
Neurospora	12.9	3.0
Drechslera	1.8	10.7
Aureobasidium	5.5	4.0
Trichoderma	-	6.1
Cunninghamella	1.8	3.0

On the eastern Mediterranean coast it was also found that dust storms in North Africa lead to increased concentrations of microorganisms in the atmosphere in Haifa, Israel (Schlesinger *et al*. 2006). Counts for culturable fungi during separate dust events were approximately two and seven times those on clear days, but bacterial counts were around nine times greater. The commonest species during the dust events was *Penicillium chrysogenum*, which was 20-30 times more abundant than on clear days. *Aspergillus fumigatus* and *P. griseoroseum* were also prominent but not detected on clear days, while *Alternaria alternata*, *A. niger*, *A. thomii and P. glabrum* counts were greater for one or other event than on the clear days. On the Mediterranean coast of Turkey, Griffin *et al*. (2007) isolated *Acremonium*, *Alternaria*,

Cladosporium, *Fusarium*, *Microsporum*, *Penicillium* and *Trichophyton* from samples of airborne dust of regional and North African origin. The maximum number of culturable fungi in the air recorded on dust event days was more than twice the non-dust day maximum and, unusually, around 12 times greater than the corresponding bacterial count. During a cruise across the Mediterranean from Tel Aviv to Istanbul, however, Waisel *et al.* (2008) detected very little particulate matter from a dust cloud originating in North Africa and moving eastwards at an altitude of 3000 m. The mean daily AVST spore count in mid-sea was 300-750 spores m^{-3} air, but was much higher nearer the Turkish and Israeli coasts, repectively 1200-2400 and 340-1695 m^{-3} air. The major component of these counts was *Cladosporium*. The authors inferred that north-northwesterly winds at lower altitudes were probably responsible for bringing these spores from Turkey, Greece and the Balkans across the Mediterranean to Israel.

Tropical and subtropical climates

The air spora in tropical and subtropical regions is likely to be substantially different from that in temperate regions of the world. This is exemplified by a study of Oliveira *et al.* (1993), who used settle plates to study the air spora at five locations around the city of Natal in tropical Brazil over a one-year period. A similar incidence was observed at all of the sites, each of which was sampled for 15 min every two weeks. The lack of volumetric sampling did not allow estimations of spore concentrations, and the incidence of fungi is expressed as percentage frequency of fungal isolation (Table 4), but from this it is apparent that the relative incidence of fungi in Natal is quite different from that reported in Copenhagen (Larsen 1981, Larsen and Gravesen 1991). *Aspergillus* and *Penicillium* were isolated with a very high percentage frequency especially during the dry season and there was a dramatic increase in *Fusarium* during the rainy season.

In contrast, *Cladosporium*, which dominates the air spora in temperate regions had a comparatively low frequency, although this was not true for all cities in Brazil and the authors quote percentage frequencies from other cities where the incidence of *Cladosporium* was much greater, but overall the incidence of *Aspergillus* and *Penicillium* throughout the surveys quoted tends to be higher than that reported in temperate regions.

In subtropical Taiwan, Li and Hsu (1995) used an Andersen sampler with malt extract agar culture plates to compare spore concentrations within and outside the Taipei homes of atopic and non-atopic children between July and September (Table 5). The numbers of airborne CFU m^{-3} air recorded were comparatively low, with *Cladosporium* having a lower incidence than *Aspergillus* and *Penicillium*. Hurtado *et al.* (1989) sampled the air in the mildly tropical climate of Bogota in Colombia for pollen and spores for a year and found that *Cladosporium* was the most frequently recorded genus, with the combination *Penicillium/Aspergillus* being next. The findings in the tropical and subtropical surveys show a different pattern of fungal spore incidence, and spores of *Aspergillus* and *Penicillium* appear to be more prevalent in the air than in temperate regions of Europe and North America.

Table 5. Concentration of viable fungi (colony forming units) collected by Andersen N6 impaction sampler from outdoor air of homes of asthmatic, atopic and control children in Taiwan (after Li and Hsu 1995). Geometric mean concentration (CFU m^{-3} air)

Type	Asthmatic	Atopic	Control
	(n = 46)	(n = 20)	(n = 26)
Alternaria	1.7	1.5	1.8
Aspergillus	17.2	22.5	23.6
A. clavatus	1.1	1.1	1.2
A. flavus	4.7	2.7	5.9
A. fumigatus	1.1	1.1	1.2
A. niger	4.7	6.7	5.9
Cladosporium	19.5	7.3	3.8
Curvularia	1.7	1.3	1.9
Fusarium	4.3	1.8	5.5
Paecilomyces	2.2	1.5	2.0
Penicillium	47.6	55.5	101.0
Trichoderma	1.1	1.1	1.2
Yeasts	70.3	73.8	72.5
Total	547	449	668

Although aspergilli and penicillia are generally less abundant in outdoor air in temperate regions, a range of species in both genera may be isolated by culture plate sampling for spores in the atmosphere (Hudson 1969, Fradkin *et al.* 1987). The opportunistic respiratory pathogen *Aspergillus fumigatus*, for example, may be detected. In a comparative study of *A. fumigatus* in the air of St Louis in USA and Cardiff in UK (Fig. 13) over a 12-month period using Andersen samplers, sampling onto Blakeslee's malt extract agar culture plates which were incubated at 37°C to eliminate other fungi, average concentrations recorded were 13.5 CFU m^{-3} in St Louis and 11.3 CFU m^{-3} in Cardiff and the seasonal incidence of the fungus was similar

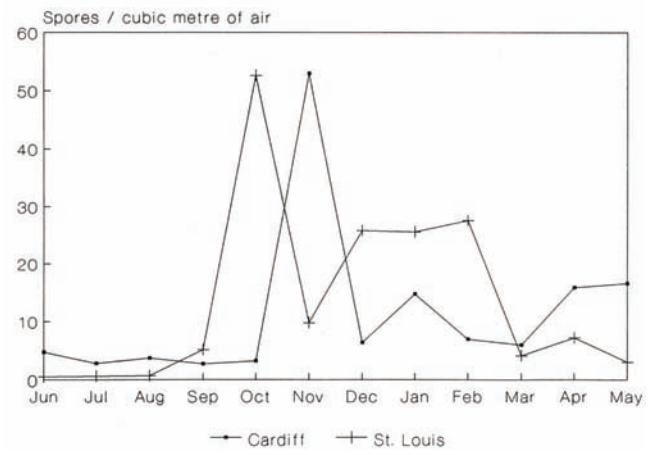


Fig. 13. Outdoor culturable *Aspergillus fumigatus* concentrations in Cardiff and St. Louis (after Mullins, Hutcheson and Slavin 1984)

at both sites (Mullins *et al.* 1984).

In order to sample for *A. fumigatus* in the air both culture media and incubation conditions were modified to favour growth of this fungus at the expense of any competing fungi. In particular, richer media, such as Blakeslee's malt extract agar, favour *Aspergillus* and *Penicillium*. Blakeslee's 1915 formula for the agar contains 2% malt extract, 2% glucose and 0.1% peptone, as opposed to the unamended 2% malt extract usually recommended for isolation of the general run of fungi from air. In all surveys of the air spora the sampling method chosen will favour sampling of certain fungi and it should be remembered, as mentioned previously, that the sampling techniques used can have a major influence on the results of the survey.

Owing to the important effect of weather conditions on spore release, daily spore counts can vary considerably; in some fungi spore discharge is associated with drying whereas in others damp conditions are required. Raindrops can discharge basidiospores from puffballs, and Walkey and Harvey (1968a,b) observed that ascospore discharge through the ostiole of the ascocarps in pyrenomycetes (flask fungi) was stimulated by rainfall. Many spores are thrown up by rain splash, e.g. in *Chaetomium* (Harvey *et al.* 1969), so that certain spore types are characteristically associated with damp conditions and others with dry conditions. The variability of conditions during the day can affect spore concentrations and it was found in UK that fungi such as the yeast *Sporobolomyces* tend to occur in high concentrations during the night when humidity is highest, whereas *Cladosporium* has its highest concentration in the day when humidity is at its lowest (Hirst 1953, Gregory and Sreeramulu 1958). This was also noted in Ontario by Li and Kendrick (1995). Even light can act as a contributory factor in spore release, and Ingold (1971) has described light and dark induced rhythms of spore discharge in a number of fungi.

The regular seasonal cycles of the year determine which fungal spores are likely to be available for release into the air. Thereafter the development of the fungi and the subsequent release of their spores will depend on weather conditions. The concentration will further be dependent on location and prevailing winds.

Table 6. Culturable fungi isolated from tropical air in mid-Atlantic Ocean (~15° N, 45°W) and identified using nucleic acid sequencing methodology (Griffin *et al*., 2006).

GenBank closest relative
Alternaria brassicae, Embellisia sp., *Pezizaceae*
Alternaria dauci
Alternaria sp.
Aureobasidium pullulans or *Discosphaerina fagi*
Cladosporium sp.
Cladosporium sp. or *Trimmatostroma macowanii*
Dendryphion sp.
Lojkania enalia
Lithothelium septemseptatum
Massaria platani
Myriangium duriaei
Myrothecium sp., *Letendraea helminthicola, Cucurbidothis pityophila, Paraphaeosphaeria* sp. *(P. pilleata* or *P. michotii)*
Neotestudina rosatii
Penicillium chrysogenum, P. glabrum, Penicillium sp. or *Talaromyces leycettanus, Penicillium* sp., *Uscovopsis* sp., *Thysanophora* sp., *Chromocleista malachitea, T. leycettanus* or *Eupenicillium* sp.
Phoma herbarum
Pleosporaceae
Preussia terricola
Setosphaeria monoceras, Pleospora herbarum or *Embellisia* sp.
S. monoceras, S. rostrata or *Cochliobolus sativus*
S. rostrata
Stachybotrys kampalensis
Trichophyton mentagrophytes or *T. rubrum*
Ulocladium botrytis
U. botrytis, Clathrospora diplospora or *Alternaria sp.*

BACTERIA IN OUTDOOR AIR

Surveys of outdoor air for bacteria are much less common than for fungi. The interest in airborne bacteria tends to be confined to the possible presence of pathogens, but even when sewage sludge is being applied to land (Pillai *et al*. 1996) bacterial pathogens appear to be absent from the outdoor air, although they may possibly be present at levels below the limits of detection of the methods employed.

Counts of airborne culturable bacteria vary widely, and great differences can be noted over very short time intervals. For example, Lighthart and Shaffer (1994) observed as much as a 14-fold difference over 2 min. Numbers are affected by variables such as location, the time of day and the season, meteorological conditions, and the type of sampler and isolation medium employed. Differences between locations were demonstrated in Oregon by Shaffer and Lighthart (1997). The average count for a major thoroughfare in Corvallis (725 CFU m^{-3} air) was higher than for a ryegrass field and a fir forest, and more than six times greater than at a headland on the Pacific coast that was exposed to onshore sea breezes during sampling days. In the rye-grass field, when the grass was mature the average count was 127 CFU m^{-3} air, but after harvesting the seed by combine it had risen to 704 CFU m^{-3}. Chaff, seed and cut straw scattered on the ground by combining were likely to have been sources contributing to the increased numbers. Contrasting with these findings, when Köck *et al.* (1998) sampled at seven sites around Graz in Austria they found that, at 327 CFU m^{-3} air, bacterial counts in an agricultural area were four times higher than in a suburban residential area. In the vicinity of a composting facility the counts were 29% higher than in the residential area, but at an industrial and business site affected by heavy traffic numbers were twice those at the composting facility.

The well-known shortcomings of methods involving culture in assessing the fungal burden in air that have already been mentioned (Burge *et al*. 1977) also apply to airborne bacteria. Lighthart (2000) concluded from studies by his group using a wet cyclone bioaerosol sampler in Oregon that the culturable numbers were on average only around 1% of the total bacterial burden in the atmosphere, but ranged from as low as 0.01% to as much as 75% of the total. In a dust plume downwind of a combine harvesting grain, 73% of the 2.9×10^6 cells m^{-3} air were culturable (Lighthart and Tong 1998). Other counts in midsummer were 8.8×10^2 to 5.9×10^5 m^{-3}, but only 0.02-10.6% of the bacteria were culturable. Diurnal periodicity was observed, with both total counts and the culturable percentage (39%) being highest during the day and lowest (0.5-2% culturable) at dawn and dusk (Lighthart and Tong 1998). However, in other studies (Lighthart 2000) peak concentrations were noted at dawn in inland forested, rural-agricultural and urban areas, although not at coastal sites.

Table 7. Culturable bacteria isolated from air on a clear day and during dust events in St John, US Virgin Islands, and identified to genus or species by 16S/18S rDNA gene sequencing (Griffin et al., 2001).

		No. of isolates	
GenBank closest relative	Gram stain	Clear day	Dust events
Arthrobacter globiformis	G+ve	2	
Arthrobacter sp.	G+ve		1
Bacillus megaterium	G+ve	1	1
B. pumilis	G+ve		1
Curtobacterium albidum	G+ve		1
C. citreum	G+ve	2	5
C. luteum	G+ve		4
Curtobacterium sp.*	G+ve		1
Kocuria erythromyxa	G+ve		1
Microbacterium arborescens	G+ve		3
M. testaceum	G+ve		6
*M. testaceum**	G+ve		1
Microbacterium sp	G+ve		2
Paracoccus aminovorans	G-ve		1
Paracoccus sp.	G-ve		1
Pseudomonas alcalophila	G-ve		1
Ps. oleovorans	G-ve		1
Ps. riboflavina	G-ve		1
Sinorhizobium sp.	G-ve		1
Sphingomonas pruni	G-ve		4
S. trueperi	G-ve		1
Sphingomonas sp.	G-ve	1	4
Sphingomonas sp.*	G-ve		1

*Unidentified isolates with homology to named taxon

Most investigations have indicated that culturable bacteria in outdoor air are predominantly Gram-positive. For example, Fang *et al*. (2007) recently reported that in Beijing, China, counts at three locations within the city ranged from 71 CFU m^3 to 2.2×10^4 CFU m^3, and 165 species in 47 genera were identified. Gram-positive bacteria comprised on average 84% of the total, 53% being cocci and 31% rods. At nearly 27% of

Table 8. Species of culturable bacteria isolated from air in Bamako, Mali, during four dust events and identified to genus or species by 16S rNA/18S rDNA gene sequencing (Kellog *et al.*, 2004)

Acinetobacter calcoaceticus[e]	*Gordonia terrae*[a]
Acinetobacter sp. phenon 2[e]	*Kocuria erythromyxa*[b]
Agrococcus jenensis	*K. polaris*[c]
Arthrobacter nicotianae	*K. rosea*
A. protophormiae	*Kocuria sp.*
Aureobacterium liquifacens[d]	*Microbacterium barkeri*
Bacillus aminovorans	*Micrococcus luteus*
B.endophyticus	*Micrococcus* sp. (2 different)
B. flexus	Paenibacillus illinoiensis
B. firmus	*Paenibacillus* sp.
B. kangii	*Paracoccus* sp.
B. megaterium	*Planococcus* sp.
B. mycoides	*Planomicrobium koreense*
B. niacini	*P. mcmeekinii*
B. pumilus[b]	*Rhodococcus ruber*
B. subtilis[b]	*Saccharococcus* sp.
Bacillus sp. (5 different)	*Staphylococcus gallinarum*
Corynebacterium cf. *aquaticum**[c]	*S. xylosus*
Corynebacterium sp.	*Streptomyces* sp.[a]
Deinococcus erythromyxa[d]	*Zoogloea ramigera*[e]
Dietzia sp.[a]	

[a] Actinobacteria, [b] Also isolated from non-dust air sample, [c] Only isolated from non-dust air sample, [d] Identified by fatty acid profiling, [e] Gram-negative.

the total, the dominant genus was *Micrococcus*, with *Staphylococcus* (12%), *Bacillus* (7%), *Corynebacterium* (4%) and the Gram-negative genus *Pseudomonas* (4%) the most prominent among the other genera.

