THE IMPACT OF H.V.A.C. SYSTEM CLEANING ON LEVELS OF SURFACE DUST AND VIABLE FUNGI IN DUCTWORK

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ABSTRACT

Samples of surface dust were collected from ducts before and after an HVAC system cleaning project in an office complex in Canada. Dust levels were quantified gravimetrically and concentrations of viable fungi were determined (1) using a standard dilution plating method from vacuum-collected surface dust samples; and (2) by the collection of surface samples on commercially available agar contact slides. A decrease in surface dust concentrations from 120.9 ±50.1 mg/100cm^2 to less than 0.1 mg/100cm^2 was observed throughout the HVAC systems following cleaning procedures. The reduced dust levels within the HVAC system ductwork coincided with lower RSP concentrations within the office building. Surface concentrations of viable fungi as determined by contact sampling procedures were reduced from a pre-cleaning average of 118 (±35) CFU/100cm^2 to 23 (±4) CFU/100cm^2 following cleaning. Dilution plate analysis of pre- and post-cleaning vacuum-collected dust samples showed a similar overall decrease. However, this technique resulted in higher variance and considerably lower species diversity. We propose that desiccation due to the high flow rate required by vacuum collection procedures negatively affected the viability of fungal propagules, accounting for the discrepancy.

INTRODUCTION

Sampling and analysis for total dust and fungal contamination was performed within the unlined sheet metal ductwork of the Heating, Ventilation and Air Conditioning (HVAC) systems of an office complex in Victoria, British Columbia, Canada. The office complex comprised three interconnected buildings served by five independent HVAC systems.

Surface sampling was undertaken both before and after HVAC system cleaning in June 1998. The study was designed to meet three objectives: (1) to satisfy cleanliness verification testing requirements specified by the building owners; (2) to gather data for use in drafting HVAC system cleaning specifications and performance criteria for Canadian federal buildings, under development by Public Works and Government Services Canada; and (3) to investigate the suitability of two sampling techniques, contact slide and dilution plating of vacuum-collected samples, for the assessment of surface fungal loading in ductwork.

The building owners specification for successful HVAC system cleaning required that (a) the HVAC systems should have no visible contaminants present as determined by visual inspection; and (b) the aggregate weight of debris (dust and fibres) should not exceed 0.5 milligrams per 100 square centimetres (mg/100cm^2). The project performance criterion for total dust (0.5 mg/100cm^2) exceeded the stringency of the North American Duct Cleaners Association (NADCA) Standard 1992-01, in which a maximum surface debris loading of 1.0 mg/100cm^2 is specified [1].

An acceptance threshold for surface fungal loading was not specified by the building owner. However, the performance criteria in development for Canadian federal buildings based upon sampling for culturable fungi expressed in colony forming units (CFU) as a function of the total surface area sampled, have tentatively suggested a guideline level of 100 CFU/100cm^2 as a preliminary indicator of duct cleanliness, with the additional qualifications that (a) the species in the ducts comprise those found typically in outdoor air (e.g. phylloplane "leaf surface" taxa); and that (b) the presence of an indoor fungal amplifier is unacceptable regardless of the extent to which it contributes to the surface loading or airborne fungal concentration. Acceptance of these tentative criteria shall be based upon interpretive guidelines set forth by the American Industrial Hygiene Association (AIHA) [2]. It must be noted that this guideline is tentative, and requires further field validation.

MATERIALS AND METHODS

Total surface dust

Surface sampling for total dust and viable fungi was performed at 21 locations within the five HVAC system areas before and after HVAC system cleaning. Sheet metal surfaces in both the air handing units and the air distribution ductwork were tested. Sampling locations were accessed through pre-existing openings as well as new openings installed as part of the current HVAC system cleaning. The sampling and analysis for surface dust was performed using gravimetric methods according to Dust Vacuum Sampling Protocol described in NADCA Standard 1992-01 [1]. Sample media consisted of pre-weighed PVC membranes housed in 37 mm cassettes. Samples were collected by using the open faced cassette to vacuum through a standard NADCA template configured with two, 2 cm by 25 cm slots (total sampling area of 100 cm^2), at a flow rate of 10 L/min. Areas exposed through the template were vacuumed twice.

