CHAPTER 1

INTRODUCTION TO MICROBIOLOGICAL GROWTH AND CONTAMINATION INDOORS

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1.1. INTRODUCTION

Humans in modern society have been reported to spend 80% or more of their time indoors, whether at work or at home.[1] Indoor air quality has become an important public health concern since the mid-1970s, at least. Among various indoor pollutants, microbiologicals are one of the most important.[2] It has been estimated that one-third of indoor air quality (IAQ) complaints may be due to microbial contamination.[3]

Among the many microbial contaminants, fungal growth and contamination in the indoor environment has been the focus of news media reports since the mid-1990s as cases of infant death from acute pulmonary hemorrage/hemosiderosis in Cleveland, Ohio were associated with mold growth, specifically *Stachybotrys chartarum*.[4–7] Later a review by a panel of outside experts convened by the Centers for Disease Control and Prevention (CDC) found shortcomings in the implementation and reporting of the investigation. CDC concluded, on the basis of the review, that a possible association between acute pulmonary hemorrage/hemosiderosis in infants and exposure to molds, specifically *S. chartarum*, was not proved.[8] Unfortunately, there was no explanation for the cause(s) of the infant's death in the CDC review, and there has been no cause offered so far.

Indoor microbiological contamination is not new. Leviticus 14:33–45 of the *Old Testament* is often cited as the earliest known reference to a "mold problem" in the indoor environment. Although it is reasonable to believe that the reference of "plague" means mold, it can also be fungi, mildew, bacteria, algae, or any combination of these microorganisms due to moisture problems. Straus described it as putting forth a detailed protocol for the remediation of contaminated structures.[9]

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It did imply a wet or damp condition for microbial growth. In the East, the Chinese have traditionally associated "mushroom growing and becoming moldy" (*sen gu fa mei*; 生菇發黴 or 生菇发霉) on materials or in the living environment as an unhealthy sign. These references are significant because they indicate microbial growth due to wet and damp environments as unhealthy.

The interest in and concern for indoor microbiological growth and contamination has led to many books and publications, including reference books from the American Industrial Hygiene Association and American Conference of Governmental Industrial Hygienists.[10–13] There have been other reference books and conference proceedings related to the topic published since the mid-1980s. A list of selected books includes Refs. 2 and 14–24. The scope of these books varies widely. Some are texts with certain scientific depth and were written by research or practicing scientists. Some are how-to books for practicing professionals. In addition, most or all of the books offer basic, standardized approaches to assessing mold or microbial growth and contamination indoors. Furthermore, some books focus exclusively on mold even though bacterial growth and other biological contaminants do occur in water-damaged conditions. Chapters of this book are written by several scientists with advanced academic degrees and extensive practical experience in areas of indoor microbial contamination. The book intends to bridge the gap between the sciences and reality.

In additional to reference books, substantial numbers of publications (e.g., reviews, position papers, peer-reviewed original articles) have accumulated in the literature. This book is not designed to extensively review the entire literature. Instead, useful and relevant references with thoughts and depth that are authored by reputable and credible scientists or professionals are selected. Governmental and professional guidance documents are referenced where they are appropriate. These documents are often guidelines but not standards, even though some are titled standards. Such guidance documents may be designed to serve certain constituents and can become out-of-date rather quickly because it often takes a long time to prepare and produce such documents.

In 2004, the Institute of Medicine (IOM) published a report titled *Damp Indoor Spaces and Health* based on "a comprehensive review of the scientific literature regarding the relationship between damp or moldy indoor environments and the manifestation of adverse health effects, particularly respiratory and allergic symptoms."[25] The primary backdrop for the review was public health concerns due to fungal growth from damp environments as well as visible mold in homes with recent water damage. The report, however, emphasized the importance of a comprehensive approach toward microbial assessment in the indoor environment. This approach would include bacteria, fungi, and components of microbial agents, such as allergens of microbial origin, structural components of fungi and bacteria, microbial volatile organic compounds (MVOCs), and potentially toxic products of microbial secondary metabolism. Allergens of dust mites and cockroaches can also be important factors in a damp environment. Two previously published books by IOM[2,26] also emphasized the importance of various microbiologicals as allergens and asthma triggers in the indoor environment.

