Application of Surface Sampling to Survey Microflora in Building Indoor Environmental Quality (IEQ) Investigations.

Clifford A. Cooper, Chem-Safe Inc., Pullman/Spokane, WA

Our understanding of the association of building microflora and symptoms reported by building occupants has increased steadily within the past decade. In many of the most serious outbreaks of building-related illness, where a cause and source have been identified, a biological source has been found responsible. [1,2]

Building-related illnesses (diseases) are comprised of infections, including legionellosis and humidifier fever, and more common non-specific intoxications (sometimes termed "hypersensitivity" diseases[3])for which a single causative agent often is not identified. This later case has become labeled "sick-building syndrome" or, "tight building syndrome". It is apparent that we must include biological factors among our considerations in assessing health-related factors in indoor environmental quality.

Microflora Ecology

A basic understanding of microflora ecology is necessary in order to understand the role of the *biological* environment in building-related disease. **Aerobiology** has developed as a science and provides basic information on many aspects of the airborne spread of disease. [3,4,5,6] By combining knowledge gained in this field and knowledge of the ecology of microorganisms in the natural environment, a model can be derived to describe the interaction of a building's microflora with their indoor environment in relation to the sudden onset of hypersensitivity disease symptoms in non-atopic (not predisposed to allergy) building occupants. According to Gregory[4], a concentration of allergens must be present, regularly or occasionally, either indoors or outdoors in sufficient concentration to sensitize or provoke symptoms in ordinary (non-atopic)

persons (Table I). Gregory also notes that the severity of the symptoms is related to the quantity of allergen inhaled.

Table I

TYPICAL REACTIONS TO AEROALLERGENS

Subjects affected:	*Atopic persons (± 10% of population)*		*Non-atopic persons (majority of population)*	
Sensitization:	By normal exposure to inhalant, often multiple		By massive exposure, not typically multiple	
Antibodies:	Reaginic (non-precipitating, transferable) IgE		Precipitating (non-transferable), IgG and IgM	
Reaction:	Immediate (Type I) as a rule (but a night attack may result from daytime exposure) No complement needed for reaction		Late (Type III) after 5 to 7 hours Reaction needs complement	
Skin test:	Urticarial weal in 10–15 min, gone in 2 hours		Often negative (or extensive oedema in 3 to 4 hours, gone in 24 hours)	
Examples of Aeroallergens:	POLLENS Grasses (Gramineae) Ragweed (Ambrosia) Nettle (*Urticaria*, and *Parietaria*)	LARGER SPORES *Alternaria* *Cladosporium* Basidiospores Ascospores	*Aspergillus fumigatus* (is also pathogenic)	SMALLER SPORES *Cryptostroma corticale* *Aspergillus clavatus* *Micropolyspora faeni* *Thermoactinomyces vulgaris* *Thermoactinomyces sacchari*
Site of reaction:	NOSE	UPPER RESPIRATORY TRACT (trachea, bronchi and bronchioles)	LOWER RESPIRATORY TRACT (respiratory bronchioles, alveolar ducts, alveoli)	
Condition:	Rhinitis	Typically asthma	Typically alveolitis	

Source: (4)

Cook and Rayner[7] describe stress and disturbance conditions (destructive and enhancement) which may promote rapid growth and dissemination of microorganisms. Three primary ecological strategies -- Stress-tolerance (S-selective), Combative (C-selective), and Ruderal (R-selective) describe a microorganism's response to its environment.(Figure 1)

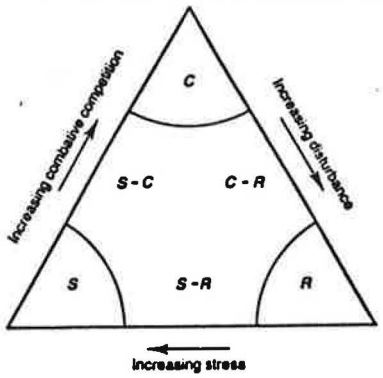

Figure 1: Model of location of primary and secondary strategies in relation to the relative importance of combative competition, stress and disturbance. Primary strategies: C, combative; S, stress-tolerant; R, ruderal. Secondary strategies:C-R, combative ruderal; S-R stress-tolerant ruderal; S-C, stress-tolerant combative. (From Cook & Rayner (7))

C-selected organisms indicate vitality but not necessarily rapid growth or high production rates of spores or somatic cells. The indoor environment characterized by a predominance of these organisms would be a stable environment containing readily available and easy to assimilate nutrients, and in culture would be identified as a slow-to-moderately growing polyculture. R-selected organisms are indicated by a rapidly growing monoculture that may be seen to "take over" the growing medium. S-selected organisms typically inhabit more hostile environments which require the organism to adapt to conditions of stress in order to survive. Examples of such environmental stress include lack of free available water or nutrients, air velocity, temperature, presence of biocides, and substrate availability. Cultures from samples taken under these conditions typically show a slow growing monoculture. According to our (Cook and Rayner's) model we should be most interested in identifying microorganisms which exhibit the C-selected and R-selected strategies; C-selected to indicate a stable nutrient-rich environment, and R-selected to indicate "enhancement disturbance" conditions. Either of these environmental conditions can lead to abnormally high (severe) microflora levels.

