PAPER

Intervention study of airborne fungal spora in homes with portable HEPA filtration units

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The concentrations and composition of airborne fungal spores in homes fitted with portable HEPA filtration units were examined to provide information to evaluate the importance of varying levels of fungal spores in residential environments in Perth, Australia. A novel method for simulating activity/impaction on carpeted environments was also investigated. Reductions in fungal (35%) and particulate (38%) levels were achieved in the air filter homes. *Penicillium*, *Cladosporium* and yeasts were the most common and widespread fungi recovered indoors and outdoors. Fungal range decreased over the study period but this could be due to an overall reduced dissemination of spores (less spores in the air).

Introduction

Over the last few years, there has been increasing public interest and research emphasis placed on the role of indoor fungi and their by-products in indoor environments. This is partly due to increasing health concerns and to scientific research linking indoor air quality problems and indoor fungi with various major respiratory health effects like asthma, hypersensitivity, pneumonitis and sick building syndrome symptoms.[1–6]

Substantial indoor air quality research programmes particularly focusing on residential environments and health outcomes have been conducted in the USA, Canada and the European countries of Sweden, Finland, Denmark and the Netherlands.[7–10] In contrast, in Australia there are limited studies.[11–17] Furthermore, there is a lack of baseline fungal data for Australian residential conditions in comparison with international studies.

While outdoor sources of contaminants are mainly determined by climatic and local factors,[18,19] at least some of the indoor contaminants and propagules, especially those associated with indoor fungi, can be prevented through manipulation of building design and operation by occupants or building managers.[20–22]

The three main strategies for controlling and reducing indoor air pollutants are removal of the source or control of its emissions, ventilation and air cleaning. Source control is the most cost-effective strategy and involves the elimination or reduction of individual sources of pollutants or their emission. The disadvantages are that not all pollutant sources can be identified and practically eliminated or reduced. Ventilation involves the exchange of indoor air with outdoor air, by opening windows and doors, utilisation of mechanical ventilation systems or exhaust fans, with or without heat recovery ventilators (air-to-air heat exchangers). The disadvantages are that costs for heating or cooling incoming air can be significant, and outdoor air itself may contain undesirable levels of contaminants or allergens. Air cleaning may serve as an adjunct to source control and ventilation. However, the use of air cleaning devices alone cannot assure adequate air quality, particularly where significant concentrations are present and ventilation is inadequate.[23–26]

Air cleaning devices are designed to remove particles from the air stream. They include medium efficiency filters (fibrous) for the removal of larger particles, to high efficiency filters (HEPA) or electrostatic precipitators, which can remove small respiratory particles of sub-micron sizes. Air cleaners may be installed in the ducts of central heating or air-conditioning systems in homes, or are portable stand alone units that treat the air in one room only.[23,27]

The overall effectiveness or efficiency of air cleaners in removing pollutants from the air depends on both the efficiency of the device itself (*e.g.*, the percentage of the pollutant removed as it goes through the device) and the amount of air handled by the device.[25,26] The efficiency of particle size collection ranges from 0.1 to 1.0 μm. High efficiency particulate air (HEPA) filters are characterized by efficiencies in excess of 99.97% at a minimum particle size of diameter 0.3 μm.[28]

The use of air cleaners and various other air filtration devices has increased significantly in recent years, with many medical practitioners commonly recommending their use for patients with asthma and allergic rhinitis.[29,30] Despite their increasing use, relatively few studies have been conducted regarding the efficacy of portable air cleaners and air filtration devices. The majority of these studies have been conducted in a laboratory setting and focused on particulate or allergen removal.[31–33] Furthermore, very few studies have been conducted in non-complaint home environments.[29,34–36]

The collection of air samples before and after activity/disturbance of a potential source of a biological agent may provide useful information on the potential of the source to contribute to the bioaerosol burden in the space.[6] Studies have shown that carpets can serve as a reservoir of biocontamination, providing a sustainable microenvironment for fungal growth and reproduction, especially under conditions of moisture.[37–39] Impaction or normal daily activity on carpets could lead to resuspension of particulates including fungi into the air, leading to possible respiratory symptoms and health effects.[5,12,38] Various studies have been conducted simulating various human activities in carpeted environments and have consistently found increased levels following impaction/simulated activity.[40–42] These simulated activities range from simply walking across the flooring to complicated dance steps/routines to specific instruments with falling weights.[43,44] A simple, inexpensive, and standardised method was developed in this study to simulate potential activity/disturbance on carpeted environments.