It is extremely important to emphasize that fungi are never the only group of organisms to proliferate in an indoor environment in which moisture is not controlled. This agrees with the three IOM reports as well as basic principles of biology. Various microbes, from bacteria, fungi, and slime molds, to algae and protozoa, have been associated with such an environment.[2,25,26] Therefore, fungi and other microbes, particularly bacteria, and their byproducts should be taken into consideration when conducting an assessment of a water-damaged environment. Furthermore, some environmental bacteria, including *Legionella* species and *Pseudomonas aeruginosa*, are common in water associated with building systems, such as hot water, sewage, or cooling water of the HVAC system, and are opportunistic pathogens that can cause death on infection without proper treatment. Reports of outbreaks of sporadic and nosocomial infections of Legionnaire's disease are not uncommon. Nosocomial infection by *P. aeruginosa* is one of the major infection control issues in healthcare facilities.[27]

In a water-damaged environment, bacterial, fungal, and other biological (e.g., insects and mites) growth is likely to occur depending on the duration of water damage. Expertise and experience from a variety of scientists and professionals are necessary to address the complex problem. This book is intended to be multi- and interdisciplinary, including authors who have the best and most thorough knowledge as well as extensive experience in diagnosing complex indoor microbial growth and contamination issues. The authors include microbiologists, mycologists, public health scientists, environmental professionals, and certified industrial hygienists with advanced academic degrees.

In a modern building, whether residential, commercial, or industrial, construction is complex and contains many components and different systems for various purposes, from functionality and comfort to necessity. These components and systems may be microbiological reservoirs or may become the cause of or a contributor to microbial exposure. For example, heating–ventilating–air-conditioning (HVAC) systems have been known to contain mold growth, requiring remediation.[28–30] Materials used in a building can also be part of the problem because of their nutritional value and hygroscopicity, or ability to absorb moisture. Wood and paper products are notoriously susceptible to moisture, leading to problems of fungal infestation and decay (see also Chapters 8 and 10).

1.2. HEALTH EFFECTS OF INDOOR FUNGAL AND BACTERIAL GROWTH

It is well established that fungi and bacteria have known health effects in humans.[3,12,25] Many species of fungi and bacteria can cause infections. They also produce a wide range of chemical byproducts, from microbial volatile organic compounds (MVOCs), endotoxins, and fungal glucans, to mycotoxins. Fungi include allergens and triggers of asthma. Fungal glucans are inflammatory in the lung and have been reported to be associated with headaches. Mycotoxins have a wide range of health effects, primarily in ingestion exposures. Although airborne mycotoxin exposures and health effects have been widely reported,[31] their impact is controversial within the medical community.[25] Gram-negative bacteria are producers of endotoxins, which have a wide range of health effects, from mild fever and flulike symptoms to death in extreme exposure conditions. Some medical clinicians question health effects other than allergy-related diseases from such exposures. Nonetheless, the American Industrial Hygiene Association took the position that the significant presence of fungi in indoor air that are not present or are a minor component of the outdoor air is unacceptable from a health (and building performance) perspective.[10,11]

In addition to the health impact on humans, fungal and bacterial growth can cause unsightly stains on building materials, degradation of building materials, and wood decay.[31–34] In fact, fungi are well documented biodeteriorating agents of many foodstuffs and paper and wood products.[34–36]

1.3. TEAM AND INDIVIDUAL EXPERTISE

A modern indoor environment is designed to accommodate many human needs and comfort, and has many built-in components and systems to fulfill such needs. Because of the complexity of such an environment, a team of professionals with various areas of expertise is often necessary in a comprehensive investigation and assessment, including sampling and analysis for microbiological organisms. Individual professionals and their expertise and experience in such activities are discussed below.

