

Understanding and managing risks associated with fungal contamination in indoor environments

Sharyn Gaskin, Michael Taylor, Richard Bentham and Dino Pisaniello

Occupational & Environmental Hygiene, Public Health, School of Population Health,
University of Adelaide

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Executive Summary

Purpose of the study

The majority of people spend most of their working time in office environments. The assessment of indoor work environments is important because it potentially involves a range of hazards such as chemicals, lighting, and indoor air quality issues. Microbial contamination (such as fungi and moulds) of the indoor working environment is an increasingly common OHSW concern, yet there are no published studies investigating typical office environments in Australia. This paucity of survey information limits the comparisons that can be made against measurements taken in workplaces that have indoor microbial air quality issues. Exposure to airborne fungal spores has the potential to exacerbate asthma, act as irritants and allergens and infrequently cause disease in susceptible individuals.

There is limited information available for OHS professionals and others involved in indoor air quality assessments and investigations, to guide the interpretation and communication of biological air quality results.

The **aim** of this study was to obtain profiles of indoor airborne fungi in typical South Australian office environments, including seasonal variation and indoor-outdoor air relationship. The **purpose** of the study and this document is to provide guidance on how to interpret data/results gained from indoor air quality monitoring of fungal contamination (akin to noise data interpretation), and recommend approaches to investigate potential health complaints/issues.

Expected benefits

Expected benefits of data gathered include: translation of evidence for those involved in routine IAQ assessments (e.g. workplaces, SafeWork SA Inspectors; Building owners/managers, Occupational Hygienists, OHS Professionals). This guidance document outlines workplace assessment outcomes and information for background “normal” or “typical” conditions in well-maintained buildings. A proposed workshop will be held for professional skill development of stakeholders and would include how to interpret information gained from IAQ monitoring of fungal contamination. Information gathered may also help to address lack of knowledge and inform local guidelines on indoor microbial air quality under Australian conditions.

Methodology and scope

Air monitoring was conducted at representative South Australian indoor office workplaces in order to identify and enumerate fungi common to indoor work environments. A range of workplaces across

metropolitan Adelaide were sampled to ensure buildings were of varying age, usage, geographical distributions, and neighbour proximity and type (industry, residential, commercial, landfill etc). A total of eighteen buildings were assessed between Spring 2011 and Winter 2012, yielding a total of 89 indoor office air samples, and 39 outdoor air samples for the study period. Concurrent measurements of general indoor air quality indicators (temperature, relative humidity, carbon dioxide) were monitored at each sampling location.

Summary of outcomes and Conclusions

The outcomes of this study can be broadly summarised as follows:

- Indoor fungal counts were generally 75% lower than corresponding outdoor samples, with an average spore concentration (CFU/m³) of 131 (\pm 120) for indoor environments and 509 (\pm 459) for outdoor environments.
- Indoor and outdoor fungal concentrations in air fluctuated seasonally, with the highest counts obtained in warmer months, Summer and Autumn.
- No single indoor air quality parameter (temperature, relative humidity, carbon dioxide) was strongly correlated with fungal concentration in indoor environments, but weak trends/associations were notable and may be useful measurements when carrying out assessments.
- Similar dominant fungal genera (*Aspergillus*, *Penicillium*, *Cladosporium* and *Alternaria*) were isolated from the majority of workplaces, regardless of their use or location, and broadly mirrored results obtained from outdoor environments.

Conclusions deriving from this research:

- In general, indoor levels should be lower than those outdoors, and diversity should be similar indoors and out. In addition, fungi recovered indoors should be those commonly present in outdoor air at that season.
- Low diversity and high concentrations may indicate a potential indoor source of contamination. High diversity and moderate/high concentration may not strictly be an indicator of a specific indoor issue depending on its relationship to outdoor results, with seasonal variations taken into account.
- International guidelines which suggest a fungal concentration of <500 CFU/m³ is a reasonable threshold for indoor environments, may be appropriate for South Australian conditions. This is subject to satisfying the criteria outlined above with respect to seasonal variation and relationship to outdoor levels.
- The issue of fungal contamination in indoor air environments cannot necessarily be resolved by simple visual observation in the workplace because moulds can be inaccessible, and/or spores

may come from a source off-site. Furthermore, visual and/or olfactory assessment may or may not accurately indicate the true extent of the problem.

Recommendations for OHS professionals

Target audience for these recommendations includes SafeWork SA Inspectors; Building owners/managers, Occupational Hygienists, and OHS Professionals.

- Care should be taken when appointing a professional to conduct indoor air quality assessments and investigations as it requires specialised expertise and experience.

When interpreting results from indoor air quality investigations the following recommendations are given:

- When assessing fungal contamination of indoor air, both concentration (CFU/m³) and diversity play a role in identifying potential problems and evaluating the biological quality of the air.
- Indoor levels must be interpreted with respect to control environments, such as outdoor air and/or interiors with no complaints or symptoms, because health effect dose-response data are not available for fungal spore exposure.
- Use of geographically relevant baseline information (for example outcomes from the current study) for typical outdoor and indoor environments may also assist professionals when evaluating indoor air quality.
- Concurrent measurement of indoor air quality parameters (e.g. temperature, humidity, carbon dioxide) may inform efforts to locate and remediate mould contaminated materials. However, there is not sufficient evidence to suggest a single parameter can inform the bioaerosol content of the air.
- When conducting indoor air quality assessments in the workplace, where fungal contamination is suspected, assessors should undertake an occupant survey of health symptoms. Survey responses together with air monitoring results may help inform recommendations while also identifying possible sensitive individuals requiring special consideration.
- On-site assessment of HVAC (heating, ventilation, and air conditioning) condition and maintenance will help inform the assessor of potential sources of fungal contamination.
- When designing a monitoring strategy, inclusion of multiple sample replicates should be considered, where budgetary and time allowances are available. As a minimum, a sample of the indoor complaint area, an indoor non-problem area and outdoors should be performed. Where multiple replicates of each are not performed, care should be taken in interpreting single sample results due to the natural variability of microbial load in the air.

1.0 Introduction

Fungal intrusion into indoor environments is a uniquely modern phenomenon. The construction of buildings designed to contain a temperature controlled, relatively static volume of air can create numerous microclimates favourable to either the growth of fungi, or to the accumulation and deposition of fungal spores (Airaksinen et al., 2004, Rowan et al., 1999). These effects are particularly apparent in large, carpeted office buildings, designed to minimise external air exchange and improve energy efficiency. Temperature buffered, enclosed air environments often are unable to mitigate large changes in humidity either through moisture intrusion in roof spaces or large increases in relative humidity. These humid microclimates provide the opportunity for condensation or standing water to form, fostering the growth of fungi (Singh, 1994).

