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Martin County Courthouse and Constitutional Office Building
Stuart, Florida

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PREFACE

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Health Hazard Evaluation Report 93-1110-2575
Martin County Courthouse and Constitutional Office Building
Stuart, Florida
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SUMMARY

On September 9, 1993, the National Institute for Occupational Safety and Health (NIOSH) received a request for a Health Hazard Evaluation (HHE) from the Martin County Board of County Commissioners in Stuart Florida. NIOSH was asked to evaluate potential worker exposures to toxigenic fungi during renovation of the microbiological-contaminated areas of the Martin County Courthouse Complex. The courthouse complex was severely contaminated with predominantly *Aspergillus*, *Penicillium*, and *Stachybotrys*. The Courthouse complex had been unoccupied since December 1992, due to occupant health complaints.

In September 1993, NIOSH investigators conducted an initial environmental assessment at the Courthouse complex before remediation activities began. Follow-up site visits were conducted in October and November 1993, during remediation activities of the Martin County Courthouse (MCC) and the Constitutional Office Building (COB). A final site visit was conducted in June 1994, after the completion of the remediations. Environmental monitoring included air sampling for culturable (viable) fungi, total fungal spores, total particulates, and ergosterol (the major constituent of the cell walls of fungi). Viable microorganisms in bulk samples were also identified.

Remediation activities were performed in asbestos abatement-type containment areas with dedicated supply and exhaust ventilation systems equipped with high efficiency particulate air (HEPA) filters. Initial air sampling using culturable sampling techniques resulted in unquantifiable concentrations of fungi due to overgrowth; therefore, filtration sampling techniques were utilized. Personal breathing zone (PBZ) and general area air samples were collected during remediation activities with polycarbonate filters which were subsequently analyzed for (1) total spore counts via microscopic analysis and (2) total fungal biomass which involved the determination of ergosterol (the major sterol constituent of most fungi).

PBZ sampling was conducted inside containment areas, immediately outside containment, and in distant parts of the building. While containment areas appeared to reduce the dissemination of spores, potentially toxigenic fungal spores were identified on 56% of all filter samples collected outside containment areas. The escape of these spores most likely occurred during the removal of contaminated building materials from the containment areas.

Workers removing microbiologically-contaminated building materials were exposed to a potential health hazard. While the containment in this study appeared to reduce the dissemination of spores, potentially toxigenic fungal spores were identified on 56% of all filter samples collected outside containment areas. The escape of these spores most likely occurred during the removal of contaminated building materials from the containment areas. The application of both viable and non-viable sampling methods in this study proved to be extremely useful in monitoring potential fungal exposures. The results of this study indicate that construction workers or building maintenance staff performing renovations or remediations may unknowingly put themselves and other occupants at risk of exposures to toxigenic fungi.

Keywords: SIC 9211 (Courts), indoor environmental quality, bioaerosols, fungi, *Stachybotrys*, microbial contamination, IEQ, IAQ.

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INTRODUCTION

On September 9, 1993, the National Institute for Occupational Safety and Health (NIOSH) received a request for a Health Hazard Evaluation (HHE) from the Martin County Board of County Commissioners in Stuart, Florida. NIOSH was asked to evaluate potential worker exposures to toxigenic fungi during remediation activities of the Martin County Courthouse Complex. Martin County planned to renovate the microbiologically-contaminated areas of the complex. The complex had been unoccupied since December 1992.

On September 21- 22, 1993, NIOSH investigators conducted an initial environmental assessment at the Courthouse complex before remediation activities began. Follow-up site visits were conducted on October 12-14, 1993, and November 15-18, 1993, during remediation activities of the Martin County Courthouse (MCC) and the Constitutional Office Building (COB), respectively. A final site visit was conducted on June 22-23, 1994, after the completion of the remediations. Environmental monitoring included air sampling for culturable fungi, total fungal spores, total particulates, and ergosterol (a major constituent of the cell walls of fungi). Culturable microorganisms in bulk samples were also identified.

BACKGROUND

The Martin County Courthouse complex includes three connected buildings, the MCC, the COB, and the Public Defender Building. The MCC and COB were built in 1988, and the Public Defender Building dates back to 1975. The Public Defender Building was not evaluated by NIOSH as part of this study. The COB has four floors; each floor consists of approximately 15,000 square feet (ft^2). The second and third floors house county offices and the first floor contains public business areas, such as the tax collector's department and the motor vehicles department. The MCC has three floors (each floor is about 15,000 ft^2). The MCC has courtrooms and judges' chambers on the second and third floors, while the first floor contains public access areas, including family services and a jury assembly room. The evaluated areas of the two buildings are shown in Figures 1 and 2.