Environmental Sampling

Most methods for assessing microflora populations in buildings are indirect and depend on developing colonies from samples incubated on or in nutrient agar. These colonies are taken to be a measure of populations.[7] Sampling the indoor environment for microflora is utilized most appropriately to identify basic characteristics of the indoor microenvironment, in particular the extent to which it supports populations of microorganisms, as well as the diversity and vitality of the organisms present. Alternative environmental sampling for infectious organisms is generally inappropriate principally because sampling efficiency for infectious organisms in the indoor environment is poor. [3,4,6]

Areal Sampling

Techniques for environmental microflora sampling include "gravity" collectors, impingement, impaction, filtration, precipitation, and surface sampling techniques. With the exception of surface sampling techniques, all of the techniques listed are areal sampling techniques and all suffer drawbacks of variable collection efficiency dependant on such factors as particle size, collection media, airflow velocity, time and frequency of sampling and sampling duration. Such factors make it imperative that the operator of a bioaerosol collection system and the biologist who interprets the results are well trained in the system. All personnel involved in project design, field sampling, laboratory analysis and data assessment should follow a well thought out quality assurance program developed to minimize the factors which lead to loss of precision in sample measurements. However, even under controlled measurement conditions, a sample collected from short-term areal sampling may not reflect actual environmental conditions, which in most cases are dynamic and can vary significantly both temporally and spatially. [5,6,7,8,9] Figure 2, adapted from Ottauni and Franceschetti[6] represents colony count data from 30 consecutive areal measurements and indicates the variability associated with sampling in a "normal" environment.

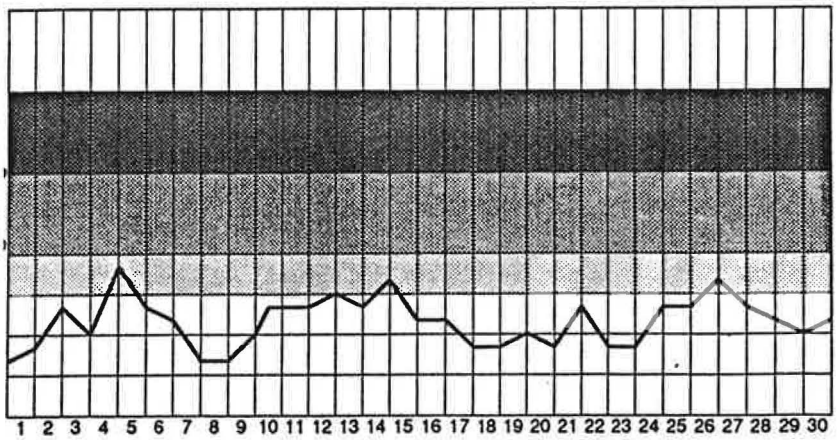

Figure 2. Typical "counts" using areal sampling techniques for 30 consecutive samples under "normal" conditions. Note the variability in the range of counts between samples. (Adapted from (6))

Surface Sampling

The sampling of building surfaces which are in continuous contact with building supply air can indicate the recent history as well as present conditions in a building's microenvironment. When incorporated into a general building IEQ investigation, surface sampling can provide information on the possible role of microflora in reported health complaints, and can be used to isolate the source(s) of these organisms.

The surface sampling method we use is a plastic test strip which contains a small absorbent pad on the end having selective growth media for either aerobic bacteria or fungi (yeasts and molds). To sample, the test strip is first moistened with a neutralizer solution to prevent inhibitory effects from residual sanitizers or with distilled water. The strip is used in the same manner as swabs to sample a defined area by gently pressing the pad to the surface and rotating in a circular motion or drawing the pad across the surface. The test strip is then put into a sterile transparent zip-lock pouch and incubated. After incubation the density of microorganisms on the test strip is compared to a standard growth chart and colony count is determined.(Figure 3)

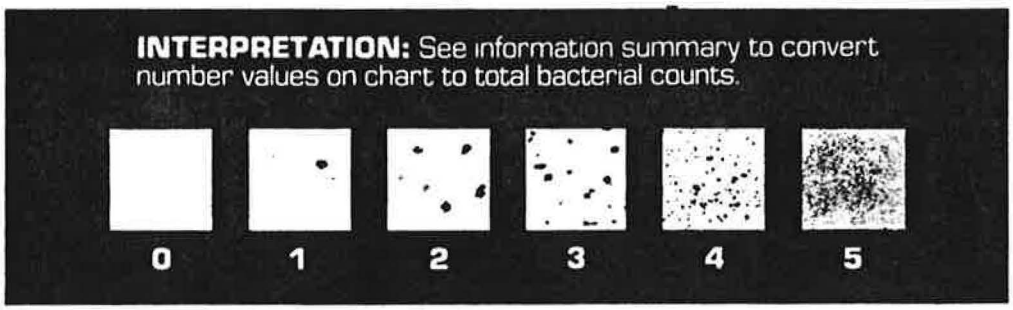

Figure 3. Example test strip comparison chart used for obtaining colony counts from surface sample measrements.