Many assessments and investigations are often initiated by complaints or reports of illness related to the indoor environment. More recently, surveys for fungal and microbial contamination have become routine as a part of due diligence in real estate transactions, whether residential, commercial, or industrial. If health complaints, personal injury, or health-related issues are alleged, medical and public health professionals, including physicians, must be included in the team. Specialty board-certified physicians, such as occupational health physicians, allergists, immunologists, pulmonolgists, infectious disease specialists, and epidemiologists, are some of the medical experts considered. Most physicians have limited experience in diagnosing patients with mold or microbial exposure. In fact, the University of Connecticut Health Science Center published a document titled *Guidance for Clinicians on the Recognition and Management of Health Effects Related to Mold Exposure and Moisture Indoors* in 2004, with a grant from the U.S. Environmental Protection Agency.[37] The title of the document unambiguously states that it is designed to assist clinicians to recognize and manage health effects related to mold exposure. A more recent position paper published in 2006 by the American Academy of Allergy, Asthma and Immunology (AAAAI) reaffirms that the allergy community requires better professional education and training on this topic.[38]

The 2006 AAAAI position paper on the medical effects of mold exposure intended to provide a state-of-the-art review of the role that molds are known to play in human disease.[38] The paper includes a section on measurement of molds and mold product exposure in the patient's environment and makes the following three conclusions: (1) sampling of both indoor and outdoor air for mold spores provides a measure of potential exposures and can be useful in certain conditions but has many shortcomings; (2) bulk, surface, and within-wall cavity measurement of mold or mycotoxins, although having potential relevance for other purposes, cannot be used to assess exposure; and (3) testing for airborne mycotoxins in non-agricultural environments cannot be used to diagnose mold exposure. Because physicians lack the education, understanding, training, and experience necessary to assess the extent of microbial growth within buildings, the conclusions beg further discussion and clarity than this book intends to provide. The results of properly conducted sampling and testing can be used to identify and determine the extent of fungal growth and contamination indoors. Such information can be used to evaluate and index occupants' exposures qualitatively as well as to determine the environmental control treatment of patients' living and work environments. The results typically consist of qualitative and quantitative data. Qualitative data include the identification of fungal taxa. By expert and logical analysis of the data, one can determine whether fungi are actively growing indoors. Clearly, the allergy community has very little training and background in mycology, sampling, and testing for fungi, or in result interpretation of data derived from such testing. Allergists could benefit from learning how to work with environmental professionals and use such information and evaluations in their practice.

Spores of *Alternaria alternata* are often cited as evidence of sensitization to the fungus[39,40] and links to the presence, persistence, and severity of asthma.[38,41] It was the only fungal allergen included in the National Health and Nutrition Examination Surveys (NHANES) II and III studies and reported by Arbes et al.[40] The report indicated that 54.3% of the U.S. population is sensitive to one or more allergens by skin testing 10 common allergens.[40] Prevalences to indoor allergens are as follows: dust mite 27.5%, German cockroach 26.1%, cat 17.0%, and at least one indoor allergen 43%.[40] *Alternaria alternata*, which was considered to be one of the outdoor allergens in the study, was the only fungal allergen tested. It is known to grow on water-damaged indoor environments. Among the population, 12.9% are allergic to *A. alternata*.

Spores of *A. alternata* are considered ubiquitous but as a minor component in abundance in both outdoor and indoor air. Because it is possible to measure its allergens in dust, it has been widely measured indoors[42] and because it is always present, although typically at low numbers compared with other funal spores, questionable epidemiological conclusions have been drawn and permeate the literature. Furthermore, the spore size of *A. alternata* varies from $7-18 \times 16-63$ μm[43], $7-18 \times 18-83$ μm,[15] $8-12 \times 20-40$ μm,[36] $8.5-14 \times 18-66$ μm,[44] to $9-18 \times 20-63$ μm.[45] The average size is 13×37 μm according to Ellis.[45] These spore sizes and ranges are inhalable but not respirable and, therefore, unlikely to reach deep into the respiratory system and the lungs. Andersen et al. studied and concluded that *A. alternata, A. longipes*, and *A. gaisen* are different species but often mis-identified as *A. alternata*.[46] In addition, Nielsen believed that growth and spores of *A. tenuissima* are far more common indoors than *A. alternata*.[47] *Alternaria* *alternata* is considered a rare species and is often misidentified simply because it manifests black *Alternaria*-like spores.[48] This raises the issue regarding the significance and importance of the spores as a human allergen. This further suggests that this type of study is multidisciplinary and calls for the input and involvement of mycologists in such important studies.