The aims of this study are threefold. Firstly, to establish baseline ambient indoor and outdoor fungal levels and determine fungal compositions in Western Australian residential

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environments. Secondly, to investigate the influence of portable HEPA filtration units on indoor fungal numbers and composition in these environments. And thirdly, to examine the effect of simulated activity/impaction of carpeting on indoor fungal conditions.

Materials and methods

As part of a larger study on childhood asthma (Asthma 2000), 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 occupants who were non-smokers.[45] Of the 52 eligible participants in the asthma study, ten were selected for this air filter intervention study. Five participants (air filter homes) operated a portable HEPA air filtration unit (Defender™, Captiva filtration, HMI Industries) in the bedroom of the asthmatic child for the study period of 18–20 weeks beginning mid-May through to mid-October 2000. The homes of the five other participants were monitored as controls, with no filtration unit installed. The ten residential homes were predominantly detached, single storey and naturally ventilated dwellings. No specific instructions were given to the occupants with regard to the opening or closing of windows or doors in the bedroom of the asthmatic participants.

Portable HEPA filtration unit

The portable air filtration unit uses a fan to draw particle and microbial-laden air through a HEPA filter. Because air enters and disperses from the unit at 360 degrees, the filtration unit was placed above ground level (0.5 m) in the subject's bedroom at least a metre from obstructions such as walls and furniture. The HEPA filter cartridge was independently tested, to an efficiency of 99.98% (particle diameter of 0.1 μm), EU 9/F 9 (ASHRAE-Standard 52.1–1992, Entsprechend DIN EN 779). The recommended replacement schedule for the HEPA filter cartridge is every 12 months, so for the purpose of this study period (18–20 weeks), no maintenance was needed.

Various studies have suggested that some mycotoxins and fungal volatile organic compound (VOC) emissions emitted from fungi are extremely toxic to humans and animals and may also act as potent synergizers (substances that can enhance the potency of other toxins in the environment).[7,46,47] The unit is also fitted with an activated charcoal filter to minimise volatile organic compounds, odours and gases.

The unit has three operational speeds: low, medium and high. For the purpose of this study, participants were asked to keep the unit on medium to high speed throughout the day and on low at night thus reducing the inconvenience of noise when the child was sleeping. The airflow rates were 1.08 m^3 min^{-1}, 1.89 m^3 min^{-1} and 4.725 m^3 min^{-1} while on low, medium and high speeds, respectively (Defender™ owner's manual). Throughout the study period, homeowners maintained their normal household cleaning practices (regular weekly–fortnightly vacuuming of carpets and dust cleaning).

Monitoring protocol

Prior to the installation of the portable air filtration units, baseline air samples were obtained from the bedrooms of all ten children. The baseline air quality parameters measured included particulate matter (particles m^{-3}), temperature, relative humidity and airborne fungal levels. Outdoor air quality parameters were concurrently monitored for comparison with indoor levels. The air filter and control homes were monitored a further four times after the installation of the portable air filtration units, at 3 weeks, 7 weeks, 11 weeks and 15 weeks after the air filter intervention. The portable HEPA filtration units were removed after the 11th week.

Monitoring was conducted in the bedroom of each participant. Airborne viable indoor fungal spore sampling was conducted in the morning, in the middle of the bedroom with duplicate N-6 Andersen multi-hole impactor samplers (Andersen Instruments Inc., Atlanta, GA, USA) co-located at a height of 1–1.5 m above the ground or floor surfaces (reflecting normal breathing-zone levels), for two minutes at a flow rate of 28.3 L min^{-1}. Outdoor control samples were collected outside the house (2 m away from house) to represent the air that may enter the buildings through open windows and doors,[6] for comparison with indoor samples. Monitoring of homes scheduled on a particular day was completed within two hours, to maintain similar monitoring time period constraints. In order to enumerate a broad spectrum of fungi, duplicate side-by-side sampling of airborne indoor fungi was conducted on malt extract agar (MEA, DIFCO) (broad spectrum medium) and Dichloran 18% Glycerol Agar (DG18, Oxoid) (slow growing fungi, low water activity, a_w) plates.[6,48,49] Both media were amended with Chloramphenicol (Sigma) to limit bacterial growth.