As the majority of people in the 21st century now spend most of their working time in office environments the control of fungi within these spaces is of increasing importance (Mendell et al., 2002). Although Australian workplaces are commonly monitored for a range of chemical and physical hazards, particularly in industrial settings, microbial contamination of indoor working environments is often neglected unless health symptoms are reported and require investigation. In recent years, Indoor Air Quality (particularly bioaerosol) assessments in non-industrial workplace environments have been an increasing portfolio of Occupational Hygienists and other workplace OHSW assessors in Australia (Bird et al., 2012). Currently there are no published studies investigating typical background fungal levels in office work environments in Australia. Kemp *et al.* (2002) reported the only airborne fungi monitoring study in Australian workplace buildings, which focussed on the education sector in metropolitan Perth. The study confirmed that counts alone are not able to determine healthiness as it does not detect changes in the mixture of fungi from outdoor to indoor air and will not detect hazardous fungi. Some assessment of airborne fungal spore profiles in Australian domestic dwellings has been published (Garrett et al., 1997; Hargreaves et al., 2003; CSIRO 2010). However, even in the absence of widespread survey information, the results of work conducted in homes and other air-conditioned buildings in Australia indicate fungi are an indoor pollutant of significant concern.

The paucity of workplace survey information limits the comparisons that can be made against measurements taken in workplaces that have reported indoor microbial air quality issues. There are currently no national standards in Australia for allowable or acceptable

concentrations of indoor airborne fungi, and typically European WHO guidelines (World Health Organization, 2009) are adopted when interpreting bioaerosol monitoring results in occupational settings. These are not designed for arid Australian conditions, such as in South Australia, where a high prevalence of asthma is evident (Wilson et al., 2006). Airborne fungal spores have been demonstrated to exacerbate asthma, act as irritants and allergens and infrequently cause disease in susceptible individuals. These effects are rarely seen in well maintained buildings, however water intrusion/damage or inappropriate humidity control can cause dramatic spikes in fungal spore loads (Singh et al., 2010).

This study is intended to be viewed as an initial investigation into airborne fungal profiles of typical “non-problem” office environments, with broad insights into seasonal variation of abundance and genera identified. The results obtained through this pilot study are intended to establish a baseline for indoor fungi encountered in workplace building investigations in Adelaide, South Australia. In line with similar investigations conducted both nationally (dwellings) and internationally (Garrett et al., 1997, Li and Kendrick, 1995, Mitakakis and Guest, 2001), the results of this work are intended to inform Occupational Hygienists, building inspectors, OHS professionals and health care officials and contextualise the results they obtain during both routine and complaint indoor air quality workplace assessments. Information gathered may also help to address lack of knowledge and inform local guidelines on indoor microbial air quality under Australian conditions.

2.0 Methods

2.1 Sampling location selection

A range of locations were selected for sampling to ensure buildings were of varying age, usage, geographical distributions, and neighbour proximity and type (industry, residential, commercial, landfill etc). Sample locations were intentionally dispersed over a wide geographical range (approximately 232 square kilometres) encircling the city of Adelaide, South Australia. This distribution was selected to ensure as wide a sample of potential meteorological and surrounding land usage conditions were met whilst remaining within the designated Adelaide metropolitan environs. Buildings were selected primarily based upon the most prevalent building types located within this area, specifically offices, followed by light industrial and commercial facilities. Where offices abutted a warehouse or factory or other industrial structure, a separate sample/s in these areas were also collected (treated as a separate data set). A total of eighteen buildings were assessed between Spring 2011 and Winter 2012. This yielded a total of 89 indoor office air samples, and 39 outdoor air samples for the study period.

2.2 Air sampling equipment and procedure

At each site, a series of air samples (typically 6 indoor and 2 outdoor, depending on building size) were collected onto malt extract agar (MEA) plates using a BioStage® single-stage viable cascade impactor attached to a SKC QuickTake®30 Sample Pump (Airmet, Victoria). Each air sample was collected for 2 minutes at a sampling flow rate of 28.3 L/min (NIOSH 1998). The sampling pump with representative sampler in line was calibrated before each use using a rotatmeter, and the impaction aperture cleaned with sterile alcohol wipes between sampling events to minimise cross contamination between locations. Samples were kept cool and transported promptly to the laboratory for analysis.

Indicators of general indoor air quality (temperature, relative humidity, carbon dioxide) were concurrently monitored at each sampling location using a direct-reading instrument TSI® Q-Track Indoor Air Quality Meter Model 8551 (Kenelec Scientific, Victoria). The instrument was allowed to equilibrate in the sample environment for a period of ten minutes prior to readings. Final reading values for each sample site were a composite of ten minute data acquisitions.

2.3 Fungal growth and identification

Samples were assessed in the laboratory for enumeration and identification of viable fungi. Agar plates were incubated at 25 °C (Brainchild BTC-1010, SEM scientific equipment, Adelaide) and observed for growth between 3-5 days after sampling. Resulting fungal spore load per sample was expressed as colony forming units (CFU) per cubic meter of air (m³):

$$\text{CFU/m}^3 \text{ calculated by: } \frac{\text{Number of colonies}^{\#} \times 1000}{\text{Sampling time (min)} \times \text{Flow rate (L/min)}}$$

adjusted for multiple-jet impactions using the positive hole correction table (Macher, 1989)

Fungal colonies were identified to genus level under microscopy (Nikon Eclipse Ci; Coherent Scientific, Adelaide), using lactophenol blue stain (Sigma-Aldrich, Australia).

2.4 Feedback of results to workplaces

Air monitoring results obtained for individual workplaces was communicated back to the organisation via a confidential report, including interpretation of results and recommendations (where appropriate). An example of workplace reports is given in Appendix A (without identifying information).

2.5 Statistical analysis

Calculated CFU/m³ fungal concentrations were statistically analysed using GraphPad Prism v.5.0 and SPSS v.19. Paired Samples T-tests were conducted to determine difference between outdoor and indoor concentrations, across all seasons. Analysis of variance (ANOVA) at $\alpha=0.05$ was conducted to determine seasonal significance, and separated using Tukey Multiple Comparisons Test at 0.05 level of significance. Correlation analysis was performed to determine relationship between CFU/m³ and IAQ parameters.