Fungi grow and reproduce by spore production. In their lifecycle, fungi also produce chemical byproducts, many of which are associated with fungal spores. Fungal spores are biologically designed for easy dispersal by various means, such as minute air movements and wind, water, insects, small rodents, and tiny creatures. Airborne dispersal is the primary dissemination route for most fungal spores. The presence of active fungal growth indoors is correlated with human exposures,[49] although the exposure dosages or quantities are difficult to measure because of sampling and testing difficulties, the biological complexity of fungi, and spatial and temporal variations.

The selection of physicians who are open-minded and diligent in conducting a review of accurate and up-to-date medical literature and research is important. If a physician, in examining a patient, determines that a specific group of infectious or allergic agents, their byproducts, or both is responsible for the patient's conditions or symptoms, this information will enable the investigator to design a sampling–testing strategy to maximize the probability of finding those etiologic agents in the patient's environment. A physician may also review medical opinions offered by opposing medical professionals.

Architects and various engineering professionals may play an important role in a team that is investigating a building for moisture leading to microbial problems. These professionals may include architects, civil engineers, mechanical engineers, structural engineers, roofing specialists, and geotechnical engineers. A team of architects and engineers are often involved in building design and construction. It is, therefore, important to have architects and engineers with background and training in design, construction, and maintenance of buildings on the team participating in the assessment and investigation of water-damaged buildings. Sometimes, architects and engineers with experience and acumen in forensic investigations play pivotal roles. Although an architect or an engineer with a professional license (such as AIA, PE, or SE) is important, it does not necessarily guarantee that this individual is the most competent professional available. Experience and knowledge are critically important. The same architects and engineers or other professionals may also be asked to provide solutions to the structural problems and assist in designing a microbial remediation project.

Industrial hygienists and environmental professionals usually play an important role in microbial assessments indoors. They may include certified industrial hygienists (CIHs), other environmental consultants, and possibly home and building inspectors. They are often called on to investigate, sample, and test for microbial contaminants indoors. They should review all the relevant facts pertinent to the building, perform an independent assessment of the subject building, and render an opinion on the likelihood of the cause of the mould and bacteria as it overlaps with engineers also involved. They should include an opinion on the implications of their findings on occupant health, and offer specifications to remediate the property and restore it to its previous condition to the extent possible. Although many environmental consultants are very competent in providing these services, a CIH with experience in microbial assessment is often preferred. Certified industrial hygienists are certified by a process that requires educational prerequisites, professional experience, and an examination administered by the American Board of Industrial Hygiene (ABIH). It is a professional certification that has gained wide recognition by many, including local and federal governmental agencies. On the other hand, a CIH may be educated and trained in chemistry, engineering, public health, or areas of environmental science but not in biology, mycology, or microbiology. They may practice broad general industrial hygiene, which may include ergonomics, noise, asbestos, lead, hazardous-site assessment, indoor air quality assessment and mould assessment. They may acquire skill and knowledge in environmental microbial sampling and analysis from literature, references, or by attending seminars and professional development courses. Other consultants may include home and building inspectors, registered sanitarians, or other individuals, all of whom provide such services for a fee. There are few regulations, professional requirements, certifications, or licensing procedures for these consultants. Their competency is usually highly variable and depends heavily on the individual's educational background, training, and experience. Several trade and professional organizations have set up "certifications" for residential mold inspectors or indoor air quality specialists. The credibility and usefulness of such "certifications" are questionable and highly uncertain at this time. It is strongly recommended that potential candidates, whether they are CIHs or other environmental consultants, should be interviewed and evaluated for their competence and experience in microbial assessment and sampling as well as their ethics.