Duplicate viable outdoor air fungal samples were collected concurrently for comparison with indoor levels. In total, ten airborne viable fungal samples were taken per house per sampling occasion.

Temperature, relative humidity and suspended particulate matter were considered as possible predictors of indoor fungal levels and were measured in tandem with airborne fungal sampling. 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 μm. The concentration range for the P-Trak was 0 to 5×10^5 particles per cubic metre (particles m^{-3}). Temperature and relative humidity were measured with an indoor humidity gauge thermometer (accuracy ± 1 °C, $\pm 5\%$ RH) (Model 63-1013, InterTAN Inc.).

Simulated activity/impaction

A novel and new technique for simulating indoor activity was developed to determine the influence of human activity on indoor fungal numbers and composition in the carpeted environment. To maintain quality control, the methodology developed had to be simple, repeatable and use inexpensive and readily available equipment. A standard, fully inflated (30 psi) basketball was dropped from a height of 1.5 m in a grid-like pattern over the entire exposed carpeted area for a period of 60 s. Duplicate viable airborne fungal samples were subsequently taken following the impaction/simulated activity for both MEA and DG18 media. This technique was utilised to simulate activity during the monitoring period.

Incubation and counting

The duplicate samples collected on the MEA and DG18 plates were transported in an insulated container to the laboratory for incubation and analysis, within two hours of sampling. The ten replicate culture plates were incubated for five days at 22 °C (± 1 °C) and 30% RH ($\pm 5\%$ RH) in a darkened climate controlled incubation room. Once incubated, the total concentration of viable culturable fungal colonies was determined with a counting loop and binocular and compound microscopes and reported as mean colony forming units per cubic metre of air (CFU m^{-3}). Since replicate plates were collected, the data were averaged.

After counting, a subset of the fungal samples was identified to genus and species level to determine the fungal composition. Subsets were taken from samples prior to installation of the portable units (pre-filter), whilst the filtration units were

operational (with filter) and after the filtration units were removed (no filter). The morphological characteristics of the fungi were determined microscopically at 40× and 100× objective magnification. Fungi were identified to genus and species level with the aid of taxonomic texts,[50–52] the Hughes–Tubaki–Barron system and Saccardo system.[53] Fungal colonies that did not produce spores or conidia were classified as sterile mycelia. Fungal species that are not classed as potential human pathogens[52] and did not make up significant numbers were grouped as 'others'.

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 possible associations or relationships between the mean air temperature, relative humidity, particulate matter and total fungal colony forming units.

Results

Two hundred and twenty airborne viable fungal samples were taken from the five air filter homes and two hundred and forty-five samples from the control homes over the 18–20 weeks of the study period beginning mid-May and ending mid-October.

Baseline ambient airborne viable fungal levels and air quality parameters for Australian conditions

The average ambient baseline indoor fungal levels of the ten Australian residential homes (pre-intervention) were 443 CFU m^{-3}. Corresponding outdoor fungal levels were 473 CFU m^{-3}. The average indoor fungal level following the simulated activity/impaction was 566 CFU m^{-3}. Ambient indoor conditions in the ten test homes at the start of the study period included an average temperature of 20.2 °C, 61.8% RH and 1.25×10^{-2} particles m^{-3} airborne fine particulates. Corresponding outdoor conditions included an average temperature of 19.4 °C, 62.2% RH and 1.58×10^{-2} particles m^{-3} airborne fine particulates. There were no strong or statistically significant correlations observed between indoor fungal levels with temperature ($r = 0.136$, $P = 0.06$), relative humidity ($r = -0.304$, $P = 0.07$) and airborne particulate matter (particles m^{-3}) ($r = -0.106$, $P = 0.051$).

Fungal composition

Due to the substantial increase in sample numbers following the interventions, only those species identified before the intervention were targeted to track any changes. New species following the interventions were not identified to species level unless they occurred at substantial concentrations.

