VARIANT INDOOR FUNGAL LEVELS IN RESIDENTIAL ENVIRONMENTS FOLLOWING A CLEANING INTERVENTION ON CARPETS AND SOFT FURNISHINGS

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ABSTRACT

Changes in indoor fungal levels in residential environments were investigated over a 16-week study period following a cleaning intervention on carpets and soft furnishing. Comparable indoor and outdoor air quality and fungal levels were observed in the study homes. On average, the test homes had a 26% reduction in airborne indoor fungal levels, and a 28% reduction in corresponding outdoor levels. Data did not reveal substantial verification that the cleaning intervention resulted in reductions in indoor fungal levels, rather that the variant indoor fungal levels could be attributed to changes to fluctuations in outdoor levels.

INDEX TERMS

Indoor fungi, Carpets, Soft furnishings, Cleaning intervention, Outdoor levels

INTRODUCTION

Carpets and soft furnishings are one of the most important reservoirs and potential sources of aeroallergens, pollen, bacteria, fungi and other components of dust in residential and commercial environments (Lewis *et al.*, 1998; ACGIH, 1999). Poor cleaning and maintenance leads to an accumulation of microbial contaminants, while water damage incidents provide moisture and nutritional substrates (dust, carpet material, padding/underlay, glue) for microbial organisms to proliferate to problematic levels (ACGIH, 1999).

Microbial organisms may then be re-suspended into the air, during normal everyday activities such as walking and vacuum cleaning, leading to respiratory symptoms and diseases like asthma and sick building syndrome (Cole *et al.*, 1994; Kemp *et al.*, 1998; Norback *et al.*, 1994). To avoid these problems, carpets and soft furnishings can be removed and replaced with hard surfaces like wooden floors or furniture. However, this can add considerable financial and economical burden to the building occupant and significantly change the characteristics of the home. An alternative is to clean and treat the carpets and soft furnishings *in situ* (Etkin, 1994).

Current and past studies on airborne indoor fungal levels have focussed mainly on problem buildings or the remediation of obviously mould-contaminated buildings after complaints (Koskinen *et al.*, 1999; Hyvarinen, 1993). Few studies have focused on environments where water or mould damage is not apparent. Even in environments where moisture or mould damage is not apparent. Even in environments where moisture or mould damage is not apparent, elevated indoor fungal presence and indoor air quality (IAQ) problems can still occur as shown in a study by Nevalainen *et al.* (1994). Our aim in this 16-week study was to investigate indoor fungal levels following a cleaning intervention on carpets and soft furnishings in the homes of asthmatic children in residential environments.

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METHODS

As part of a larger study (Asthma 2000) on childhood asthma, asthmatic children between the ages of 6 and 11, were recruited from metropolitan state schools in Perth, Western Australia. Children had to have a clinical history of asthma, be living in a home with the majority of its floor space carpeted, and its occupants to be non-smokers (Jones, 2001). Nine participants (test homes) had their carpets, bedding and soft furnishings (sofas and lounges) in all rooms of their homes professionally cleaned prior to treatment with a polymer-based antimicrobial agent. The homes of five other participants were monitored as controls.

Cleaning intervention

The carpets, bedding and soft furnishings in all rooms of the nine homes were thoroughly dry vacuumed with a vacuum cleaner (Filter Queen, HMI Industries) meeting the Australian Standard 3733 and equipped with a hospital grade filtration system, to trap and remove particulate soil and dust (Standards Australia, 1995). Accredited professional carpet cleaners then performed hot water extraction and restoration or 'steam cleaning', to suspend soils and dirt with detergents and hot water in a solution, which was then removed with wet vacuuming. Industrial sized air movers (Sahara Turbo Drier Model, Drieaz) were then used to facilitate the quick drying of the damp materials.