The previously mentioned investigations of the air spora associated with Saharan dust clouds has also provided us with much additional information on the nature of bacteria in the atmosphere. In the first of these (Griffin *et al*. 2001a), only seven strains of bacteria were cultured before dust events on the island of St John, all pigmented and therefore likely more resistant to solar radiation (Table 7). More than 40 isolations were made during the dust events, 60% of which were Gram-positive. Most were pigmented, including six of the 10 Gram-negative types, i.e. isolates of *Sphingomonas* sp., *Pseudomonas alcalophila* and *Ps. oleovorans*. On St Thomas, Griffin *et al*. (2003) isolated a wider range of bacteria, 60% of these being Gram-positive, including four species in the Actinobacteria. The members this phylum have a higher GC content in their DNA than other Gram-positive bacteria. Within the phylum a range of species form branching filaments that resemble the hyphae of fungi and are classified in the order Actinomycetales. This name derives from them formerly being classified among the fungi as the Actinomycetes, and they are still frequently referred to as actinomycetes. The relatively large proportion of Gram-negative types in this and the earlier study appears to suggest local rather than Saharan origin. This would be supported by the rather different balance in mid-Atlantic (Griffin *et al*. 2006), where only two isolates (possibly of marine origin) were Gram-negative. The average count of airborne culturable bacteria here was only 0.1 - 0.4 CFU m^{-3} air, representing a roughly 10^4 reduction from levels measured during dust storms in Mali, West Africa (Kellogg *et al*. 2004). With a single exception, the levels in Mali were also one to two orders of magnitude greater than those during dust events in the Virgin Islands (Griffin *et al*. 2001a, 2003). Many of the bacterial colonies were highly pigmented, and 96% of isolates were Gram-positive (Table 8). Many of the Gram-positive species were spore formers commonly found associated with soil and dust. The predominance of Gram-positive bacteria in investigations where culture methods are involved can be attributed to the greater susceptibility of Gram-negative bacteria to solar radiation and desiccation.

As a review by Peccia and Hernandez (2006) shows, our perception of the composition of the bacterial burden in the atmosphere changes markedly when PCR-based methods are applied directly in identification, i.e. without any cultivation step and therefore being based on dead as well as living cells. For example, when Brodie *et al.* (2007) investigated urban air in two cities in Texas, San Antonio and Austin, they found at least 1,800 types of bacteria, a phylogenetic diversity approaching that in some soil-borne communities. Statistical analysis revealed that location was less important as a factor in explaining variability in the composition of the bacterial burden than were temporal and meteorological influences. An investigation in Colorado, during which 4-h samples were collected into liquid in an SKC Biosampler and DNA was extracted directly from the samples, further illustrates this bacterial diversity (Fierer *et al*. 2008). A survey of the small-subunit ribosomal RNA gene sequences generated indicated a preponderance of Gram-negative bacteria in the samples. The putative identities of the most abundant of these

were *Flavobacterium/Chryseobacterium, Flexibacter,* and *Hymenobacter* in the *Cytophaga-Flavobacterium-Bacteroides* (CFB) group, *Acidovorax*, *Acinetobacter*, *Bradyrhizobium, Burkholderia*, *Caulobacter*, Comamonadaceae, Enterobacteriaceae, *Methylobacterium*, *Nitrosovibrio*, *Pseudomonas*, *Rhizobium*, *Rhodobacter* and *Sphingomonas*. The Gram-positive genera *Arthrobacter* (an actinobacterium) and *Planococcus* (*Planomicrobium*) were among the most abundant bacteria on one of the five sample days. In relation to Gram-negative bacteria, the Gram-positive genera *Micrococcus* and *Bacillus* were relatively rare (<2% of bacterial sequences). It should be mentioned that, applying the same methodology, Fierer *et al*. (2008) also noted that the airborne mycobiota was dominated by fungi in the Hypocreales.

Clearly, these findings show that the differences in results that are associated with employment of different sampling and analytical procedures not only for fungi apply equally to airborne bacteria. They also confirm the conclusions of Miquel (1899) from his sampling in Paris, that the main source of outdoor bacteria is the surface of the ground.

CONCLUSION

Hyde (1969) characterized the air spora as being an "expression of climate, a reflex of the vegetation as a whole, and an essential factor or complex of factors in the general environment". As this chapter has shown, such essential factors include weather, prevailing winds, the local vegetation, topography and human activity.

What we learn about the outdoor air spora depends very much on sampling techniques, which have a major influence on the results obtained. The use of different techniques in different surveys can make comparisons difficult. There is, as yet, and there may never be, a sampling technique to satisfy all the requirements of aerobiologists, and the sampling technique chosen will depend on resources, the nature and number of sites to be sampled, the facilities available and the information which is sought from the survey. It continues to be necessary to have regard to the sampling technique when considering the results from any survey, and to be cautious when making comparisons between surveys.

REFERENCES

Airaudi, D. and Marchisio, V.F. (1996) Fungal biodiversity in the air of Turin. *Mycopathologia*, **136**, 95-102.

Allitt, U. (1986) Identity of airborne hyaline, one-septate ascospores and their relation to inhalant allergy. *Trans. Br. Mycol. Soc.*, **87**, 147-154.

Andersen, A.A. (1958) New sampler for the collection, sizing and enumeration of viable airborne particles. *J. Bact*., **76**, 471-484.

Battarbee, J.I., Rose, N.L., and Long, X. (1997) A continuous high resolution record of urban airborne particulates suitable for retrospective microscopical analysis. *Atmos. Environ.*, **31**, 171-181.

Bouza, E., Pelaez, T., Perez-Molina, J., *et al*. (2002) Demolition of a hospital building by controlled explosion: the impact on filamentous fungal load in internal and external air. *J. Hosp. Infect.*, **52**, 234-242.

Brodie, E.L., DeSantis, T.Z., Moberg Parker, J.P., *et al*. (2007) Urban aerosols harbor diverse and dynamic bacterial populations. *PNAS*, **104**, 299-304.

Bulloch, W. (1938) *The History of Bacteriology*. Clarendon Press, Oxford.

Burge, H.P., Boise, J.R., Rutherford, J.A., and Solomon, W.R. (1977) Comparative recoveries of airborne fungus spores by viable and non-viable modes of volumetric collection. *Mycopathologia*, **61**, 27-33.

Cadham, F.T. (1924) Asthma due to grain rusts. *JAMA*, **83**, 27.

Carnelley, T., Haldane, J.S., and Anderson, A.M. (1887) The carbonic acid, organic matter and micro-organisms in air, more especially in dwellings and schools. *Phil. Trans. Royal Soc., Series B*, **178**, 61-111.

Chamberlain, A.C. (1966) Transport of *Lycopodium* spores and other small particles to rough surfaces. *Proc. Roy. Soc., A*, **296**, 45-70.

Corden, J.M., Millington, W.M., and Mullins, J. (2003) Long-term trends and regional variation in the aeroallergen *Alternaria* in Cardiff and Derby UK – are differences in climate and cereal production having an effect. *Aerobiologia*, **19**, 191-199.

Cox, G.M., Schell, W.A., Scher, R.L., and Perfect, J.R. (1994) First report of involvement of *Nodulisporium* species in human disease. *J. Clin. Microbiol*., **32**, 2301-2304.

Davies, R.R. (1969a) Aerobiology and the relief of asthma in an Alpine valley. *Acta Allergol*., **24**, 377-395.

Davies, R.R. (1969b) Climate and topography in relation to aeroallergens at Davos and London. *Acta Allergol*., **24**, 296-409.

Delfino, R.J., Zeiger, R.S., Seltzer, J.M., *et al*. (1997) The effect of outdoor fungal spore concentrations on daily asthma severity. *Environ. Health Perspect.,* **105**, 622-635.

Dobell, C. (1932) *Antonie van Leeuwenhoek and his "Little Animals"*. Bale and Danielsson, London.

Emberlin, J., Adams-Groom, B., and Tidmarsh, J. (1999) *The Dispersal of Pollen from Maize,* Zea mays. *A Report Based on Evidence Available from Publications and Internet Sites*. The Soil Association, Bristol, UK.

Fang, Z., Ouyang, Z., Zheng, H., *et al*. (2007) Culturable airborne bacteria in outdoor environments in Beijing, China. *Micro. Ecol*., **54**, 487-496.

Fernández, D., Valencia, R.M., Molnár, T., *et al*. (1998) Daily and seasonal variations of *Alternaria* and *Cladosporium* airborne spores in León (North-West Spain). *Aerobiologia*, **14**, 215-220.

Fierer, N., Liu, Z., Rodriguez-Hernandez, M., *et al*. (2008) Short-term temporal variability in airborne bacterial and fungal populations. *Appl. Environ. Microbiol.*, **74**, 200-207.

Fitzpatrick, F., Prout, S., Gilleece, A., *et al*. (1999) Nosocomial aspergillosis during building work – a multidisciplinary approach. *J. Hosp. Infect*., **42**, 170-171.

Fradkin, A., Tobin, R.S., Tarlo, S.M., *et al*. (1987) Species identification of airborne molds and its significance for the detection of indoor pollution. *JAPCA*, **37**, 51-53.

Fröhlich-Nowoisky, J., Pickersgill, D.A., Després, V.R., and Pöschl, U. (2009) High diversity of fungi in air particulate matter. *PNAS*, **106**, 12814-12819.

Giner, M.M., Garcia, J.S.C., and Camacho, C.N. (2001) Airborne *Alternaria* spores in SE Spain (1993-98): occurrence patterns, relationship with weather variables and prediction models. *Grana*, **40**, 111-118.

Gonianakis, M., Neonakis, I., Darivianaki, E., *et al*. (2005) Airborne Ascomycotina on the island of Crete: seasonal patterns based on an 8-year volumetric survey. *Aerobiologia*, **21**, 69-74.

Goudie, A.S., and Middleton, N.J. (2001) Saharan dust storms: nature and consequences. *Earth. Sci. Rev.*, **56**, 179-204.

Gravesen, S., and Schou, C. (1997) *Allergi og Anden Overfølsomhed*. Munksgaard, Copenhagen, Denmark

Gregory, P.H. (1973) *Microbiology of the Atmosphere*, 2nd Ed. Leonard Hill, Aylesbury, Bucks, UK.

Gregory, P.H., and Sreeramulu, T. (1958) Air spora of an estuary. *Trans. Br. Mycol. Soc.*, **41**, 145-156.

Griffin, D.W., Garrison, V.H., Herman, J.R., and Shinn, E.A. (2001a) African desert dust in the Caribbean atmosphere: microbiology and health. *Aerobiologia*, **17**, 203-213.

Griffin, D.W., Kellog, C.A., and Shinn, E A. (2001b) Dust in the wind: long range transport of dust in the atmosphere and its implications for global public and ecosystem health. *Global Change & Human Health*, **2**, 20-33.

Griffin, D.W., Kellog, C.A., Garrison, V.H., *et al*. (2003) Atmospheric microbiology in the northern Caribbean during African dust events. *Aerobiologia*, **19**, 143-157.

Griffin, D.W., Westphal, D.L., and Gray, M.A. (2006) Airborne microorganisms in the African desert dust corridor over the mid-Atlantic ridge, Ocean Drilling Program, Leg 209. *Aerobiologia*, **22**, 211-226.

Griffin, D.W., Kubilay, N., Kocak, M., *et al*. (2007) Airborne desert dust and aeromicrobiology over the Turkish Mediterranean coastline. *Atmos. Environ.*, **41**, 4050-4062.

Harvey, R.R. (1967) Air spora studies at Cardiff. I. *Cladosporium*. *Trans. Br. Mycol. Soc*., **50**, 479-495.

Harvey, R.R. (1970) Spore productivity in *Cladosporium*. *Mycopath. Mycol. Appl*., **41**, 251-256.

Harvey, R.R. Hodgkiss, J.J., and Lewis, P.N. (1969) Air-spora studies at Cardiff. II. *Chaetomium*. *Trans. Br. Mycol. Soc*., **53**, 269-278.

Herrero, A.D., Ruiz, S.S., Bustillo, M.G., and Morales, P.C. (2006) Study of airborne fungal spores in Madrid. *Aerobiologia*, **22**, 135-142.

Herxheimer, H., Hyde, H.A., and Williams, D.A. (1969) Allergic asthma caused by basidiospores. *Lancet 2*, 131-133.

Hirst, J.M. (1952) An automatic volumetric spore trap. *Ann. Appl. Biol*., **39**, 257-265.

Hirst, J.M. (1953) Changes in atmospheric spore content: diurnal periodicity and the effects of weather. *Trans. Br. Mycol. Soc*., **36**, 375-393.

Hirst, J.M., Stedman, O.J., and Hurst, G.W. (1967) Long distance spore transport: vertical sections of spore clouds over the sea. *J. Gen. Microbiol*., **48**, 357-377.

Hudson, H. J. (1969) Aspergilli in the air-spora at Cambridge. *Trans. Brit. Mycol. Soci.,* 52, 153-159.

Hurtado, I., Leal Quevedo, F.J., Rodriguez Ciodaro, A., *et al*. (1989) A one year survey of airborne pollen and spores in the neotropical city of Bogota (Colombia). *Allergol. Immunopathol. (Madrid)*, **17**, 95-104.

Hyde, H.A. (1969) Aeropalynology in Britain – an outline. *New Phytol*., **68**, 579-590.

Ingold, C.T. (1971) *Fungal Spores: Their Liberation and Dispersal*. Clarendon Press, Oxford.

Kellogg, C.A., Griffin, D.W., Garrison, V.H., *et al*. (2004) Characterization of aerosolized bacteria and fungi from desert dust events in Mali, West Africa. *Aerobiologia*, **20**, 99-110.

Köck, M., Schlachter, R., Pichler-Semmelrock, F.P., *et al*. (1998) Airborne microorganisms in the metropolitan area of Graz, Austria. *Cent. Eur. J. Public Health*, **6**, 25-28.

Kramer, C.L., and Eversmeyer, M.G. (1984) Comparisons of airspora concentrations at various sites within a ten kilometer radius of Manhattan, Kansas, USA. *Grana*, **23**, 117-122.

Larsen, L.S. (1981) A three-year-survey of microfungi in the air of Copenhagen 1977-79. *Allergy*, **36**, 15-22.

Larsen, L.S., and Gravesen, S. (1991) Seasonal variation of outdoor viable microfungi in Copenhagen, Denmark. *Grana*, **30**, 467-471.

Levetin, E., and Dorsey, K. (2006) Contribution of leaf surface fungi to the air spora. *Aerobiologia*, **22**, 3-12.

Li, C.S., and Hsu, L.Y. (1995) Fungus allergens inside and outside the residences of atopic and control children. *Arch. Environ. Health*, **50**, 38-43.

Li, D.W., and Kendrick, B. (1995) A year-round outdoor aeromycological study in Waterloo, Ontario, Canada. *Grana*, **34**, 199-207.

Lighthart, B. (2000) Mini-review of the concentration variations found in the alfresco atmospheric bacterial populations. *Aerobiologia*, **16**, 7-16.

Lighthart, B., and Shaffer, B. T. (1994) Bacterial flux from chaparral into the atmosphere in mid-summer at a high desert location. *Atmos. Environ*., **28**, 1267-1274.

Lighthart, B., and Tong, Y. (1998) Measurements of total and culturable bacteria in the alfresco atmosphere using a wet-cyclone sampler. *Aerobiologia*, **14**, 325-332.

Lugauskas, A., Šveistytė, L., and Ulevičius, V. (2003) Concentration and species diversity of airborne fungi near busy streets in Lithuanian urban areas. *Ann. Agric. Environ. Med.*, **10**, 233–239.

May, K.R. (1945) The cascade impactor: an instrument for sampling coarse aerosols. *J. Sci. Instrum*., **22**, 187-195.

