MEASURING THE EFFICACY OF MOLD REMEDIATION ON CONTAMINATED DUCTWORK

JD Krause[1,2*] and YY Hammad[1]

[1]College of Public Health, University of South Florida, Tampa, FL, USA
[2]Indoor Air Solutions, Tampa, FL, USA

ABSTRACT
Published guidelines on mold remediation do not specify sampling protocols to measure the efficacy of remediation efforts. The purpose of this study was to evaluate fungal remediation of contaminated ducts by comparing the amount of residual surface contamination to the amount in new ducts. Fungal contamination of galvanized metal and rigid fibrous glass ducts were evaluated using fluorometric and microscopic methods. Fungal contamination was measured in newly installed ducts in addition to pre- and post-remediation. Newly installed ducts had low levels of fungal debris. Findings demonstrated that both fluorometry and microscopy methods detected fungal surface contamination. After cleaning, metal ducts were statistically less contaminated than when new. Fungal contamination of rigid fibrous glass ducts was reduced by ~90%, but was statistically more contaminated than when new. The fluorometric method performed as well as the microscopic method in detecting fungal contamination and provided faster results for monitoring efficacy of fungal remediation.

INDEX TERMS
Mold remediation, Clearance sampling, Fluorometry, Microscopy, Mold detection.

INTRODUCTION
Public health officials have acknowledged the myriad of health effects attributed to fungal exposures in indoor environments. Because fungal growth inside the ductwork of heating ventilation and air-conditioning (HVAC) systems pose a risk of releasing spores and other products into the occupied space the need for effective remediation is recognized. Studies have demonstrated that fungal growth in HVAC systems can contribute to higher air concentrations of fungal spores in the occupied areas they serve (Stetzenbach, 1999; Kulp, 1995). Published guidelines on mold recommend the immediate mitigation of microbial growth found on materials in the indoor environment. The usefulness of post-remediation sampling to determine the efficacy of remediation efforts is acknowledged, however no specific methods or clearance criteria are discussed (NYC DOH Guidelines, 2000; US EPA 2001; Macher, 1999).

Assessing the efficacy of fungal remediation in HVAC system ducts by microscopy is difficult due to its highly subjective nature. Published guidelines for acceptable surface concentrations of fungi have not been established due to the large variability in sampling data and poor correlations with air concentrations and inhalation exposures (Macher, 1999). By using the level of fungal enzyme (Miller, 1998) and fungal structures present on newly installed duct surfaces as reference levels, remediation efficacy could be assessed in relation to new materials. This approach follows published guidance for judging remediation

* Contact author email: dkrauseiaq@msn.com

effectiveness in that "Concentrations of biological agents in surface samples should be similar to what is found in well-maintained buildings and on construction materials" (Macher, 1999).

The purpose of this study was to evaluate the efficacy of fungal remediation of contaminated ducts by comparing the amount of residual contamination to that found in new ducts of the same material. Due to a number of factors HVAC system ducts are often found to have fungal contamination and growth (Bearg, 1993; US EPA, 1992; Hansen, 1997). Two materials commonly used in the construction of HVAC ducts are galvanized metal and rigid fibrous glass. Some remediation guidelines call for removal and replacement of contaminated porous materials like rigid fibrous glass ducts, while others recommend removing it only if the material cannot be sufficiently cleaned (Macher, 1999; US EPA, 1997). The absence of a published protocol that details field and laboratory methods for measuring remediation efficacy makes this determination difficult. This study created a methodology to sample fungal contamination of ductwork and compared the results from two analytic methods to characterize the interior surfaces of newly installed, contaminated, and post-remediation condition of galvanized metal and rigid fibrous glass ducts.

METHODS

Study Design

Newly installed galvanized duct from a Tampa, Florida office building under construction, and newly installed rigid fibrous glass duct from a Tampa, Florida home under construction were sampled to determine levels of existing fungal contamination. Twenty samples were collected from each of the new ducts before operation of the HVAC system.