Once dry, a light application of an antimicrobial agent (RS1957) was applied wet with an airless pressure-type spray applicator (Unijet Model, Spraying Systems Inc.), onto the surface of the carpets, bedding and soft furnishings in a sweeping motion from left to right, then right to left. This was again dried with the assistance of the air movers. Occupants were told to keep off the carpets, bedding and soft furnishings for 12 hours or until drying was completed. Thereafter, homeowners maintained their normal household cleaning practices for the duration of the study period.

Monitoring protocol

Baseline air samples were obtained from the subject's bedroom, prior to the cleaning intervention. The air quality parameters measured during the baseline-monitoring phase included particulate matter (particles per cubic centimeter), temperature, relative humidity (RH) and airborne fungal levels. Outdoor air quality parameters were concurrently monitored for comparison with indoor levels. The nine homes with the cleaning intervention and the five control homes were monitored a further three times over the 16-week study period.

Monitoring was conducted in the bedroom of each asthmatic child. The level of activity was kept at a minimum at each sampling frame. Airborne viable indoor fungal spore sampling was conducted in the middle of the room with two N-6 Andersen multi-hole impactor samplers, placed on beds, for two minutes at a flow rate of 28.3 L min⁻¹. In order to enumerate as wide as possible spectrum of fungi, duplicate side-by-side sampling was conducted for malt extract agar (MEA - DIFCO) (broad spectrum medium) and Dichloran 18% Glucose Agar (DG-18 - Oxoid) (specific for xerophilic fungi – low water activity, a_w) plates. Both media were amended with Chloramphenicol (4 ml Chloramphenicol in 6 ml acetone per 1 litre agar media - Oxoid) to limit bacterial growth. Outdoor air fungal samples were collected concurrently for comparison with indoor levels. Culture plates were incubated for five days in the dark at 22°C ($^{-1}$ °C) and 30%RH ($^{-5}$ %RH) in a climate controlled incubation room. Once incubated, the total concentration of viable culturable fungal colonies was determined and reported as colony forming units per cubic meter of air (CFU m⁻³).

particulate matter was measured with a P-Trak Ultrafine Particle Counter (Model 8525, TSI Inc.), capable of detecting particles in the size range 0.02 to 1.0 micrometer. The concentration range for the P-Trak was 0 to 5 x 10^{5} particles per cubic meter (particles m⁻³). Temperature and relative humidity were measured with an indoor humidity gauge thermometer (accuracy $\pm 1^{\circ}$ C, $\pm 5^{\circ}$ RH) (Model 63-1013, InterTAN Inc.).

Statistical analysis

Analysis of indoor air quality data collected for this study was performed using the MS Excel V5.0 Statistical Add-ins Package. Paired t-tests assuming equal variance (P) and alpha = 0.05 and ANOVA calculations were performed for analysis of variance. Pearson product moment correlation analysis was used to investigate associations or relationships between the mean air temperature, relative humidity, particulate matter and total fungal colony forming units.

RESULTS

Air quality parameters

Results of air quality monitoring showed comparable indoor and outdoor air quality levels (Table 1). There was a 22.4% and 24.6% reduction in corresponding airborne indoor and outdoor particulate matter (particles m⁻³) 16 weeks after the cleaning intervention. Particulate levels were always higher indoors than outdoors. Over this time, temperatures increased by 2.3°C indoors and 3.7°C outdoors whereas relative humidity decreased 1.9% indoors and increased 1.1% outdoors (Table 1).

Weak but statistically significant correlations were observed between indoor fungal levels and with temperature (r = -0.279, P = 0.02) and relative humidity (r = 0.285, P = 0.025). There was a strong negative and statistically significant correlation between airborne particulate matter (particles cm⁻³) and indoor fungal levels (CFU m⁻³) (r = -0.984, P = 0.021).