3.0 Results

3.1 Building parameters

All buildings sampled were considered non-complaint, with no active cause for investigating either water intrusion events or fungal contamination of indoor materials or air. Buildings sampled were primarily multi-story (80% having two or more stories) and were serviced by reverse-cycle ducted air conditioning systems in 60% of cases. The remainder were serviced by a mixture of split systems (15%) or fresh air/no HVAC system (1 case). Building ages varied from historic constructions (85 years) to recently renovated and modern six-star energy efficient buildings (less than 18 months old). Building occupancy also varied widely from as few as four in smaller offices to greater than 35 in large, multi-story workplaces in the Adelaide CBD and industrial districts.

3.2 Indoor air quality parameter monitoring

Average seasonal results for both indoor and outdoor air quality measurements are presented in Table 1. Indoor air quality parameters measured at each workplace showed no gross differences, with all results falling within recommended Australian guidelines. Any seasonal variations in temperature and humidity were generally less apparent in indoor environments. In comparison, outdoor measurements displayed slightly larger variations, as would be expected. On average indoor CO₂ measurements tended to be slightly higher than outdoor measurements, but did not exceed the specified guideline levels (as shown in Table 1).

Correlations observed between IAQ parameters and fungal spore concentrations in indoor air are discussed in section 3.4.

Table 1: Mean seasonal variation in air quality parameters obtained from office buildings across metropolitan Adelaide.

Parameter	Units	Spring	Summer	Autumn	Winter	Suggested Indoor Air Quality Guidelines /Limits (NHMRC)	SafeWork Australia Exposure Standard (8 hour time-weighted average)**
INDOORS							
Temperature	°C	23.6	26.7	21.9	21.5	20-24	
Relative humidity	%	36.4	41.3	45.8	39.9	30-60	
Carbon dioxide	ppm	347	644	675	580	1000*	5000
OUTDOORS							
Temperature	°C	25.5	29.3	20.9	17.7		
Relative humidity	%	26.4	40.3	47.6	42.8		
Carbon dioxide	ppm	377	511	510	469		

*1000 ppm as a surrogate indicator of sufficient outside air provision.

** Exposure Standards represent airborne concentrations of individual chemical substances, which according to current knowledge, should neither impair the health of, nor cause undue discomfort to nearly all workers.

3.3 Variations in indoor and outdoor fungal concentrations

Average fungal CFU/m³ were calculated for indoor and outdoor environments by pooling all results collected during the course of the study. This information is presented in Figure 1. Indoor fungal concentrations were on average 75% lower than outdoor levels. Mean indoor fungal concentration was 131 ± 120 CFU/m³, and equivalent outdoor samples had a mean CFU/m³ of 509 ± 459. Outdoor samples tended to display a much wider variability in gross CFU/m³.

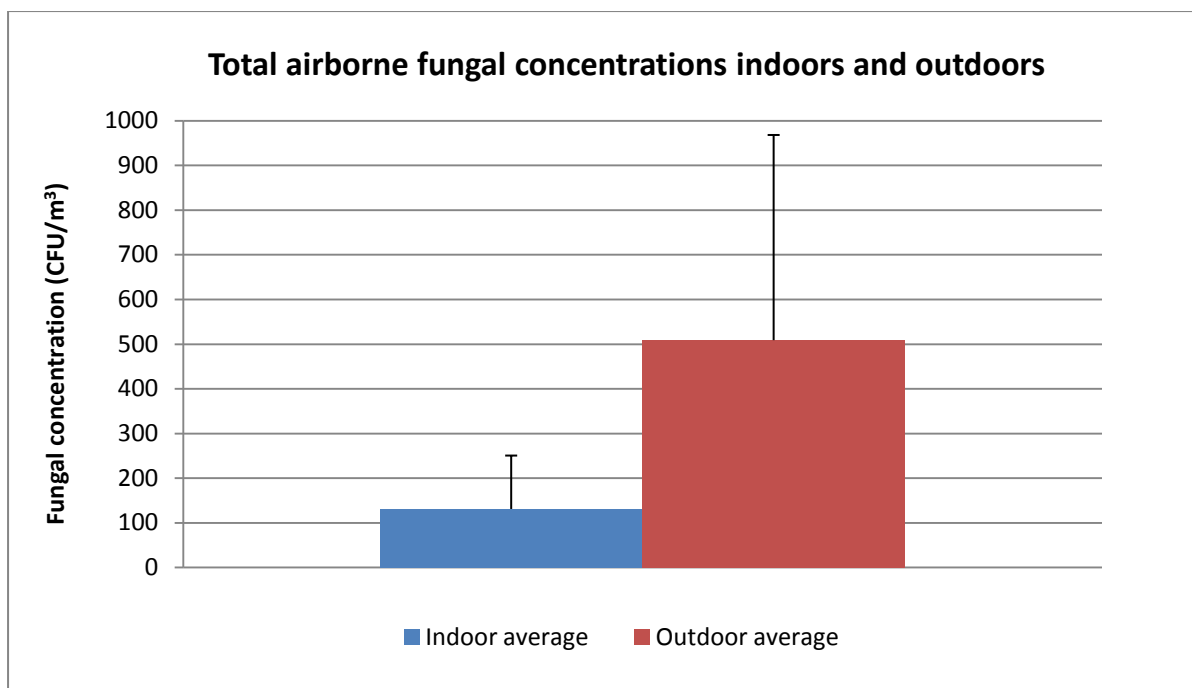


Figure 1: Comparison of total indoor (n=89) and outdoor (n=39) fungal concentration (CFU/m³) in air samples obtained from offices across metropolitan Adelaide.

Results were further examined seasonally. Overall, seasonal indoor results broadly reflect the trend highlighted for all combined data (as shown in Figure 1), that is, lower average indoor concentrations than outdoor levels across all seasons. Figure 2 highlights seasonal variation in fungal concentrations in indoor and outdoor environments.

A significant seasonal influence was observed for fungal concentrations indoors ($p=0.0011$). Significantly higher fungal concentrations were recorded in autumn (186 ± 118 CFU/m³) and summer (218 ± 131 CFU/m³), compared with lower concentrations in spring (71 ± 93 CFU/m³) and winter (61 ± 56 CFU/m³). However, there was no significant difference between summer compared to autumn, nor winter compared to spring for indoor data.

A similar seasonal influence was noted in outdoor fungal concentrations (CFU/m³) ($p=0.021$); samples collected in autumn were significantly higher (751 ± 596 CFU/m³) than samples collected in spring ($170 \pm$ CFU/m³). Higher variability (often by a factor of ten) in outdoor fungal concentrations was noted.

