CHAPTER 3

Biological Pollutants in the Indoor Environment

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ABSTRACT

Studies carried out by Healthy Buildings International (HBI) found that in over one third of the more than 400 buildings studied, the major pollutants included allergenic fungi, and in greater than two thirds, air supply systems were contaminated with dust, dirt, and microbes. This paper discusses the recognition of sick building syndrome (SBS) as an accepted malady and the possible association of microbial contaminants with SBS. The different types of microbes are described along with the problems they can produce. Sources and spread of microbes within buildings is discussed along with descriptions of methods for sampling from surfaces, water, and indoor air. Special mention is made of sampling for and identification of *Legionella pneumophila,* using a new rapid assay technique, and the importance of correct interpretation of microbial findings against available standards.

INTRODUCTION

In 1982 the World Health Organization[1] made an attempt to define sick building syndrome and, at least since then, it has been recognized as a malady affecting a proportion of people in certain problem buildings. By its nature, SBS is difficult to define, and despite an ever-increasing number of publications on the subject, no single cause has been identified.[2] The indicators that a building may be sick usually are increased staff complaints of minor health symptoms and of stuffy air, intermittent odors, and visible increases in dust levels. Other factors may be uneven temperature zones, noticeable smoke accumulation, and dirt coming out of air supply diffusers. Management may also be aware of increased staff absenteeism and reduced productivity. The symptoms that affected people usually complain of are groups of almost trivial problems such as eye, nose, and throat irritation; headache, rhinitis, and sinusitis with skin irritation; cough, shortness of breath, and general lassitude; and dizziness, nausea, and mental confusion. One common factor often associated with these nebulous groups of symptoms is that they occur on entering the problem building and usually clear shortly after leaving it; another is that they are felt least in the morning and tend to become worse in the afternoon.

The symptoms associated with SBS are quite different from another set that occurs with what has come to be called building-related diseases, although there may be overlaps between them. Building-related disease symptoms, however, usually comprise much more definite and serious symptoms with recognizable medical signs and positive laboratory findings, with the causative agent being much more frequently identified. Such illnesses include Legionnaires' disease, humidifier fever, hypersensitivity pneumonitis, asthma and allergic rhinitis, all of which are usually excluded from descriptions of SBS.[2]

Studies carried out by HBI since 1980[3] have found that in more than one third of the buildings studied, one of the major pollutants was fungi, which is known to be allergenic to susceptible people. The heating, ventilating, and air conditioning (HVAC) systems in 70% of these buildings were either grossly or moderately contaminated with dust, dirt, and associated microbes. The airborne pollutants that may be present in the indoor environment comprise a complicated mixture of living and inanimate materials and may be gases or vapors, fibers, dusts, or microbes. The bacteria that have been isolated from indoor environments, both from HVAC internals and other surfaces as well as from air samples, have included a wide range of species. In some cases species known to cause infections are found, but the vast majority of species identified are generally regarded as harmless to humans. In the case of the fungi, many of the types found are saprophytic and a number that are known to cause allergic reactions and also infections in susceptible people are frequently isolated.

The biological pollutants present in the indoor environment come from a variety of sources, some of which are predominantly found outdoors but many of which occur both indoors and outdoors. In terms of routine building studies that include an assessment of biological pollutants, it is impractical to sample and test for all possible ranges of types that might be present. As far as building surveys are concerned, therefore, we look for those types that can be reliably isolated and identified using well tried and tested sampling and laboratory methods. Thus, we sample and check for the presence of a wide range of fungi and bacteria from surfaces and water and in the air of occupied areas of a building. In the case of biological pollutants such as protozoans, viruses, and chlamydia it is possible to isolate them, but they are more difficult and expensive to culture; this would usually only be done under special circumstances, such as might be found when medical evidence strongly indicates that this is required. Microbes or microorganisms are minute particles of living matter that occur in three main forms generally known as viruses, bacteria, and fungi.