Miquel, P. (1878-99) Annual reports, in *Annu. Obs. Montsouris*. Baulthier-Villars, Paris.

Mitakakis, T., Ong, E.K., Stevens, A., *et al*. (1997) Incidence of *Cladosporium, Alternaria* and total fungal spores in the atmosphere of Melbourne (Australia) over three years. *Aerobiologia*, **13**, 83-90.

Mitakakis, T.Z., and Guest, D.I. (2001) A fungal spore calender for the atmosphere of Melbourne, Australia, for the year 1993. *Aerobiologia*, **17**, 171-176.

Mullins, J., Harvey, R., and Seaton, A. (1976) Sources and incidence of airborne *Aspergillus fumigatus* (Fres.). *Clin. Allergy*, **6**, 209-217.

Mullins, J., Hutcheson, P., and Slavin, R.G. (1984) *Aspergillus fumigatus* spore concentrations in outside air: Cardiff and St. Louis compared. *Clin. Allergy*, **14**, 351-354.

Oliveira, M.T.B., Santos Braz, R.F., and Ribeiro, M.A.G. (1993) Airborne fungi isolated from Natal, State of Rio Grande do Norte – Brazil. *Rev. Microbiol*., *São Paulo*, **24**, 198-202.

Pady, S.M., and Kramer, C.L. (1960) Kansas aeromycology, VI: hyphal fragments. *Mycologia*, **52**, 681-687.

Pasteur, L. (1861) Mémoire les corpuscles organisés qui existent dans l'atmosphère. Examen de le doctrine des générations spontanées. *Ann. Sci. Nat. (Zool.), 4e Sér.*, **16**, 5-98.

Peccia, J., and Hernandez, M. (2006) Incorporating polymerase chain reaction-based identification, population characterization, and quantification into aerosol science: a review. *Atmos. Environ.*, **40**, 3941-3961.

Pillai, S.D., Widmer, K.W., Dowd, S.E., and Ricke, S.C. (1996) Occurrence of airborne bacteria and pathogen indicators during land application of sewage sludge. *Appl. Environ. Microbiol.*, **62**, 296-299.

Prospero, J.M., Blades, E., Mathison, G., and Naidu, R. (2005) Interhemispheric transport of viable fungi and bacteria from Africa to the Carribean with soil dust. *Aerobiologia*, **21**, 1-19.

Pyrri, I., and Kapsanaki-Gotsi, E. (2007) A comparative study on the airborne fungi in Athens, Greece, by viable and non-viable sampling methods. *Aerobiologia*, **23**, 3-15.

Rantio-Lehtimäki, A. (1988) Yeasts in rural and urban air in southern Finland. *Grana*, **27**, 313-319.

Raynor, G.S., Ogden, E.C., and Hayes, J.V. (1970) Dispersal and deposition of ragweed pollen from experimental sources. *J. Appl. Meteorol.*, **9**, 885-895.

Richardson, M.J. (1996) The occurrence of airborne *Didymella* spores in Edinburgh. *Mycol. Res.*, **100**, 213-216.

Rodriguez-Rajo, F.J., Iglesias, I., and Jato, V. (2005) Variation assessment of airborne *Alternaria* and *Cladosporium* spores at different bioclimatical conditions. *Mycol. Res.*, **109**, 497-507.

Schlesinger, P., Mamane, Y., and Grishkan, I. (2006) Transport of microorganisms to Israel during Saharan dust events. *Aerobiologia*, **22**, 259-273.

Schulz, B., and Boyle, C. (2005) The endophytic continuum. *Mycol. Res.*, **109**, 661-686.

Shaffer, B.T., and Lighthart, B. (1997) Survey of culturable airborne bacteria at four diverse locations in Oregon: urban, rural, forest, and coastal. *FEMS Microbiol. Ecol.*, **34**, 167-177.

Shelton, B.G., Kirkland, K.H., Flanders, W.D., and Morris, G.K. (2002) Profiles of airborne fungi in buildings and outdoor environments in the United States. *Appl. Environ. Microbiol.*, **68**, 1743-1753.

Stakman, E.C., and Hamilton, L.M. (1939) Stem rust in 1938. *Pl. Dis. Reptr.*, **117** (Suppl.), 69-83.

Stepalska, D., and Wolek, J. (2005) Variation in fungal spore concentrations of selected taxa associated to weather conditions in Cracow, Poland, in 1997. *Aerobiologia*, **21**, 43-52.

Streifel, A. J., Lauer, J. L., Vesley, D., *et al*. (1983) *Aspergillus fumigatus* and other thermotolerant fungi generated by hospital building demolition. *Appl. Environmental Microbiol.*, **46**, 375-378.

Unterseher, M., and Schnittler, M. (2009) Dilution-to-extinction cultivation of leaf-inhabiting endophytic fungi in beech (*Fagus sylvatica* L.) – Different cultivation techniques influence biodiversity assessment. *Mycol. Research*, **113**, 645-654.

Waisel, Y., Ganor, E., Epshtein, V., *et al*. (2008) Airborne pollen, spores, and dust across the Mediterranean Sea. *Aerobiologia,* **24**, 125-131.

Wakefield, A.E., Stringer, J.R., Tamburrini, E., and Dei-Cas, E. (1998) Genetics, metabolism and host specificity of *Pneumocystis carinii*. *Med. Mycol.*, **36** (Suppl. 1), 183-193.

Walkey, D.G.A., and Harvey, R. (1966) Spore discharge rhythms in Pyrenomycetes. *Trans. Brit. Mycol. Soc.*, **49**, 583-592.

Walkey, D.G.A., and Harvey, R. (1968a) Spore discharge rhythms in Pyrenomycetes. IV. The influence of climatic factors. *Trans. Brit. Mycol. Soc.*, **51**, 779-786.

Walkey, D.G.A., and Harvey, R. (1968b) Spore discharge rhythms in Pyrenomycetes. V. The effect of temperature on spore discharge in *Sordaria macrospora*. *Trans. Brit. Mycol. Soc.*, **51**, 787-789.

West, J.S., Atkins, S.D., and Fitt, B.D.L. (2008) Detection of airborne plant pathogens: halting epidemics before they start. In J.S.West and P.J. Burt, (eds.), *Aspects 89: Applied Aspects of Aerobiology,* Association of Applied Biologists, Wellesbourne, Warwick, UK, pp. 11-14.

Williams, D.A., and Higgins, I.T.T. (1959) The prevalence of asthma in two communities in South Wales. *Acta Allerg.*, **13**, 126-127.

Womiloju, T.O., Miller, J.D., Mayer, P.M., and Brook, J.R. (2003) Methods to determine the biological composition of particulate matter collected from outdoor air. *Atmos. Environ.*, **37**, 4335-4344.

This page intentionally left blank

Chapter 1.2

MICROORGANISMS IN INDOOR AIR

Brian Flannigan

Scottish Centre for Pollen Studies, Napier University, Edinburgh, UK.

INTRODUCTION

Bioaerosols in indoor environments comprise a range of microscopic biological particles of animal, plant and microbial origin that can be inhaled and impinge on human health. Allergenic animal particulates include dander and dried saliva of pets, such as cats and dogs, and other furred animals; fragments of insects such as cockroaches, fleas and clothes moths; fragmented bodies and faeces of storage and house dust mites; and amoebae and other unicells belonging to the Protozoa. Plant particulates include unicellular algae and fragments of multicellular algal filaments; spores of lower plants such as mosses and ferns; and minute fragments and whole pollen grains of higher plants, which are discussed in Chapter 1.3. Although protozoans and unicellular algae are microorganisms, the most medically important microbial particulates in indoor air belong to neither of these groups, but are the cells and spores of fungi and bacteria, and viruses. In this chapter, the different categories of microorganism (including algae and protozoa) present in indoor air are described in general terms. Because of the diversity of forms, fungi are dealt with in more detail than the other categories. Where the health implications of individual groups are not dealt with in detail in later chapters, they are discussed briefly here.

COMPONENTS OF BIOAEROSOLS IN INDOOR ENVIRONMENTS

Algae

Nature of algae in indoor air and house dust

Although it has been shown that they can behave as inhalant allergens and cause rhinitis and asthma, microalgae comprise a largely ignored category of microorganism in indoor environments. These organisms contain chlorophyll and are therefore photosynthetic, unlike the heterotrophic fungi and bacteria to be considered later. The types most often isolated are green (the plant division Chlorophyta) and blue-green algae (now recognized as being bacteria and therefore classified as Cyanobacteria), and diatoms (Chrysophyta). They may be unicells, simple chains of cells, cylindrical filaments or colonies (composed of up to 50,000 unicells organized into coherent structures). Some are macroscopic membranous or tubular structures composed of very large numbers of cells. Unlike the other types, the unicellular diatoms are enclosed by a two-piece wall of crystalline silica, which has been likened to a Petri dish. The shape of these diatoms varies, with centric diatoms being radially symmetrical and pinnate diatoms being bilaterally symmetrical.

Although algae may be generated indoors, the most usual sources of the organisms in indoor environments are unicells or fragments of filaments in air infiltrating from outside and dust blown in or carried in on clothing or animal fur. These algae normally grow in water or in soil, and on rocks and the bark of trees. They are aerosolized by wind, mechanical disturbance of soil or dust, bubble phenomena or forced aeration of aqueous environments ranging from aquaria to sewage disposal plants (Sharma *et al*. 2007). The role of the wind in dispersal has been observed by Torma *et al.* (2001) when they continuously sampled the outdoor air of the city of Badajoz (SW Spain). Most of the airborne algae were members of the order Chlorococcales and small centric diatoms (particularly *Cyclotella*). Some filamentous green algae and even coenobia (a coenobium is a colony of

undifferentiated cells) of the colonial type *Pediastrum*. The concentrations of airborne algae correlated positively with temperature, and negatively with relative humidity, but wind speed also appeared to have a positive effect on concentrations of diatoms and the coenobia of members of the Chlorococcales. When Chrisostomou *et al.* (2009) investigated diversity of air-dispersed algae from a Greek river-reservoir system, they also found evidence that, at least over short distances, the wind was an important agent of dispersal of the algae, of which *Chlorella* was among the most frequent and abundant, with the larger types *Mougeotia* and *Ulothrix* also being frequent.

Counts of algae in the air are not likely to exceed those of pollen grains or fungal spores (Tiberg 1987). In USA, the most prominent types detected by cultural methods in house dust collected during the indoor heating season in homes were the unicellular green algae *Chlorella*, *Chlorococcum*, *Chlamydomonas* and *Planktosphaeria* and the filamentous cyanobacteria *Anabaena* and *Schizothrix* (Bernstein and Safferman 1970). In Sweden, 88% of dust samples collected by Tiberg *et al*. (1984) from carpets and beds in the homes of atopics contained viable algae. The genera were mostly the same as those found outdoors, but with cyanobacteria being found in slightly higher frequency. The most frequent types were the unicellular green algae *Chlorococcum*, *Chlorella* and *Stichococcus* and the cyanobacteria *Nostoc*, *Phormidium* and *Anabaena*. The same types dominated in dust from a health care centre, a recreation centre and several nursery schools. In the nursery schools, the diatoms *Hantzschia* and *Navicula* were occasionally isolated. Although counts of green algae in these schools peaked in June, the cyanobacteria showed no marked seasonal differences.

Health effects of algae

Since the allergenic properties of algae are discussed in Chapter 3.1, it is sufficient to note here that it is more than 40 years since it was first reported that they were a cause of respiratory allergy in children and that in skin tests they reacted positively in patients with histories of inhalant allergies (McElhenny *et al*. 1962, McGovern *et al*. 1966, Bernstein and Safferman 1967). It is now recognized that, in addition to members of the Chlorophyta such as *Chlorella* and *Chlorococcum* (Tiberg *et al.* 1984,1995), cyanobacteria such as *Anabaena*, *Nostoc* and *Phormidium* are allergenic (Tiberg 1987, Sharma and Rai 2008).

Although there appear to be no reports of algae in air or house dust causing dermatitis, a case of contact dermatitis has been reported in a swimmer exposed to *Anabaena*, one of a number of species forming "algal blooms" in freshwater lakes (Cohen and Reif 1953). There are also rare human cases of cutaneous or disseminated infections by unicellular *Prototheca*. For example, a disseminated infection by achlorophyllous *P. wickerhami* in a male with a specific deficiency in cell-mediated immunity was reported from New Zealand (Cox *et al*. 1974). However, *Prototheca* is generally found in soil and it is thought that soil contact is the major cause of infection.

Protozoa

Nature of Protozoa in indoor environments

Motile free-living members of this group of unicellular animals are found scavenging on organic debris in soil and water outdoors, and may also be found indoors in bodies of water such as aquaria, humidifier reservoirs and HVAC drainage pans (Schlichtung 1969), and also in plumbing systems (Steinert *et al.* 1997). Although some have cilia and others flagella that propel them through water, most of the free-living protozoans that impinge on human health are amoeboid, moving and engulfing nutrient particles by means of protoplasmic extensions, pseudopodia. They become aerosolized by mechanisms ranging from forced aeration of aquatic environments such as sewage ponds or aquarium tanks and dispersion of water droplets by humidifiers to scouring of drying soil by wind. An example of soils being the most likely source of airborne protozoa is provided by an investigation of outdoor air in Rapid City, South Dakota, in which Rogerson and Detwiler (1999) collected the cysts of 25 morphologically different protozoans from the air. The commonest of these were flagellates and naked amoebae, while ciliates were rare. This reflected their natural occurrence in the soil. In general, the concentration of cysts increased as a function of total airborne particulates. The concentration of both particulates and cysts exhibited a wide range of variability, but the highest concentrations of cysts and total particulates were recorded on days with higher winds and lower relative humidity.

The types of protozoan that have been isolated from air in a variety of investigations have been collated by Schlichtung (1969), and the types that may be present in indoor environments have been indicated by Rohr *et al*. (1998). Amoebae in hospital hot water systems were identified as *Hartmannella vermiformis* and species of *Echinamoeba, Saccamoeba*

Table 1. Abbreviated classification of some fungi found as spores in indoor air.

Phylum	Sub-phylum	Genera
Ascomycota	Pezizamycotina	*Emericella, Eurotium, Peziza, Alternaria*, Aspergillus*, Botrytis*, Epicoccum*, Penicillium*, Phoma*, Stachybotrys**
	Saccharomycotina	*Saccharomyces*
Basidiomycota	Agaricomycotina	*Agaricus*, *Coprinus, Ganoderma, Sistotrema, Serpula*, *Filobasidiella* (*Cryptococcus*)
	Wallemiomycetes†	*Wallemia*
Zygomycota	Mucoromycotina	*Absidia, Cunninghamella, Mucor, Rhizopus*

*Anamorphic genus, †Class

and *Valkampfia*, and species of *Acanthamoeba, Hartmanella, Naegleria, Valkampfia* and *Vanella* were isolated from moist areas in bathrooms and showers, sink drains and water taps (Rohr *et al.* 1998). *Hartmannella castellani* has been isolated from the air of a respiratory care unit (Kingston and Warhurst 1969).

Health effects of Protozoa in indoor environments

As far as health is concerned, the protozoans best known as affecting human health are those parasites that are spread by insect bite, causing diseases such as malaria, sleeping sickness, Chaga's disease and leishmaniasis. However, there are free-living protozoans that may occasionally cause disease. The widespread species *Naegleria fowleri* is occasionally pathogenic, causing a fatal meningoencephalitis. It is an amoeboflagellate, existing in flagellate form in water and as an amoeba in human tissues. In nature, it also forms cysts that are protected from adverse environmental conditions by a thick wall. Species of *Acanthamoeba* are also widespread and may cause keratitis and encephalitis, most particularly in immunocompromised individuals (Curry *et al.* 1991). They also cause ulceration of the skin and cornea (Baron *et al*. 1994). The cysts of these amoebae are readily dispersed by air currents, contaminating aqueous environments ranging from brackish water to hot tubs, and also soil and dust. It seems probable that infection with *N. fowleri* is most often the result of contaminated water entering the nose, for example during swimming, but it is considered that in the case of acanthamoebae inhalation may be responsible as well as direct contact with contaminated waters or soil. Rohr *et al.* (1998) found six potentially pathogenic strains of *Acanthamoeba*, but none of *Naegleria*, colonizing shower heads, drains, walls and tiles in bathrooms. They postulated that infection might occur by inhalation of aerosolized *Acanthamoeba* in sprayed water.