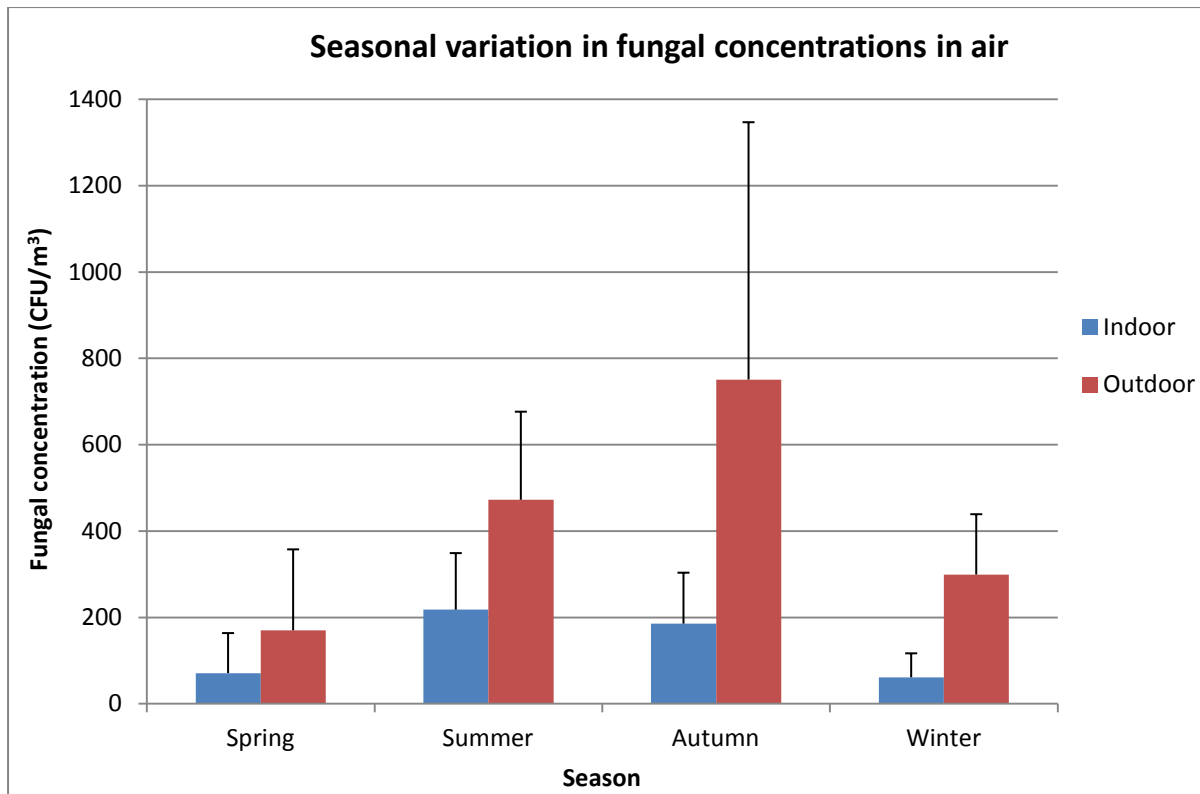


Figure 2: Seasonal variation in fungal concentrations (CFU/m³) in indoor and outdoor environments in air samples obtained from sites across metropolitan Adelaide.

A subset of samples was collected comprising the interface between office environments and warehouse/loading-depot/factory and storage areas. These results (n= 14) were separated from the original pool of indoor samples to determine if these environments were significantly different from office indoor locations, and outdoor locations. Figure 3 compares these “warehouse/industrial” areas with true indoor and outdoor samples. The calculated fungal concentration (CFU/m³) for these spaces showed average concentrations significantly higher ($p < 0.001$) than samples taken indoors, but lower than samples collected from true outdoor environments (not significantly different). These variations may be explained by a higher, unfiltered air exchange rate with outdoor air sources within these spaces compared to indoor air.

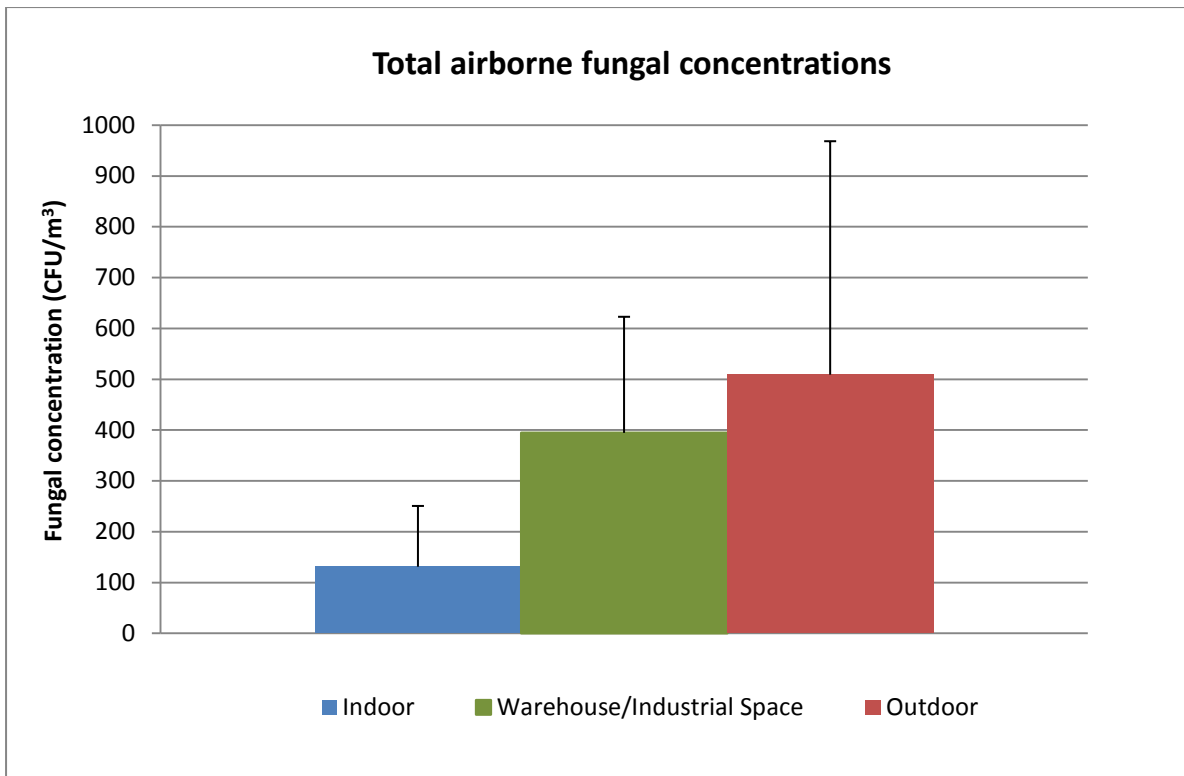


Figure 3: Comparison of total indoor, warehouse/industrial space and outdoor fungal concentrations (CFU/m³) in air samples obtained from sites across metropolitan Adelaide.