In total, seventeen fungal genera were identified in the viable airborne indoor and outdoor samples, 11 to genus level (*Cladosporium, Penicillium, Aspergillus, Alternaria, Fusarium, Botrytis, Aureobasidium, Rhizopus, Epicoccum,* yeast, *Nigrospora*) and 6 to species level (*Neurospora crassa, Trichoderma viride, Chaetomium globosum, Ulocladium chartarum, Wallemia sebi, Mucor heimialis*). Between five and six different fungal genera were commonly isolated in each of the indoor and outdoor samples for the 10 residential homes. *Penicillium* (95.1%), *Cladosporium* (92.7%) and yeast (90.2%) were the most commonly found fungi in the indoor samples, along with *Alternaria* (53.7%) and *Aspergillus* (48.8%) species (Table 1). There was a similar fungal composition in the outdoor samples with *Cladosporium* (97.6%) and *Penicillium* (95.1%) the most commonly found species with yeast (65.8%), *Alternaria* (58.5%) and *Aspergillus* (48.8%). With activity/impaction resuspending dust and particulates from the carpet/floor, a greater percentage and number of fungal species were isolated, with *Penicillium* (100%) found in all samples, along with *Cladosporium* (92.7%), yeast (95.1%), *Alternaria* (78.0%) and *Aspergillus* (60.9%) (Table 1). *Cladosporium* and *Penicillium* were the two dominant fungal genera, making up 72–75% of the total indoor fungal composition in the control and air filter homes.

Table 1 Prevalence of fungal genera in air filter and control samples expressed as a percentage (%)

Fungal genera	Indoor ($n = 41$)	Activity ($n = 41$)	Outdoor ($n = 41$)
Cladosporium	92.7	92.7	97.6
Penicillium	95.1	100	95.1
Aspergillus	48.8	60.9	48.8
Alternaria	53.7	78.0	58.5
Yeast	90.2	95.1	65.8
Botrytis	34.1	34.1	36.6

Air filter intervention

The installation of portable air filters brought about immediate reductions in indoor fungal levels in the air filter homes. A 35% reduction in indoor fungal levels (647 to 424 CFU m^{-3}) was recorded in the air filter homes. This compared to a 24% increase (238 to 294 CFU m^{-3}) in indoor fungal levels recorded in the control homes during the same period. After the removal of the air filters, indoor fungal levels in the air filter homes increased 132% (1504 CFU m^{-3}), compared to a 62% reduction (91 CFU m^{-3}) in indoor fungal levels in the control homes (Fig. 1). However the large average increase during the no filter period in the air filter homes was due to a significant increase in one of the homes (H5: 1033 to 4101 CFU m^{-3}). Removing this data set, the indoor fungal levels in the air filter homes after the removal of the air filters was 68.3% lower than pre-filter levels (647 to 205 CFU m^{-3}) and reflected a similar reduction to that of the control homes.

There was a 38% reduction (1.14 to 0.70×10^{-2} particles m^{-3}) in airborne fine particulate levels in the homes with the air filters operational, compared to a 210% increase (1.36 to 4.22×10^{-2} particles m^{-3}) in airborne fine particulate levels in the control homes. Subsequent to the removal of the air filters, particulate levels in the air filter homes continued to drop. Similar results were seen in the control homes, with particulates returning to pre-filter levels (Table 2).

Change in fungal composition over time

A change in fungal composition can be seen in the outdoor samples over time, with fungal dominance changing from *Cladosporium* dominance in the beginning of the study (*Clad.* 59% *Pen.* 27%) to *Penicillium* dominance at the end of the study (*Clad.* 13% *Pen.* 76%) in the air filter homes (Table 3).

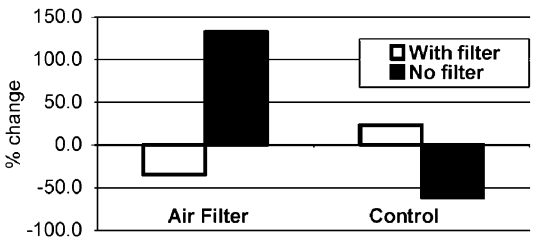

Fig. 1 Percentage change in indoor fungal levels in the air filter and control homes.

