In 2007, scientists estimated the direct cost of diseases associated with mould and dampness on the US population to be in the range of 4 billion dollars, and the indirect costs of lost work and school days are gauged even higher. The US Centers for Disease Control recently concluded that elimination of moisture and mouldy materials in the home definitively results in improved health. Unfortunately, problems of accurate assessment and precise identification plague the full understanding of the effects of mould on human health.

Addressing exposure assessment and identification, *Microorganisms in Home and Indoor Work Environments, Second Edition: Diversity, Health Impacts, Investigation and Control* discusses the methodology for and conduct of investigations on indoor environments, including details on key fungi and actinobacteria, and reflects advances in predicting their occurrence in buildings in various parts of the world.

**FEATURES:**
- Examines the difficulties and novel solutions in measurement, identification, and assessment.
- Includes new chapters on Pollen in Indoor Environments, Molecular Methods of Investigation, and Analysis for Toxins and Inflammatory Compounds.
- Updates information on microorganisms in outdoor air, bacteria and other bioaerosols in industrial workplaces, and respiratory tract infections caused by indoor fungi.
- Discusses epidemiological and case-related evidence on building-related illness.

Bringing together the state-of-the-science in this health-critical field, this accurate and timely book offers researchers, public health officials, and industrial hygienists crucial information on specific microorganisms in the built environment, along with current measurement and assessment solutions to clean up indoor air and keep residents and workers healthy in the future.
For Section 3.1 the copyright is retained by the British Crown.
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 leav- ing 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)
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.
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Chapter 1. Microorganisms in air
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,
Microorganisms in outdoor air 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:

\[ V = \frac{\sigma \cdot \rho g r^2}{\mu} \]

where

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

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 m³ 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.
Microorganisms in outdoor air

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...
**Microorganisms in Home and Indoor Work Environments**

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).

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-