Members of the Protozoa growing within buildings have also been implicated in humidifier fever. Edwards *et al*. (1976) isolated both amoebae and ciliates from a contaminated humidifier serving an office in which a number of workers suffered this febrile disease. Extracts of the amoebae, but not the ciliates, reacted with the sera of affected workers, and antigenic identity between extracts of the amoebae, dust on a suspended ceiling and a culture-collection strain of *Naegleria gruberi* was demonstrated. Protozoa such as *Acanthamoeba, Hartmannella* and *Naegleria* have been isolated from plumbing systems contaminated with bacteria in the genus *Legionella* (Steinert *et al.* 1997) and the presence of the bacterium in amoebae such as *A. polyphaga* has been demonstrated (Newsome *et al*. 1998). Some 13 amoebae and two ciliates were shown to provide an environment in which legionellae can replicate prolifically (Kwaik *et al*. 1998), and it was also shown that two species of *Acanthamoeba* can expel vesicles 2.1-6.4 μm in diameter containing living *L. pneumophila* (Berk *et al*. 1998). Aerosolization of such vesicles, which are of respirable size, may be a means of transmission of this agent of legionellosis. Another bacterium for which *Acanthamoeba* can act as environmental host is *Mycobacterium avium*, a pathogen which like *Legionella pneumophila* is widespread in aquatic environments, including municipal drinking water. However, *M. avium*, a primary health threat to AIDS patients, is found in the cyst wall of the amoeba rather than in vesicles (Steinert *et al*. 1998).

The potential for free-living *A. culbertsoni,* and *Paramecium caudatum* and two other ciliated protozoans, to provide a predatory mechanism for removing *Cryptosporidium parvum* from aquatic ecosystems, including wastewater treatment plants, was investigated by Stott *et al.* (2003). While the *Acanthamoeba* ingested the cysts of this waterborne protozoan parasite of the human intestine, the ciliates showed greater predatory activity.

Filamentous Fungi

Structure and classification of filamentous fungi

Like those in outdoor air, the fungi found in indoor air as spores and cells fall into two basic categories, filamentous fungi and yeasts. Yeasts are regarded conventionally as unicells and are dealt with in the next section of this chapter. The typical filamentous fungus develops from a germinating spore, which extends into a germ tube. As a result of continued apical growth this develops into a roughly tubular hypha, which branches as it grows, forming an extending network of hyphae known as a mycelium.

The hyphae are incompletely divided by septa into communicating compartments (the equivalent of cells) along their entire length, giving septate mycelium. In members of the Zygomycota (Table 1), however, the mycelium is described as non-septate, although occasional septa occur. These are in older areas of mycelium and where reproductive structures are formed. The mycelium in members of the Basidiomycota is characterized by clamp connections at the septa which give the hyphae a nodular appearance absent in other septate fungi.

An extremely important fundamental characteristic of fungi is that they produce dispersible spores. In microfungi, including those frequently referred to as moulds, a microscope is needed to examine the spores and the specialized structures on or in which they are produced and thus enable the species to be identified. In other fungi, aggregations of hyphae organized into complex macroscopic sporing structures may arise from a vast mycelium. Because fungi were originally regarded as plants and were described and classified by botanists, these macroscopic structures tend still to be known as fruiting bodies. They include the cups, mushrooms and brackets (or conks) of species that are referred to as macrofungi.

The greatest number of filamentous fungi found indoors were formerly allocated to an artificial grouping of species for which no sexual reproductive phase had been found, but there are in indoor air also spores of species which can be assigned to one or other of three phyla with a sexual phase (Table 1). The fungi lacking sexual (or perfect) stages have at various times in the past been categorized as belonging to the Fungi Imperfecti, Deuteromycetes, Deuteromycotina or Deuteromycota, but these taxonomic categories are now considered not acceptable, since they are artificial groupings of diverse fungi that have not evolved from a common ancestral group (Kirk *et al*. 2001). In modern classification, most of them have been allocated to the subphylum Pezizomycotina of the Ascomycota. Nevertheless, very often they are still informally referred to as deuteromycetes, and indeed sometimes by the earlier term Fungi Imperfecti. The term "mitosporic fungi" has also been applied to these fungi because the spore is a mitospore, its nuclei being the product of the type of nuclear division associated with vegetative growth and asexual reproduction, i.e. mitosis, and not of the reduction division, meiosis, which is part of sexual reproduction (Kirk *et al*. 2001). However, as was mentioned in Chapter 1.1, the spore is most often called a conidium. The structure bearing conidia is a conidiophore. The wide range of morphological variation in both conidia and conidiophores which enables different mitosporic fungi isolated from the air or from surfaces in buildings to be identified is fully evident from the illustrated descriptions in Chapter 4.2 and Chapter 5. In earlier classifications, fungi in the Deuteromycetes were classed according to whether the conidiophores were naked (Hyphomycetes) or were enclosed in sporing bodies (Coelomycetes), e.g. *Phoma*. There are also some well-characterized fungi which have never been found to produce spores. These sterile fungi, or Mycelia Sterilia, were formerly allocated to the Agonomycetes.

As mentioned in Chapter 1.1, filamentous fungi in the three phyla informally referred to as ascomycetes, basidiomycetes and zygomycetes (Table 1) are recognizable by the characteristics of their sexual phase, the teleomorph. They may also have an asexual phase, or anamorph, in which they produce mitospores. Because the anamorph may be more frequently encountered, the anamorphic name may be more widely used than that of the teleomorph, e.g. in the two ascomycetes mentioned later in this paragraph. Most of the fungi referred to as deuteromycetes are either fungi which in the process of evolution have lost their teleomorphic phase, or are anamorphs for which no teleomorph has yet been found. Based on morphological and/or physiological similarities and particularly comparison of DNA sequences, most of these anamorphic fungi are now allocated to the ascomycete subphylum Pezizomycotina. Although the reproductive structures in ascomycetes such as *Emericella nidulans* (anamorph: *Aspergillus nidulans*) or *Eurotium herbariorum* (anamorph: *Aspergillus glaucus*) are microscopic, the teleomorphs of many others are characterized by complex macroscopic fruiting bodies, ascocarps, e.g. the cup fungi in the genus *Peziza*. Likewise, although the teleomorphs of basidiomycetes like *Sistotrema brinkmannii* are microscopic, those of the dry rot fungus *Serpula lacrymans* indoors

Table 2. Abundance of principal spore types in indoor and outdoor air of 15 homes in Ontario, presented as mean concentration for 132 sampling days over 22 months and percentage of total air spora (after Li and Kendrick 1995).

	Indoor		Outdoor	
	No. m^{-3} air	%	No. m^{-3} air	%
Alternaria	44	1.9	74	2.1
Aspergillus/Penicillium	457	19.8	131	3.8
Cladosporium	895	38.8	1479	42.5
Coprinus (basidiospores)	41	1.8	78	2.3
Epicoccum	7	0.3	20	0.6
Ganoderma (basidiospores)	59	2.6	111	3.2
Leptosphaeria (ascospores)	182	7.9	547	15.7
Unidentified ascospores	65	2.8	138	4
Unidentified basidiospores	152	6.5	310	8.9
Other unidentified spores	206	8.9	301	8.7
Hyphal fragments	146	6.3	112	3.2

and of the beech bracket *Ganoderma applanatum* outdoors are conspicuously macroscopic basidiocarps. The ascospores and basidiospores produced by ascomycetes and basidiomycetes are dispersed by air currents, but the zygospores in the zygomycetes are larger and their function is regarded as being survival rather than dispersal. Under suitable conditions, a zygospore will germinate to produce an anamorphic structure, the sporangium, which contains sporangiospores that are dispersed.

Outdoor air as a source of filamentous fungi in the indoor air spora

The wide diversity of fungal spores in outdoor air between early summer and autumn, when vegetative growth and sporulation of fungi are at their greatest, has been mentioned in Chapter 1.1. The outdoor air is for indoor air an important source of living spores and hyphal fragments, which act as propagules from which mycelium can develop. These propagules infiltrate naturally ventilated buildings and strongly influence the indoor fungal burden both qualitatively and quantitatively (Sneller and Roby 1979). Factors such as the proximity of a building to vegetation and organic debris that support fungal growth and spore production, and shade that enhances survival, lead to increased numbers of airborne propagules penetrating buildings (Kozak *et al*. 1979). Among other authors, Ackermann *et al*. (1969) and Sneller and Roby (1979) have reported that indoor concentrations of mould propagules broadly parallel those outdoors during the summer, but at a lower level and with a time lag before peak outdoor concentrations are reflected.

In the extensive regional investigation in USA, mentioned in Chapter 1.1, Shelton *et al*. (2002) noted that, overall, the mean and median concentrations of culturable fungi indoors correlated with the corresponding outdoor concentrations. Like those outdoors, the median indoor concentrations were highest in the summer and autumn, and lowest in winter and spring. The indoor/outdoor ratio only ranged from roughly 0.1 to 0.5, and there was no significant variation in the ratio either seasonally or from year to year. Corresponding to the situation outdoors, the highest indoor concentrations occurred in the Southeast, Southwest and Far West regions and the lowest in the Northwest region, with those in the Midwest and Northeast being intermediate. In arctic and temperate climates, of course, outdoor fungal growth and sporulation are minimal in winter because of the lower temperature. Consequently, winter counts of spores indoors are normally higher than those outdoors, particularly when there is snow cover (Reponen *et al.* 1992), which precludes re-entrainment of settled spores in dust and soil and on outdoor surfaces.

Naturally, the degree and type of ventilation employed greatly affects the air spora in buildings. An investigation in UK (Adams and Hyde 1965) found that, as with pollen, simply closing the windows and doors of a room in a naturally ventilated home during summer could exclude 98% or more of the spores present in outdoor air. Provided that it is not circumvented by opening doors and windows, central air-conditioning reduces spore counts in houses by 50% or more (Spiegelman *et al.* 1963). Window air-conditioning units can reduce numbers of *Alternaria* and *Ganoderma*

spores, and also pollen grains, to around 5% of the outdoor levels (Solomon *et al*. 1980). It has also been reported that central electrostatic filtration can reduce indoor air concentrations of total propagules to 3% of those experienced without such filtration, and *Cladosporium* to <1% (Kozak *et al.* 1979).

The lower numbers of airborne spores indoors relative to outdoor air in summer is, with some exceptions, well illustrated by a Canadian study (Table 2) in which spores were collected on coated glass slides and counted under a microscope (Li and Kendrick 1995). It should be said here that Table 2 also shows that air contains spores of types which do not appear in counts obtained by so-called viable sampling methods that are more frequently employed in studies of indoor air. Although there are large discrepancies, which will be discussed later (see Chapter 4.1), counts of the phylloplane fungi *Alternaria*, *Cladosporium* and *Epicoccum* can be made by either method. However, numbers of basidiospores of the macrofungi *Coprinus* and *Ganoderma* and the ascospores of *Leptosphaeria* (a plant pathogen and an important cause of late summer asthma) in indoor air are not enumerated by viable count methods. More detailed analysis of the Canadian data (Li and Kendrick 1996) showed that from May to October the relationships between numbers of airborne spores and hyphal fragments (and the diversity of fungi) indoors and outdoors were very strong (especially so for *Alternaria* and *Leptosphaeria*), probably because of windows being opened during the summer months. Path analysis indicated that, as might have been expected, *Alternaria, Leptosphaeria*, unidentified ascospores and *Coprinus* and *Ganoderma* basidiospores, came mainly from outdoor sources.

However, because of their large size, the spores of some fungi may not penetrate buildings in any quantity. This is instanced in a study of basidiospore distribution of a white-spored variety of the toadstool known as the fly agaric (*Amanita muscaria* var. *alba*). Li (2005) used an Allergenco MK-3 sampler at ground level 30 cm from basidiocarps, which were 6 m from a house. For the first three days in which the sporing surfaces were fully exposed the daily average concentration of airborne spores adjacent to the basidiocarps was $>10^4$ m^{-3} air. Small numbers of basidiospores infiltrated the house occasionally each day, the daily averages in the indoor air were <0.1% of the spores dispersed from the basidiocarps. There was a positive correlation between the indoor concentration of airborne spores and windows and doors being left open, but there was no correlation with the activity of the occupants. Li (2005) has suggested that the relatively large size of the basidiospores (in the early phase of release $12.5 \pm 2.6 \times 7 \pm 1.4$ μm, and later $10.5 \pm 2.2 \times 7 \pm 1.4$ μm) and their shape (broadly ellipsoid to elongate) may be major factors in their rapid deposition oudoors and, consequently, limited potential for infiltrating buildings.

Table 3. Rank order of 30 most abundant airborne fungi inside and outside 50 non-problem houses in Atlanta, GA (after Horner *et al.* 2004).

Indoor (600 air samples)	Outdoor (200 air samples)
Cladosporium cladosporioides	*Cladosporium cladosporioides*
Cladosporium spp.	*Cladosporium* spp.
C. sphaerospermum	*C. sphaerospermum*
Penicillium spp.	*Penicillium chrysogenum*
P. sclerotiorum	*Penicillium* spp.
Epicoccum nigrum	*P. corylophilum*
P. brevicompactum	*P. brevicompactum*
P. decumbens	*Aspergillus niger*
Yeasts	*P. citrinum*
Aspergillus niger	*P. variabile*
Non-sporulating hyaline fungi	Non-sporulating hyaline fungi
Alternaria alternata	*Epicoccum nigrum*
P. pinophilum	*P. commune*
Curvularia spp.	*P. decumbens*
P. corylophilum	*P. glabrum*
P. glabrum	*Curvularia* spp.
Non-sporulating fungi	*P. citreonigrum*
Arthrospore- forming fungus	*P. pinophilum*
Non-sporulating pigmented fungi	Yeasts
P. citrinum	Non-sporulating fungi
P. variabile	*P. sclerotiorum*
Aureobasidium pullulans	*P. aurantiogriseum*
P. crustosum	*Alternaria alternata*
Alternaria sp.	Arthrospore- forming fungus
Bipolaris sp.	*Aspergillus versicolor*
Aspergillus fumigatus	*P. crustosum*
P. purpurogenum	*P. purpurogenum*
P. solitum	*P. paxilli*
P. chrysogenum	Non-sporulating pigmented fungi
Aspergillus versicolor	*P. rugulosum*

Effect of growth of filamentous fungi indoors on the air spora

Since indoor temperatures are usually favourable for fungi, growth and sporulation of fungal contaminants

Table 4. Species of *Aspergillus* and *Penicillium* reported present as airborne viable particles in homes of asthmatic children and adults in Mexico City (Garcia *et al.* 1995; Rosas *et al*. 1997).