VIRUSES

Viruses are the smallest of the group, although there are overlaps in all the definitions with regard to size, shape, content, and activity of this widely varied and enormous group of living things. Viruses range in size from about 0.3 to 0.02 µm. They consist of a core of nucleic acid (either RNA or DNA but not both) within one or two protein coats and do not grow or reproduce but replicate within host cells. Viruses are incapable of producing their own enzymes to break down complex compounds to simpler substances for their nutrition, and they are generally considered to survive only within living cells that may be human or other animal cells, plant cells, or even bacterial or fungal cells. Once in the cell, they change its normal function to that of a virus production factory that will ultimately destroy the cell and liberate many more virions into the host body. Because of this cellular requirement, it is difficult to conceive that the air conditioning system plays a large role in their multiplication and dissemination. This cannot be completely ruled out, however, as it is known that certain viruses are transmitted from person to person by droplets in the air, as with influenza and measles viruses, or in association with materials shed from the body, such as scabs from the pocks produced by the smallpox and chicken pox viruses. It is possible, therefore, that some viral species may survive long enough in the circulating air protected in some way by other particles or cells and be able to infect building users.

BACTERIA

Bacteria range in size from about 5 to 0.5 µm and are single-celled structures that can only be seen singly under the microscope. When they are cultured artificially, however, they form colonies that are composed of millions of single cells and are readily visible to the naked eye. Bacteria are procaryotic, which means that throughout the cell cycle there is no membrane that separates the nuclear material from other cell components. Some species possess flagellae that allow them to swim through liquid media or swarm over moist surfaces. Bacteria occur in almost every environment, particularly in dusty, dirty places inhabited by humans or other animals. Many of the species of bacteria isolated from buildings are harmless and frequently include members of the genera *Bacillus* and *Micrococcus* and also diphtheroid bacilli. Species that have been isolated from HVAC systems and other parts of buildings and can cause problems are *Pseudomonas* spp., especially *P. aeruginosa*, *Flavobacterium* spp., *Staphylococcus pyogenes*, *Serratia marscescens*, and *Legionella pneumophila*. More than thirteen serotypes of *L. pneumophila* have now been identified, all of them pathogenic but some causing the less lethal version of the disease known as

Pontiac fever, after the first definitive episode which occurred in that county of Michigan in 1969. The presence of a mixture of even the harmless species of bacteria in the various parts of an HVAC system may be taken as a sign that the conditions for microbial growth are being supplied, and steps should be taken to clean out dirt accumulations and remove standing water.

FUNGI

The group of microorganisms whose cells have a membrane separating the nuclear material from the rest of the cell protoplasm are classified as eucaryotic and include fungi, yeasts, and molds. These are single- or multi-celled or filamentous organisms whose cell walls are well defined structures containing polysaccharides, and sometimes polypeptides and chitin. They reproduce sexually and asexually often, with an abundance of spores that can be carried in air streams. They are ubiquitous in nature and inevitably enter buildings from the outdoors to set up colonies wherever conditions for growth are favorable. They are very resistant, and once they have established themselves in a niche in a building are very difficult to completely eradicate. Most species are saprophytic; many have been found to be the cause of both infections and allergies in office building occupants and are frequently isolated from various parts of buildings and their HVAC systems and associated ducts, chambers, and voids. From the results of about 200 surveys made in various parts of the world of outdoor airborne spores,[4] the same genera, *Cladosporium, Alternaria, Penicillium,* and *Aspergillus,* accounted for the highest mean percentages. A survey of 11 Florida homes[5] showed a high incidence of these same genera in circulating air. From data based on skin reactivity studies, these four genera also constituted those most prevalent in allergic respiratory disease.[4] Approximately 85% of patients found to be allergic to molds will react to one or more of these fungal allergens. Many other fungal species are routinely isolated from the internals of HVAC systems and their relationship to staff allergies is less clear.[6] Fungal species known to cause infections are *Aspergillus* spp., especially *A. niger* and *fumigatus* which classically cause serious lung infections.