Table 2 Average airborne fine particulate levels in the five air filter and five control homes

Average airborne particulates	Air filter homes $\times 10^{-2}$ particles m^{-3} (SD)	Control homes $\times 10^{-2}$ particles m^{-3} (SD)
Pre-filter	1.14 (0.42)	1.36 (1.33)
With filter	0.70 (0.84)	4.22 (1.73)
No filter	0.36 (0.24)	1.55 (1.23)

Whilst the air filters were operational, there was a reduction in the dominance of *Cladosporium* and *Penicillium* (62%). This was also reflected in outdoor samples (51%). Following the removal of the air filters, *Cladosporium* and *Penicillium* resumed their dominance of the total fungal composition in both the indoor (79%) and outdoor (89%) samples.

The number of fungal genera (biodiversity) identified in the samples decreased over time for the air filter homes. At the beginning of the study, on average six fungal genera were found in the indoor samples in the air filter homes. With the air filtration units operating, on average five different genera were found. At the end of the study only four fungal genera on average were found in the indoor samples of the air filter homes. There was a reduction in total fungi numbers recovered in the control homes at the end of the study period (winter/spring) compared to the beginning (autumn).

Indoor–outdoor fungal ratios (I : O ratios)

Fungal levels can be influenced by a variety of environmental and human conditions present at the time of sampling.[54] Since such differences can cause large variations from house to house, indoor/outdoor (I : O ratios) data comparisons were made to determine if the indoor flora composition was amplified beyond the corresponding outdoor flora.[78] I : O ratios are a direct numerical comparison of indoor fungal levels with outdoor levels.

I : O ratios were below 1.0 for most of the samples in the air filter and control homes. On average the pattern of the I : O ratio in the air filter homes showed a reduction whilst the air filter was running followed by an increase in the I : O ratio following the removal of the filter (Table 4). An example in an air filter home (Home 5) shows a baseline I : O ratio of 2.39, which was reduced to 0.84 with the filter running, but increased to 4.06 subsequent to the filter being removed. In the control homes, there was a greater range and variation in I : O ratios with the average pattern showing an increase in the I : O ratio during the 'with filter' period followed by a return to baseline conditions during the 'no filter' period. An example of the range in I : O ratios in a control home (Home 9) had an occasion where the I : O ratio increased from 0.52 to 11.69 followed by a drop to 0.22.

Indoor–activity fungal ratios (I : A ratios)

I : A ratios are direct numerical comparisons of indoor fungal levels pre-activity/impaction with indoor levels following simulated activity/impaction. I : A ratios less than one generally indicate that the carpet/floor is a contributor/source of indoor fungi to the indoor environment. The lower the I : A ratio, the more significant the contribution of the carpeting/flooring, which together with fungal speciation data, could then give investigators/researchers another tool to better establish where possible indoor sources of fungi can be located.

I : A ratios equal or greater than one generally indicate that the carpet/floor is not a significant contributor/source of indoor fungi to the indoor environment and may on the other hand be acting as a potential sink (retaining and accumulating fungal spores).

I : A ratios calculated in this study were consistently below 1.0 for most of the air filter and control homes, indicating that the simulated activity/impaction on the carpet/floor were consistently yielding more fungal spores. In general, I : A ratios were higher in the control homes than in the air filter homes (Table 4). The standardised method developed in this study to simulate activity produced consistent and reproducible results.

I : O ratios, I : A ratios, fungal differentiation and effectiveness of air filters

An example utilising I : O ratios, I : A ratios and fungal differentiation in investigating the effectiveness of air filters in this study can be seen in Table 5.

In this example, at baseline conditions (pre-filter) I : O ratios were relatively high, indicating that indoor levels of fungi were significantly higher than outdoor levels. The I : A ratio was less than 1.0 suggesting that the carpet/floor contributed to an increased level of fungi following activity/impaction. Species composition of indoor pre-activity and post-activity were similar with *Cladosporium*, the dominant species. The presence of a single dominating species generally indicates a potential source of contamination.