An office building with extensive fungal growth and contamination in its HVAC systems and ductwork was chosen to evaluate the remediation efficacy of galvanized metal duct and rigid fibrous glass duct. An area of contaminated galvanized metal duct and an area of rigid fibrous glass duct were each randomly chosen for pre- and post-remediation sampling. Pre-remediation samples (n=10) were collected from each type of duct to characterize the level of fungal contamination present. Post-remediation samples (n=20) were collected from each type of duct to assess the level of fungal contamination remaining on the internal surfaces.

Sampling Approach

Side-by-side random samples were collected from each type of duct material for fluorometric and microscopic analysis. A $1m^2$ grid was divided into equal squares and sequentially numbered from 1 to 100. Sample locations were selected within the grid by random number generation. Two grid sites were used for each type of duct. All side-by-side samples were collected using sterile swab and adhesive tape lift collection methods immediately adjacent to one another. Adhesive tape lift samples were collected using 3M Crystal Clear Tape, approximately 2 cm x 5 cm and placed onto Pre-Cleaned Microscope Slides, 2.5 x 7.6 cm. Tape lift samples were collected as described in the *ACGIH Bioaerosols Assessment and Control* (Macher,1999). Swab samples were collected from surfaces adjacent to the tape lift sample using a 9 cm^2 template and moistened sterile cotton swab (Reeslev, 2000). Samples were collected by rubbing the surface area with the moist sterile cotton swab as described in the *ACGIH Bioaerosols Assessment and Control* (Macher,1999).

Measurement Methods

Adhesive tape sample preparation for light microscopy involved staining the slides with lactophenolcotton blue to highlight fungal structures. Slides were examined using a bright field microscope under 400X magnification to determine the number of identifiable fungal

structures within a 1cm^2 area. Samples were examined and placed into one of 4 categories: Category I (< 10 fungal structures/ cm^2) indicates a clean surface; Category II (10-100 fungal structures/ cm^2) indicates light deposition of fungal structures including hyphal fragments and spores; Category III (100-1,000 fungal structures/ cm^2) indicates accumulation of fungal structures; Category IV (greater than 1,000 spores/ cm^2) indicates heavy accumulation of fungal structures and possible amplification. The presence of conidiophores and hyphae were used as indicators of past or present fungal growth.

Sample preparation for fluorometric analysis involved reaction of fungal enzyme collected onto swabs with a fluorescent substrate and transferring a sample of the media to a developer cuvette for measurement in a fluorometer. Swab samples were analyzed for fungal enzyme activity according to instrument specifications (MycoMeter, 2001). Results were corrected for blank values and reported as Fluorometric Values (FV). The reported limit of detection for this method is 14 FV. Sample results below 14 were classified as Below Detection Limit (BDL). Sample values were assigned categories representing a continuum of the amount of surface contamination detected. Category A (≤ 25 FV) represents clean surfaces, including samples below the detection limit (<14 FV) and samples with low levels of fungal spores and fungal fragments (14-25 FV). Category B (26 – 450 FV) indicates accumulation of fungal spores, hyphal fragments or dormant fungal colonies, but not active growth. Category C (>450 FV) indicates active fungal growth (Reeslev, 2000).

Statistical Methods

Surface swab sample data from areas with fungal contamination were log normally distributed therefore; data were log transformed to approximate the normal distribution prior to statistical analysis. Fluorometric sample results of less than 14 FV were below detection limit (BDL) and assigned a value of 7 (½ the detection limit) for statistical analysis. Range, geometric mean (Mg) and geometric standard deviation (σg) was calculated. The Student's t-test was used to compare the geometric means of log transformed fluorometric data. The non-parametric Mann-Whitney test was used to compare the categorical data from microscopic analysis of adhesive tape lift samples. The binomial distribution of each sample set was used to compare the ability of each sample method to detect fungal contamination. "Pass" and "Fail" outcomes were determined for each sample set and the resulting z-score was used to calculate the probability associated with each outcome.