 Table 1. Indoor (In) and Outdoor (Out) air quality parameters before (Bf) and 16 weeks after (Af) cleaning intervention

| | Air Temperature (°C) | | | | Relative humidity (% RH) | | | | Airborne particulate matter (particles m ⁻³) | | | |
|---------------------------------------|-------------------------|----|------|------|-----------------------------|------|------|------|---|------|------|------|
| l | | | | | | | | | | | | |
| · · · · · · · · · · · · · · · · · · · | Bf Af Bf Af | | | Bf | Bf Af Bf | Af | Bf | Af | Bf | Af | | |
| | In | In | Out | Out | In | In | Out | Out | In | In | Out | Out |
| Mean | 16.7 | 19 | 16.6 | 20.3 | 61.3 | 59.4 | 54.6 | 55.7 | 2.52 | 1.96 | 2.15 | 1.62 |
| % change | 13.8 | | 22.3 |] | -3.1 |] | 2.0 | | -22 | | -25 | |
| Min | 13 | 16 | 13 | 17 | 36 | 46 | 34 | 43 | 0.42 | 0.45 | 0.62 | 0.32 |
| Max | 20 | 20 | 20 | 25 | 81 | 77 | 81 | 73 | 5.85 | 8.30 | 4.53 | 4.59 |

Airborne fungal colony forming units

Large fluctuations in airborne indoor fungal levels were observed in both the test homes and control homes over the 16-week study period. Reductions of between 14.8% and 88.1%, to increases of up to 1410% in indoor fungal levels were observed in the test homes over the duration of the study period (Table 3). On average, there was a 25.9% reduction in indoor fungal levels in the homes with the cleaning interventions, compared with a 19.6% increase in the control homes (Table 2). Average outdoor fungal levels at both the test homes and control homes yielded reductions of 28.3% and 50.4% respectively.

| Time period | Test home | 25 | Control homes | |
|--------------------------------------|----------------------|---------|----------------|---------|
| | (CFU m ⁻³ |) | $(CFU m^{-3})$ |) |
| | Indoor | Outdoor | Indoor | Outdoor |
| Before cleaning intervention | 516 | 720 | 295 | 481 |
| 16 weeks after cleaning intervention | 382 | 516 | 353 | 238 |
| Percentage change | - 25.9% | - 28.3% | + 19.6% | - 50.4% |

Table 2. Average airborne fungal colony forming units

Large increases ("hot spots") in indoor fungal colony forming units was observed in two test homes, H3 after 16 weeks (2808 CFU m³ compared to 742 CFU m⁻³) and in H7 after 6 weeks (5333 CFU m⁻³ compared to 353 CFU m⁻³) (Table 3). Corresponding outdoor fungal levels recorded reductions of 30% & 98%, respectively. Fungal samples from the two homes were differentiated and showed that the large increase in the indoor sample in H3 was made up of 99% *Penicillium* species (2790 CFU m⁻³) and in H7, 95% *Cladosporium* species (5138 CFU m⁻³). Corresponding outdoor fungal samples for H3 was made up of 38% *Cladosporium*, 15% *Penicillium*, 12% *Botrytis* and the rest made up of *Aspergillus*, *Alternaria*, and sterile species, whilst H7 was made up of 50% *Cladosporium*, 25% *Alternaria* and 25% *Botrytis* species. No further attempt was made to investigate the source of the microbial proliferation.

Table 3. Indoor (in) and outdoor (out) fungal levels (CFU m³) and percentage (%) change in the nine test homes (H1-H9) before (B), at 2 days, 6 weeks and 16 weeks after the cleaning intervention

| | HI | HI | H2 | H2 | H3 | H3 | H4 | H4 | H5 | H5 |
|----------|------|------|-----|-----|------|-----|-----|------|-----|------|
| | in | out | in | Out | in | out | in | Out | in | out |
| Before | 1183 | 1801 | 335 | 477 | 742 | 653 | 335 | 406 | 477 | 1077 |
| % change | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 2 days | 212 | 388 | 159 | 194 | 88 | 971 | 247 | 971 | 406 | 406 |
| % change | -82 | -78 | -53 | -59 | -88 | 49 | -26 | 65 | -15 | -17 |
| 6 weeks | 159 | 388 | 159 | 194 | 477 | 371 | 88 | 388 | 335 | 441 |
| % change | -87 | -78 | -52 | -59 | -35 | -43 | -73 | -4.3 | -30 | -13 |
| 16 weeks | 653 | 583 | 335 | 265 | 2808 | 459 | 124 | 65.2 | 212 | 371 |
| % change | -45 | -68 | 0.0 | -44 | 279 | -30 | -63 | -61 | -56 | -13 |

