CONCENTRATIONS OF VIABLE FUNGAL SPORES ON PAPER DOCUMENTS

INTRODUCTION

This work was performed as part of a broader Indoor Air Quality (IAQ) response action, which lasted in excess of eight months. A significant number of microbiological samples, including airborne, bulk, wipe, vacuum, and contact samples were collected. The subject building is a 13 story office building located in Washington, DC. The response action was initiated due to employee complaints of upper respiratory distress, skin rash, and other symptoms commonly associated with potential IAQ problems. During group interviews, some employees working in localized areas of the building associated the occurrence of red, irritated skin on their hands, forearms, face, and upper chest with handling the papers in their offices.

The objectives of this study were two-fold. The relative humidity (RH) often reached 60 percent (%) in the subject building during the summer months. Individual pieces of papers were often limp due to the high RH. Paper, with its high cellulose content, was evaluated as a potential growth medium for fungal spores. In addition, one might expect airborne spores to settle on papers that had been lying on a desk top for several days, with paper functioning similar to an integrating sampler. The ability of the fungal loading on paper surfaces to reflect the recent history of airborne fungal spores in the workplace was also evaluated.

The surfaces of papers lying on desk tops, in file cabinets, and in boxes in employee offices were sampled for viable fungal spores. Samples were collected in both the complaint areas and non-complaint areas of the subject building using contact (Rodac) plates. Samples were also collected in two control buildings (CB): (1) an older building [CB # 1] similar in age to the subject building [27 years old], and (2) a newer building [CB # 2] where the symptomatic employees had been relocated. The results of the survey, as well as the concentrations of fungal spores on paper surfaces, are discussed.

METHODS AND MATERIALS

Contact samples were obtained by gently pressing a 60 millimeter (mm) diameter Rodac plate containing Malt Extract Agar (MEA) against the surface of the paper to be sampled. The plate was held against the paper surface for approximately 5 seconds, removed, sealed, and labeled. The collected samples were put in paper bags, and placed in an insulated container over sealed ice packs for overnight shipment. The samples were analyzed by P&K Microbiology Services, Inc., Cherry Hill, N.J.

Subject Building

In non-complaint areas, a stratified random sampling pattern was used to collect a similar number of samples from interior and exterior offices, from each of the four corridors on the main floors, and from each floor. These data, which were collected using a random sampling plan, are

used to compare the subject building to the control buildings.

Between 16 and 25 samples were collected on each of the 13 floors of the subject building, for a total of 221 samples from non-complaint areas. A number of surfaces besides papers were sampled, including light fixtures, wall surfaces, carpets, and tree leaves. However, the discussion is limited to the 176 samples collected randomly from paper surfaces. Almost all the papers sampled in the non-complaint areas were those that were exposed on desk tops, file cabinets, book cases, etc.

An additional 123 samples were collected from the complaint areas, with 111 of the samples collected from paper surfaces. The papers sampled in complaint areas were in the following locations: 65 on desk tops; 38 in file cabinets; and 8 in cardboard boxes stored on the carpeted floor. The sampling in the complaint areas could be described as judgment sampling, and was performed at the direction of the employees. A similar emphasis was placed on sampling pieces of papers that had been left on the desk top for at least several days.

Control Building # 1

CB # 1 was an older seven story office building located near the subject building. The building appeared to be well maintained, the RH was in the recommended range, and none of the papers were limp due to high moisture content. Four pieces of paper, which were found lying on desk tops, were sampled on each floor, for a total of 28 samples.

Control Building # 2

Those employees who indicated they were potentially affected by contact dermatitis were relocated to a relatively new office building in Washington, DC (CB # 2). This building was included as a control because the employees found the papers and conditions in that space to be acceptable. They occupied one suite of offices on one floor of the building, and a total of 16 samples were collected from papers lying on desk tops in the various offices within the suite.

RESULTS

When comparing the subject building to the control buildings, the data for the non-complaint areas are used. These samples were collected using a random sampling plan, and are considered to be more representative of conditions in the subject building. In addition, the samples in CB # 1 were collected randomly throughout the building, and the building is similar in age to the subject building. Therefore, CB # 1 is considered to be the primary control building for purposes of comparison.

Comparison of Complaint and Non-complaint Areas

Table 1 contains a comparison of fungal loadings in the complaint and non-complaint areas in the subject building, as well as CB # 1. In comparing the complaint and non-complaint areas of the subject building based on average cfu/plate, concentration ratios exceeded a factor of two for *A. flavus, A. versicolor,* and *Paecilomyces*. In addition, the average cfu/plate in both the complaint and non-complaint areas of the subject building exceeded the average value in CB # 1 by a factor of two.