PROTOZOANS

The protozoans are generally more advanced in their structure with more differentiation of parts for feeding, locomotion, and reproduction. Their importance in the indoor environment is that they are able to colonize stagnant water in humidifier reservoirs and improperly drained condensate trays. Aerosolization of their cells occurs directly from the contaminated water as it stands or when the humidifier sprays are reactivated; if the contaminated water dries up, either in its holder or on other parts of the system following carryover, the desiccated cells are

entrained in the air supply. Potent allergens may be formed, which if inhaled by susceptible people, give rise to a hypersensitivity response resulting in pneumonitis, humidifier fever, asthma, or allergic rhinitis. Additionally, it is now known[7,8] that these protozoans feed on bacteria, including *Legionella* sp., and that the bacteria can survive, sequestered within the cell protected from biocides in the water, or other unfavorable conditions, to multiply and emerge when better conditions return. Normally these tiny animals will not occur on their own in these stagnant waters within the HVAC systems but appear to require the presence of other microorganisms for nutritional purposes. In most case studies where building-associated hypersensitivity has been reported,[9-11] examination of the humidifier water has shown it to resemble a kind of "living organic stew," with representatives of all the different kinds of microorganisms present. The best way to avoid the problems caused by microbes and protozoans in buildings, therefore, may be to prevent any significant build-up of their numbers in the air supply systems, which may become their incubators and disseminators, by keeping such systems clean and free of water.

TYPES OF MICROBIAL PROBLEMS

Fungi and bacteria isolated from heating, ventilating, and air conditioning systems and other parts of buildings can cause problems in susceptible people in two ways: either by causing an infection or by causing an allergic reaction. With an infection the living organism penetrates the body's defenses and actively colonizes tissues, such as conjunctivae or the respiratory tract. The symptoms may range from slight eye, nose, and throat irritation to multiple deaths from acute pneumonia, as happened in Philadelphia in 1976 with what came to be called Legionnaires' Disease. With allergic reactions, susceptible individuals become sensitized to antigenic material and suffer from symptoms that can range from slight "hay fever type" symptoms, as noted in allergic rhinitis, to the much more serious reactions which may occur with allergic asthma, hypersensitivity pneumonitis, and "humidifier fever" cases. This sensitization may be caused by living microbial or protozoan cells, or by fragments of dead cells, or by toxic waste products produced by them. The affected individuals usually show acute symptoms such as malaise, fever, shortness of breath, dizziness, coughing, rhinitis, and muscular aches and pains, which are reduced on leaving the building for several days and then start up again on returning to it.

SOURCES

Microorganisms may gain entry into a building in many ways. Indeed, they may be built into it, for construction dust and debris along with microbes may contaminate HVAC system components and internals from the time of installation. Since microbes and their spores are small enough and light enough to be carried on air currents, the outside air taken into a building may contain large numbers. Usually they do not travel alone in the air as single microbial cells but tend to clump together in groups and, more frequently, become attached to dust and other particles many times larger than themselves. More should, therefore, be trapped in the medium efficiency filters recommended for installation in commercial buildings than if they were travelling as single cells. Even so, a proportion will still pass the filters, and the more there are in the air presented to filters, the higher the numbers that will pass them. The fresh air intake of an HVAC system is an obvious point for any foreign bodies to enter the system, and it should be protected with a grille of fine enough mesh to prevent birds, moths, butterflies and other insects, as well as dead leaves and other plant materials, from being drawn in. If these living things or their remains are allowed to putrefy, the numbers of microbes present may increase many times and may include species more pathogenic than might normally be found.