Due to the wide range of building types, ages, design, occupancy level etc, it is not possible to derive specific correlations between indoor air quality data and fungal concentrations with building parameters.

3.4 Correlation between IAQ parameters and fungal spore concentration in indoor air

Similar trends were observed between fungal concentrations and measured indoor air quality parameters across seasons (Figure 4). However, a significant correlation was only found between carbon dioxide and fungal concentration ($p = 0.014$, $R=0.259$).

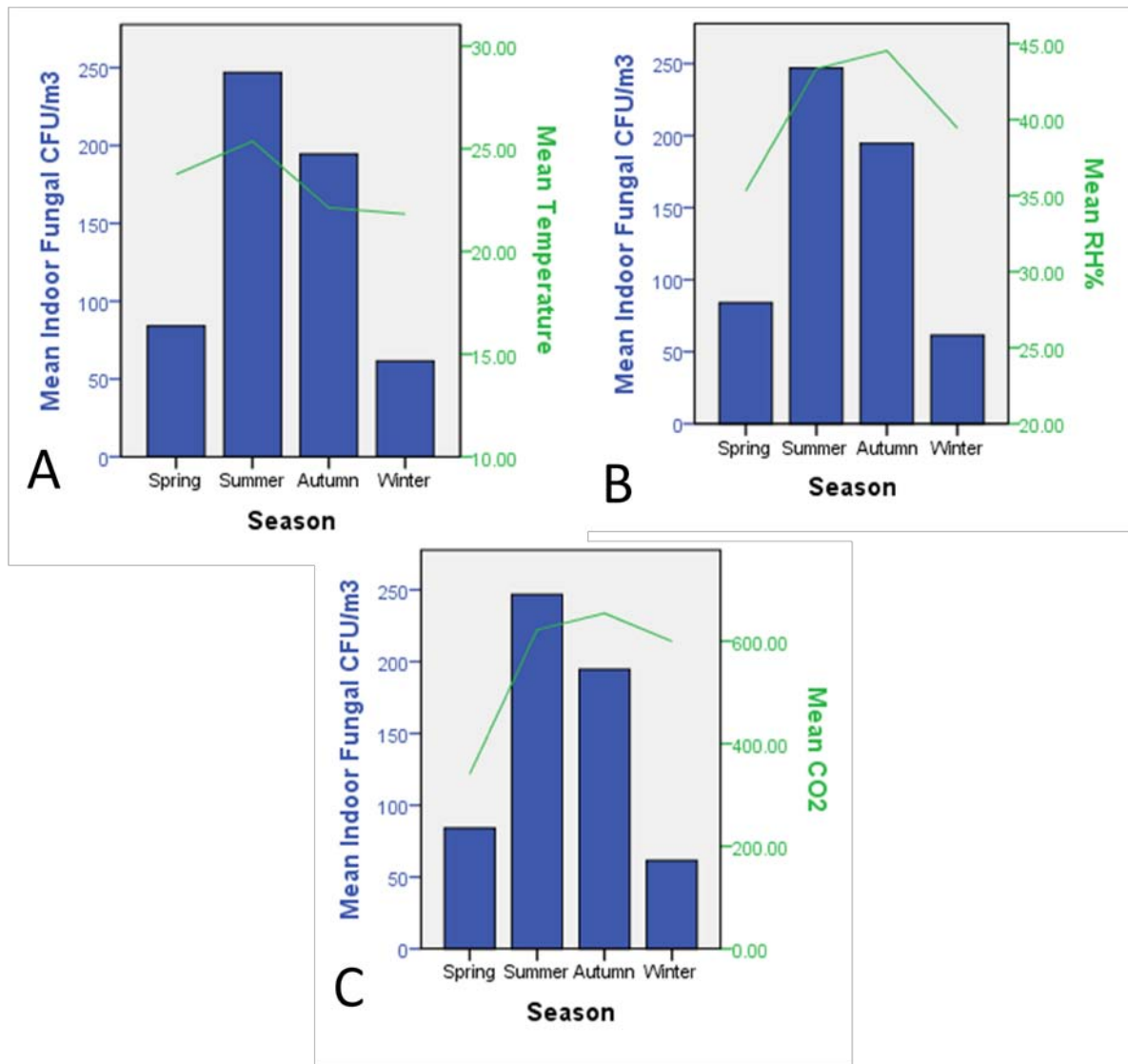


Figure 4: Seasonal trends in fungal concentration (CFU/m³) and IAQ parameters: Temperature °C (A), relative humidity %RH (B) and CO₂ ppm (C).

3.5 Fungal diversity

The prevalence of specific fungal genera was determined for both indoor and outdoor environments by calculating the percentage of samples in which each genus was found. These results are presented in Figure 5. The top four most commonly detected fungi in indoor air were *Penicillium* (53.9%), *Aspergillus* (48.3%), *Cladosporium* (38.2%) and *Alternaria* (12.4%). The same genera were also noted as top four most commonly detected in outdoor air, although rank order was slightly different outdoors *Aspergillus* (89.7%), *Penicillium* (outdoor 74.4%), *Cladosporium* (51.3%) and *Alternaria* (38.5%).

Diversity results, in terms of rank order of prevalence, were generally consistent between indoor and outdoor samples. *Alternaria* tended to appear more frequently in outdoor environments, however the indoor prevalence of *Alternaria* still consistently placed it within the most frequently isolated fungi. Overall, a higher diversity of fungal genera was noted in outdoor air compared with indoors. Non-sporulating (or sterile mycelia) colonies were not included in sample final results.

No fungal genera regarded as toxigenic were isolated from any workplace. Infrequently detected genera in indoor samples only (e.g. *Stemphylium*, *Malbranchea*, *Chaetomium*) whilst interesting, were too infrequently isolated to draw conclusions. In no cases were these genera the dominant organisms present and were generally detected as single colonies.