Shortly after the opening of the Martin County Courthouse complex in January 1989, employees began complaining of relative humidity (RH) levels above 60%, visible mold growth under the wall paper on the perimeter walls of the building, and a variety of symptoms including eye and throat irritation, fatigue, headaches, and allergies. County officials responded to these complaints by hiring consultants to assess the indoor environmental quality. The consultants recommended such actions as dehumidification, upgrades in the ventilation system, and overall cleaning of the building. The original ventilation system (before renovations in the fall of 1992) did not properly dehumidify the outdoor air supplied to the occupied spaces. The courthouse complex was vacated in December 1992, due to continuing health complaints.

The two buildings had suffered moisture problems since their original construction, both from water leakage through the perimeter walls of the building and overall high RH levels. An environmental evaluation by a private consultant indicated that the interior, air-conditioned surfaces of many of the walls were serving as amplification (growth) sites for fungi. The specific site of growth was between the surfaces of the gypsum wallboard and the vinyl wall covering (which acted as a vapor barrier). The predominant fungal taxa identified were *Aspergillus versicolor* and *Penicillium* sp. In addition, ceiling tiles throughout the buildings were contaminated with *Stachybotrys* due to condensed moisture dripping from the above-ceiling ventilation system components which were not properly insulated. These types of fungi are capable of producing toxic metabolites (mycotoxins). Since the presence of these specific fungi in indoor environments represented a potential health hazard, it was recommended that officials remove all contaminated, porous materials.

Remediation activities were performed by an asbestos abatement contractor in asbestos abatement-type containment areas with dedicated supply and exhaust ventilation systems equipped with high efficiency particulate air (HEPA) filters. Prior to the remediation, all asbestos abatement workers were trained concerning the hazards of exposures to toxigenic fungi. Workers wore appropriate protective clothing, including disposable coveralls, gloves, and NIOSH-approved full-face respirators with HEPA-filter cartridges.

Contaminated areas of the building were designating as either Level 1 or Level 2 remediation areas. The more highly contaminated areas (Level 1) were located around the perimeter walls of the building where moisture incursion through the building envelope had occurred. Areas which were less contaminated (Level 2) were found in the interior parts of the building. Contaminated materials removed from the buildings included vinyl wall covering, underlying gypsum wallboard and insulation, ceiling tiles, carpeting, and ventilation system components. Microbiologically-contaminated materials were placed in double-sealed bags inside the containment areas. Bags were then passed through the first airlock system of the abatement area entrance. In the second airlock system, known as the equipment room, the outside surface of the bags was vacuumed with a HEPA-filtered vacuum cleaner (the vacuum was also used to clean workers). Materials were then triple-bagged in the clean room of the airlock system and removed from the building.

METHODS

Prior to Remediation

The purpose of the initial site visit was to characterize concentrations of fungi prior to remediation activities. To determine the concentrations of culturable airborne fungi, an Anderson 2-stage viable cascade impactor was used at a calibrated flow rate of 28.3 liters per minute (lpm). All culturable samples were collected over a sample time of 10 minutes. The 50% effective cutoff diameter for the Anderson two-stage sampler is 8 micrometers (μm), therefore, larger, non-respirable particles are collected on the top stage and smaller, respirable particles are collected on the bottom stage. Both Malt Extract agar (MEA) and Rose-Bengal agar (RB) were used for the enumeration of fungi. RB agar was used as a selective media for *Stachybotrys* sp. Sample plates were incubated at 30°C. The taxa of the collected microorganisms were determined by morphological characteristics.

During each sample run with the Anderson samplers, simultaneous samples were collected for total spores and total particulates using the Burkhard Personal Volumetric Air Sampler and the Met One Model 227 Hand-Held Particle Counter, respectively. The Burkhard sampler collects fungal spores at a flow rate of 10 lpm onto a greased glass slide. All spore samples were collected over a sample time of 20 minutes. The Met One, a direct-reading instrument, counts airborne particles using a solid state laser diode in two simultaneous size ranges (0.3 and 1 μm were selected for this survey). Total particle count samples were collected over a sample time of ten minutes at a flow rate of 2.8 lpm. Particulates greater than 1 μm in diameter were used for analysis.