| | H6 | H6 | H7 | H7 | H8 | H8 | H9 | H9 |
|----------|------|-----|------|-----|-----|------|-----|-----|
| | in | out | in | out | in | out | in | Out |
| Before | 230 | 424 | 353 | 441 | 777 | 583 | 436 | 553 |
| % change | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 2 days | 300 | 353 | 300 | 124 | 547 | 441 | 247 | 212 |
| % change | 31 | -17 | -15 | -72 | -30 | -24 | -43 | -62 |
| 6 weeks | 300 | 371 | 5333 | 141 | 265 | 353 | 371 | 371 |
| % change | 30.8 | -13 | 1410 | -98 | -66 | -39 | -15 | -33 |
| 16 weeks | 124 | 371 | 494 | 406 | 865 | 1130 | 247 | 459 |
| % change | -47 | -12 | 40 | -8 | 11 | 94 | -43 | -17 |

* % change is compared to initial baseline ("Before") level

Only a small subset of samples was differentiated for the test homes. Prior to the cleaning intervention, the most common indoor fungi found in the test homes were *Penicillium* spp., yeast species, *Cladosporium* spp., *Alternaria* spp. and *Botrytis* sp. Sixteen weeks after the cleaning intervention the most common fungi in the indoor air were yeasts, *Penicillium* spp. and *Cladosporium* spp., with corresponding outdoor samples showing *Cladosporium* spp. and *Penicillium* spp., the most common outdoor fungi.

DISCUSSION

Comparable indoor and outdoor air quality levels were observed in the study homes for particulate matter, temperature, relative humidity and fungal colony forming units. Many studies have indicated that temperature, relative humidity and suspended particulate matter can be used as possible predictors of indoor fungal levels (Dharmage *et al.*, 1999; Fang *et al.*, 1998; Pasanen *et al.*, 1991). Results from the current study support these previous studies, confirming relationships between indoor fungal numbers and temperature, relative humidity and airborne particulate matter.

Data collected in this study did not reveal substantial verification or evidence that the cleaning intervention resulted in reductions in indoor fungal levels, rather that the variant indoor fungal levels could be attributed to changes to fluctuations in outdoor levels. Numerous studies have shown outdoor air as the main source of fungi in indoor air and a major contributor of external contamination to the levels of indoor fungi (Kemp *et al.*, 2001; Verhoeff *et al.*, 1992). Our results agreed with these observations with six of the eight homes on Day 2, which yielded lower indoor fungal levels, corresponding to similar declines in outdoor levels. This study showed similar decreases in both average airborne outdoor fungal levels (28%) and corresponding airborne indoor fungal levels (26%) in the majority of the test homes. Fungal differentiation of a subset of indoor and outdoor samples (16 weeks after the cleaning intervention) from the test homes also showed similar fungal species composition (*Penicillium* spp. and *Cladosporium* spp.) in both the indoor and outdoor environment.

The study raised the possibility that the cleaning intervention itself may have resulted in microbial proliferation with two homes recording substantial increases, not immediately attributable to outdoor sources. A possible explanation of the increased microbial levels could be attributed to moisture availability due to cleaning with wet methods and inefficient drying in certain areas resulting in "hot spots" of potential microbial proliferation. One of the acknowledged limitations in this study is the failure to investigate such point sources or "hot spots" in more detail. Fungal differentiation of test versus control homes as well as both indoor versus outdoor samples could have provided further information as to the source of the fungi indoors. The immediate question of which influence is more significant; the outdoor constraint or the influence of cleaning interventions on indoor levels, or indeed prevailing point sources within the homes, still remains for current and future research.