"Microbiologist" is a collective term used to include scientists who are specialists in various disciplines of virology, bacteriology, mycology or parasitology. Some microbiologists may specialize in the biology of a single microbial species, such as *Escherichia coli*. On the other hand, there are microbiologists who have a broad interest in all aspects of microbiology. It is important to understand and evaluate the background and research interest of individual microbiologists.

Bacteriologists are the scientists who study bacteria. Bacteria are *prokaryotic*, which means that their cellular organization is structurally simpler than eukaryotic organisms, such as fungi, plants, animals, and humans, and their cellular functions are simpler also. Bacteria do not have a nucleus, mitochondria, or other organelles. Their DNA, RNA, and enzymes are dispersed in the cell. In evolution, eukaryotic organisms are considered much more advanced than prokaryotes. The differences between prokaryotes and eukaryotes are many and profound. This underscores the importance of using mycologists in analyzing fungi and bacteriologists for analyzing bacteria.

Mycologists are the scientists who study fungi. Mycology is a unique branch of microbiology and biology, and only mycologists have a true understanding of the science. There are very few colleges and universities offering degreed study in mycology. Therefore, there are not many trained, degreed mycologists.

Furthermore, very few mycologists have expertise in the group of molds and fungi that are found in water-damaged moldy environments. For example, there are mycologists who specialize in wild mushrooms, which are seldom found growing indoors. The mycologist who specializes in basidiomycetes, including mushrooms and wood-decaying bracket fungi and polypores, may not be familiar with ascomycetes and deuteromycetes, which include most microfungi growing in water-damaged environments. It is important to find the right expertise. The expertise of mycologists is different from that of virologists, bacteriologists, parasitologists, or microbiologists. Mycologists may play a role in assisting with planning a field investigation, sampling, laboratory analysis of samples, assisting with interpretation of the data, remediation, or any combination of these actitivies because they know the biology of fungi. On the other hand, mycology is a very broad field. It covers five major groups of fungi: zygomycetes, ascomycetes, basidiomycetes, deuteromycetes, and myxomycetes. Mycologists may offer information and guidance about the identification, detection, health effects, eradication, and control of fungi. On a few occasions, biologists with various areas of expertise may be called on to address issues caused by other biological agents, such as dust mites, cockroaches, pollens, and insects.

Microbiologists, bacteriologists, and mycologists are scientists who are seldom subject to certification or licensing requirements. However, there are certifications required for practicing public health and medical microbiologists, bacteriologists, and mycologists. They are usually affiliated with public health and medical laboratories. Their expertise is in medically important microbial species. Their practices are often of limited use in the study of microorganisms from the indoor environment.

Other professionals who may be helpful in sampling and testing for microbes and their byproducts can include biochemists, some of whom specialize in mycotoxins or other biochemical toxicants, and toxicologists, some of whom specialize in the toxic effects of mycotoxins and other biochemical toxicants. However, the biochemistry and toxicology of microbial byproducts are highly specialized. There are very few scientists and professionals who have expertise in these areas. A careful selection of experts is critical.

1.4. APPROACH OF THIS BOOK

In an assessment for fungal growth and contamination indoors, it is important to start with the building structure and the water history of the building. An engineer or a professional with such training and experience should conduct a detailed inspection and survey of the building and its systems for design, construction, installation, and maintenance issues that can lead to leaks, floods, and condensation. A building inspector should understand the control of moisture in three different forms: liquid water, water vapor, and condensation. Liquid water includes water from pipe bursts, overflow, flood, or leaks. Water vapor in a building may travel with the airflow and by diffusion. In a hot, humid environment, outdoor airflow into a building can carry large amounts of water vapor and increase both the moisture content or a_w of building materials and the likelihood of condensation. A high dew-point temperature is an indication of high moisture load in the air, which can increase the possibility of condensation on cold surfaces. In cold-climate buildings, high humidity load, particularly in late fall and early winter, can also result in condensation on and around cold surfaces, such as windows. If an elevated humidity load maintains steady through the entire winter as a result of human activities, such as cooking and washing, condensation can continue. The importance of moisture to indoor microbial growth is such that readers will find discussions of moisture in several chapters in the book. It is recommended that readers of this book further consult the references listed at the end of this chapter, including those by L'stiburek and Carmody[50] and by Harriman et al.[51] for more information on understanding and dealing with moisture indoors.