Media strips have been evaluated by a number of laboratories and correlation between test strips and plate counts is reported to be within the 95% confidence level.[11] As a result this method has found acceptance in

the U.S. for a variety of commercial and industrial applications.[11,12,13] In most cases, surface colony count data can be related directly to building environmental quality utilizing a semi-quantitative index which relates surface colony count data to severity of contamination present at the sampling site. Once combined with sampling site data from throughout the building, a picture emerges which describes the microflora environment of the building. Table II provides an example of the application of this scheme from a recent IEQ study of a 16-story office building in Spokane, Washington.

Table II

Results of Microflora Sampling of a 16-Story Office Building using surface sampling test strip technique.

Floor	Zone *	Bacteria Colony count/in^2	Value	Yeast/Mold Colony count/in^2	Value
16	Interior	<10	low	<10	low
	Exterior	<10	low	<10	low
15	I	100	low/mod.	<10	low
	E	10	low	10	low
14	I	10	low	<10	low
	E	10	low	<10	low
12	I	10	low	10	low
	E	10	low	<10	low
11	I	10-100	low	<10	low
	E	100	low/mod	10	low
10	I	100	low/mod	100	low/mod
	E	100	low/mod	100	low/mod
9	I	100	low/mod	<10	low
	E	10	low	<10	low
8	I	<10	low	<10	low
	E	--			
7	I	1,000	mod.	<10	low
	E	10	low	<10	low
6	I	1,000	mod	10	low
	E	<10	low	<10	low
5	I	<10	low	<10	low
	E	100	low/mod	<10	low
4	office	10	low	<10	low
		1000	mod	10,000+	severe
BANK	Safety Dep	<10	low	10	low
	Mezzanine	<10	low	10	low

* Two-zone HVAC system

Data Correlation between Surface and Areal Sampling

There are at present no published standards associating risk with exposure to non-pathogenic microflora. Burge. et. al.[3] suggest that indoor air levels of fungal spores be less than one-third of outdoor levels which, during the growing season, typically range from 100-100,000/M^3 of air. Ottaviani and Franceschetti[6] have proposed "Acceptability Limits" for environmental food plant surveys based on seven grades of sanitation, with grade 1 being a sterile environment and grade 7 indicating extreme contamination. (Figure 4) They suggest total microflora counts under "normal" indoor conditions, without air conditioning, should be between 300 and 500 microorganisms per cubic meter, and suggest this as a recommended maintenance level for most indoor environments.

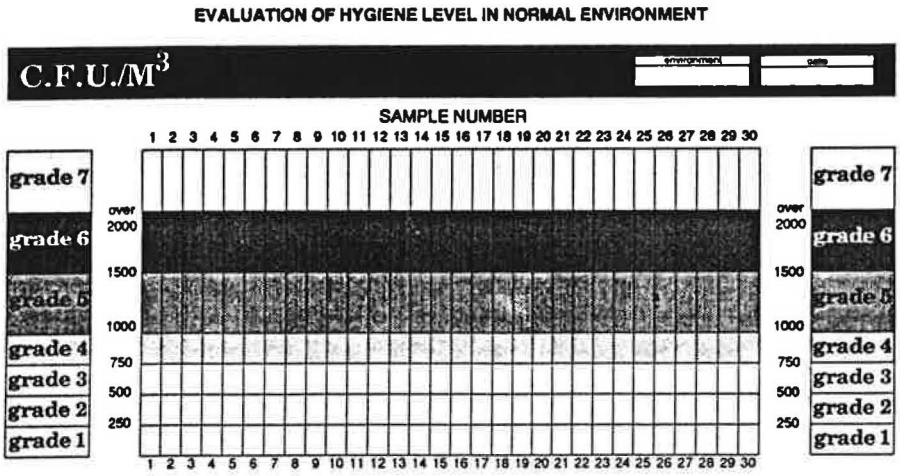

Figure 4. Example contamination index and plot used for areal sampling. (Source: (6))

In attempting to relate colony counts from surface sampling to airborne counts numerous problems exist and an empirical approach must be taken. It must be kept in mind, too, that in such comparisons we look to relate surface sampling to peak rather than mean bioaerosol measurements. This

is principally because surface samples will include spores, vegetative cells and somatic cells all of which may indicate recent past history of events which have resulted in bioaerosol development as well as present activity. From our earlier discussion it is apparent too that it can be at times the peak rather than average bioaerosol concentration most associated with adverse health effect in non-atopic populations. By collecting the sample from an appropriate surface rather than areal sampling, the information obtained may also represent potential for future population growth and activity.

Summary

Like areal sampling, surface sampling records what are essentially spore and somatic cell numbers and can therefore be useful in relation to microflora population assessment. Advantages of surface sampling include low-cost reproducible results that reflect simultaneously both past reproductive effort and potential for future population growth and activity. In applying this or any technique for indoor assessment of building microflora it is necessary to understand factors which may be present and playing a role in the micro-ecology of the indoor environment, and the limitations and sensitivities of the measurement technique applied.

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