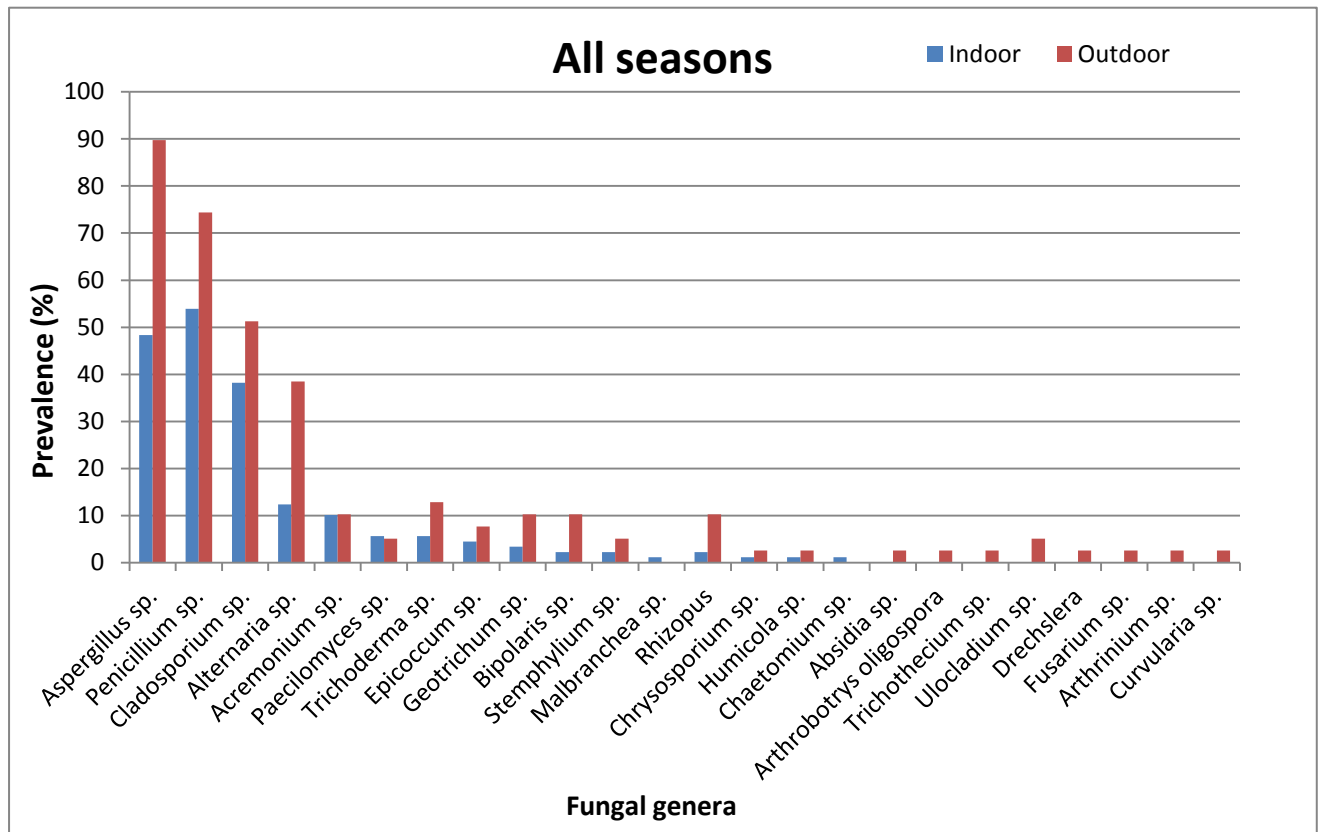


Figure 5: Prevalence of fungi in indoor (n=89) and outdoor (n=39) air samples obtained from office buildings across metropolitan Adelaide.

Examination of seasonal variation in diversity revealed consistent trends in the top four most commonly isolated fungi in both indoor and outdoor environments (i.e. *Penicillium*, *Aspergillus*, *Cladosporium* and *Alternaria*), across all seasons (data not shown).

3.6 Representative examples of fungal colonies on agar plates

For reference, included in Figures 6-8 are common examples of varying fungal diversity and abundance obtained from indoor and outdoor air samples.

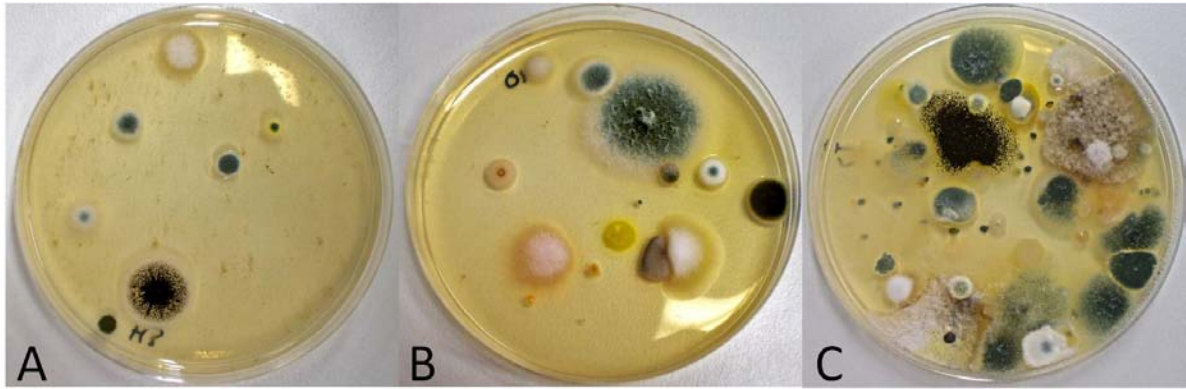


Figure 6: Examples of indoor air sample results showing low fungal concentration (Plate A, ~120 CFU/m³), moderate concentration (Plate B, ~265 CFU/m³), and high concentration (Plate C, ~700 CFU/m³). NOTE: Low to Moderate concentrations are most commonly measured in non-complaint indoor environments.

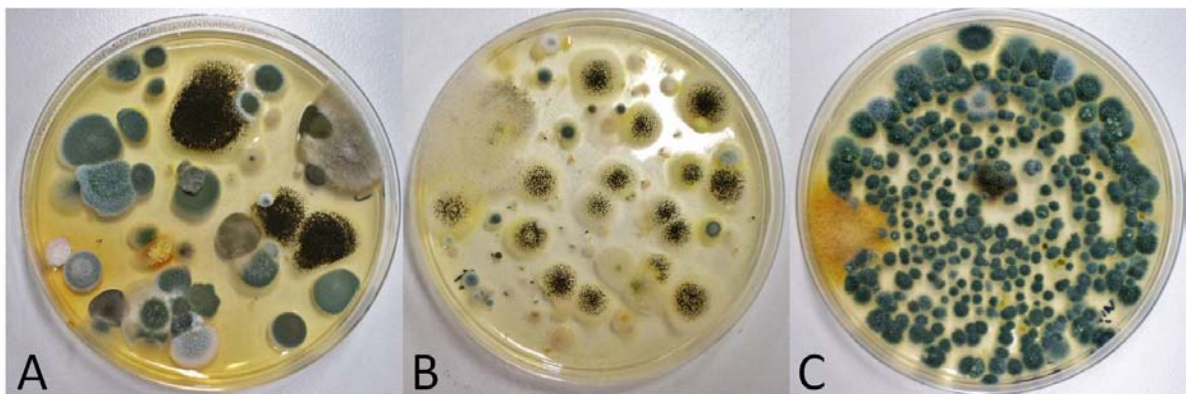


Figure 7: Examples of varying diversity in air sample results: Plate A shows a highly diverse sample from outdoor air, Plates B and C show low and very low diversity from indoor air samples with apparent single dominant species on each.