Category	Frequency (Percentage of samples)	
	Adults (n =30)†	Children (n = 8)*
Aspergillus spp.	32	48
A. candidus	3	-
A. flavus	9	10[a]
A. fumigatus	3	-
A. glaucus (Eurotium herbariorum)	1	63
A. melleus	-	27[b]
A. niger	10	16
A. ochraceus	6	-
A. parasiticus	2	-
A. versicolor	4	56[c]
A. wentii	1	-
Penicillium spp.	97	100
P. aurantiogriseum	58	88
P. brevicompactum	13	-
P. chrysogenum	15	79
P. citrinum	9	-
P. crustosum	1	1
P. griseofulvum	4	-
P. janthinellum	2	-
P. mineoluteum	-	2
P. oxalicum	1	-
P. purpurogenum	3	7
P. spinulosum	-	41
P. verrucosum	2	-
P. viridicatum	20	-

Burkard personal sampler, malt extract agar; † Andersen 2-stage sampler, dichloran-18% glycerol agar; - Not reported; [a]Plus *A. sydowi;* [b]Plus *A. flavofurcatis;* [c]Plus *A. petrakii*

are likely to occur in any damp areas in buildings and consequently modify the relationship between the indoor and outdoor aeromycota (see Chapter 2.1). The major exceptions to the tendency for indoor counts in summer to be lower than the corresponding outdoor counts are *Aspergillus* and *Penicillium*. The failure of Li and Kendrick (1996) to detect any functional or causal relationship, winter or summer, between *Aspergillus/Penicillium* conidia in indoor and outdoor air is in line with the generally held belief that the conidia of fungi in these two genera are primarily of endogenous origin. Path analysis also suggests indoor sources of *Cladosporium*, *Epicoccum*, unidentified basidiospores and "other unidentified spores" (Table 2), although they also have outdoor origins (Li and Kendrick 1996). Along with *Penicillium*, *Cladosporium* is usually one of the commonest genera isolated from mould patches in damp homes (Grant *et al*. 1989).

As in other studies involving counting of spores under the microscope, the category *Aspergillus/Penicillium* was used by Li and Kendrick (1996) because the spores of only a very few species in these two large genera are sufficiently distinctive for them to be recognizable in a field sample under the microscope, and even then only by an extremely skilled mycologist. Although the quantitative data indicate the relative abundance of aspergilli and penicillia (taken together), it should be realized that different species of *Aspergillus* and *Penicillium* are quite different in their physiology, ecology and significance for health. It is therefore important that in investigating indoor environments species should be accurately identified, and that involves culture of the fungi isolated. An example of the diversity in *Penicillium* spp. that may be encountered in indoor air can be seen in Table 3. The results presented are for the fungi in the air inside and outside a set of 50 houses in Atlanta in which there was either minimal or no water damage or associated mould growth (Horner *et al*. 2004). Although both indoors and outdoors *Cladosporium* spp. ranked highest among the 30 most abundant taxa, some 12 species of *Penicillium* were abundant in indoor air and 16 in outdoor air. Table 4 presents the results of two studies carried out in Mexico City using different samplers and different isolation media, illustrating a greater diversity of aspergilli than in Table 3, and also a diverse range of penicillia. The species in these two genera given in Tables 3 and 4 are by no means the only ones that have been reported in studies. For example, in their nationwide study Shelton et al. (2002) noted *A. caespitosus, A. carneus*, *A. restrictus*, *A. sydowii*, *A. terreus*, *A. unguis* and *A. calidoustus* (= *A. ustus*) among the aspergilli in indoor air, and yet others are mentioned elsewhere in this volume.

It has to be realized, however, that not all of the species contributing to total counts of *Aspergillus/Penicillium* obtained in investigations such as that of Li and Kendrick (1995), or the collective viable counts for *Aspergillus* spp. or *Penicillium* spp. in many other published investigations may actually be more abundant indoors. Fradkin *et al.* (1987) found that, whilst the collective viable counts of penicillia indoors were twice those outdoors, the very common species *Penicillium chrysogenum* was more abundant outdoors. This study also found that some *Cladosporium* spp.

were less abundant indoors during the summer, but the concentrations of others were double those outdoors. There are notable differences in the ranking of the penicillia in the Atlanta investigation (Horner *et al*. 2004, Table 3) that also point to the fundamental importance of species identification in aerobiological studies. For example, whilst *P. chrysogenum* was the most abundant of the penicillia in outdoor air, it ranked lowest among those in the 30 most abundant taxa in indoor air. Conversely, *P. sclerotiorum* was the most abundant *Penicillium* species in indoor air, but it was of much lower rank in outdoor air. Further, five species among the most abundant taxa in outdoor air – *P. commune*, *P. citreonigrum*, *P. aurantiogriseum*, *P. paxilli* and *P. rugulosum* – were not among those most abundant in indoor air, while *P. solitum* appeared in the top 30 taxa in indoor air, but not in outdoor air.

The sporocarps of macrofungi growing within buildings can also contribute to the air spora of the indoor environment. For example, it has been shown that the concentration of basidiospores near to the basidiocarps of the dry rot fungus, *Serpula lacrymans,* may be as high as $3.6 \times 10^5\ m^{-3}$ air (Hirst and Last 1953). This is of the same order as the peak spore concentration of approx. $2.8 \times 10^5\ m^{-3}$ recorded by Li (2005) outdoors close to the *Amanita muscaria* var. *alba* basidiocarps mentioned earlier. It has been shown that such concentrations of *S. lacrymans* can be responsible for development of asthma and hypersensitivity pneumonitis (extrinsic allergic alveolitis) in susceptible occupants of buildings where there is extensive dry rot of wood (O'Brien *et al.* 1978).

A further source of microbial contamination has been demonstrated in mechanically ventilated buildings in Finland. If the floor of first-floor apartments is not airtight, air exhausted from the apartments is replaced by air from the crawl space. Airaksinen *et al.* (2004a,b) found that the air in that space usually had a greater concentration of airborne spores than the outside air, and was at its greatest in summer. Spores of an unspecified *Acremonium*, not regarded by these workers as typically having a source indoors, were found to be present in larger numbers during summer in the crawl space than in the air outdoors. The numbers in the apartments correlated with those in the crawl space, and it was concluded that in summer the warm damp unfiltered air entering the crawl space produced conditions favourable for growth and sporulation of *Acremonium*, leading to more spores infiltrating the apartments through the floor. No such correlation was found between the numbers of the most abundant category, the large genus *Penicillium*, possibly for reasons mentioned in the second last paragraph to this.

Table 5. Rank order of most abundant fungi in 2-5 mg samples of indoor dust from 50 non-problem houses in Atlanta, GA, directly plated over entire surface of malt extract agar (MEA) and dichloran-18% glycerol agar (DG18) (after Horner *et al*. 2004).

MEA	DG18
Cladosporium cladosporioides	*Cladosporium* spp.
Yeasts	*C cladosporioides*
C. sphaerospermum	*Penicillium* spp.
Cladosporium spp.	*C. sphaerospermum*
Penicillium spp.	*Aspergillus niger*
Aureobasidium pullulans	*P. chrysogenum*
Aspergillus niger	*P. brevicompactum*
Epicoccum nigrum	*Aspergillus versicolor*
P. chrysogenum	*Aspergillus* spp.
P. glabrum	*P. citrinum*
P. aurantiogriseum	*P. glabrum*
P. sclerotiorum	*P. aurantiogriseum*
P. citrinum	*Aspergillus ochraceus*
P. purpurogenum	Non-sporulating fungi
Alternaria alternata	*Rhodotorula* spp.
Rhodotorula spp.	*P. expansum*
Aspergillus spp.	*P. variabile*
Curvularia spp.	*P. spinulosum*
Aspergillus versicolor	*Aspergillus sydowii*
P. spinulosum	*Aspergillus unguis*
P. decumbens	*P. crustosum*
P. brevicompactum	Yeasts
P. variabile	*Eurotium amstelodami*
P. citreonigrum	*Aureobasidium pullulans*
P. corylophilum	*Alternaria* spp.
Non-sporulating fungi	*Syncephalastrum racemosum*
Non-sporulating pigmented fungi	*Alternaria alternata*
Trichoderma harzianum	Unidentified
Unidentified	*P. solitum*
Pithomyces charatarum	*Eurotium herbariorum*
Bipolaris spp.	Non-sporulating hyaline fungi

Overall, the range of species that may be encountered in indoor air is wide. Zyska (2001) showed this in compiling from available literature a list of fungi reported as either being present in the air of European indoor environments or growing on structural or other materials within these environments and therefore

Table 6. Dermatophytes and other fungi isolated from floor dust of student houses (Maghraby *et al*. 2008).

Dermatophytes and related fungi:	
Aphanoascus fulvescens	*Chrysosporium lucknowense*
Aphanoascus sp.	*Gymnoascus uncinatus* (anamorph *Chrysosporium merdarium*)
Arthroderma cuniculi	*Trichophyton rubrum*
Other fungi:	
Alternaria alternata	*Mucor circinelloides*
A. citri	*M. hiemalis*
Aspergillus alutaceus	*M. racemosus*
A. flavus	*Nectria haematococca*
A. flavus var. *columnaris*	*Paecilomyces lilacinus*
A.fumigatus	*Penicillium brevicompactum*
A. niger	*P. camemberti*
A. ochraceus	*P. chrysogenum*
A. parasiticus	*P. citrinum*
A. sulphureus	*P. duclauxi*
A. terreus	*P. funiculosum*
A. ustus	*P. griseofulvum*
Candida albicans	*P. oxalicum*
Cladosporium cladosporioides	*P. purpurogenum*
C. sphaerospermum	*P. rubrum*
Cunninghamella echinulata	*Rhizopus stolonifer*
C. elegans	*Rhodotorula rubra*
Emericella nidulans	*Syncephalastrum racemosum*
Geotrichum candidum	Non-sporing isolates
Gibberella pulicaris	

likely to contribute to the airborne fungal burden. Of the 227 species isolated from residential and public buildings (libraries) and work environments (sawmills and deep coal mines) nearly 80 were known mycotoxin producers and 17 had been isolated from human tissues. In a later compilation, Zyska (2004) listed 434 species, of which 73% are anamorphic.

The presence of hyphal fragments in outdoor air has been demonstrated in Table 5 of Chapter 1.1, and in indoor air in Table 2 of the present chapter, and the possibility of these fragments acting as propagules has also been mentioned. However, there are fragments which are so badly damaged that they are non-viable or are too small to contain intact nucleate compartments that will grow into mycelium. Despite this, the emphasis in most investigations of the airborne mycobiota has been on spores, and hyphal fragments and subcellular particles have been ignored. Górny *et al*. (2002), however, examined the release of spores and fragments of three species, *Aspergillus versicolor*, *Penicillium melinii* and *Cladosporium cladosporioides*, from agar plate cultures and ceiling tiles. They found that up to 320 times more fragments were released than spores, and that they contained the same antigens. Fungal particulates smaller than conidia released from ceiling tiles on which the toxigenic species *Stachybotrys chartarum* had grown have been shown to contain trichothecene mycotoxins (Brasel *et al*. 2005). These investigations therefore have implications for assessment of human exposure to allergens and mycotoxins.

Effect of human behaviour and activity on the indoor air spora

Spores and hyphal fragments that have infiltrated buildings by the airborne route add to those brought in on contaminated clothes and fur by humans and their animal pets. Li and Kendrick (1996) suggested that firewood and fuel wood chips, vegetables, and clothes and tools brought indoors after garden work, were possible sources of *Epicoccum*. Unwashed root vegetables and birch firewood were found to be abundant sources of *Cladosporium* and *Penicillium* spores contaminating indoor air Lehtohnen *et al*.

(1993), and fuel wood chips have been reported as a potential source of spores of the toxigenic fungus *Trichoderma* (Miller *et al*. 1982). Pasanen *et al*. (1989) presented circumstantial evidence for carriage on clothes of *Acremonium*, *Alternaria*, *Botrytis* and *Chrysosporium* spores from cow sheds into farm houses during the Finnish winter, and (Lehtonen *et al.* 1993) noted transport of *Aspergillus* on riding clothes. By whatever route they enter, or whatever their indoor source, airborne propagules are rapidly disseminated throughout naturally ventilated buildings by air currents (Christensen 1950), although the closer to an endogenous source the higher are airborne counts likely to be (Hunter *et al*. 1988).

House dust usually contains large numbers of microorganisms that have sedimented out from the air. For example, in a health-related investigation of settled dust in schools during winter, Meyer *et al.* (2004, 2005) recorded levels that ranged from 8.3×10^2 to 3.1×10^6 CFU g^{-1} dust. One can therefore expect that indoor activities that raise dust will also affect the indoor air spora. This was elegantly demonstrated clearly 120 years ago in a study of houses and schools in a Scottish mill town, Dundee (Carnelley *et al*. 1887). This paper, which incidentally made observations on antibiosis that pre-date Fleming's serendipitous discovery by half a century, should be prescribed reading for anyone carrying out surveys of indoor air. Comparison of corresponding male and female classes in school showed that under normal conditions "boys tend to make the air of a room more impure than girls do" because they "are more restless, and so raise more dust, which necessarily contains micro-organisms". When Carnelley *et al.* (1887) subsequently got a class of boys to stamp on their classroom floor, the total number of microorganisms in the air increased 15-fold. In more recent times, using a continuous recording volumetric spore trap Millington and Corden (2005) reported a five-fold increase in the total count of airborne fungi after re-entry to a closed room in a modern house, and also an elevated total on return from work in the evening to the house after it had been unoccupied during the day. In an older cottage, in which the proportion of *Aspergillus*/*Penicillium spores* was much higher than in the modern house, the passage of people to and fro boosted the airborne count by more than 20 times (Millington and Corden 2005). Various other investigators have shown that constructional/demolition work (Maunsell 1952, Hunter *et al.* 1988, Goebes *et al.* 2008), floor sweeping (Lehtonen *et al.* 1993), cleaning carpets using a vacuum cleaner lacking an exhaust filter (Hunter *et al.* 1988) and changing bedclothes (Lehtonen *et al*. 1993) all temporarily elevate airborne mould counts. The source strength for house dust raising activity is a function of the type and vigour of the activity and the number of people performing the activity, and also the type of flooring (Ferro *et al*. 2004).

Dybendal *et al.* (1991) noted that carpeted floors accumulated more dust and pollen and mould allergens (and by inference pollen grains and fungal spores/hyphal fragments) than smooth floors, indicating why the same activity raises more particulate matter from a carpeted floor than from a wooden floor (Ferro *et al*. 2004). Cho *et al*. (2006) also found that antigen level was greater in carpeted rooms than in those without carpeting. In a birth cohort study, these workers surveyed the child's primary activity room and found *Alternaria* antigen in the collected floor dust of nearly 90% of 777 homes. The level of *Alternaria* antigen was not associated with visible mould/water damage. Taken together with this, their observing the antigen levels were highest (a) in the autumn and (b) in homes with dogs led Cho *et al*. (2006) to conclude that transport of *Alternaria* from the outdoor environment was the source of the antigen in floor dust. Culturable *Alternaria* has certainly been noted in house dust. Horner *et al.* (2004) found that in 85% of samples of dust from non-problem homes in Atlanta 20% or more of the colonies on isolation plates were *Alternaria* and other phylloplane fungi, although collectively penicillia, aspergilli and other fungi shown in Table 5 predominated. At the other end of the scale, Maghraby et al. (2008) found that among the culturable fungi listed in Table 6 *Alternaria* spp. were only present in 22% of dust samples from university student houses in Egypt and accounted for only 1.6% of the total numbers. Although the species *Aspergillus* and *Penicillium* listed made up about 40% of the mean total of >38500 CFU g^{-1} dry dust, and the yeasts *Candida albicans* and *Rhodotorula rubra* approx. 11%, dermatophytic fungi comprised approx. 33%. The most abundant of these dermatophytes were *Chrysosporium lucknowense* and the two *Aphanoascus* spp. (altogether, around 26%).

Further consideration of the mycobiota in dust is given in Chapter 2.1, in which fungal growth in indoor environments is discussed.

It is clear, then, that human behaviour and activity have a pronounced impact on the indoor air spora. Variations in the degree and type of activity of adults and children, and of pets, in occupied buildings are responsible for large temporal and spatial differences in the numbers of fungi (and bacteria) in indoor air.

Marked differences can be seen even when counts are taken within minutes of each other (Hunter *et al.* 1988, Verhoeff *et al*. 1990, Mouilleseaux and Squinazi 1991).