The sample locations in the MCC included MCC-1 and MCC-2 on the first floor, and MCC-3 and MCC-4 on the second floor. The sample locations in the COB included COB-1, COB-2, COB-3, and COB-4, all on the first floor (see Figures 1 and 2 for sample locations). Sample location COB-2 was in the computer room and was used as a control area for comparison to contaminated areas. Samples were collected on September 21, 1993, in the MCC and on September 22, 1993, in the COB. At each location, an attempt was made to replicate six samples for culturable fungi (three using MEA and three using RB), two samples for total spores, and three samples for total particulates, within the same approximate one-hour time frame. Nine bulk samples were collected, of materials which were visibly contaminated with fungi, to identify culturable microorganisms.

During Remediation

Martin County Courthouse (MCC)

Airborne samples for culturable fungi, total spores, and total particulates were collected with the Anderson samplers, Burkhard spore traps, and Met-Ones, respectively. The same sampling methodology, as described for the initial site visit, was used during remediation activities in Level 1 and Level 2 containment areas. Due to the anticipated higher concentrations of airborne fungi during removal activities, sample times were shortened (culturable samples and spore samples were collected over sample times of two to five minutes, and total particulate samples were collected over a sample time of ten minutes).

Sample locations included the second floor of the MCC (Level 2 remediation area) and MCC-2 on the first floor (Level 1 remediation area). Sampling was not conducted at location MCC-1, since it had been remediated prior to the site visit. Samples were collected over a three-day period from October 12 to 14, 1993. At each location, an attempt was made to replicate six samples for culturable fungi (three using MEA and three using RB), two samples for spores, and three samples for total particulates within the same approximate one-hour time frame. Four bulk samples were collected of materials which were visibly contaminated with fungi to identify the microorganisms which were present in the interior wall cavity.

Personal breathing zone (PBZ) and area air samples were collected side-by-side for total spores and ergosterol with personal sampling pumps at a flow rate of 2.5 lpm; the pumps were calibrated immediately before and after sampling with a mass flowmeter which had been calibrated with a primary standard (bubble flowmeter). The means of the measured pre- and post-sampling flow rates were used to calculate sample volumes. Samples for total spores were collected by drawing air through 0.2 micron (μm) pore size, 37 millimeter diameter polycarbonate filters in open-faced cassettes; samples for ergosterol were collected by drawing air through 0.2 μm pore size, 37 millimeter diameter polycarbonate filters in close-faced cassettes. Filter samples were analyzed for (1) total spore counts via microscopic analysis and (2) total fungal biomass which involved the determination of ergosterol (the major sterol constituent of most fungi). Samples were collected inside containment areas, immediately outside containment areas, and in a control location for comparison to contaminated areas.

Constitutional Office Building

The sample locations in the COB included COB-1, COB-2 (control location), COB-3, and COB-4; all sampling was performed during Level 1 remediation activities (see Figure 1 for sample locations). Filter samples were collected over a three-day period, from November 15 to 18, 1993, for total spores and ergosterol. Sampling was not performed using the Anderson samplers, Burkhard spore samplers, and Met One particle counters during this site visit.

After Remediation

During the period of June 22 to 23, 1994, follow-up air sampling was conducted to compare concentrations of culturable fungi (using four Anderson samplers), total spores (using two Burkhard samplers), and total particulates (using two Met One samplers) to concentrations measured prior to the remediation of the buildings. In addition, air sampling for ergosterol (using polycarbonate filters) was conducted. Sample locations in the MCC included MCC-1 and MCC-2 on the first floor, and MCC-3 and MCC-4 on the second floor. The sample location in the COB include COB-1, COB-2 (control location), COB-3, and COB-4, all on the first floor (see Figures 1 and 2 for sample locations). At each location, an attempt was made to replicate four samples for culturable fungi (two using MEA and two using RB), four samples for spores, and six samples for total particulates within the same approximate one-hour time frame.

Acceptable levels of airborne microorganisms have not been established. Evaluation criteria concerning

exposures to bioaerosols are discussed in Appendix A.

RESULTS AND DISCUSSION

Prior to Remediation

Bulk Samples

The results from the analysis of bulk samples for the presence of culturable fungi are presented in Table 2. Total concentrations of fungi in bulk samples from the MCC and the COB ranged from 1.00×10^5 to 6.97×10^8 colony forming units per gram (CFU/gm). Fungal genera identified included species of *Aspergillus*, *Penicillium*, *Cladosporium*, and yeasts. *Aspergillus* was the predominant fungus in six of the nine samples. Previous analytical results of similar bulk samples collected by a private consultant reported that *Aspergillus versicolor* was the predominant species of fungi identified. The elevated concentrations of yeast in two of the vinyl wall covering samples is characteristic of chronic moist conditions. These results indicate that the water-damaged materials of the perimeter walls of the MCC and COB were serving as amplification sites for fungi. Previous building investigations also identified ceiling tiles contaminated with *Stachybotrys* throughout both buildings. NIOSH did not collect bulk samples of contaminated ceiling tiles, since most of these sources had been removed prior to the site visit.