Culture of vacuum-collected dust

Following gravimetric analysis, each membrane was washed aseptically with 40 mL of 2% peptone broth by vortex mixing the membrane for 1 min. Triplicate aliquots of 20, 200 and 1000 µL of rinsate were plated on Rose Bengal agar and incubated in darkness at room temperature for 7 to 10 days, following which fungal colonies were identified, enumerated and final concentrations were corrected to standard units (i.e. CFU/100cm^2).

Collection of surface fungi on agar contact slides

The surface fungal testing was performed using commercially available contact slides containing Rose Bengal agar medium. Samples were collected by removing a contact slide with a surface area of 26 cm^2 from a sealed package aseptically, and pressing the agar surface of the slide onto the duct surface. Contact slides were incubated and enumerated in a manner similar to cultures of vacuum-collected dust (see above).

Respirable suspended particles

Measurements of respirable suspended particulates (RSP) were taken within the occupied space of the office complex before and after HVAC system cleaning. RSP concentrations were determined by direct measurement using a light-scattering photometer fitted with a cyclone separator with a 50% cut-point at 4 µm, aerodynamic equivalent diameter.

HVAC system cleaning procedures

The cleaning of the internal surfaces of the HVAC systems was performed by non-destructive mechanical and manual scrubbing and abrasion. Loose materials were removed from the internal surfaces of the HVAC systems using mechanical disruption and vacuuming

procedures. Negative pressure was maintained within the ductwork at all times during cleaning to prevent the propagation of aerosolized debris into occupied areas.

RESULTS

Visual inspection of the HVAC systems prior to cleaning showed considerable accumulation of dust and debris on system components such as fans, coils, drain pans, turning vanes and sheet metal ductwork. Parallel visual observations following cleaning verified the removal of conspicuous debris. These observations were confirmed by objective indicators of cleaning effectiveness measured as reductions in total surface dust (*see* Tables 1, 5) and surface burden of culturable fungi (*see* Tables 2-3, 5). Contaminant burdens (e.g. total dust and culturable fungi) on surfaces within the HVAC systems were reduced substantially by rigorous cleaning procedures (*see* Table 5).

Total surface dust

Gravimetric analyses of surface dust samples taken pre- and post-cleaning are summarized in Table 1. The limit of detection (LOD) for this method was 0.1 mg/100cm^2 [1]. Statistical calculations assumed the value of the LOD for samples where the loading fell below the LOD. A highly significant reduction in surface dust was observed following HVAC system cleaning. Pre-cleaning dust levels ranged from 0.8 to 592.0 mg/100cm^2 with an average of 120.9 mg/100cm^2. Following cleaning, concentrations of surface dust ranged from the LOD to 0.2mg/100cm^2 with a mean concentration below the LOD.

TABLE 1. Effect of cleaning on surface dust levels within HVAC systems

HVAC system area	Average dust loading ±SE* mg/100cm^2 *(n)*	
	Pre-cleaning	**Post-cleaning**
Building 1 north	**8.3** ±4.0 *(3)*	**<LOD**** *(4)*
Building 1 south	**93.3** ±39.3 *(3)*	**<LOD** *(4)*
Building 2	**64.4** ±49.1 *(3)*	**0.2** ±0.1 *(4)*
Building 3 main	**301** *(1)*	**<LOD** *(2)*
Building 3 upper	**325.7** ±266.4 *(2)*	**<LOD** *(4)*
Overall	**120.9** ±50.1 *(12)*	**<LOD** *(18)*

*SE = standard error of the mean

**LOD = limit of detection (0.1 mg/100 cm^2), after NADCA [1]

Fungal analyses of agar contact slides

The results of the enumeration of culturable fungi from the contact slide samples are summarized in Table 2. The limit of detection (LOD) for this method was calculated to be 4 CFU/100cm^2. Pre-cleaning values ranged from 8 to 644 CFU/100cm^2 with a mean concentration of 118 ±35 CFU/100cm^2. Following the completion of the HVAC system cleaning, surface concentration of culturable fungi ranged from 6 to 75 CFU/100cm^2, with a mean concentration of 23 ±4 CFU/100cm^2. Thus, a significant decrease in the burden of culturable fungi on HVAC system surfaces was obtained by cleaning. The profile of fungal taxa observed in contact slide samples remained similar in pre-and post-cleaning samples. In both cases, the predominant taxa were *Alternaria* spp., *Cladosporium cladosporioides* and other *Cladosporium* spp., *Epicoccum nigrum*, *Penicillium* spp., and *Ulocladium* spp. (*see* Table 3). A significant proportion of the colonies obtained in both pre-and post-cleaning samplings were non-sporulating mycelial isolates (approx. 7 %). This method showed an average pre-cleaning viable fungal concentration of 2.9 CFU/mg dust based upon pre-cleaning samples by corroboration of these data with parallel gravimetric results (*see* Table 2).