Health effects of filamentous fungi in indoor environments

The health impact of filamentous fungi, as sources of allergens, mycotoxins and β-glucans, and as pathogens is dealt with in Chapter 3.1-3.3 and Chapter 4.5 of this book.

Yeasts

Nature of yeasts in indoor environments

Yeasts are usually defined as unicellular fungi reproducing vegetatively by budding, i.e. producing buds that develop into daughter cells, which either separate from the mother cell or remain adherent so that clusters or branched chains of cells are formed. In some genera (*Sterigmatomyces* and *Fellomyces*), the buds are produced on short stalks. Not all yeasts bud, however. In *Schizosaccharomyces*, reproduction is by fission (one or more cross-walls divide the cell into segments which split off and develop independently). In some budding yeasts, such as *Candida,* under a range of growth conditions the budded cells are elongated and remain attached in chains, forming pseudohyphae. In *Geotrichum* and *Trichosporon*, there are true hyphae which split up into loosely joined chains of cylindrical cells.

As well as reproducing vegetatively, yeasts such as *Saccharomyces* reproduce sexually, forming ascospores, and are therefore allocated to the ascomycete subphylum Saccharomycotina. Other sexually reproducing yeasts are basidiomycetes. One such is *Rhodosporidium sphaerocarpon*, which is best known because of its anamorphic state *Rhodotorula glutinis*. *R. sphaerocarpon* produces teliospores from which basidiospores are derived. The genera *Sporobolomyces* and *Bullera*, which multiply vegetatively by budding, also produce spores which are forcibly discharged (ballistospores). These two genera are also basidiomycetes; teleomorphs have been found for some species of *Sporobolomyces*.

Although yeasts are frequently mentioned in reports of investigations of the indoor air spora, they are seldom identified even to generic level. The reason for this is that identification of individual species, and even genera, requires both experience and skill and is extremely time-consuming, involving morphological examination and a battery of physiological or biochemical tests (see Chapter 4.5). Consequently, many reports present only total viable counts, sometimes subdividing them according to colony colour into "pink" and "white" yeasts. Mention of pink or reddish yeasts as *Sporobolomyces* or *Rhodotorula* in reports should always be treated with caution, unless full identification procedures have been followed.

A detailed examination of three urban apartments and a rural farm-house in Finland did, however, reveal that members of the basidiomycetous genera *Cryptococcus*, *Rhodotorula* and *Sporobolomyces* were the most numerous airborne yeasts indoors (Rantio-Lehtimäki 1988). Both indoors and outdoors, other yeasts (mostly *Debaryomyces hansenii*, *Williopsis californica* and *Wingea robertsii*) only accounted for around 2% of the total trapped on Andersen sampler plates. As with outdoor air, numbers of yeasts were greater in the autumn than at other times of the year. *S. roseus*, *S. holsaticus* and *S. salmonicolor* were found indoors during autumn, and small numbers of *S. hispanicus* were noted. Cryptococci appeared to be more abundant in indoor air than outdoors (or in similar numbers in the case of the farmhouse) and showed little seasonal variation, with *C. albidus* and *C. laurentii* as the most frequent species. *Rhodotorula* spp. were also more frequently isolated from indoor air, with *R. graminis* and *R. glutinis* being isolated in summer and *R. pilimanae* in late autumn. Elsewhere, Mouilleseaux and Squinazi (1994) found that airborne yeasts were primarily unspecified *Rhodotorula* and *Torulopsis,* although *Candida* also appeared occasionally, and Solomon (1974) reported that in USA *Sporobolomyces roseus* and *Rhodotorula* spp., and also *Geotrichum candidum*, were abundant in air humidified by a cold-mist vaporizer.

Various authors have reported that yeasts are abundant in house dust, e.g. Flannigan *et al*. (1993) and Verhoeff *et al*. (1994), and the predominant species found in dust from both mattresses and floors have been *R. glutinis*, *R. minuta*, *R. mucilaginosa*, *C. albidus* and *C. laurentii* (Hoekstra *et al*. 1994).

Health effects of yeasts in indoor environments

In general, it appears that very few of the yeasts isolated in homes or non-industrial work places are either fermentative or ascomycetous (Rantio-Lehtimäki 1988), but in work environments such as bakeries, breweries and distilleries, the fermentative ascomycete *Saccharomyces cerevisiae* (baker's or brewer's yeast) may be detected in the air spora in addition to cereal-borne fungi. The enolase of this species is

Table 7. Viable bacteria isolated from air in air-conditioned and naturally ventilated sites in two office buildings (after Austwick *et al.* 1989).

Gram-positive	Gram-negative
Micrococcus spp.	*Acinetobacter calco-aceticus* var. *lwoffii*
Staphylococcus aureus	*Aeromonas hydrophila*
Staph. epidermidis	*Flavobacterium* sp.
Streptococcus spp.	*Moraxella* sp.
	Pasteurella haemolytica
	P. pneumotropica
	Pseudomonas aeruginosa
	Ps. cepacia
	Ps. fluorescens
	Ps. paucimobilis
	Ps. vesicularis

a major allergenic component, and a wider range of patients other than bakery workers with inhalant allergies to fungi show sensitivity to this enzyme (Baldo and Baker 1988), which cross reacts with that from *Candida albicans*. Cross reactivity between allergens from different yeast genera has also been reported by Koivikko *et al.* (1988). Although the range of yeasts which have been implicated in allergic disease is much smaller than for filamentous fungi, *Rhodotorula*, *Sporobolomyces* and *Tilletiopsis* are other yeasts which have been reported to cause allergic reactions (Jackson 1984, Rantio-Lehtimaki and Koivikko 1984, Burge 1989). Hodges *et al.* (1974) noted precipitating antibodies to both *Rhodotorula* sp. and *Cryptococcus* sp. (and a range of other fungi isolated from a home cold-mist vaporizer) in the serum of a patient with hypersensitivity pneumonitis (HP), and a *Rhodotorula* sp. has been recorded as a cause of HP in the occupant of a house with a heavily contaminated basement (Gravesen 1994). Sensitivity to *Candida* has been recorded in children with asthma (Koivikko *et al*. 1991), and *Trichosporon cutaneum* is the cause of a summer HP in Japan (Yoshida *et al*. 1989).

Bacteria

Numbers of bacteria in indoor air are usually, but not always, greater than the numbers of fungi in the air of homes (Nevalainen *et al*. 1988), schools (Mouilleseaux *et al*. 1993) and non-industrial workplaces such as air-conditioned offices (Mouilleseaux *et al.* 1993)**.** Total numbers of bacteria may, for example, be as high as 15×10^6 m^{-3} air in homes (Kujundzic et al. 2006). Ventilation and overcrowding affect the numbers of airborne bacteria, as was demonstrated more than 120 years ago in a study of homes and schools by Carnelley *et al.* (1887). Carnelley and his colleagues noted that numbers of viable airborne bacteria were higher in naturally ventilated than in mechanically ventilated schools. The ratio of bacteria to fungi was 132:1 under natural ventilation as opposed to only 29:1 with mechanical ventilation. Counts of culturable bacteria in domestic air were shown to rise with occupation density and the ratio of bacteria to fungi also increased, from 21:1 in spacious houses to 49:1 in overcrowded houses. In more recent times, it has been shown that even in much larger buildings human numbers and activity have an important bearing on counts of airborne bacteria. Despite filtration and air-conditioning in the Vatican, counts of airborne bacteria (and fungi) correlated positively with the numbers of visitors to, and their presence in, the Sistine Chapel (Montacutelli *et al.* 2000). The increased bacterial numbers observed during visiting hours were comprised largely of human-shed *Staphylococcus* spp.

As Tables 7 and 8 show, any investigation of airborne bacteria in buildings generally leads to the isolation of a diverse range of bacteria, but a limited number of types shed by the occupants normally predominate among the culturable bacteria. These dominant types are principally members of the Micrococcaceae; among these Gram-positive bacteria *Staphylococcus epidermidis* associated with skin scales is frequently most prominent. The shedding of staphyloccoci by humans accounts for their airborne numbers increasing proportionally more than other bacteria during visiting hours at the Sistine Chapel (Montacutelli *et al.* 2000). Other bacteria in the Micrococcaceae which are most likely to be present are *Staph. aureus* shed from the nasal membranes and skin and *Micrococcus* spp., such as *M. luteus,* originating on the skin (Table 8). Although Gram-positive endospore-forming rods in the genus *Bacillus* are commonly isolated from a wide range of indoor (and outdoor) environments, they are seldom isolated from indoor air in substantial numbers. Two other Gram-positive elements that may found in indoor air (Austwick *et al*. 1986) are the irregularly shaped, non-sporing rods of coryneform actinobacteria and the spores and mycelial fragments of filamentous actinobacteria in the order Actinomycetales.

Only rather small numbers of culturable mesophilic *Streptomyces* and other filamentous actinobacteria are normally found in indoor air (Nevalainen *et al*. 1988, Nevalainen 1989). They are commoner in homes and other buildings with dampness prob-

Table 8. Culturable airborne bacteria found in 11 homes in Central Scotland (after Flannigan *et al.* 1999).

Gram-positive cocci	Gram-negative cocci
*Aerococcus viridans**	Various, unidentified
Micrococcus spp.[1]*	**Gram-negative rods**
*Staphylococcus aureus**	*Achromobacter* spp.
*Staph.epidermidis**	*Acinetobacter* sp.*
Streptococcus spp.	*Aeromonas hydrophila**
Gram-positive rods	*Agrobacterium* sp.*
Actinomyces spp.	*Alcaligenes denitrificans*
Arthrobacter sp.	*Enterobacter agglomerans*
Bacillus spp.[2]*	*Flavobacterium* sp.
Corynebacterium spp.*	*Klebsiella* spp.*
Erysipelothrix sp.*	*Moraxella lacunata**
Kurthia sp.	*Proteus* sp.
Lactobacillus sp.	*Pseudomonas* spp.[3]*
Mycobacterium sp.	

[1] *M. luteus*, *M. roseus*, *M. varians* and *M. viridans,* [2] including *B. lichenlformis*, *B. megaterium* and *B. subtilis,* [3]Including *Ps. fluorescens*, *Ps. mallei*, *Ps. inendocin*a, *Ps. oryzihabitans*, *Ps. paucimobilis*, *Ps. pickettii*, *Ps. pseudomallei*, *Ps. putida*, *Ps. stutzeri* and *Ps. vesicularis,* * Also detected on surface of walls

lems, and have been particularly associated with complaints of odour in a range of types of building (Nevalainen *et al*. 1990). Ström *et al.* (1990) found streptomycetes in 25% of building material samples taken from sick buildings, and Hyvärinen et al. (2002) recorded unspecified mesophilic actinobacteria in association with fungi on a range of visibly damaged building materials. In a house with water damage in a basement bathroom, roof and outdoor walls Peltola *et al.* (2001) detected in the basement approx. 10^3 culturable bacteria m^{-3} air, of which 64 m^{-3} were spore-forming actinobacteria, including toxigenic strains of *Streptomyces* and *Nocardiopsis*. In an investigation of schools with visible moisture/mould problems, Meklin *et al.* (2002) found 0-7 mesophilic actinobacterial CFU m^{-3} air (GM = 0.1) in concrete/brick reference schools and 0-43 CFU m^{-3} (GM = 1.3) in corresponding index schools. In wooden schools the respective ranges were 0-47 (GM = 5.7) and 0-2700 CFU m^{-3} (GM = 6.3). It is not rare to isolate thermophilic actinobacteria such as *Saccharopolyspora* (*Faenia*) *rectivirgula* and *Thermoactinomyces candidus* or *Th. vulgaris* from HVAC equipment (Fink *et al*. 1971, Kreiss and Hodgson 1984).

Culturable Gram-negative bacteria are usually found to be much less abundant than Gram-positive species in investigations of indoor air. Elevated counts of Gram-negatives are usually indicators of conditions wet enough to allow proliferation, e.g. in HVAC humidifier reservoirs and drainage pans or on very damp surfaces. In such cases, *Pseudomonas, Acinetobacter, Alcaligenes* and *Flavobacterium* are likely to be among the most common culturable bacteria, but other Gram-negative rods such as *Achromobacter, Aeromonas, Agrobacterium, Enterobacter, Klebsiella, Moraxella* and *Proteus*, and also Gram-negative cocci, may also be present (Table 8).

It is well known that the proportion of the bacterial total in indoor air that can be cultured is small; for example, Flannigan *et al*. (1996) found that <1% of the total bacterial burden in a set of houses was culturable. A strategy that can be employed as a marker of the total level of Gram-negative bacteria is to assay air samples collected on membrane filters for lipopolysaccharide (LPS), or endotoxin. This is of medical significance because it is an immunomodulator, and is present in the outer membrane of the cell walls of both culturable and non-culturable Gram-negative bacteria, but not in Gram-positive bacteria. Two examples of the use of endotoxin measurement in investigations of indoor air are those of Kujundzic *et al*. (2006), who used it to assess seasonal differences in the air in non-problem homes, and Rao et al. (2007) to compare the air in residences with different degrees of flood damage.

Endotoxin has also been used as a marker of Gram-negative bacteria in investigations of house dust. In a study of some 400 European houses, Bischof *et al*. (2002) found that endotoxin levels in house dust were higher in old houses; the lower storey of houses; in houses with longer occupancy; high utilisation; infrequent vacuum cleaning of carpets; and an indifferent attitude to ventilation. Among other workers, Wickens *et al.* (2003) and Giovannangelo *et al.* (2007) have noted higher levels in houses with more occupants or pet cats and dogs, and Giovannangelo *et al.* (2007) also reported up to 3.4 times more endotoxin in dust from carpeted floors than from floors without carpets. Instanes *et al*. (2005) and Hyvärinen *et al*. (2006) recorded more endotoxin in floor dust than in dust from mattresses. The presence of pets and contact with animals outside have been found to be factors contributing to greater levels of endotoxin in mattresses (Gehring *et al*. 2004).

The value of PCR-based methods in determining the identity of airborne bacteria, irrespective of whether the bacteria are culturable or not, has been mentioned in connection with outdoor air (Chapter 1.1). These methods have also been employed in the investigation of bacteria in house dust, for instance by Pakarinen *et al*. (2008), who carried out their work in Russian and Finnish Karelia, where living conditions on opposite sides of the national border are fundamentally different. By DNA cloning these workers

were able to identify 94 different genera of dustborne bacteria, more than observed in any earlier study in houses. Compared with Finnish Karelia, dust from Russian Karelia contained up to 20 times more muramic acid, a marker for Gram-positive bacteria, and two-thirds of the bacterial DNA clones represented Gram-positives, approximately double the number in Finnish Karelia. They were predominantly members of the Staphylococcaceae and Corynebacteriaceae. Among members of the former family in Russian Karelia were species that are typically associated with animals. In Finnish Karelia, where the number of households with cats was 50% of that on the other side of the border, no such staphylococci were present and the only animal-associated species detected was the actinobacterium *Dietzia maris*. In the Russian houses 14 species (including staphylococci) typically associated with animals were found. In contrast to the Russian houses, Gram-negatives (mainly Proteobacteria) predominated in the house dust from the Finnish houses, and the endotoxin levels were higher. The majority of the protobacterial DNA sequences represented species associated with plants, and the number of clones of plant-associated species was three times that in Russian Karelia.

The effect of the numbers of human occupants and the presence of pets in the premises, the types of floor covering and the nature and intensity of associated activity have been mentioned as being among the factors quantitatively affecting the airborne microbial burden indoors. The investigation carried out by Pakarinen *et al*. (2008) illustrates that variation in the types of bacteria in house dust will lead to qualitative differences between exposures.

