INTERPRETING MICROBIAL MEASUREMENT AND ANALYSIS

T Nathanson*

Public Works and Government Services Canada, Ottawa, Canada

ABSTRACT
The potential for microbial contamination exists in every building and within every HVAC system. The two basic requirements for microbial growth are first, a moisture source, and second, a nutrient. Fungal spores and bacteria are prevalent outdoors and indoors and so are nutrients such as organic materials including dust and soil. Given the right conditions, microbials will grow on any surface. Therefore control of moisture and good housekeeping will reduce the possibility for microbial growth within buildings. A rapid response to water damage using established remediation procedures will prevent microbial contamination (Nathanson, 2001).

Visible mould within buildings must be remediated. In most cases however, mould growth is not evident (hidden) and airborne sampling needs judicious interpretation. There are no ‘threshold limit values’ for microbial exposure, measurement methods vary, analysis is difficult, and there are other factors which can greatly influence the results. However, there is still value in taking airborne microbial samples.

INDEX TERMS
Microbial measurement, Interpretation of results, Microbial exposure guidelines.

INTRODUCTION
Micro-organisms such as fungi, bacteria, viruses and pollen, are natural and ubiquitous components of the outdoor and indoor environment. Fungi, often called ‘mould’, or ‘mildew’, originate on plants, leaves and in soil. Yeasts are also fungi. Over 100,000 species of fungi are known to exist and many have yet to be classified; most produce spores that are designed to be transported through the air.

Microbial evaluation of indoor air started in the late 1950s when secondary (nosocomial) infections of patients were reported. Later, an industrial case of exposure to airborne contaminated cutting fluids (Pontiac fever) and cases of exposure to contaminated cooling towers (legionellosis, 1976) and humidifiers (humidifier fever) were documented. In Canada, microbial contamination affects approximately 20 % of buildings with indoor air quality problems (Nathanson, 1995). The first measured case by Public Works and Government Services Canada (PWGSC) occurred in 1986.

Mould should not grow in indoor environments. However, spores will germinate anywhere where both moisture and nutrients exist. Therefore, strategies to prevent microbial growth must include the avoidance of wet surfaces, keeping relative humidity levels below 60%, effective filtration of particulates, proper HVAC system operation and maintenance, and good housekeeping.

* Contact author email: Tedd.Nathanson@pwgsc.gc.ca
Floods and leaks do occur in buildings and established procedures should be followed to avoid contamination (Nathanson, 2001). When areas do become contaminated, often the mould is not visible and odours may be absent. Release of spores can take place months after water has disappeared. Air sampling is undertaken in order to characterise the extent of the problem and to establish a remediation plan.

Microbials can be measured by a variety of methods; bulk samples, surface samples using a swab, contact strip or tape, and air-borne samples using a pump and collection medium. The samples can be inspected microscopically or can be cultured on a nutrient (agar). An accredited and experienced laboratory should do the identification and numeration of species. The Canadian Federal Government’s protocol uses a Reuter Centrifugal Sampler (RCS), Rose-Bengal agar, and a 4-minute sampling time to measure airborne microbials. Swab samples are taken to locate the source of contamination.

FUNGAL EXPOSURE REGULATIONS
There are no regulated exposure threshold limit values or standards for microbials (ACGIH, 1999). Several reasons for this are as follows:

• It is not possible to collect all bio-organisms using a single sampling method. The methods used to collect, culture and analyse samples vary greatly. Sampling equipment is size-specific, for example, settle plates will collect only large microbials, and centrifugal samplers will miss the larger spores. Microbials may be culturable, nonculturable, and non-viable. Fungal and bacterial fragments can be allergenic. Different agars will support the growth of different fungal species, depending on the agar formulation and moisture availability. Incubation time and temperature also favour selective organisms.

• Collection methods do not reflect actual human exposure. Microbial concentrations in air will vary by several orders of magnitude in one location and between sites. Short 'grab' samples (4 minutes for the RCS) cannot represent real exposure values over a work period.

• Information relating both viable and non-viable microorganisms is presently insufficient to establish dose-response relationships. Very few epidemiological studies have been done. The issue is further complicated be the secondary by-products that many microbial species produce, such as mycotoxins, endotoxins, volatile organic compounds, antigens, 1,3-glucan, etc. that may be more potent than the microbial itself. These 'indicator' measurements do not accurately reflect total exposure.

• There is a wide variation in individual susceptibility to microbials and various factors such as genetics, age, personal habits, health, pre-existing conditions, medication, and previous exposure, will affect people’s reaction. Furthermore, building occupants are exposed to a large variety of complex and variable chemical and biological mixtures at work, outdoors and at home. Consequently, exposure information is imprecise because agents, other than those identified and measured, will also be present and may be responsible for some of the health responses by exposed persons. Biological markers of exposure to fungi are largely unknown.
INTERPRETATION OF RESULTS
Using data collected since 1986 (over 6,000 samples in hundreds of buildings), the Federal-Provincial Advisory Committee on Environmental and Occupational Health published interpretation guidelines for microbial measurements in building environments (Nathanson, 1995, and Federal-Provincial Advisory Committee on Environmental and Occupational Health, 1995). Other cognisant authorities such as the World Health Organisation, The American Industrial Hygiene Association (AIHA, 1996), and the American Conference of Governmental Industrial Hygienists (ACGIH, 1999) have referenced these Canadian guidelines.