RESULTS

Newly Installed Duct Materials

In new galvanized metal duct the Mg of swab samples analyzed by fluorometry was 10.1 FV, with 90% falling within Category A and 65% below the detection limit. Ninety percent of adhesive tape lift samples from new duct were below Category III and 55% in Category I. Rigid fibrous glass duct swab samples analyzed by fluorometry were all below the detection limit (BDL). Microscopy results were all below Category III, with 90% in Category I. Analysis results for each duct material are presented in Table 1.

Pre-Remediation Duct Materials

Surface samples from galvanized metal duct analyzed by fluorometry had a Mg of 62.0 with 70% of samples in Category B. Seventy percent of adhesive tape lift samples analyzed by microscopy were in Categories III and IV. Fluorometry samples from rigid fibrous glass duct had higher fungal contaminant levels than from metal duct, with 70% in Category C. Microscopy samples showed similar contamination with 80% of samples in Category IV.

Table 1. Results of Sample Analyses

Material Description n : number of samples Mg: Geometric Mean Fluorometry σg: Geometric Standard Deviation	Fluorometry Results # of samples per Category				Microscopy Results # of samples per Category			
	BDL[1]	Cat. A	Cat. B	Cat. C	Cat. I	Cat. II	Cat. III	Cat. IV
	< 14 FV	14-25 FV	26-450 FV	> 450 FV	< 10/cm^2	10-100/ cm^2	100-1,000/ cm^2	> 1,000/ cm^2
Newly Installed Materials								
	BDL	**Cat. A**	**Cat. B**	**Cat. C**	**Cat. I**	**Cat. II**	**Cat. III**	**Cat. IV**
Galvanized Metal Duct n = 20, Mg = 10.1, σg = 1.83	13	5	2	-	11	7	2	-
Rigid Fibrous Glass Duct n = 20**,** Mg = 7, σg = 1	20	-	-	-	18	2	-	-
Pre-Remediation Materials								
Galvanized Metal Duct n=10**,** Mg = 62.0, σg = 4.39	2	1	7	-	1	2	4	3
Rigid Fibrous Glass Duct n =10**,** Mg = 591, σg = 3.5	-	-	3	7	-	-	2	8
Post Remediation Materials								
Galvanized Metal Duct n = 20**,** Mg = 7, σg = 1	20	-	-	-	19	1	-	-
Rigid Fibrous Glass Duct n = 20**,** Mg = 62.8, σg = 3.41	3	1	16	-	5	10	5	-

[1] BDL : Below Detection Limit

Post Remediation Duct Materials

In post-remediation samples of galvanized metal duct analyzed by fluorometry all samples were below the detection limit. All adhesive tape lift samples from post-remediated metal duct surfaces were below Category III with 95% in Category I. Eighty percent of post-remediation samples from rigid fibrous glass duct analyzed by fluorometry were in Category B. Twenty five percent of microscopy results from post-remediation rigid fibrous glass duct were in Category III, and 75% were in Categories I and II.

Statistical Analysis

Statistical analysis of fluorometric sampling data from newly installed ducts and pre-remediation (visibly contaminated) ducts showed that galvanized metal duct had significantly higher levels of fungal contamination than newly installed duct materials ($p < .0001$) confirming the presence of fungal contamination. Microscopy data also demonstrated a statistically greater amount of fungal contamination on pre-remediation surfaces in metal duct than in newly installed duct. New rigid fibrous glass duct was found to have no detectable fungal contamination when analyzed by fluorometry. Fungal contamination detected by microscopy samples of pre-remediation rigid fibrous glass duct was significantly greater than samples from new ducts ($\alpha = 0.05$), confirming the presence of fungal contamination.