Health effects of bacteria in indoor environments

Inhalation of the LPS or endotoxin present in the wall of Gram-negative bacteria is known to cause ill health in work and domestic situations (see Chapters 2.2, 4.1 and 4.6). The Gram-negative bacterium of greatest concern is the respiratory pathogen *Legionella pneumophila,* which has been mentioned above in relation to free-living protozoa. *Legionella* has been the subject of reviews in many recent publications, e.g. Cianciotto *et al.* (2007) and Hoffman *et al.* (2009), to which the reader is referred. Although some coryneforms are harmless saprotrophs in soil and water, many are pathogens. Among these, *Mycobacterium tuberculosis* and related species are responsible for tuberculosis, a disease now reported to claim more victims than malaria and AIDS. Like *Mycobacterium tuberculosis, Corynebacterium diphtheriae* is considered to invade the host mainly as a result of inhalation of droplet nuclei from a carrier of the disease and probably does not survive long in indoor air. The spores of thermophilic actinobacteria dispersed from massively contaminated HVAC systems may cause HP among building occupants (Banaszak *et al*. 1970, Fink *et al.* 1971).

The importance for health of exposure to various bacteria in work environments is discussed in Chapter 2.2.

Viruses

It is generally assumed that, under normal circumstances, viruses pass from person to person by direct contact or by forcible expulsion of droplets during coughing and sneezing. The largest droplets expelled are of an aerodynamic diameter (d_a) >100 µm and settle in the environment within seconds. They may have a role in infection by settling on fomites such as towels, clothing and upholstery from which viruses may infect victims by contact. Large droplets, d_a =10-100 µm, are expelled by coughing and sneezing are inspirable by individuals in the proximity, but are largely restricted to the upper airways. Airborne transmission of droplet nuclei of d_a <10 µm is still a matter of some contention, but if it occurs it could lead to the lower respiratory tract. Weber and Stilianakis (2008) have suggested that all three modes of transmission are involved in the spread of influenza. Fabian *et al*. (2008) have recently reported that coughing and sneezing are not the only source of infective viruses. Individuals infected with influenza A or B exhaled influenza virus RNA during normal tidal breathing at rates ranging from <3.2 to 20 particles min^{-1}. More than 87% of these particles were <1 µm in diameter and would therefore reach the lower respiratory tract.

Studies in which coughing has been simulated have shown that forcibly expired droplets can be dispersed well beyond a metre, the currently viewed limit of risk of droplet infection. When in a hospital ward equipped with ceiling intake diffusers and exhaust vents Wan *et al*. (2007) simulated human coughs using artificial saliva droplets similar in size distribution (peak size 12 µm) and airflow rate (0.4 L sec^{-1}) to a natural cough, they demonstrated that the exhaust vents had significant impact on the dispersion pattern of expiratory droplets, not unexpectedly enhancing lateral dispersion in the direction of the vents. Wan and Chao (2007) further noted that the time taken for droplets and droplet nuclei to be transported to exhaust vents or deposition surfaces for removal differed according to the ventilation flow pattern. In

the same air-conditioned hospital ward as Wan *et al*. (2007), Sze To *et al.* (2008) examined the distribution of a simulated respiratory fluid containing a known concentration of a benign bacteriophage aerosolized in artificial coughs. The air was sampled using a six-stage Andersen sampler containing culture plates seeded with *Escherichia coli* and plaque counts made on incubation of the exposed plates. The direction of coughing had a significant effect on the airborne transport of the phage; plaque counts decreased with lateral distance from the "infector" when the cough was directed vertically up, but were constant or even increased with distance when the cough was directed sideways.

It has been widely held that viruses do not survive in infective form for any length of time outside the host organism, but in reviewing the available literature on environmental inactivation of the influenza A virus Weber and Stilianakis (2008) noted that aerosolized influenza viruses appear to be stable at low RH and low to moderately high temperatures. They noted that whilst the daily inactivation rate constants are of the order of 10^3 on hands they are in the range 1-10^2 in aerosols and on inanimate surfaces, i.e. the virus can survive on hands for only minutes, but when airborne it can remain infective for some hours – the half-life of influenza A viruses in aerosols is 1-16 h.

The increased risk of airborne infection that is to be expected with overcrowding and poor ventilation is not confined to buildings. Air travel presents examples. In one instance, 72% of passengers on a commercial flight became infected with influenza during a 3-h delay on the ground, during which the ventilation system was not operational (Moser *et al*. 1979). Another, more recent, example is of a 3-h commercial flight from Hong Kong which carried one passenger symptomatic for severe acute respiratory syndrome (SARS). The result was that 22 of the other 119 passengers became infected with the SARS Coronavirus (SARS-CoV). Illness with this febrile respiratory disease was related to the physical proximity of the victims to the index patient (Olsen *et al.* 2003). In contrast, after a 90-min flight carrying four symptomatic passengers, two of whom had been coughing, only one other person reported fever and respiratory symptoms, but this was not reported as having been a probable case of SARS. On another 90-min flight, this time carrying a presymptomatic SARS patient, no illness was documented among other passengers (Olsen *et al.* 2003).

Robust evidence of airborne transport of viruses as a result of air movement within a building was presented by Wehrle *et al.* (1970), who reported on a nosocomial smallpox outbreak in a 3-storey hospital in Meschede, Germany, in which 17 patients became infected from the index patient in a ground-floor room. Smoke tests indicated that the virus particles were disseminated from the ground floor room to other floors as air currents created by the radiators rose up the stairwell, so infecting patients on the upper floors. There is also evidence that viruses can be distributed by mechanical ventilation systems, and a paper frequently cited as indicating such a spread is that by Brundage *et al*. (1988) concerning an outbreak of acute febrile respiratory disease among trainee soldiers. Significantly greater numbers of recruits in recently constructed mechanically ventilated barracks were affected than in similar, but older, naturally ventilated barracks. It was later found that the risk in mechanically ventilated barracks was reduced significantly by prophylactic treatment with adenovirus vaccine (Brundage *et al*. 1988). In a report of an outbreak of measles in a school, the spread of infection from infected children in one classroom to others in different classrooms was attributed to recirculation of air via the central ventilation system (Riley *et al*. 1978). Another measles outbreak was associated with a paediatric practice, where the disease was transmitted from a vigorously coughing child in an examining room to children elsewhere in the practice (Bloch *et al*. 1985). The authors suggested that that the evidence indicated that the measles virus survived for at least 1 h when airborne, and speculated that tightly insulated modern offices with a substantial proportion of recirculated air might predispose to airborne transmission. In addition to this paper, a paper by Gustafson *et al.* (1982) revealed that the employment of isolation rooms at positive pressure relative to other areas is ill-advised. They reported on an outbreak of chickenpox in a hospital where the airborne spread was from an immunocompromised child kept in strict isolation. The isolation room was at a higher pressure than in a corridor through which the varicella zoster virus was transmitted to other rooms, where susceptible children developed chickenpox. Another, more recent, example of a nosocomial disease outbreak has been reported by Li *et al.* (2004). The index patient in this case had SARS and was in one semi-enclosed cubicle of a ward of a Hong Kong hospital in which 70% of the air supply was recirculated. The non-functional return air outlet for this cubicle enhanced the spread of aerosolized SARS-CoV to other cubicles in the ward.

Whilst there is conflicting evidence, the overall conclusion of an international interdisciplinary pan-

el (Li *et al*. 2007) that reviewed 40 relevant original studies carried out between 1960 and 2005, including some of those mentioned above, is that there is sufficient strong evidence to confirm that there is an association between ventilation, air movement in buildings and the transmission/spread of infectious diseases such as measles, chickenpox, smallpox and SARS.

REFERENCES

Ackermann, H., Schmidt, B., and Lenk, V. (1969) Mycological studies of the outdoor and indoor air in Berlin. *Mykosen*, **12**, 309-320.

Adams, K.F., and Hyde, H.A. (1965) Pollen grains and fungal spores indoors and out at Cardiff. *J. Palynol.,* **1**, 67-69.

Airaksinen, M., Kurnitski, J., Pasanen, P., and Seppanen, O. (2004a) Fungal transport through a building structure. *Indoor Air*, **14**, 92-104.

Airaksinen, M., Pasanen, P., Kurnitski, J., and Seppanen, O. (2004b) Microbial contamination of indoor air due to leakages from crawl space: a field study. *Indoor Air*, **14**, 55-64.

Austwick, P.K.C., Davies, P.S., Cook, C.P., and Pickering, C.A.C. (1986) Comparative microbiological studies in humidifier fever. In C. Molina, (ed.), *Maladies des Climatiseurs et des Humidificateurs*, Colloque INSERM, Paris, pp. 155-164.

Austwick, P.K.C., Little, S.A., Lawton, L., *et al*. (1989) Microbiology of sick buildings. In B. Flannigan, (ed.), *Airborne Deteriogens and Pathogens*, The Biodeterioration Society, Kew, Surrey, UK, pp. 122-128.

Baldo, B.A., and Baker, R.S. (1988) Inhalant allergies to fungi: reaction to bakers' yeast (*Saccharomyces cerevisiae*) and identification of bakers' yeast enolase as an important allergen. *Int. Arch. Allergy Appl. Immunol.*, **86**, 201-208.

Banaszak, E.F., Thiede, W.H., and Fink, J.N. (1970) Hypersensitivity pneumonitis due to contamination of an air conditioner. *N. Engl. J. Med.*, **283**, 271-276.

Baron, E.J., Chang, R.S., Howard, D.H., *et al*. (1994) *Medical Microbiology*. Wiley-Liss, New York.

Berk, S.G., Ting, R.S., Turner, G.W., and Ashburn, R.J. (1998) Production of respirable vesicles containing live *Legionella pneumophila* cells by two *Acanthamoeba* spp. *J. Allergy Clin. Immunol.*, **64**, 279-286.

Bernstein, I.L., and Safferman, R.S. (1967) Sensitivity of skin and bronchial mucosa to green algae. *J. Allergy*, **38**, 166-173.

Bernstein, I.L., and Safferman, R.S. (1970) Viable algae in house dust. *Nature*, **227**, 851-852.

Bischof, W., Koch, A., Gehring, U., *et al*. (2002) Predictors of high endotoxin concentrations in the settled dust of German homes. *Indoor Air*, **12**, 2-9.

Bloch, A.B., Orenstein, W.A., Ewing, W.M., *et al*. (1985) Measles outbreak in a paediatric practice: airborne transmission in an office setting. *Paediatrics*, **75**, 676-683.

Brasel, T.L., Douglas, D.R., Wilson, S.C., and Strauss, D.C. (2005). Detection of airborne *Stachybotrys chartarum* macrocyclic mycotoxins on particulates smaller than conidia. *Appl. Environ. Microbiol.*, **71**, 114-122.

Brundage, J.F., Scott, R.M., Lednar, W.M., *et al*. (1988) Building-associated risk of febrile acute respiratory diseases in army trainees. *J. Amer. Med. Assoc.*, **259**, 2108-2112.

Burge, H.A. (1989) Airborne allergic fungi: classification, nomenclature, and distribution. *Immunol. Allergy Clin. N. Am.*, **9**, 307-319.

Carnelley, T., Haldane, J.S., and Anderson, A.M. (1887) The carbonic acid, organic matter and micro-organisms in air, more especially in dwellings and schools. *Phil. Trans. Roy. Soc., Series B*, **178**, 61-111.

Cho, S.-H., Reponen, T., Bernatein, D.I., *et al.* (2006) The effect of home characteristics on dust antigen concentrations and loads in homes. *Sci. Total. Environ*., **371**, 31-43.

Chrisostomou, C., Moustaka-Gouni, M., Sgardelis, S., and Lanaras, T. (2009) Air-dispersed phytoplankton in a Mediterranean River-Reservoir System (Aliakmon-Polyphytos, Greece). *J. Phycol. Research***, 31**, 877-884.

Christensen, C.M. (1950) Intramural dissemination of spores of *Hormodendrum resinae*. *J. Allergy*, **21**, 409-413.

Cianciotto, N.P., Kwaik, Y.A., Edelstein, P.H., *et al.* (2007) *Legionella: State of the Art 30 Years after Its Recognition*. ASM Press, Washington, DC.

Cohen, S.G., and Reif, C.B. (1953) Cutaneous sensitization to blue-green algae. *J. Allergy*, **24**, 452-457.

Cox, G.E., Wilson, J.D., and Brown, P. (1974) Protothecosis: a case of disseminated algal infection. *Lancet* 2, 379-382.

Curry, A., Turner, A.J., and Lucas, S. (1991) Opportunistic protozoan infections in human immunodeficiency virus disease: review highlighting diagnostic and therapeutic aspects. *J. Clin. Pathol.*, 44, 182-193.

Dybendal, T., Wedberg, W.C., and Elsayed, S. (1991) Dust from carpeted and smooth floors: IV. Solid material, proteins and allergens collected in the different filter stages of vacuum cleaners after ten days of use in schools. *Allergy*, **46**, 427-435.

Edwards, J.H., Griffiths, A.J., and Mullins, J. (1976) Protozoa as sources of antigen in humidifier fever. *Nature*, **264**, 438-439.

Fabian, P., McDevitt, J.J., DeHaan, W.H., *et al*. (2008) Influenza virus in human exhaled breath: An observational study. *PLoS ONE*, **3**, e2691.

Ferro, A.R., Kopperud, R.J., and Hildemann, L.M. (2004) Source strengths for indoor human activities that resuspend particulate matter. *Environ. Sci. Technol*., **38**, 1759-1764.

Fink, J.N., Resnick, A.J., and Salvaggio, J. (1971) Presence of thermophilic actinomycetes in residential heating systems. *Appl. Microbiol*., **22**, 730-731.

Flannigan, B., McCabe, E.M., Jupe, S.V., and Jeffrey, I.G. (1993) Mycological and acaralogical investigation of complaint and non-complaint houses in Scotland. In *Indoor Air '93, Proceedings of the Sixth International Conference on Indoor Air Quality and Climate*, Vol. 4, Indoor Air '93, Helsinki, pp. 143-148.

Flannigan, B., McCabe, E.M., and Jupe, S.V. (1996). Quantification of air- and dust-borne deteriogenic microorganisms in homes. In *Biodeterioration and Biodegradation*, *Proceedings of the Tenth International Biodeterioration and Biodegradation Symposium,* DECHEMA, Frankfurt am Main, pp. 377-384.

Flannigan, B., McEvoy, E.M., and McGarry, F. (1999) Investigation of surface and airborne bacteria in homes. In G. Raw, C. Aizlewood, and P. Warren (eds.), *Indoor Air '99, Proceedings,* Vol. 1. Construction Research Communications Ltd., London, pp. 844-849.

Fradkin, A., Tobin, R.S., Tarlo, S.M., *et al*. (1987) Species identification of airborne molds and its significance for the detection of indoor pollution. *J. Air Pollut. Control Assoc.*, **37**, 51-53.

Garcia, B.E., Comtois, P., and Borrego, P.C. (1995) Fungal content of air samples from some asthmatic children's homes in Mexico City. *Aerobiologia*, **11**, 95-100.

Gehring, U., Bischof, W., Borte, M., *et al*. (2004) Levels and predic-

tors of endotoxin in mattress dust samples from East and West German homes. *Indoor Air*, **14**, 284-292.

Giovannangelo, M., Gehring, U., Nordling, E., *et al*. (2007) Determinants of house dust endotoxin in three European countries – the AIRALLERG study. *Indoor Air*, **17**, 70-79.

Goebes, M.D., Baron, E.J., Mathews, K.L., and Hildemann, L.M. (2008) Effect of building construction on *Aspergillus* concentrations in a hospital. *Infect. Control Hosp. Epidemiol*., **29**, 462-464.

Górny, R.L., Reponen, T., Willeke, K., *et al*. (2002). Fungal fragments as indoor air biocontaminants. *Appl. Environ. Microbiol*., **68**, 3522-3531

Grant, C., Hunter, C.A., Flannigan, B., and Bravery, A.F. (1989) Water activity requirements of moulds isolated from domestic dwellings. *Int. Biodet*., **25**, 259-284.

Gravesen, S. (1994) Allergic and non-allergic manifestations related to indoor fungal exposure – management of cases. In R.A. Samson, B. Flannigan, M.E. Flannigan, *et al*., (eds.), *Health Implications of Fungi in Indoor Environments*, Elsevier, Amsterdam, pp. 241-248.