Air Samples

Culturable fungi and total particulate ($\geq$1 µm in diameter) sampling results are presented in Figure 3 for the MCC and in Figure 4 for the COB. The left y-axis of the figures refers to the vertical bars in the graph which display the concentrations (logarithmic scale) of culturable fungi and total particulates. The concentrations of culturable fungi and total particulates are presented as colony forming units per cubic meter (CFU/m^3) and particles per cubic meter (particles/m^3), respectively. Parenthetical values indicate the respirable fractions of the culturable fungi. Air sampling data was assumed to conform to a log-normal distribution based on previous bioaerosol sampling data; therefore, all concentrations are reported as geometric means.

A graphical summary of the culturable fungi sampling results for the MCC is presented in Figure 3. The concentration of culturable fungi for the control location was 36 CFU/m^3; whereas, the concentrations at other sample locations inside the building ranged from 77 to 370 CFU/m^3. Sample location MCC-1 had a mean fungal concentration of 370 CFU/m^3, which was higher than the concentration found outside (213 CFU/m^3). Sixty-three to 89% of the culturable fungal particles were in the respirable range which was indicative of the spore sizes of the predominant genera. The taxonomic rank was different among the samples collected outdoors and indoors. The predominant fungi identified in four of the five indoor samples was *Aspergillus*; whereas, 50% of the fungi identified in the outdoor sample was *Penicillium*. Identification of plated fungal samples showed a random distribution consisting primarily of unidentified species of *Aspergillus*, *Penicillium*, *Cladosporium*, and yeasts (as identified in the bulk samples). Similar trends were observed when comparing total particulate concentrations (collected with the Met One sampler) to culturable fungi concentrations (collected with the Anderson samplers).

A graphical summary of the culturable fungi sample results for the COB is presented in Figure 4. The mean fungal concentrations at various locations inside and outside of the building ranged from 36 (control location) to 727 CFU/m^3 (COB-3). The outdoor fungal concentration was 213 CFU/m^3. However, *Stachybotrys* was cultured from sample location COB-3 (where 88% of the fungal genera consisted of *Aspergillus*). The respirable fraction of culturable fungi in the indoor samples ranged from 85 to 89%. The taxonomic rank was different among the samples collected indoors and outdoors. The predominant fungi identified in the indoor samples were *Aspergillus* and *Penicillium*; whereas,

only 3% of the fungal genera identified in the outdoor sample was *Aspergillus*. Identification of plated fungal samples showed a random distribution consisting primarily of unidentified species of *Aspergillus*, *Penicillium*, *Cladosporium* sp, and yeasts. Similar trends were observed when comparing the total particulate and the culturable fungi data.

Results from total spore counts (collected with the Burkhard samplers) are not presented because the analytical laboratory reported that slides could not be counted due to presence of excess particulate matter. This was an initial attempt to use a local laboratory which had not done previous work with airborne samples. Future samples, collected as part of this study, were sent to a NIOSH contract laboratory.

During Remediation

Martin County Courthouse (MCC)

Bulk Samples

The results from the analysis of bulk samples of various materials from sample location MCC-2 for culturable fungi and bacteria are presented in Table 3. Total concentrations of fungi ranged from 6.50×10^5 to 4.50×10^8 CFU/gm. The fungal genera identified included unidentified species of *Aspergillus*, yeasts, and *Cladosporium. Aspergillus* was the predominant fungi in the two samples of insulation material and in the sample of the vinyl wall covering. An unidentified yeast, indicative of chronic moisture, was the predominant fungi in the fiberglass duct liner. An unclassified gram-positive bacteria (CDC) was the predominant bacteria identified in a sample of fiberglass duct liner, as well as in a sample of insulation from the interior wall cavity. Most of the materials in the interior wall cavity (insulation) which were exposed during the remediation activities, were saturated with moisture.