Table 1. Comparison of T&O Aspergillus Concentrations for the Subject Building and Control Building # 1.

	COMPLAINT (111 Plates)		NON-COMPLAINT (164 Plates)		CONTROL # 1 (28 Plates)	
FUNGI	**Plates (%)**	**cfu/Plate (avg)**	**Plates (%)**	**cfu/Plate (avg)**	**Plates (%)**	**cfu/Plate (avg)**
A. flavus	3.60	0.054	1.22	0.024	0	0
A. fumigatus	2.70	0.036	3.05	0.031	3.57	0.036
A. niger	22.52	0.72	21.95	0.567	21.43	0.536
A. ochraceus	1.82	0.018	4.27	0.043	3.57	0.036
A. ustus	0	0	3.05	0.073	0	0
A. versicolor	5.41	0.063	1.83	0.024	7.14	0.071
Cladosporium	55.86	8.37	70.12	8.10	42.86	1.14
Fusarium	4.51	0.045	3.66	0.043	0	0
Paecilomyces	2.70	0.054	1.83	0.018	0	0
Penicillium	43.24	2.41	41.46	2.40	39.29	3.39
Total Fungi	70.27	16.41	75.00	15.27	78.56	7.04

Fungal concentrations in the non-complaint areas of the subject building, relative to the concentrations detected in CB # 1, were possibly amplified for *Cladosporium, A. flavus, A. ustus*, and *Fusarium*. Table 2 contains a comparison of fungal loadings in non-complaint and complaint areas in the subject building for total fungi and *Cladosporium*. No significant differences in the loadings for total fungi were detected between the non-complaint and complaint areas in the subject building. However, *Cladosporium* concentrations, based on the geometric mean (GM), average concentration, and 95th percentile concentration, were somewhat higher in the complaint areas.

Table 2. Comparison of Fungal Loadings in Non-complaint and Complaint Areas in the Subject Building for Total Fungi and *Cladosporium*.

	TOTAL FUNGI		CLADOSPORIUM	
(cfu/Plate)	**NON-COMPLAINT**	**COMPLAINT**	**NON-COMPLAINT**	**COMPLAINT**
< LOD (%)	25.0 %	29.7 %	13.0 %	17.7 %
GM	5.5	5.8	5.5	8.4
GSD	5.8	5.3	3.4	2.7
Mean	15.3	16.4	11.6	15.0
95^{TH} Percentile	54	60	32	51
Maximum	94	157	72	128

A comparison of loadings for total fungi and *Cladosporium* between the subject building and the two control buildings is contained in Table 3. The percentages of plates with no-growth in Table 3 were similar in each building, with 21 % in CB # 1, 25 % in the Subject building, and 31 % in CB # 2. Total fungal concentrations, based on the mean and 95^{th} percentile concentrations, were 2 to 3 times higher in the subject building compared to the two control buildings. *Cladosporium* concentrations were 2 to 4 times higher in the subject building based on the mean and 95^{th} percentile concentrations. These results are consistent with the results from numerous airborne and bulk samples, which indicated that *Cladosporium* was amplified in the subject building.

Table 3. Comparison of Fungal Loadings in the Non-Complaint Subject and Control Buildings for Total Fungi and *Cladosporium*.

	TOTAL FUNGI			CLADOSPORIUM		
(cfu/Plate)	**SUBJECT**	**CB # 1**	**CB # 2**	**SUBJECT**	**CB # 1**	**CB # 2**
< LOD (%)	25.0 %	21.4 %	31.3 %	13.0 %	57.1 %	62.5 %
GM	5.5	3.0	1.0	5.5	NC	NC
GSD	5.8	3.6	10.3	3.4	NC	NC
Mean	15.3	7.0	5.1	11.6	2.7	3.0
95^{TH} Percentile	54	19	17	32	7.2	3.7
Maximum	94	49	22	72	8	4

Table 4 compares the concentrations of *Aspergillus sp.* detected on papers in the subject and control buildings. As indicated, no significant differences in concentration were detected between the subject and control buildings. Therefore, as for the data in Table 1, it would not be possible to ascribe the reported symptoms to *Aspergillus sp.*

Table 4. Comparison of Fungal Loadings in the Subject and Control Buildings for *Aspergillus niger* and T&O *Aspergillus sp.*

	ASPERGILLUS NIGER			T&O ASPERGILLUS SP.		
(cfu/Plate)	SUBJECT	CB # 1	CB # 2	SUBJECT	CB # 1	CB # 2
< LOD (%)	78.0 %	78.6 %	93.8 %	88.4 %	89.3 %	81.3 %
Mean	0.57	0.54	0.06	0.20	0.14	0.25
95^{TH} Percentile	2.0	3.6	0.2	0.60	0.80	0.50
Maximum	19	5	1	4	2	2

Comparison of Complaint and Non-complaint Areas

The rank order for the six most prevalent fungi detected in samples collected from the complaint and non-complaint areas of the subject building are contained in Table 2. As indicated in Table 2, no significant difference was detected in the rank order between the six most prevalent fungi in the complaint and non-complaint areas.