Apart from outdoor sources, there are also sources of microbial contamination within a building. We ourselves are a prime source of both animate and inanimate pollution, for whenever we move the action of our clothes rubbing against our skin causes the outer, dead layers of skin to be shed. It has been calculated that on average we each shed about seven million particles and cells per minute and that each of these carries with it an average of four microbial cells.[12] This happens all the time to some extent, but it may increase under particularly dry air conditions. Additionally, we are constantly shedding hair, and under warm conditions we perspire. These processes release parts of ourselves into the environment, and these parts are inevitably accompanied by microbes of one type or another from our bodies. One purpose of an HVAC system is to circulate air, and anything as light as these items are also carried in the air. Thus the return air drawn back to an air handling unit in an office building may be heavily loaded with airborne particulate and microbial contamination. If it is a good, well designed system, this air will be diluted with fresh air and efficiently filtered, and its airborne content will be significantly reduced. If not, then a large number of microbes may be passed through the unit's various pieces of equipment, and some may come to rest in the chill coils, condensate tray, or in the fan chamber, or be carried through to be offered again in the air to be breathed by the occupants. A proportion of those microbes that are deposited within the unit will die, but some will survive until growth conditions are favorable. For some this may simply mean the addition of free water which may be readily available from the condensation of humid air as it passes across chill coils; in a very short time, maybe even a few hours, a small number of microbes will have multiplied to a healthy colony actively spreading across a damp condensate tray. In the same way humidifier reservoirs and any other areas of moisture in the system exposed to the circulating air are likely to become contaminated. Particularly prone to this process is internal glass fiber insulation dampened by water carryover from condensate trays, spray humidifiers, or even steam humidifiers. With its indentations and rough surface, internal glass fiber insulation acts as a dust trap, and when damp forms an ideal matrix for microbial growth.

Once one species of microorganism establishes a foothold in an air handling unit, it is not long before others follow because among these lifeforms there occurs a certain amount of parasitism and symbiosis, some living on others, their waste products, or their dead cells. This is particularly true of periodically stagnant bodies of water that can occur in humidifier reservoirs; at least one outbreak of humidifier fever (or Monday sickness) has been shown[9] to be caused by antigenically active cells, or parts of cells, of a species of amoeba, *Naegleria gruberi,* that lives in the water phagocytosing bacteria. The contaminated water is introduced into the supply air by atomization through spray jets, and some water will fall outside the humidifier where it may evaporate. The amoeba will dry up and either die or sporulate. Dust coming off this area will now contain either spores or dried up fragments of the protozoan that are antigenically active and, if breathed in by susceptible individuals in the occupied area, may cause them to become sensitized, or, if they already are sensitized, they may suffer from an allergic response in the form of the fevers already described. Many other species cause reactions in people in this fashion, including the thermophilic actinomycetes, such as *Thermoactinomyces vulgaris,* often isolated from humidifier systems and air washers.[10]

SAMPLING METHODS

A variety of sampling methods have been developed and recommended for establishing the microbial load present on the internal surfaces of buildings and HVAC systems and in the air they are supplying.[13] Rodac (random organism detection and counting) plates containing trypticase soy agar for bacteria and malt extract agar for fungi may conveniently be used for sampling from surfaces; although certain problems may be encountered in some cases because of high numbers of microbes, such cultures are useful in showing which species of bacteria and fungi are present. These plates are normally incubated at 25°C for 48 h for bacteria and up to 7 d for fungi, with enumeration and identification being done by standard microbiological methods. Incubation at 30°C may be useful for the recovery of bacteria if fungal overgrowth occurs at the lower temperature. Counts may be expressed in the numbers of colony forming units per square inch or square centimeter of sampling area. Additional plates may be incubated at 56°C for several days for the isolation of thermophilic bacteria. Swabs moistened with an isotonic solution may also be used to sample from defined surface areas with subsequent inoculation to appropriate culture media and incubation and examination as before.

Bulk samples of water, ideally of at least 1 l, are required from stored water reservoirs, condensate trays, humidifier water, and cooling tower water for the culture of *Legionella* sp. and protozoans where this is required. Sampling for other bacterial species from water may be done using agar slides dipped in the water, or filter slides backed by an absorbent dried pad of culture medium activated by the sample water. After suitable incubation and identification, the results are given as the numbers of viable bacteria per milliliter of water. This is an effective method to obtain routine total bacterial counts from water and is more convenient than the traditional sampling and shipping to the laboratory of water for serial dilution and culture on solid media.

Several instruments are available for airborne microbial sampling. Three of the more reliable in our hands are the Andersen sampler (six stage and two stage), the Casella Slit Sampler, and the Reuter Centrifugal Sampler. Of these, the Centrifugal Sampler is the most portable and gives sufficiently accurate results for routine sampling. Nutrient agar is used for bacterial culture and a suitable medium, such as malt extract agar, for fungi. The numbers of microbes isolated are expressed in colony forming units per cubic meter of air. The manufacturer's instructions as to calibration and sterilization must be carefully followed to ensure accurate results from all sampling equipment. Gravitational or sedimentation methods of sampling for airborne microbes using settle agar plates or similar are not considered to give accurate representations of the airborne microbial load, either qualitatively or quantitatively, and are not recommended.