REFERENCES

- American Conference of Governmental Industrial Hygienists (ACGIH). 1999. Bioaerosols: Assessment and Control. ACGIH, Cincinnati, Ohio, USA.
- Cole, E.C., Foarde, K.K., Leese, K.E., Green, D.L., Franke, D.L. and Berry, M.A. 1994. Assessment of fungi in carpeted environments. In: *Health Implications of Fungi in Indoor Environments*, Samson, R.A., Flannigan, B., Flannigan, M.E., Verhoeff, A.P., Adan, O.C.G. and Hoekstra, E.S., eds.. Air Quality Monographs. Vol 2. Elsevier, Amsterdam, pp 103-128.
- Dharmage, S., Bailey, M., Raven, J., Mitakakis, T., Thien, F., Forbes, A., Guest, D., Abramson, M. and Walters, E.H. 1999. Prevalence and residential determinants of fungi within homes in Melbourne, Australia. *Clinical and Experimental Allergy*, 29, 1481-1489.
- Etkin, D.S., 1994. Biocontaminants in Indoor Environments. *Indoor Air Quality Update*. Cutter Information Corp, Arlington, MA.
- Fang, L., Clausen, G. and Fanger, P.O. 1998. Impact of temperature and relative humidity on perception of indoor air quality. *Indoor Air* 8, 80-90.

- Hyvärinen, A, Reponen, T, Husman, T, Ruuskanen, J. and Nevalainen, A. 1993. Characterizing mould problem buildings - concentrations and flora of viable fungi. *Indoor Air* 3, pp 337-343.
- Jones, J. 2001. Improving asthma symptoms through better cleaning practices. Honours Thesis. School of Environmental Science. Murdoch University. Western Australia.
- Kemp, P.C., Dingle, P. and Neumeister, H.G. 1998. Particulate Matter Intervention Study: A Causal Factor of Building-Related Symptoms in an older Building. *Indoor Air* 8, 153-171.
- Kemp, P.C., Neumeister-Kemp, H.G., Lysek, G. and Murray, F. 2001. Survival and growth of microorganisms on air filtration media during initial loading. *Atmospheric Environment*. (in print)
- Koskinen, O, Husman, T, Meklin, T, Nevalainen, A. 1999. Adverse health effects in children associated with moisture and mould observations in houses. *International Journal of Environmental Health Research*, Vol. 9, pp 143-156.
- Lewis, R.D., Breysse, P.N., Lees, P.S.J., Diener-West, M., Hamilton, R.G. and Eggleston, P. 1998. Factors affecting the retention of dust mite allergen on carpet. *AIHA Journal* 59, 606-613.
- Nevalainen, A., Hyvarinen, A., Pasanen, A.L. and Reponen, T. 1994. Fungi and bacteria in normal and mouldy dwellings. In: *Health Implications of Fungi in Indoor Environments*, Samson, R.A., Flannigan, B., Flannigan, M.E., Verhoeff, A.P., Adan, O.C.G. and Hoekstra, E.S., eds. Air Quality Monographs. Vol 2. Elsevier, Amsterdam, pp 163-168.
- Norback, D., Edling, C. and Wieslander, G. 1994. Asthma symptoms and the sick building syndrome. The significance of microorganisms in the indoor environment. In: *Health Implications of Fungi in Indoor Environments*, Samson, R.A., Flannigan, B., Flannigan, M.E., Verhoeff, A.P., Adan, O.C.G. and Hoekstra, E.S., eds. Air Quality Monographs. Vol 2. Elsevier, Amsterdam, pp 229-239.
- Pasanen, A.L., Pasanen, P., Jantunen, M.J. and Kalliokoski, P. 1991. Significance of air humidity and air velocity for fungal spore release into the air. *Atmospheric Environment* 25 (A), 459-462.
- Verhoeff, A.P., van Wijnen, J.H., Brunekreef, B., Fischer, P., van Reenen-Hoeskstra, E.S. and Sampson, R.A. 1992. Presence of viable mould propagules in indoor air in relation to house damp and outdoor air. *Allergy* 47, 83-91.