Remediation efficacy was demonstrated by the difference between pre- and post-remediation samples. For galvanized metal duct samples analyzed by fluorometry, statistical analysis showed that the post remediation samples were at least 90% lower than pre-remediation samples, demonstrating a significant reduction in fungal contaminant levels as indicated by enzyme activity ($p<0.0001$). Comparison of adhesive tape lift sample results demonstrated that post-remediation samples of metal duct had significantly less fungal contamination than pre-remediation samples. For rigid fibrous glass duct samples analyzed by fluorometry, statistical analysis showed that the post remediation samples were approximately 90% lower than pre-remediation samples, demonstrating a significant reduction in fungal contaminant levels as indicated by enzyme activity ($p<0.0001$). Comparison of adhesive tape lift sample results demonstrated that post-remediation samples of fibrous glass duct had significantly less ($\alpha = 0.05$) fungal contamination than pre-remediation samples.

Comparison of post-remediation samples with samples from newly installed ducts was performed to assess remediation efficacy. For galvanized metal duct samples analyzed by fluorometry, statistical analysis showed that the post remediation samples were significantly lower than samples from new ducts ($p= .0094$). Comparison of adhesive tape lift sample results demonstrated that post-remediation samples of metal duct had significantly less fungal contamination than samples from new duct ($\alpha = 0.05$). For rigid fibrous glass duct samples analyzed by fluorometry, statistical analysis showed that the post remediation samples were significantly higher than samples from newly installed ducts ($p<.0001$). Comparison of adhesive tape lift sample results demonstrated that post-remediation samples of fibrous glass duct were significantly higher than ($\alpha = 0.05$) samples from newly installed ducts.

To determine if both sample methods detected fungal contamination equally well a two-tailed test for significance was conducted on the binomial distribution of each sample set. Sample data from each analytical method were categorized as either “Pass” or “Fail”. Pass criteria for fluorometry sample results was ≤ 25 FV (Category A). Pass criteria for microscopy sample results was ≤100 fungal structures per cm^2 (Categories I and II). Comparing the means of the binomial distributions showed no significant difference ($\alpha=0.05$) between test methods on either duct material for new or pre-remediation surfaces. Analysis of samples collected from post-remediation galvanized metal ducts showed no significant difference between methods. A significant difference between methods was detectable for samples taken from post-remediation rigid fibrous glass duct ($p<0.0001$) where microscopy classified more samples in the “Pass” category than fluorometry. (See Table 1)

DISCUSSION

Remediation efficacy of duct cleaning was evaluated using the sampling methods described. Sample results used in this study to indicate acceptable levels of residual surface contamination were ≤ 25 FV for the fluorometric method and ≤100 fungal structures/cm^2 for microscopy. Using these criteria both sample methods were equally effective at detecting fungal contamination on new and contaminated duct surfaces of both types. The two methods differed in their ability to detect residual fungal contamination only on rigid fibrous glass duct post remediation. The most likely source of this disagreement between fluorometry and microscopy results is interference with microscopic analysis due to debris and fibers loosened by the cleaning process. Since sample results from the two methods agreed on new fiberglass ducts a false positive result due to some constituent of the fiberglass is unlikely.

Recommendations for HVAC duct cleaning provided by the US EPA describe visual cleanliness as the way to determine efficacy of cleaning (EPA, 1997). The inherent limitations

of detecting microscopic organisms with the unaided eye are highlighted in this study by the post-remediation sample results of rigid fibrous glass duct. These surfaces were visibly clean but still had significantly elevated levels of fungal contaminants. The level of fungal contamination remaining on the rigid fibrous glass duct after cleaning was equivalent to the fungal contamination on galvanized metal duct before cleaning. While visual assessment of gross contamination may be sufficient to warrant remediation the same may not be true for assessing post-remediation efficacy.

CONCLUSION AND IMPLICATIONS

The analysis of surface samples to measure the efficacy of mold remediation has been demonstrated using both fluorometry and microscopy sample analysis methods. In the absence of risk-based criteria for clearance sampling of mold remediation projects, comparison with the mean value of new materials provided a useful measure of efficacy. The fluorometric method performed as well as the microscopic method in detecting fungal contamination on new and contaminated surfaces and provided more timely results for monitoring efficacy of fungal remediation.

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