Whenever indoor airborne microbial counts are made, several outdoor counts should also be made to act as controls.[13] These outdoor counts should be taken well away from known sources but should also be taken adjacent to the outdoor air intake of the building and close to any potential generators of bioaerosols that may affect the indoor air, such as cooling towers and exhaust stacks. The indoor samples should be collected in areas where no complaints have been recorded as well as known problem areas, ideally, at different times during the day and on different days.

For total fungus spore counts, assessments can be made over time with Hirst-type spore traps. Another method is to use the more readily available filter cassette and personal sampling pumps that allow the spores to be washed off, centrifuged, resuspended in a known volume of liquid, and counted in a hemocytometer.

LEGIONELLA SPECIES

The first identified outbreak of Legionnaires' disease occurred at a convention in a Philadelphia hotel in 1976 when, of 221 people infected, 34 died from a fatal pneumonia. The final identification of the causative organism and its route of infection were not made until many months after the event. Many other cases of *Legionella* have now been identified using immunoserological methods to pinpoint the causative agent, and some sources report that the Communicable Disease Center at Atlanta estimates that more than 40,000 cases occur in the U.S. every year. The *Legionella* bacilli occur naturally in soil and water and may contaminate untreated water used in cooling towers or other water systems where large volumes are stored. For their survival and multiplication, *Legionella* require certain substances to be present in the water, such as iron and an amino acid, cysteine, that may be supplied by other microbes. People have been known to become infected by breathing contaminated aerosols from cooling tower spray drift, from seldom used shower heads in institutions, and from contaminated HVAC systems.

Until recently, diagnosis of *Legionella* contaminated water samples was usually done using culture methods that may take from 4 to 14 days to give results. Now the Legionella Rapid Assay (LRA)[14] test is available which gives a result within one working day from receipt in the laboratory of the water sample. The test is specific for *L. pneumophila* serogroup one, subtype Pontiac, which is responsible for more than 90% of outbreaks, and it can detect as few as 1000 organisms. The assay is a colorimetric monoclonal antibody method based on the well tried and tested Enzyme Linked Immunosorbant Assay (ELISA) principle using antibodies specific to the virulent Pontiac strain of serogroup one conjugated to an enzyme that reacts with a substrate to produce a colored response. The LRA achieves an accuracy comparable with other assay methods presently available, such as immunofluorescence antibody (IFA) techniques, and compared with culture methods it produces more positive results because it can identify the presence of viable but nonculturable *Legionella*. The LRA test is relatively easy to carry out, and a batch of five or ten samples can easily be analyzed in a modestly equipped laboratory within one working day. Test water samples are concentrated, either by filtration or centrifugation, and then inactivated by exposure to 80°C for 10 min. The various solutions and conjugates are prepared and the vacuum manifold set up. The bacteria are then filtered through the sample filter units where they are focussed into a central spot. The filter is then incubated in a solution of monoclonal antibody specific for *L. pneumophila* SG1 (subtype Pontiac) which has been conjugated with the enzyme alkaline phosphatase. Unbound conjugated antibody is washed away and a substrate that turns blue and precipitates in the presence of alkaline phosphatase is then added. The color intensity of the central blue spot indicates the concentration of *L. pneumophila* SG1 (subtype Pontiac) present in the sample. The results from the first 100 samples examined in the HBI Laboratories using the Micro-C LRA test gave 47 positives, of which 18 were from 41 samples of cooling tower water.