With the HEPA filtration units operational, I : O ratios were less than 1.0 and I : A ratios greater than 1.0. I : O ratios less than 1 as explained earlier, generally indicate a "healthy" indoor air balance. A decrease in the dominant fungal species results in a relative increase in other fungal species. A wider selection and fungal range generally indicates healthier fungal spora and balance than one dominated by a single species. With the filtration units operational, there was a reduction in the *Cladosporium* dominance and a greater variety of species isolated reflecting a similar composition to outdoor samples. In this situation the outdoor air was the main source of fungi in the indoor air. The range of fungi and levels found post-activity increased during this time as reflected in the increase in I : A ratio (0.90 to 1.48). When the HEPA filtration units were removed, the I : O ratio increased significantly (11.33) and the I : A ratio was 0.83. The species composition shifted back to *Cladosporium* being the dominant species.

Discussion

Baseline culturable indoor fungal levels

The baseline culturable indoor fungal levels reported in this Australian study (mean = 443 CFU m^{-3}) were in the same range as those reported in studies of airborne indoor fungi levels in residential Australian houses (median = 421 CFU m^{-3}, 495 CFU m^{-3}, and 812 CFU m^{-3} (rural area)).[13,15,17] As

Table 3 *Cladosporium* and *Penicillium* composition change in air filter homes expressed as a percentage of the total colonies counted

AF ($n = 5$)	Indoor			Outdoor		
	Clad.	*Pen.*	*Clad. + Pen.*	*Clad.*	*Pen.*	*Clad. + Pen.*
Pre-filter	57	18	75	59	27	86
With filter	23	39	62	25	26	51
No filter	41	38	79	13	76	89

Table 4 Indoor (I), outdoor (O) and activity (A) ratios for air filter and control homes

	Air filter homes (SD)		Control homes (SD)	
Average ratios	I : O ratio	I : A ratio	I : O ratio	I : A ratio
Pre-filter	0.98 (0.74)	0.74 (0.20)	0.84 (0.40)	0.93 (0.18)
With filter	0.86 (0.51)	0.96 (0.39)	1.10 (4.96)	1.15 (0.27)
No filter	0.55 (0.11)	0.68 (0.28)	0.85 (0.52)	1.11 (0.58)

a comparison with international conditions, levels reported in this study were in a similar range to that of Californian houses (mean = 480 CFU m^{-3}) which have a similar climatic condition to that of Perth, and complaint houses in Scotland (median = 624 CFU m^{-3}), but higher than those reported in Finland (generally under 100 CFU m^{-3}, colder conditions) and lower than those reported in non-complaint houses in Iowa (1200 CFU m^{-3}, farming environment).[55–58] The comparison of our results and those internationally supports the hypothesis of higher levels of airborne viable fungi homes in farming or rural areas compared to those in urban residential areas.[13,59]

The range of airborne viable indoor fungi concentrations reported in this study was in the order of 10–10^3 CFU m^{-3}. This range is consistent with urban indoor fungal range, compared to other studies located in rural and farming environments, where the upper range of indoor air concentrations was an order of magnitude higher, at 10^4 CFU m^{-3}.[59,60]

Studies by Dharmage *et al.*,[14] Fang *et al.*,[61] Toftum *et al.*,[62] and Pasanen *et al.*,[63] have indicated that temperature, relative humidity and suspended particulate matter may have an influence on levels of airborne indoor fungi found indoors. Analyses of correlations in the present study however, showed no strong or significant correlations between the air quality parameters and measured indoor fungal levels. This was similarly found in a study by Li and Kendrick.[64]

Fungal composition

Penicillium, *Cladosporium, Aspergillus, Alternaria* and yeasts were the most common and widespread fungal taxa recovered indoors and outdoors in our study, reflecting similar findings in fungal composition recovered in residential environments in other Australian, US and European studies.[13,16,37,58,64–66]

The change in fungal species composition from *Cladosporium* dominance in the beginning of the study (Autumn season) to *Penicillium* dominance at the end of the study (wetter Winter/Spring season) is supported by studies by Chew *et al.*,[37] Koch *et al.*,[67] Hirsch and Sosman[68] and Solomon and Platts-Mills,[69] who similarly found significant decreases in occurrence of *Cladosporium* and *Alternaria* in the winter period, but is contrary to studies by Takatori *et al.*[70] and Solomon,[71] which show *Cladosporium* species dominating wetter time periods/seasons (Table 3). As a direct comparison, the Australian study by Godish *et al.*[16] found much higher levels of *Penicillium* species indoors (85.8%) rather than outdoors (65%), whereas in our study *Penicillium* species indoors were lower than outdoors (Table 3).