TABLE 2. Levels of culturable fungi on surfaces inferred by contact slide testing

HVAC system area	Mean culturable fungi ±SEM*			
	by area CFU/100cm^2 *(n)*		by mass of dust CFU/mg *(n)*	
	Pre-cleaning	**Post-cleaning**	**Pre-cleaning**	**Post-cleaning**
Building 1 north	**69** ±26 *(4)*	**31** ±10 *(5)*	**8.3** ±6.5 *(3)*	> 310 *(4)*
Building 1 south	**69** ±10 *(5)*	**13** ±2 *(5)*	**0.7** ±0.3 *(3)*	> 130 *(4)*
Building 2	**115** ±20 *(3)*	**33** ±15 *(4)*	**1.8** ±0.4 *(3)*	165 *(4)*
Building 3 main	**62** ±54 *(2)*	**8** ±4 *(3)*	**0.2** *(1)*	> 80 *(2)*
Building 3 upper	**325** ±160 *(3)*	**27** ±3 *(3)*	**1.0** ±0.6 *(2)*	> 270 *(3)*
Overall	**118** ±35 *(17)*	**23** ±4 *(20)*	**2.9** ±1.9 *(12)*	**> 200** *(17)*

*SEM = standard error of the mean

TABLE 3. Summary of fungi sampled by agar contact slide method

Fungal taxa	Average concentration of viable fungi by area (CFU/100cm^2)									
	Pre-cleaning					Post-cleaning				
Location*	**B1n**	**B1s**	**B2**	**B3m**	**B3u**	**B1n**	**B1s**	**B2**	**B3m**	**B3u**
Acremonium sp.		1				2				
Alternaria spp.	5	6	7		5			3		3
Aspergillus oryzae		1								
Aspergillus sydowii						1				
Aspergillus versicolor								1		
Aureobasidium pullulans					4	1		3		5
Cladosporium cladosporioides	16	5	5		14	2		9		5
Cladosporium herbarum		4				1	1	2	1	
Cladosporium spp.	3	14	10	29	9	8				
Cunninghamella sp.						1				
Epicoccum nigrum	5	4	17	4	8		1			
Eurotium sp.						2				
Fusarium sp.								1		
Geotrichum sp.										1
Mucor plumbeus				2						
non-sporulating isolates	8	9	3		36	6	2	4	3	3
Penicillium spp.	11	7	10	21	5	7	8	7	4	7
Phoma sp.		1	1							
Rhizopus oryzae		2		2						
Rhizopus sp.	1			4				1		
Scopulariopsis brevicaulis							1			
Trichoderma sp.	1									
Ulocladium spp.	17	4	4		3					1
unknown ascomycete			1			1				
yeast	4	13	56		242	2		1		1
TOTAL**	**71**	**71**	**114**	**62**	**326**	**34**	**13**	**32**	**8**	**26**

* **B1n** = Bldg 1, north; **B1s** = Bldg 1, south; **B2** = Bldg 2; **B3m** = Bldg 3, main; **B3u** = Bldg 3, upper
**Totals vary from Table 2 due to rounding error

Dilution plating of vacuum-collected dust

An overall decrease in the surface burden of viable fungi was observed by this method, similar to that which was observed in agar contact slide samples, (*see* Table 4). The limit of detection (LOD) for this method in the present study was calculated to be 40 CFU/100cm^2. Pre-cleaning samples showed a mean surface concentration of 207 ±152 CFU/100cm^2. Samples taken following cleaning failed to detect levels in excess of the LOD. However, positive culture results were observed in only three HVAC systems during pre-cleaning sampling (buildings 2 and 3, main and upper) (*see* Table 4). Furthermore, the levels observed ranged from 40 to 1,560 CFU/100cm^2, and overall greatly exceeded levels shown using the

agar contact slide method. The fungal taxa determined by culture of vacuum collected dust comprised species of *Penicillium*, with only a single record of the yeast-like fungus *Aureobasidium pullulans* (data not shown). This method showed an average viable fungal concentration of 1.9 CFU/mg dust based upon pre-cleaning samples (data not shown).