Air Samples

Results of air sampling data are presented in Figures 5, 6, and 7. Sampling was conducted during remediation activities in the MCC on October 12-14, 1993. The majority of both the area and PBZ samples represent an 8-hour time-weighted average. Figure 5 summarizes the sampling results for total spore (spores per cubic meter) and ergosterol (nanograms per cubic meter [ng/m^3]) concentrations collected during Level 2 remediation activities performed on the second floor of the MCC, while Figures 6 and 7 summarize the results collected during Level 1 remediation activities performed at sample location MCC-2 (located on the first floor) over a two-day period. Samples were collected inside the containment area, immediately outside the containment area, and in a distant part of the building which served as a control location.

A graphical summary of the air samples collected during Level 2 remediation activities on the second floor of the MCC is presented in Figure 5. Remediation workers were removing contaminated carpeting during the sampling period. All other materials, including the vinyl wall covering, gypsum wallboard, ceiling tiles, and ventilation system components, were removed prior to the NIOSH site visit. The mean concentration of total particulates measured inside the containment area was 2.15×10^7 particles/m^3 (geometric mean of seven samples ranging from 7.30×10^6 to 5.95×10^7 particles/m^3). In general, samples for total particulates were higher near sample location MCC-4 compared to MCC-3 (this was found to be consistent with sampling performed during the initial site visit). A mean concentration of 3.38×10^4 spores per cubic meter (spores/m^3) was measured for the area samples collected inside the containment area (geometric mean of two samples: 1.97×10^4 and 5.78×10^4 spores/m^3). The mean concentration for the three PBZ samples collected inside the containment was 1.60×10^5 spores/m^3 with a range of 9.76×10^4 to 2.23×10^5 spores/m^3. Ergosterol was detected in two of the PBZ samples. Immediately outside the containment area, the concentration of total spores was 1.35×10^3 spores/m^3, and in the control area, the concentration was 109 spores/m^3. Ergosterol was not detected in these samples. All air samples contained *Aspergillus* and *Penicillium*-like spores.

In addition, one of the area samples and two of the PBZ samples contained *Stachybotrys*-like spores.

A graphical summary of the air samples collected during Level 1 remediation activities on the first floor of the MCC (sample location MCC-2) is presented in Figure 6. The predominant activities performed by the remediation workers during the sampling period consisted of sawing through the contaminated gypsum wallboard and double-bagging contaminated materials. The mean concentration of total particulates measured inside the containment area was 9.45×10^7 particles/m^3 (geometric mean of fifteen samples ranging from 3.41×10^7 to 2.47×10^8 particles/m^3). Immediately outside the containment area, the mean concentration of total particulates was 3.31×10^5 particles/m^3 (geometric mean of two samples: 3.17×10^5 and 3.45×10^5 particles/m^3). Although the containment appeared to reduce the dissemination of particles, the concentration measured outside the containment area during the remediation activities represented an approximate two-fold increase from the concentration measured during the initial site visit of 1.77×10^5 particles/m^3. A mean total spore concentration of 3.98×10^6 spores/m^3 was measured for the area samples collected inside the containment area (geometric mean of two samples: 3.05×10^6 and 5.21×10^6 spores/m^3). Similarly, the mean concentration for the six PBZ samples collected inside the containment was 8.62×10^5 spores/m^3 with a range of 9.17×10^4 to 2.91×10^6 spores/m^3. Ergosterol was detected in all of the area and PBZ samples with a geometric mean of 594 ng/m^3 for the area samples and 173 ng/m^3 for the PBZ samples. Immediately outside the containment area, the total spore concentration was 407 spores/m^3 for the area sample and 3.92×10^3 spores/m^3 for the PBZ sample. Ergosterol was not detected in the samples collected outside the containment area. All samples contained *Aspergillus* and *Penicillium*-like spores. All samples, except one PBZ sample collected inside the containment area, contained *Stachybotrys*-like spores including the PBZ sample collected on the remediation worker outside of the containment area. This worker was carrying the bagged, contaminated building materials from the containment site to a disposal area outside.

A graphical summary of air sample results collected during the second day of Level 1 remediation activities at sample location MCC-2 is presented in Figure 7. In general, overall exposures were lower since remediation activities consisted of cleaning the abatement area (i.e., sweeping the floor and removing double-bagged contaminated materials from the containment area). Inside the containment area, the mean concentration of total particulates was 6.18×10^7 particles/m^3 (geometric mean of four samples ranging from 3.31×10^7 to 1.17×10^8 particles/m^3). The total spore concentration measured for the area and the PBZ samples collected inside the contain-ment area was 5.61×10^5 spores/m^3 and 4.32×10^5 spores/m^3 (geometric mean of three samples ranging from 3.22×10^5 to 5.31×10^5 spores/m^3). Ergosterol was detected in the area and PBZ samples with concentrations of 8 ng/m^3 and 1 ng/m^3, respectively. Immediately outside the containment area, the concentration of total spores was 152 spores/m^3. Ergosterol was not detected in the sample collected outside the containment area. All samples contained *Aspergillus* and *Penicillium*-like spores. Two of the PBZ samples contained *Stachybotrys*-like spores.