It must be noted that not all *Penicillium* species prefer dry conditions. It is possible that a shift to wet loving *Penicillium* species could have occurred in our study. However, isolates were only identified to genera level, and therefore meaningful analysis of any species shift within the *Penicillium* species was not possible.

The overall reduction in fungal genera/biodiversity could be attributed not only to the air filter reducing fungal numbers, but the combination of increasing humidity as a result of the shift into the winter period (less spores in air) and carpets acting as a possible sink (retaining fungal spores in the carpet fibres) resulting in an overall reduced dissemination of spores.[72] This is confirmed with the broadest range of fungal genera most commonly associated with sampling periods with the highest spore counts. The higher diversity of fungal genera isolated generally reflects a greater total microbial population available/present at the time of sampling. In our study, sampling commenced in the autumn period (associated with higher fungal loads) and concluded in winter/spring period (lower airborne fungi load). This finding is supported by studies by Chew *et al.*,[37] Beaumont *et al.*[73] and Su *et al.*[74]

Effectiveness of portable air filtration units

The air filter intervention achieved an overall average reduction of 35% in airborne viable indoor fungal levels and a 38%

Table 5 Indoor (I), outdoor (O), and activity (A) ratios and fungal species distribution in an air filter home

Home 5 (air filter)	I : O ratio	I : A ratio	Indoor species[a]	Outdoor species[a]	Activity species[a]
Pre-filter	2.39	0.90	74% *Clad.* 6% *Pen.* 14% Yeast 6% Sterile	58% *Clad.* 19% *Pen.* 4% *Asp.* 8% *Alt.* 4% Yeast 8% Sterile	74% *Clad.* 9% *Pen.* 13% Yeast 4% Sterile
With filter	0.84	1.48	27% *Clad.* 43% *Pen.* 8% *Asp.* 7% *Fus.* 15% Yeast	43% *Clad.* 36% *Pen.* 2% *Asp.* 5% *Alt.* 2% *Fus.* 2% *Epic.* 10% Yeast	33% *Clad.* 22% *Pen.* 8% *Asp.* 14% *Alt.* 1% *Epic.* 23% Yeast
No filter	11.33	0.83	68% *Clad.* 25% *Pen.* 5% *Asp.* 2% Yeast	9% *Clad.* 82% *Pen.* 9% Sterile	58% *Clad.* 36% *Pen.* 3% *Asp.* 3% Yeast

[a] *Clad.* = *Cladosporium*, *Pen.* = *Penicillium*, *Asp.* = *Aspergillus*, *Alt.* = *Alternaria*, *Fus.* = *Fusarium*, *Epic.* = *Epicoccum*

reduction in airborne fine particulates, suggesting that the portable HEPA filtration units located in target areas or particular rooms in homes were relatively effective, over the study period, in reducing airborne indoor fungal and particulate levels. This result contributes to gathering knowledge and substantiation of other scientific studies, which show effective use of HEPA air cleaners in targeted areas.[33,70,75–77]

Indoor–outdoor fungal ratios (I : O ratios)

In Australian conditions, indoor–outdoor ratios (I : O) of less than or equal to 1.0 in non-problem residential environments, generally indicate a ''healthy'' indoor fungal balance with good natural ventilation and infiltration as the major source of fungi.[6,78] I : O ratios greater than one generally indicate that outdoor levels may not be a significant contributor to the levels indoors and possibly an indoor source of fungi contributing to levels. It must be noted however, that indoor fungal growth may also be present in situations where indoor concentrations of airborne fungi are equal to or lower than outdoor levels. In certain situations, unusual health risks may occur through exposure to certain kinds of fungi actively growing indoors even when total fungal concentrations are higher outdoors. Interpretation of such data is dependent on knowledge of the kinds of fungi present in indoor and outdoor environments.[6]