TABLE 4. Surface concentrations of culturable fungi on surfaces within HVAC systems inferred by dilution plating of vacuum-collected dust

HVAC system area	Mean culturable fungi ±SEM* CFU/100cm^2 *(n)*	
	Pre-cleaning	**Post-cleaning**
Building 1 north	**<LOD**** *(3)*	**<LOD** *(4)*
Building 1 south	**<LOD** *(3)*	**<LOD** *(4)*
Building 2	**68** ±27 *(3)*	**<LOD** *(4)*
Building 3 main	**120** *(1)*	**<LOD** *(2)*
Building 3 upper	**960** ±920 *(2)*	**<LOD** *(4)*
Overall	**207** ±152 *(12)*	**<LOD** *(18)*

*SEM = standard error of the mean
**LOD = limit of detection (40 CFU/100 cm^2)

TABLE 5. Average reduction of HVAC contaminants by cleaning (%)

HVAC system area	Total surface dust by gravimetric analysis	Culturable fungi by agar contact slide
Building 1 north	98.8	55.1
Building 1 south	99.9	81.2
Building 2	99.7	71.3
Building 3 main	99.9	87.1
Building 3 upper	99.9	91.7
Overall	99.6	77.3

Respirable suspended particulates

The results from the RSP measurements taken in the occupied space of the buildings before and after the HVAC system cleaning showed a reduction in RSP levels after cleaning. Prior to the implementation of the cleaning procedures, RSP concentrations ranged from 45 to 120 μg/m^3 with an average of 56 μg/m^3. After the HVAC system cleaning, RSP levels ranged from 25 to 50 μg/m^3 with a mean value of 33 μg/m^3. Outdoor RSP concentrations measured adjacent to air intakes at the rooftop level did not vary appreciably during both measurement periods.

DISCUSSION

The data from post-cleaning samples show that the cleanliness verification criteria developed by the building owner were met successfully. Specifically, (a) no visible contaminants were present in the HVAC systems; (b) the aggregate mass of interior surface dust did not exceed 0.5 mg/100 cm^2; and (c) surface fungal levels were less than 100 CFU/m^3 (the tentative guideline for cleanliness under investigation in this study)

The two methods of sampling viable fungi used in this study yielded conflicting data. We hypothesize that the inconsistency of overall counts as well as the negligible species diversity seen in cultures of vacuum-collected dust relative to agar contact slide samples is related to desiccation during sample collection. Conceivably, the high flow rate necessary for vacuum collection reduced the viability of fungal propagules, according to their individual sensitivities. In his review of fungal longevity, Sussman [3] documented the rather counter-intuitive observation that spores of species of *Penicillium* are amongst the longest-lived of

fungal propagules, greatly exceeding the viable lifetime of spores of many common phylloplane taxa. The resistance of spores of these fungi is largely due to their tolerance to desiccation. Furthermore, the genus *Pencillium* bears spores in friable, dry chains which disarticulate readily into short chains or even single spores upon mechanical disruption. Vacuum sampling may disrupt these conidial chains to a greater degree than contact slide sampling and thus explain the relatively higher levels of *Penicillium* observed in vacuum-collected samples. Given these shortcomings, the culture-based quantitation of vacuum-collected surface dust is counter-indicated.

Despite the high variance seen in the gravimetric and contact slide datasets, several intriguing trends are clear. By comparing Tables 1 and 5, post-cleaning reductions in fungal levels were related directly to the initial dust loading at the particular area sampled. In other words, greater post-cleaning reductions were obtained at locations where greater initial dust burdens were observed. As well, those areas that showed higher relative pre-cleaning dust levels tenses to harbour lower milligram-mass concentrations of viable fungi than HVAC areas with lower pre-cleaning dust levels (*see* Table2, e.g. Building 3 upper *cf.* Building 1 south). It is likely that, over time, various factors such as surface desiccation and mechanical sieving causes stratification of viable fungal propagules towards the lower extent of a dust mat within an air duct. Under these circumstances, it could be expected that the recovery efficiency of viable fungal propagules would diminish at increasing dust loads.

While the determination of viable fungi may be of interest in detecting cryptic indoor fungal amplifiers, it is clear that the presence of culturable fungi cannot provide a reliable proxy measurement of gravimetric dust-loading. Although gravimetric sampling and contact slide fungal testing both address different aspects of the cleanliness of air conveyance systems, together they provide a clearer picture of the extent and etiology of duct contamination.

ACKNOWLEDGEMENTS

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