Constitutional Office Building (COB)

Air Samples

Results of air sampling data for the COB are presented in Figures 8, 9, and 10 for sample locations COB-1, COB-3, and COB-4, respectively. Sampling was conducted during Level 1 remediation activities in the COB on November 15-18, 1993. For locations COB-1 and COB-3, samples were collected over a two-day period. The majority of both the area and PBZ samples represent an 8-hour time-weighted average. Samples were collected inside the containment area, immediately outside the containment area, and in a distant part of the building which served as a control location (COB-2).

A graphical summary of the air samples collected at sample location COB-1 over a two-day period is presented in Figure 8. The major activities performed by the remediation workers during the sampling period consisted of sawing through contaminated gypsum wallboard, and removing all porous materials in the wall cavity, as well as removing ceiling tiles. The mean concentrations, measured for total spores and ergosterol, on both days of sampling were similar, since remediation activities were the same for both days. The concentration of total spores for the area sample collected inside containment on Day 1 was 1.04 x 10^6 spores/m^3; on Day 2, the concentration for the area sample was 1.13 x 10^6 spores/m^3. Similar results were obtained for the three PBZ samples collected inside the containment on Day 1 for total spores which ranged from 1.07 x 10^6 to 2.47 x 10^6 spores/m^3. On Day 2, PBZ samples ranged from 7.88 x 10^5 to 1.07 x 10^6 spores/m^3. Ergosterol was detected in all of the samples collected inside the containment area on both days of sampling. Immediately outside the containment area on Day 1, the concentration of total spores was 1.18 x 10^4 spores/m^3, and on Day 2, the concentration of total spores was 4.82 x 10^3 spores/m^3. In the control area, the concentration of total spores was 92 spores/m^3. Ergosterol was not detected in samples collected outside the containment area or in the control location. All samples, both area and PBZ samples, contained *Aspergillus* and *Penicillium*-like spores. *Stachybotrys*-like spores were identified in samples collected both inside and outside containment areas.

A graphical summary of the air samples collected at sample location COB-3 is presented in Figure 9. The major activities performed by the remediation workers during the sampling period on Day 1 consisted of sawing through contaminated gypsum wallboard, and removing all porous materials in the wall cavity. On Day 2, overall exposures were lower since remediation activities consisted of cleaning the abatement area (i.e., sweeping the floor and removing double-bagged contaminated materials from the containment area). The concentration of total spores for the area sample collected inside containment on Day 1 was 5.90 x 10^6 spores/m^3; on Day 2, the concentration was 4.93 x 10^5 spores/m^3. Similar results were obtained for the three PBZ samples collected inside the containment on Day 1 for total spores, which ranged from 3.34 x 10^6 to 1.17 x 10^7 spores/m^3. On Day 2, PBZ samples ranged from 1.04 x 10^6 to 3.40 x 10^6 spores/m^3. Ergosterol was detected in all of the samples collected inside the containment area on both days of sampling. Immediately outside the containment area on Day 1, the concentration of total spores was 77 spores/m^3, and on Day 2, the concentration was 861 spores/m^3. In the control area, the concentration of total spores was 92 spores/m^3. Ergosterol was not detected in samples collected outside the containment area or in the control location. All samples, both area and PBZ samples, contained *Aspergillus* and *Penicillium*-like spores. *Stachybotrys*-like spores were identified in samples collected inside the containment area, only.

A graphical summary of the air samples collected at sample location COB-4 is presented in Figure 10. The major activities performed by the remediation workers during the sampling period consisted of sawing through contaminated gypsum wallboard, and removing all porous materials in the wall cavity. The concentration of total spores for the area sample was 2.24 x 10^5 spores/m^3 (geometric mean of two samples: 1.11 x 10^5 and 4.52 x 10^5 spores/m^3). The mean concentration for the three PBZ samples collected inside the containment area for total spores was 2.76 x 10^5 spores/m^3 (ranging from 1.53 x 10^5 to 5.67 x 10^5 spores/m^3). Ergosterol was detected in both the area and PBZ samples collected inside the containment area. In the control area, the concentration of total spores was 92 spores/m^3. Ergosterol was not detected at this sample location. All samples, both area and PBZ samples, contained *Aspergillus*-, *Penicillium*-, and *Stachybotrys*-like spores.