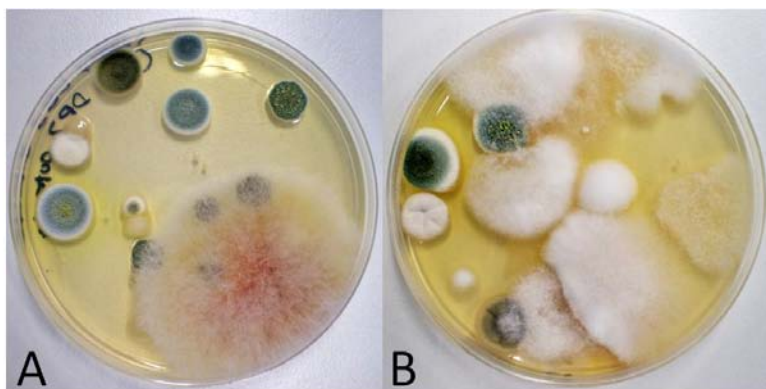


Figure 8: Plate A shows an example of colonies obscured by overgrowth, and thus may be difficult to identify; Plate B shows examples of non-sporulating sterile mycelia (white colonies).

4.0 Discussion

4.1 Intended audience and use of this study

Currently many Occupational Hygienists possess highly developed skills in recognising and assessing chemical hazards in the workplace, but generally are not as familiar with the biological contaminants in the workplace. Guidance documents exist on remediation of mould damage in buildings ('Australian Mould Guideline', Kemp & Kemp, 2005; 'The Mould Worker's Handbook: A practical guide for remediation', Kemp & Kemp, 2010), but limited information is available on interpretation and communication of biological air quality results. Most often after monitoring indoor air for biological hazards, samples are sent to analytical laboratories and Occupational Hygienists are simply presented with a calculated concentration (CFU/m³) without any greater context given. Some limited comment on fungal identification results may be provided by the laboratory. Bioaerosol monitoring results require a significant amount of interpretation which only comes from familiarity. Without a representative selection of results taken from non-complaint workplaces (i.e. baseline data), it is difficult to determine if the fungal load obtained in complaint buildings is higher than normal. More specifically, this data should ideally be sourced from studies conducted in the city in which the OHS Professional works. Published geographic variations in both fungal spore counts and particularly in the genera identified may present professionals with unreliable or inaccurate reference material.

This study aimed to provide Occupational Hygienists and other OHS Professionals undertaking fungal air quality assessments in Adelaide (South Australia), a baseline of abundance and diversity from a variety of non-complaint buildings against which their results may be compared, and to provide the much needed context and guidance to correctly interpret these results.

4.2 Fungal Abundance and Diversity – What guidance exists?

Both the fungal load (CFU/m³) and the diversity of fungal genera isolated (indoors and outdoors) together generate a picture of the overall biological quality of the air. Abundance and diversity of fungal profiles should not be used independently when evaluating a workplace indoor air quality.

There are currently no national Indoor Air Quality Standards/Guidelines in Australia for mould in air. Hence, in the absence of local guidance on fungal contamination in indoor work environments, Occupational Hygienists typically adopt international standards when assessing workplaces. There are a number of potential guidelines available, both current and historic (outlined below), and typically WHO are adopted (World Health Organization, 2009).

According to the World Health Organisation (WHO, 2009) ‘Guidelines for indoor air quality: dampness and mould’, “*as the relations between dampness, microbial exposure and health effects cannot be quantified precisely, no quantitative health-based guideline values or thresholds can be recommended for acceptable levels of contamination with microorganisms. Instead, it is recommended that dampness and mould-related problems be prevented. When they occur, they should be remediated because they increase the risk of hazardous exposure to microbes and chemicals.*” This means interpretation of fungal concentrations in indoor air samples must be interpreted with caution. Furthermore, if air monitoring for fungi is carried out, it is important to understand that care is required when evaluating one-off airborne fungal test results because the organisms are ubiquitous and variable in the environment. An outdoor sample taken on the day of test can provide a useful comparison figure for evaluation of results. Similarly, an indoor reference sample of a “non-complaint” area in the workplace can also be useful for interpreting fungal concentrations in air of ‘complaint’ environments.

The previous (1990) WHO guidelines for assessment of hazardous indoor airborne fungi used an approach, as summarised below:

Guidelines for assessing airborne fungi (WHO, 1990)		
Result of air sampling		Acceptable level
A	Confirmed pathogens (eg <i>Aspergillus fumigatus</i>) or toxigenic fungi (eg <i>Strachybotrys atra</i> and toxigenic <i>Penicillium</i> , <i>Fusarium</i> species)	Not acceptable
B	Only one species other than <i>Cladosporium</i> or <i>Alternaria</i>	< 50 CFU/m ³
C	A mixture of species reflective of outdoor flora	< 150 CFU/m ³
D	Primarily <i>Cladosporium</i> or other common phylloplane fungi	< 500 CFU/m ³

Note: CFU/m³ = colony-forming units per cubic meter of air

The guidelines given above take into consideration both fungal concentration and diversity, however it may not be immediately apparent to an OHS professional the difference between C and D, so typically they might adopt the <500 CFU/m³ approach.

In comparison, according to guidelines by the American Conference of Governmental Industrial Hygienists (ACGIH, 1989), ‘Guidelines for the Assessment of Bioaerosols in the Indoor Environment’, authors state that “*outdoor fungal air concentrations exceeding 1000 CFU/m³ were routine and that concentrations near 10,000 CFU/m³ were not uncommon in summer months. Except in specialized environments where immune suppressed persons are routinely present, levels of saprophytic fungus below 100 CFU/m³ were reported as not of concern*”.