Gustafson, T.L., Lavely, G.B., Brawner, E.R., *et al*. (1982) An outbreak of airborne nosocomial varicella. *Pediatrics,* **70**, 550–556.

Hirst, J.M., and Last, F.T. (1953) Concentrations of basidiospores of the dry rot fungus (*Merulius lacrymans*) in the air of buildings. *Acta Allergy*, **6**, 168-174.

Hirvonen, M.-R., Huttunen, K., and Roponen, M. (2005) Bacterial strains from moldy buildings are highly potent inducers of inflammatory and cytotoxic effects. *Indoor Air*, **15**, 65-60.

Hodges, G.R., Fink, J.N., and Schleuter, D.P. (1974) Hypersensitivity pneumonitis caused by a contaminated cool-mist vaporizer. *Ann. Intern. Med.*, **80**, 501-504.

Hoekstra, E.S., Samson, R.A., and Verhoeff, A.P. (1994) Fungal propagules in house dust: a qualitative analysis. In R.A. Samson, B. Flannigan, M.E. Flannigan, *et al*. (eds.), *Health Implications of Fungi in Indoor Environments*, Elsevier, Amsterdam, pp. 169-177.

Hoffman, P., Friedman, H., and Bandinelli, M., (eds.) (2009) *Legionella pneumophila: Pathogenesis and Immunity*. Springer, New York.

Horner, W.E., Worthan, A.G., and Morey, P.R. (2004) Air- and dustborne mycoflora in houses free of water damage and fungal growth. *Appl. Environ. Microbiol*., **70**, 6394-6400.

Hunter, C.A., Grant, C., Flannigan, B., and Bravery, A.F. (1988) Mould in buildings: the air spora of domestic dwellings. *Int. Biodet*., **24**, 81-101.

Hyvärinen, A., Meklin, T., Vepsäläinen, A., and Nevalainen, A. (2002). Fungi and actinobacteria in moisture-damaged building materials – concentrations and diversity. *Int. Biodet. Biodeg*., **49**, 27-37.

Hyvärinen, A., Roponen, M., Tiittanen, P., *et al*. (2006) Dust sampling methods for endotoxin - an essential, but underestimated issue. *Indoor Air*, **16**, 20-27.

Instanes, C., Hetland, G., Bernsten, S., *et al*. (2005) Allergens and endotoxin in settled dust from day-care centers and schools in Oslo, Norway. *Indoor Air*, **15**, 356-362.

Jackson, F. (1984) *Sporobolomyces; Tilletiopsis*. In K. Wilken-Jensen, and S. Gravesen, (eds.), *Atlas of Moulds in Europe Causing Respiratory Allergy*. ASK Publishing, Copenhagen, pp. 46-47.

Kingston, D., and Warhurst, D.C. (1969) Isolation of amoebae from the air. *J. Med. Microbiol.*, **2**, 27-36.

Kirk, P.M., Cannon, P.F., Minter, D.W., and Stalpers, J.A. (2001) *Ainsworth and Bisby's Dictionary of the Fungi,* (10th Ed.), CAB International, Wallingford, Oxford, UK.

Koivikko, A., Kalima, K., Nieminen, E., *et al*. (1988) Allergenic cross-reactivity of yeasts. *Allergy*, **43**, 192-200.

Koivikko, A., Viander, M., and Lanner, A. (1991) Use of the extended Phadebas RAST panel in the diagnosis of mould allergy in asthmatic children. *Allergy*, **46**, 85-91.

Kozak, P.P., Gallup, J., Cummins, L.H., and Gillman, S.A. (1979) Currently available methods for home mold surveys. II. Examples of problem homes surveyed. *Ann. Allergy*, **45**, 167-176.

Kreiss, K., and Hodgson, M.J. (1984) Building-associated epidemics. In P. J. Walsh, C. S. Dudney, and E. D. Copenhaver, (eds.), *Indoor Air Quality*, CRC Press, Boca Raton, FL, pp. 88-106.

Kujundzic, E., Hernandez, M., and Miller, S.L. (2006) Particle size distributions and concentrations of airborne endotoxin using novel collection methods in homes during the winter and summer seasons. *Indoor Air*, **16**, 216-226.

Kwaik, Y.A., Gao, L.-Y., Stone, B.J., *et al*. (1998) Invasion of Protozoa by *Legionella pneumophila* and its role in bacterial ecology and pathogenesis. *Appl. Environ. Microbiol*., **64**, 3127-3133.

Lee, T., Grinshpun, S. A., Martuzevicius, D., *et al*. (2006) Relationship between indoor and outdoor bioaerosols collected with a button inhalable aerosol sampler in urban homes. *Indoor Air*, **16**, 37-47.

Lehtonen, M., Reponen, T., and Nevalainen, A. (1993) Everyday activities and variation of fungal spore concentrations in indoor air. *Int. Biodet. Biodeg.*, **31**, 25-39.

Li, D.-W. (2005) Release and dispersal of basidiospores from *Amanita muscaria* var. *alba* and their infiltation into a residence. *Mycol. Res.*, **109**, 1235-1242.

Li, D.-W., and Kendrick, B. (1995) A year-round comparison of fungal spores in indoor and outdoor air. *Mycologia*, **87**, 190-195.

Li, D.-W., and Kendrick, B. (1996) Functional and causal relationships between indoor and outdoor airborne fungi. *Can. J. Bot*., **74**, 194-209.

Li, Y., Duan, S., Huang, X., *et al*. (2004) Role of air distribution in SARS transmission during the largest nosocomial outbreak in Hong Kong. *Indoor Air*, **15**, 83-95.

Li, Y., Leung, G.M., Tang, J.W., *et al*. (2007) Role of ventilation in airborne transmission of infectious agents in the built environment – a multidisciplinary systematic review. *Indoor Air,* **17**, 2-18.

McElhenny, T.R., Bold, H.C., Brown, R.M., and McGovern, J.P. (1962) Algae: a cause of inhalant allergy in children. *Ann. Allergy*, **20**, 739-743.

McGovern, J.P., Haywood, T.J., and McElhenny, T.R. (1966) Airborne algae and their allergenicity. II. Clinical and multiple correlation studies with four genera. *Ann. Allergy*, **24**, 145-149.

Maghraby, T.A., Gherbawy,Y.A.M.H., and Hussein, M.H. (2008) Keratinophilic fungi inhabiting floor dusts of student houses at the South Valley University in Egypt. *Aerobiologia*, **24**, 99-106.

Maunsell, K. (1952) Airborne fungal spores before and after raising dust. *Int. Arch. Allergy Appl. Immunol*., **3**, 93-102.

Meklin, T., Husman, T., Vepsäläinen, A., *et al*. (2002). Indoor air microbes and respiratory symptoms of children in moisture damaged and reference schools. *Indoor Air*, **12**, 175-183.

Meyer, H. W., Wurtz, H., Suadicani, P., *et al*. (2004) Molds in floor dust and building-related symptoms in adolescent school children. *Indoor Air*, **14**, 65-72.

Meyer, H.W., Wurtz, H., Suadicani, P., *et al*. (2005) Molds in floor dust and building-related symptoms in adolescent school children: a problem for boys only? *Indoor Air*, **15**, 17-24.

Miller, J.D., Schneider, M.H., and Whitney, W.J. (1982) Fungi on fuel wood chips in a home. *Wood and Fiber*, **14**, 54-59.

Millington, W.M., and Corden, J.M. (2005) Long term trends in outdoor *Aspergillus*/*Penicillium* spore concentrations in Derby, UK from 1970 to 2003 and a comparative study in 1994 and 1996

with the indoor air of two local houses. *Aerobiologia*, **21**, 105-113.

Montacutelli, R., Maggi, O., Tarsitani, G., and Gabrielli, N. (2000) Aerobiological monitoring of "Sistine Chapel": airborne bacteria and microfungi trends. *Aerobiologia*, **16**, 441-448.

Moser, M.R., Bender, T.R., Margolis, H.S., et al. (1979) An outbreak of influenza aboard a commercial airliner. *Amer. J. Epidemiol*., **110**, 1-6.

Mouilleseaux, A., and Squinazi, F. (1991) Contamination microbienne de l'air: strategie d'etude et exemples de differents environnements. In *Proc. Societe Francais d'Aerobiologie, 3eme Congres National*, Institute Pasteur, Paris.

Mouilleseaux, A., and Squinazi, F. (1994) Airborne fungi in several indoor environments. In R.A. Samson, B. Flannigan, M.E. Flannigan, A.P. Verhoeff, O.C.G. Adan, and E.S. Hoekstra, (eds.), *Health Implications of Fungi in Indoor Environments*, Elsevier, Amsterdam, pp. 155-162.

Mouilleseaux, A., Squinazi, F., and Festy, B. (1993) Air quality in air conditioned office buildings. In *Indoor Air '93, Proceedings of the Sixth International Conference on Indoor Air Quality and Climate*, Vol. 6, Indoor Air '93, Helsinki, pp. 615-620.

Nevalainen, A. (1989) Bacterial Aerosols in Indoor Air. PhD Dissertation (University of Kuopio), National Public Health Institute, Kuopio, Finland.

Nevalainen, A., Jantunen, M.J., Rytkönen, A.-L., *et al*. (1988) The indoor air quality of Finnish homes with mold problems. In B. Berglund and T. Lindvall, (eds.), *Healthy Buildings '88*, Vol. 2, Swedish Council for Building Research, Stockholm, pp. 319-323.

Nevalainen, A., Heinonen-Tanski, H., and Savolainen, R. (1990) Indoor and outdoor occurrence of *Pseudomonas* bacteria. In *Indoor Air '90, Fifth International Conference on Indoor Air Quality and Climate,* Vol. 2, Canada Mortgage and Housing Corporation, Ottawa, pp. 51-53.

Newsome, A.L., Scott, T.M., Benson, R.F., and Fields, B.S. (1998) Isolation of an amoeba naturally harboring a distinctive *Legionella* species. *Appl. Environ. Microbiol*., **64**, 1688-1693.

Nilsson, A., Kihlström, E., Lagesson, V., *et al*. (2004) Microorganisms and volatile organic compounds in airborne dust from damp residences. *Indoor Air*, **14**, 74-82.

O'Brien, I.M., Bull, J., Creamer, B., *et al*. (1978) Asthma and extrinsic allergic alveolitis due to *Merulius lacrymans*. *Clin. Allergy*, **8**, 535-542.

Olsen, S.J., Chang, H.L., Cheung, T.Y.-Y., *et al*. (2003) Transmission of the severe acute respiratory syndrome on aircraft. *New Engl. J. Med*., **349**, 2416-2422.

Pakarinen, J., Hyvärinen, A., Salkinoja-Salonen, M., *et al*. (2008). Predominance of Gram-positive bacteria in house dust in the low-allergy risk Russian Karelia. *Environ. Microbiol.*, **10**, 3317-3325.

Pasanen, A.-L., Kalliokoski, P., Pasanen, P., *et al*. (1989) Fungi carried from farmers' work into farm homes. *Am. Industr. Hygiene Assoc. J*., **50**, 631-636.

Peltola, J.S.P., Andersson, M.A., Haahtela, T., *et al*. (2001) Toxic-metabolite-producing bacteria and fungus in an indoor environment. *Appl. Environ. Microbiol.*, **67**, 3269-3274.

Rantio-Lehtimäki, A. (1988) Yeasts in rural and urban air in southern Finland. *Grana*, **27**, 313-319.

Rantio-Lehtimäki, A., and Koivikko, A. (1984) *Rhodotorula*. In K. Wilken-Jensen and S. Gravesen, (eds.), *Atlas of Moulds in Europe Causing Respiratory Allergy*, ASK Publishing, Copenhagen, p. 42.

Rao, C.Y., Riggs, M.A., Chew, G.L., *et al*. (2007). Characterization of airborne molds, endotoxins, and glucans in homes in New Orleans after Hurricanes Katrina and Rita. *Appl. Environ. Microbiol.*, **73**, 1630-1634.

Reponen, T., Nevalainen, A., Jantunen, M., *et al*. (1992) Normal range criteria for indoor air bacteria and fungal spores in a subarctic climate. *Indoor Air*, **2**, 26-31.

Riley, E.C., Murphy, G., and Riley, R.L. (1978) Airborne spread of measles in a suburban elementary school. *Am. J. Epidemiol*., **107**, 421-432.

Rogerson, A., and Detwiler, A. (1999) Abundance of airborne heterotrophic protists in ground level air of South Dakota. *Atmos. Research*, **51**, 35-44.

Rohr, U., Weber, S., Michel, R., *et al*. (1998) Comparison of free-living amoebae in hot-water systems of hospitals with isolates from moist sanitary areas by identifying genera and determining temperature tolerance. *J. Allergy Clin. Immunol*., **64**, 1822-1824.

Rosas, I., Calderón, C., Martinez, L., *et al*. (1997) Indoor and outdoor airborne fungal propagule concentrations in Mexico City. *Aerobiologia*, **13**, 23-30.

Schlichtung, H.E. (1969) The importance of airborne algae and protozoa. *J. Air Pollut. Control Assoc*., **19**, 946-951.

Sharma, N.K., and Rai, A.K. (2008) Allergenicity of airborne cyanobacteria *Phormidium fragile* and *Nostoc muscorum*. *Ecotoxicol. Environ. Safety*, **69**, 158-162.

Sharma, N.K., Rai, A.K., Singh, S., and Brown, R.M. (2007) Airborne algae: their present status and relevance. *J. Phycol*., **43**, 615-627.

Shelton, B.G., Kirkland, K.H., Flanders, W.D., and Morris, G.K. (2002) Profiles of airborne fungi in buildings and outdoor environments in the United States. *Appl. Environ. Microbiol*., **68**, 1743-1753.

Sneller, M.R., and Roby, R.R. (1979) Incidence of fungal spores at the homes of allergic patients in an agricultural community. I. A 12-month study in and out of doors. *Ann. Allergy*, **43**, 225-228.

Solomon, W.R. (1974) Fungal aerosols arising from cool-mist vaporizers. *J. Allergy Clin. Immunol.*, **54**, 222-228.

Solomon, W.R., Burge, H.A., and Boise, J.R. (1980) Exclusion of particulate allergens by window air cleaners. *J. Allergy Clin. Immunol*., **65**, 305-308.

Spiegelman, J., Blumstein, J., and Friedman, H. (1963) Effects of central air conditioning on pollen, mold and bacterial concentrations. *J. Allergy*, **34**, 426-431.

Steinert, M., Emödy, L., Amann, R., and Hacker, J. (1997) Resuscitation of viable but nonculturable *Legionella pneumophila* Philadelphia JR32 by *Acanthamoeba castellani*. *Appl. Environ. Microbiol.*, **63**, 2047-2053.

Steinert, M., Birkness, K., White, E., *et al*. (1998) *Mycobacterium avium* bacilli grow sporozoically in coculture with *Acanthamoeba polyphaga* and survive within cyst walls. *J. Allergy Clin. Immunol.*, **64**, 2256-2261.

Stott, R., May, E., Ramirez, E., and Warren, A. (2003) Predation of *Cryptosporidium* oocysts by protozoa and rotifers: implications for water quality and public health. *Water Sci. Technol*., **47**, 77-83.

Ström, G., Palmgren, U., Wessen, B., *et al*. (1990) The sick building syndrome – an effect of microbial growth in building constructions? In *Indoor Air '90, Fifth International Conference on Indoor Air Quality and Climate,* Vol. 1, Canada Mortgage and Housing Corporation, Ottawa, pp. 173-178.

Sze To, G.N., Wan, M.P., Chao, C.Y.H., *et al*. (2008) A methodology for estimating airborne virus exposure in indoor environments using the spatial distribution of expiratory aerosols and virus variability characteristics. *Indoor Air*, **18**, 425-438.