After Remediation

Air Samples

A graphical summary of the culturable fungi (CFU/m^3), total spore (spores/m^3), and total particulate (particles/m^3) sample results is presented in Figure 11 for the MCC and in Figure 12 for the COB. Air sampling data was assumed to conform to a log-normal distribution based on previous bioaerosol sampling data. Nondetectable concentrations of ergosterol were reported for all sample locations. The trends observed in the spore and total particulate data are similar to those observed in the data collected for culturable fungi. For both buildings, differences were observed between the outdoor sample and all other indoor sample locations. Differences were also observed between the control location (COB-2) and all other indoor sample locations.

The concentration of culturable fungi for all indoor sample locations was below 100 CFU/m^3 (concentrations ranged from 77 to 370 CFU/m^3 during the initial site visit prior to the remediation of contaminated materials). In addition to reducing the total concentration of culturable fungi, the predominant fungal taxon changed from *Aspergillus* to a common outdoor fungi (*Cladosporium*). Spores and conidiophore of *Stachybotrys atra* were observed at sample location COB-3 based on the analysis of the slides from the Burkhard sampler. During the initial site visit, *Stachybotrys* was cultured from the airborne samples collected with the Anderson samplers at this same location. The presence of *Stachybotrys*-like spores was also observed in several other samples. *Aspergillus*- and *Penicillium*-like spores were common in most of the slides from the Burkhard sampler.

SUMMARY AND CONCLUSIONS

Air monitoring should be conducted during remediation activities to determine if the dissemination of spores to areas outside containment is being prevented. Due to the extremely high concentrations of spores released during these activities, culturable bioaerosol sampling techniques were not useful. PBZ and general area air samples collected during remediation activities with polycarbonate filters and subsequently analyzed for (1) total spore counts via microscopic analysis and (2) total fungal biomass which involved the determination of ergosterol (the major sterol constituent of most fungi) were useful for estimating spore concentrations. The analytical method used to quantify ergosterol concentrations, at the time of this survey, was not sensitive enough to detect lower levels of fungal particulates as found outside of containment areas. Sampling results are briefly summarized below.

- Analytical results from bulk samples collected prior to remediation indicated that water-damaged building materials in some of the perimeter walls were serving as amplification sites for fungi in both the MCC and the COB. Total concentrations of viable fungi in the bulk samples ranged from 1.00 x 10^5 to 6.97 x 10^8 CFU/gm. *Aspergillus* (identified previously by a private consultant to be *Aspergillus versicolor*) was the predominant kind of fungus found in the vinyl wall covering samples from these samples.

- Prior to remediating the contaminated areas of both buildings, mean concentrations of culturable fungi and total particulates in the contaminated areas often exceeded the concentrations measured outside. For instance, concentrations ranged from 77 to 370 CFU/m^3 in the MCC and from 55 to 727 CFU/m^3 in the COB, while the outdoor concentration was 213 CFU/m^3. The predominant kinds of airborne fungi found in the MCC and the COB consisted of *Aspergillus* and *Penicillium;* however, the taxonomic rank among the samples collected outdoors indicated

that only 3% consisted of *Aspergillus*. *Stachybotrys* was cultured under quiescent sampling conditions from sample location COB-3.

- Area air sampling results for total spores, collected during Level 1 remediation activities in both buildings, ranged from 1.11×10^5 to 5.90×10^6 spores/m^3 inside containment areas and from 77 to 1.18×10^4 spores/m^3 immediately outside containment areas. Area air sampling results for ergosterol ranged from no detectable levels to 1.21×10^3 nanograms/m^3 inside containment areas. No detectable levels of ergosterol were identified outside containment areas. PBZ sampling results were similar to the concentrations reported for the area air samples. While the containment appeared to reduce the dissemination of spores, potentially toxigenic fungal spores (consisting of either *Aspergillus*- and/or *Stachybotrys*-like spores) were identified in over half of all filter samples collected outside containment areas.

- After the remediation of the buildings was completed, mean concentrations of culturable fungi and total particulates in previously-contaminated areas were below outdoor concentrations at all sample sites. In addition to reducing the total concentration of culturable fungi, the predominant fungal genera changed from *Aspergillus* to *Cladosporium* (a fungi commonly found outdoors). Non-culturable sampling for total spores, however, still indicated the presence of *Aspergillus*- and *Penicillium*-like spores (*Stachybotrys*-like spores were identified at sample location COB-3). Whether the presence of these fungal spores presents a health hazard to future occupants of the building or whether the fungal spores could be completely eliminated was not investigated as part of this study.