INTERPRETATION OF MICROBIAL RESULTS

The correct interpretation of microbial sampling results from the indoor environment is as important as the sampling techniques themselves. Surface culture samples from the internals of air handling units can give rise to misleading results and they must be interpreted with great care. Many variables exist in the symbiotic and pathogenic interrelationships that exist between man and microbes, not least of which is the number of infective or allergenic units necessary to produce symptoms. Other variables include susceptibility of the potential host, which can be related to such factors as age and general health; the local environment, which can include type of occupation and population density; and the mode of operation of the air supply system, which can be affected by changes in ventilation rates and the quality of the hygienic maintenance program. Such surface culture results can be very useful in a qualitative sense in demonstrating which groups of microbes are present and may in this way forewarn of potential problems. For example, if large numbers of the common, dust associated *Bacillus* sp. are identified in an air handling unit, this may not cause as much concern as a finding of only small numbers of fungal colonies identified as *Cladosporium* sp. or *Aspergillus* sp. because of the well-known propensity of the latter to cause allergic reactions and infections in susceptible people. Potential reservoirs and disseminators of microbes may be identified in this way, and comparison with the identification results from airborne samples may confirm such sources.

National standards for levels of airborne microbes in commercial buildings are not yet available, but many "safe levels" ranging over a wide scale have been proposed over the years. Experience of many building survey results[6] suggests that an acceptable level might be 750 cfu m^3 with the provision that if the total airborne count is lower, but among the species isolated are some known to cause infection or allergies in susceptible people, then even that low count is unsatisfactory and steps should be taken to reduce or eradicate the microbes. Such potentially problematical bacterial species include *Pseudomonas aeruginosa* and *Flavobacterium* sp. and among the fungi members of the genera *Alternaria, Cladosporium, Aspergillus,* and *Penicillium.*

In the more visible areas of office buildings, accumulations of dust and dirt are not normally allowed to gather because they are unsightly and unhygienic. The same attitude should be applied to areas of the building that are directly involved with the conditioning and delivery of air to be breathed by the occupants for there is no doubt that in buildings with clean and well cared for HVAC systems with effective air filtration systems and adequate outside air makeup, complaints and problems are significantly less.

In the case of the identification of *Legionella pneumophila* in cooling towers or water associated with air handling units, or in any other situation where a potential for exposure is present, the extent of the risk should be assessed. This should be based on the factors known to enhance the growth rate of *Legionella,* such as optimum temperature, pH, availability of nutritional requirements, water change rate, and the presence or absence of a water treatment program; the feasibility of airborne transmission related to exposure time and droplet size; and the total microbial population in the water, the presence of slime or sludge, and the total legionella count. Each factor is given a numerical rating, and these are compounded to give an overall score — the higher the score the greater the risk. This can be considered only as a guide, for the mere presence of *L. pneumophila* at any concentration does not imply that exposed people will succumb to infection.

However, if the results indicate that high risk levels are present, then it would be considered prudent to deal with the water promptly, ensuring that conditions are not allowed to remain that will allow bacterial or slime growth to occur.

CONCLUSIONS

The study of sick building syndrome is ongoing, and as methods continue to be developed, more will be learned about the true role of microbes and their products in the problem. For example, investigations are being done[15] into the role played by bacterial endotoxins and fungal glucans in SBS for it is known that these substances do cause inflammation and affect the immune system. Dose-response relationships have been established for symptoms produced in occupational environments,[16] but since the amounts of these substances required to produce responses in susceptible people are very small, it could be that small numbers of the appropriate organisms have so far been overlooked or discounted in building studies. Consideration has been given to the role of volatile organic compounds (VOCs) in problem buildings, and it is known that some of the odors produced by fungi are caused by products that belong in this group[17] but it is not certain what part they play in symptom production, either alone or in conjunction with other similar substances. It may be that there is an enhancing of reaction when certain compounds are grouped together. Over the years, some species of bacteria and fungi have become accepted as being "nonpathogenic" or harmless. It may be that this definition will have to be revised if new evidence comes to light showing that a slightly different method of attack has been developed by such species. Similarly, it may be that "new" species of microbes will be discovered that play a part in SBS in the same way that *L. pneumophila* has emerged. In the meantime, it is necessary to continue sampling for microbes in problem and nonproblem buildings alike, from their surfaces, water, and air, so that information can be gathered to help increase our knowledge of their role in SBS.

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