The basic, common-sense approach to interpretation is as follows:

1. Microbial growth within a building is not acceptable. Good HVAC system design, operation, and maintenance practices should follow current standards and practices to avoid microbial amplification. Moisture intrusion, visible mould, wet or soiled surfaces, must be remediated following an established protocol.

2. Fungal quantities, measured as colony forming units per cubic meter of air (CFU/m³), should be lower indoors compared to outdoors, and the 'mix' (biodiversity) should be similar.

   Dominance indoors by species of mould that are not predominant outdoors indicates an interior amplification site. This must be located and rectified. If no contaminated site or moisture source is found, then remediation is recommended; all hard surfaces wiped with a bleach solution, all fleecy surfaces vacuumed with a HEPA filtered unit. The area should be re-sampled in 3 months.

   Quantities of normal outdoor [phylloplane] species greater than 500 CFU/m³ indoors, indicates poor filtration or housekeeping.

3. The presence of more than one spore of a toxigenic fungus as defined by the Canadian Public Health Association (1987) indicates that further investigation is necessary. Visual inspection, use of a moisture meter, and air/surface sampling is usually done to locate the source. If none is found, normal remediation is recommended, and the area should be re-sampled in 3 months.

   [Similar guidelines apply to bacterial samples. Bird and bat droppings must be assumed to contain pathogenic fungi and must be removed under hazardous waste conditions.]

REMEDIAL MEASURES
While prevention and control of microbials are requisite conditions needed to maintain a healthy and comfortable workplace, remediation is necessary if there is an indoor source. Depending on the species type and level of contamination, there are protocols established by Health Canada (Federal Provincial Advisory Committee on Environmental and Occupational Health, 1995), New York City Department of Health (NYC Department of Health, 2000), and other authorities, (ACGIH, 1999 and University of Minnesota, 1998).

If microbial growth does occur, containment, cleaning and removal of contaminated materials
must proceed to reduce the risk of exposure and to avoid further deterioration. During this process, procedures must be in place so that occupant exposure is not increased. This is the first principle of remediation.

Health Canada’s protocol for mould recognition and management (Federal Provincial Advisory Committee on Environmental and Occupational Health, 1995), follows the following phases; assess the magnitude of the health problems, identify probable sources of microbial contamination, sample and identify fungal species, provide risk communication, and implement remedial action. All cases of mould contamination lead towards remediation. New York City guidelines (2000) state; ‘except in cases of widespread fungal contamination that are linked to illnesses throughout a building, building-wide evacuation is not indicated’.

The maxim that all building occupants should be protected from microbial exposure during testing and remedial action must be followed. In this regard, factors such as the extent of contamination [size] and the species type [toxigenic or not], are recognised in the guidelines and accounted for by the use of different containment strategies, equipment and methods. Another important related consideration is the isolation of the air distribution system from the remediation area so that microbials are not transported to other zones.

The following general remediation principles apply.

- Moisture control is recognised as the primary factor in controlling microbial growth. If porous materials such as fibreglass insulation, carpets, ceiling tiles, and plaster do become contaminated, it is usual to discard these materials. Hard surfaces can be salvaged using a 6 -10 % bleach solution and clean-water rinse. Biocides and antimicrobial agents may be used to decontaminate selective areas such as ducts, water reservoirs, and condensate pans. However, occupants must not be exposed to any residual compounds.

- Water damage from leaks, floods and plumbing failures should be repaired and remediated within 24 hours. Wet materials must be dried and porous materials saturated with unclean or contaminated water must be discarded. Water penetration or migration through the building envelope, and condensation within the interior or exterior wall assembly is to be avoided. Do not over-humidify the building during the winter. Dehumidify supply air in summer to 60 % maximum during occupied periods and to 70 % during downtime.

- The HVAC system can be a source of microbial contamination; avoid water vapour re-entainment from rooftop cooling towers and condensers, maintain good filter performance (small systems at 30 % efficiency, large systems at 85 %). Avoid stagnant water within the system and clean condensate pans and other wet areas such as drift eliminators, reservoirs, floors, etc., monthly. Wet porous material is to be avoided. There should be no moisture or water vapour in front of the fan as this will carry through into the supply ducts. Ensure access to all components for scheduled maintenance and cleaning. Keep a log of all activities.

CONCLUSIONS
Air sampling is not an infallible means of determining the existence of fungal contamination and any survey must rely on the skill and experience of the investigator. Information from a
A large data set has produced practical guidelines on how to interpret measurement results and how to effectively remediate the situation.

As public information on microbial contamination (and other IAQ and environmental issues) increases, research and co-operation between the various disciplines; architecture, engineering, industrial hygiene, mycology, and medicine will evolve to increase our understanding of the issues. The multi-disciplinary aspect of microbial assessment and remediation has been already demonstrated at the Third International Conference on Fungi, Mycotoxins and Bioaerosols, at Saratoga Springs, NY, in 1998, where over 300 participants, many of them medical doctors, presented an extraordinary range of papers (Johanning, 1999). Until the magnitude of the population risk is known, it would be prudent, based on current evidence, to remediate indoor sources of microbials.

REFERENCES
ACGIH. 1999. Bioaerosols: assessment and control, Cincinnati, Ohio, American Conference of Governmental Industrial Hygienists.