In conclusion, remediation of microbiologically-contaminated building materials should be conducted when affected areas are unoccupied. All renovation and construction activities involving the removal or disturbance of microbiologically-contaminated building materials should be prohibited until the dissemination of spores to other parts of the building can be prevented. As seen from the results of this study, the potential exists for exposures (both from inhalation and skin contact) to toxigenic fungi. Remediation workers should wear NIOSH-approved full-face respirators with HEPA cartridges or powered air purifying respirators, disposable coveralls, and gloves.

APPENDIX A

Evaluation Criteria for Bioaerosols

Microorganisms (including fungi and bacteria) are normal inhabitants of the environment. The saprophytic varieties (those utilizing non-living organic matter as a food source) inhabit soil, vegetation, water, or any reservoir that can provide an ample supply of a nutrient substrate. Under the appropriate conditions (optimum temperature, pH, and with sufficient moisture and available nutrients) saprophytic microorganism populations can be amplified. Through various mechanisms, these organisms can then be disseminated as individual cells or in association with soil/dust particles or water droplets. In the outdoor environment, the levels of microbial aerosols will vary according to the geographic location, climatic conditions, and surrounding activity. In an indoor environment where there is no unusual source of microorganisms, the level of microorganisms can vary as a function of the cleanliness of the heating, ventilating, and air-conditioning (HVAC) system and the numbers and activity level of the occupants. Generally, the indoor levels are expected to be below the outdoor levels (depending on HVAC system filter efficiency) with consistently similar ranking among the microbial species.[1,2]

Some individuals manifest increased immunologic responses to antigenic agents encountered in the environment. These responses and the subsequent expression of allergic disease are based on the type and extent of the exposures, and in part, on a genetic predisposition.[3] Allergic diseases typically associated with exposures in indoor environments include allergic rhinitis (nasal allergy), allergic asthma, allergic bronchopulmonary aspergillosis (ABPA), and extrinsic allergic alveolitis (hypersensitivity pneumonitis).[4] Allergic respiratory diseases resulting from exposures to microbial agents have been documented in agricultural, biotechnology, office, and home environments.[5-12] The airborne concentration levels present during the remediation of contaminated building materials would be most similar to those observed in heavily contaminated environments (i.e., agriculture settings versus office environments).

Symptoms vary with the type of allergic disease: (1) allergic rhinitis is characterized by paroxysms of sneezing; itching of the nose, eyes, palate, or pharynx; nasal stuffiness with partial or total airflow obstruction; and rhinorrhea (runny nose) with postnasal drainage; (2) allergic asthma is characterized by episodic or prolonged wheezing and shortness of breath due to bronchial (airways) narrowing. (3) ABPA is characterized by the production of IgE and IgG antibodies with symptoms of cough, lassitude, low grade fever, wheezing, and occasional expectoration of mucous.[4,13]

Heavy exposures to airborne microorganisms can cause an acute form of extrinsic allergic alveolitis which is characterized by chills, fever, malaise, cough, and dyspnea (shortness of breath) appearing four to eight hours after exposure. Onset of the chronic form of extrinsic allergic alveolitis is thought to be induced by continuous low-level exposure, onset occurs without chills, fever, or malaise but is characterized by progressive shortness of breath with weight loss.[14]

Acceptable levels of airborne microorganisms have not been established, primarily due to the varying immunogenic susceptibilities of individuals. Relationships between health effects and environmental microorganisms must be determined through the combined contributions of medical, epidemiologic, and environmental evaluation.[15] The current strategy for on-site evaluation involves a comprehensive inspection of the problem building to identify sources (reservoirs) of microbial contamination and routes of dissemination. In those locations where contamination is visibly evident or suspected, bulk samples may be collected to identify the predominant species (fungi, bacteria, and thermoactinomycetes). Air samples may be collected to document the

airborne presence of a suspected microbial contaminant. Airborne dissemination (characterized by elevated levels in the complaint area, compared to outdoor and non-complaint areas, and anomalous ranking among the microbial species) correlated to occupant symptomatology may suggest that the contamination may be responsible for the health effects.

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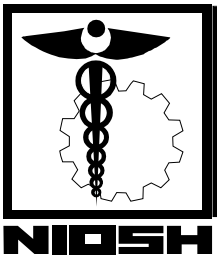

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