Results from this study showed that the majority of the Perth study homes (71.2% of samples) had I : O ratios below 1.0, indicating infiltration and outdoor air as the primary source of indoor fungi.[79–82] This was confirmed in the species differentiation, which showed similar composition of *Cladosporium, Penicillium*, yeast, *Alternaria* and *Aspergillus* species in the indoor and outdoor samples. The similarity in fungal composition found in this study is confirmed in numerous other studies, which have shown outdoor air as the main source of fungi in indoor air and a major contributor of external sources to the levels of indoor fungi.[12,15,19,83]

US and Canadian studies refer to I : O ratios of around 0.3–0.5 for healthy indoor levels.[6,84–86] However, these studies specifically refer to US and Canadian conditions where homes are either mechanically ventilated or are naturally ventilated and closed up for the majority of the time due to colder conditions. This is compared to Australian conditions where open windows and doors and a greater reliance on natural ventilation results in similar indoor and outdoor fungal levels and composition, and therefore higher indoor fungal levels and I : O ratios compared to US and Canadian studies. As a comparison, a US study conducted by Meldrum *et al.*[78] found infiltration accounting for approximately 60% of indoor mould. This compared to our study where infiltration and outdoor levels contributed 71.2% of indoor mould.

The use of I : O ratios is a more effective indication of relative change in indoor levels as it takes into account the significant influence of the outdoor concentration on indoor levels. An example of this case is seen in control Home 9, which recorded indoor fungal levels of 855 CFU m^{-3} and an outdoor level of 1042 CFU m^{-3} (I : O ratio 0.82). Although during the 'with filter' period the level indoors showed a decrease of 58.7% (353 CFU m^{-3}), the actual effective change was a 13.4% increase in relative terms, with an I : O ratio of 0.93 (outdoor 380 CFU m^{-3}).

Activity *vs.* non-activity sampling (I : A ratios)

Although many studies recognise outdoor air as a major contributor to indoor levels of fungi, not all indoor fungi found indoors can be attributed to outdoor air.[19,83,87] In cases where the outdoor air is not the source of indoor fungi, other fungal sources inside the building need to be investigated. However, in many situations, there are often no obvious indoor fungal colonisation (visible/hidden mould) and no obvious indoor sources.[88]

Settled spores present on hard and soft surfaces in the indoor environment may become resuspended by air movement caused by various activities including human disturbance (walking, cleaning, foot traffic, *etc.*), or by environmental changes such as changes in air humidity and wind gusts.[58,63,68,88–90] Several studies have since shown that human activity has a significant effect on the concentrations of microorganisms isolated during sampling.[44,91] The methodology developed in this study to simulate activity/impaction was a simple, cheap and readily accessible technique that consistently produced higher concentrations of airborne viable spores.

Indoor activity ratios (I : A) were developed in this study, and can serve a similar function as I : O ratios in providing a better indication of fungal change and possible indoor sources. I : A ratios developed in this study specifically refer to simulated activity/impaction on the carpet/floor in the bedroom of the participants. In future studies, this technique could be further developed, targeting specific sources or sinks such as soft furnishings or hot spots.

The combined use of fungal ratios and differentiation as utilised in the example seems to suggest that without the filtration units, the indoor source of contamination that was initially present (baseline conditions), reasserted itself once the filters were removed (Table 5). The balance of fungi shifted from the outdoor source being the main contributor during the 'with filter' period to another indoor source, possibly the carpets, being the source of fungi indoors subsequent to the filters being removed. Comparisons of the species composition in the indoor pre-activity and post-activity confirm similar compositions suggesting that the carpet/floor is the source of the contamination in this situation.

Conclusion

In this study conducted over 18–20 weeks in 10 residential homes, the installation of portable HEPA filtration units resulted in reductions in airborne fungal (35%) and particulate (38%) levels. The fungal composition in the homes varied with time with *Penicillium*, *Cladosporium* and yeasts the most common and widespread fungi recovered indoors and outdoors. Fungal range decreased over the study period but this could be due to an overall reduced dissemination of spores. It is suggested that fungal sampling be undertaken under ambient no activity and simulated activity/impaction conditions to give a better indication of the influence indoor sources have on fungal levels. The methodology developed in this study to simulate activity/impaction on carpeted environments yielded consistently higher fungal levels post-activity. Indoor, outdoor and activity ratios, in conjunction with fungal differentiation data, together can provide investigators with a clearer indication of probable patterns and changes in fungal levels and composition.

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