In a building with moisture issues, fungal growth is often the observed result. However, whenever fungal growth is observed and identified, bacterial growth is likely to occur also. In general, bacterial cells are much smaller than fungi and react much more quickly in response to water. In fact, the 2004 IOM report points out the likelihood of bacterial growth in a damp space and the importance of a comprehensive evaluation of bacteria, fungi, and components of microbial agents in such an environment.[25] A chapter on airborne bacteria in indoor environments is included in this book (Chapter 6).

The focus on fungal growth and contamination in water-damaged indoor environments is justifiable because the likelihood of fungal growth is minimal if there are no water damage or humidity control problems. Spores of common types of fungi of outdoor origin are ubiquitous indoors.[11,52] These spores will not germinate and grow without the required a_w or moisture content in the substrates. Fungal growth indoors is a very strong indication of water damage or long-term excessive humidity. On the other hand, bacterial growth can be from humans, or on continually rewetted surfaces such as in the kitchen, bathroom, laundryroom, and toilets. Assessing fungal growth and contamination is the better approach than determining bacterial growth indoors. The primary nutrients available indoors for fungal growth are paper and wood products. In most inspections, fungal growth on lumber and wood products is often overlooked. A chapter on fungal growth on wood and wood decay (Chapter 8) provides important information on fungal growth and wood decay. Several chapters in the book look into sampling and laboratory analysis of fungi. Common and appropriate sampling techniques and strategies are discussed. Emphasis is also placed on conventional and modern laboratory analytical methodologies, statistical analysis of data, and understanding of the ecology of fungi and bacteria found indoors. It is difficult to evaluate the quality of laboratory data without an understanding of how samples are handled, processed and analyzed in the laboratories. The importance of result interpretation is emphasized here. Several chapters include result interpretation as well as how to use laboratory test results. Recipients of the test results should take full advantages of the information contained in the laboratory reports. A chapter on the retrospective and forensic approach to the assessment of fungal growth in the indoor environment

(Chapter 11) is also included. The ultimate goal and purpose of assessment, sampling, and analysis is to detect, identify, and estimate the extent of fungal growth indoors for remediation and cleanup. Therefore, a chapter on proper professional remediation and cleanup (Chapter 12) concludes the main text of this book. Each topic covered is important in the diagnosis and mitigation of moisture and fungal problems in buildings.

1.5. CONCLUSIONS

There has been tremendous interest in the effects of microbial contamination in the indoor environment. It is well understood that bacteria, fungi, and possibly other biologics, such as mites and insects, grow in water-damaged indoor environments. An understanding of the moisture dynamics is the crucial step in dealing with microbial growth and contamination indoors. An investigation without an understanding of moisture issues in the building and their impact on microbial growth and contamination cannot address and solve the problems. Sampling and analysis for fungi, bacteria, and their byproducts are often requested, required and performed during an investigation. Sampling and analysis without proper planning, sampling, laboratory analytical methodology, and statistical analysis can, in fact, lead to erroneus conclusions. The importance of using the information and data in a specific environment with focused objectives in formulating opinions is also emphasized. In most building construction, paper and wood products are widely used. They are nutrients for bacterial and fungal growth. The use of such products is selective for cellulolytic fungi and possibly wood decay fungi when they become wet and remain wet long enough. To properly address fungal growth problems, an understanding of indoor fungal ecology is very important. In situations where responsibilities are shared, an understanding of the chronology of water damage and fungal growth is also important.

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