The frequency of detection for fungi in complaint and non-complaint areas for the six most prevalent fungi are contained in Table 2. As an example, 55.8 % of the plates collected in the complaint areas (62 of 111 plates) contained *Cladosporium*, while 70.5 % (124 of 176 plates) in the non-complaint areas contained *Cladosporium*. Both *Cladosporium* and Basidiomycetes were detected at a greater frequency in the non-complaint areas.

A total of 929 cfu of *Cladosporium* were detected in 62 of the plates (15.0 cfu/plate) collected in the complaint areas. In comparison, 1,439 cfu of *Cladosporium* were detected in 124 of the plates (11.6 cfu/plate) collected in the non-complaint areas. The average number of cfu per plate for total fungi in the two areas are 0.59 and 0.55, respectively. These averages are based on the 28 fungal species that were detected in both the complaint and non-complaint areas. Therefore, the average cfu per plate are also similar for the two areas.

Comparison of Subject and Control Buildings

A total of 28 fungal genera or species were detected in the complaint areas of the subject building, while 31 were detected in the non-complaint areas. In comparison, 18 fungal genera or species were detected in CB # 1, and 17 in CB # 2. Therefore, a greater diversity of fungi was detected in the subject building compared to the two control buildings, suggesting that conditions in the subject building might be promoting amplification.

Table 5 contains the rank order for the seven most prevalent fungi in each of the three buildings. Both the mean concentration (cfu/plate) and the rank order are included in the table.

Cladosporium, Basidiomycetes, and *Penicillium* were among the four most prevalent fungi in each of the buildings. However, the mean concentration of *Cladosporium* in the subject building was about 7 times the concentrations in both of the control buildings, suggesting that *Cladosporium* was amplified on the surfaces of papers in the subject building.

Table 5. Rank Order of Fungi in the Subject and Control Buildings.

FUNGI	SUBJECT (cfu/Plate)	CONTROL # 1 (cfu/Plate)	CONTROL # 2 (cfu/Plate)
Cladosporium	8.37 (1)	1.16 (2)	1.13 (2)
Basidiomycetes	3.05 (2)	0.43 (4)	1.75 (1)
Penicillium	2.46 (3)	3.38 (1)	0.64 (3)
Alternaria	0.71 (4)	0.18 (5)	0.32 (4)
A. niger	0.65 (5)	0.54 (3)	0.06 (7)
Epicoccum	0.50 (6)	0.11 (6)	0.19 (5)
Yeast	0.40 (7)	0.11 (7)	0.19 (6)

DISCUSSION

Fungi such as *Alternaria, Cladosporium, Epicoccum*, and many others are common in the natural environment, and are generally of little concern when detected in the office environment. However, toxigenic and/or opportunistic (T&O) fungi, such as *Aspergillus flavus, Aspergillus fumigatus, Aspergillus versicolor, Aspergillus niger,* or *Stachybotrys chartarum* are often of greater concern. Some colonies of these fungi are capable of producing potent mycotoxins. In addition, contact with significant concentrations of these mycotoxins might result in contact dermatitis, and reports of skin rash.

Both the concentration and potency of mycotoxin produced by different colonies of *S. chartarum, A. flavus*, and *Penicillium*, for example, can vary by a factor of up to 100,000. This wide variation in potency is one of the factors that makes it difficult to establish an association between the presence of fungal spores on a piece of paper and reported symptomology. In addition, both viable and nonviable spores contain mycotoxin, although only viable spores were detected by the contact plates used in this study. Finally, the concentration of mycotoxin that may result in the risk of developing allergic symptoms from handling papers is not known. Therefore, fungal concentrations on office papers are not easily associated with symptomology.

The GM concentration and GSD describe a lognormal distribution. The GM, which is less affected by extreme values compared to the mean concentration, was approximately twice the concentrations for either of the control buildings. In addition, the 95 % confidence interval on the GM for the subject building was outside the ranges for both control buildings. Therefore, with 95 % confidence, the fungal loading on papers in the subject building was higher than in either of the control buildings.