Below is a table from the Annual General Meeting, Conference and Trade Show March 2007 by the Institute for Research in Construction, and the Labrador Environmental Industry Association document ‘Indoor Air Quality and Mould (Hans Schleichinger and Wenping Yang), Indoor Environment Research Program)’ outlining a range of previous guidance values for mould in air:

Canada Health Canada (1995)	Canada Public Works (2005)	WHO (1988)	OSHA (1999)	ACGIH (1989)	Hong Kong (2003)	Commission of European Committees (1993)	Singapore (1996)
<p>≤ 150 CFU/m³ OK if a mixture of the outdoor air spores.</p> <p>≤ 500 CFU/m³ OK in summer if the species present are primarily <i>Cladosporium</i> or other tree and leaf fungi.</p> <p>Contamination if >50 CFU/m³ of a single species.</p>	<p>Indoor fungal quantities should be lower compared to outside.</p> <p>Contamination if quantity of normal outdoor [phyloplane] species ≥ 500 CFU/m³ indoors.</p>	< 500 CFU/m ³	<p>Contamination indicators :</p> <p>≥ 1000 CFU/m³;</p> <p>≥ 10⁶ fungi/g dust;</p> <p>≥ 10⁵ fungi/mL stagnant water or slime.</p>	<p>< 100 CFU/m³ considered of no concern,</p> <p>200 CFU/m³ recommended as a guideline for fungal bioaerosols.</p>	500 / 1000 CFU/m ³	<p>Houses (CFU/m³):</p> <p>> 10,000 -very high</p> <p><10,000 - high</p> <p><1000- intermediate</p> <p><200 - low</p> <p>< 50- very low</p> <p>Non-industrial indoor env. (CFU/m³):</p> <p>> 2000 - very high</p> <p>< 2000 - high</p> <p>< 500- intermediate</p> <p>< 100 - low</p> <p>< 25 - very low</p>	< 500 CFU/m ³

(WHO) – World Health Organization

(OSHA) - U.S. Department of Labor, Occupational Safety and Health Administration

(ACGIH) – American Conference of Governmental Industrial Hygienists

(Hong Kong) – The Government of the Hong Kong Special Administrative Region

(Commission of European Committees) – Luxembourg: Office for Official Publications of the European Communities

(Singapore) – Ministry of the Environment Singapore

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Although the workplaces sampled during the course of this work were non-complaint, the fungal CFU/m³ obtained would in fact exceed some of the suggested lower end levels in the guidelines above. The existence of multiple, conflicting guidelines introduces significant confounding viewpoints for professionals routinely investigating workplaces. Currently, most Occupational Hygienists would adopt the WHO guidelines (i.e. <500 CFU/m³) and use of outdoor samples to interpret indoor results. The current study confirms that these recommendations would be appropriate for SA conditions (in the absence of reported health effects). In practice, OHS professionals could use discretion where results fall between background levels found in the current study for SA conditions (i.e. Mean 131 CFU/m³) and suggested guideline levels of <500 CFU/m³, assuming indoor results are lower than comparative outdoor samples. This is of course subject to the absence of reported health effects and obvious signs of moisture intrusion or other water events e.g. condensation.

Current WHO guidelines (2009) do not state specific allowable concentrations for guidance, in order to discourage the adoption of arbitrary surrogates for investigation. But OHS professionals undertaking assessments will ultimately be required to interpret results. Recommendations for interpretation of indoor fungal assessments are provide in section 5.0.

4.3 What is known about atmospheric fungal concentration and diversity in Australia?

The limited studies on fungal contamination in Australian indoor environments have found levels often substantially exceeding the guidelines (historic) proposed by the WHO (1990). Levels have been reported of up to 18,000 CFU/m³ in comparison with the WHO guidelines of between none and 500 CFU/m³, depending on the types present (Brown 2004).

According to a report 'Air toxics and indoor air quality in Australia - State of Knowledge Report (Environment Australia, 2001), "*published studies in Australian buildings have been limited. Seneviratne et al (1994) reported measurements in three Sydney office buildings with histories of sick building syndrome symptoms. Two buildings exhibited low colony counts that were primarily Cladosporium, Aspergillus and Penicillium. The third, a building found to have damp walls but no visible mould growth, exhibited similar species but at high airborne levels (600–2500 CFU/m³)*". The report further suggests typical levels ranging from 50 to 1500 CFUs per cubic metre in outside city air (Environment Australia, 2001).

A comparison of results obtained in the current study with other published airborne fungal concentrations in Australian buildings and domestic dwellings is summarised in the table below:

Comparison of indoor office building air (current study) with previous indoor and outdoor measurements in Australia:

Measurement[#]	Study location	Reference
Spring: Indoor 71 (± 93), Outdoor 170 (± 188) Summer: Indoor 218 (± 131), Outdoor 473 (± 204) Autumn: Indoor 186 (± 118), Outdoor 751 (± 596) Winter: Indoor 61 (± 56), Outdoor 299 (± 140)	18 office buildings, SA	Current study
End of Spring Indoor 126 [Min-Max 55-223], Outdoor 503 [Min-Max 25-1130]	5 education buildings, WA	Kemp et al (2002)
Winter/Spring: Indoor 1167 (± 1233), Outdoor 1797 (± 1069) Summer/Autumn: Indoor: 2089 (± 1642), Outdoor 2245 (± 1719)	40 dwellings, Vic	CSIRO (2010)
Spring: Indoor Median 479 Summer: Indoor Median 1583	80 dwellings, Vic	Garrett (1997)
Late Autumn & Winter Outdoor 1113 (± 759) Indoor (Living rooms) 810 (± 389), (Bedrooms) 692 (± 385)	14 dwellings, Qld	Hargreaves et al (2003)

expressed as Arithmetic Mean except where noted otherwise

Standard Deviation in parentheses

Our results on fungal abundance do not appear to differ greatly from the limited other published work on non-complaint buildings in Australia (as shown in the table below), and provide a more comprehensive picture of distribution seasonally.

In terms of seasonal influence, it has been shown both in the current study and elsewhere (CSIRO, 2010) that indoor levels of fungi are significantly higher during Summer/Autumn than Winter/Spring (CSIRO, 2010). This is despite previous work being from domestic

dwellings in Australia (CSIRO, 2010), and the current study focussing on office work environments. Similarly, outdoor levels of fungi in Summer/Autumn have also been reported higher than Winter/Spring. Garrett et al (1997) also noted a seasonal influence on spore levels, both indoors and outdoors, for 80 homes in Victoria. The authors reported indoor median concentrations ranging from a low of 479 CFU/m³ in early spring to a high of 1583 CFU/m³ in summer. It is important to note this is for domestic dwellings, so the relative higher indoor levels could be attributed to more indoor sources than for occupational settings, or more likely reflect a difference in the type, size and efficiency of HVAC systems in commercial buildings.

Fungal genera found indoors should reflect those in the ambient environment, although relative abundances may change where the indoor environment is favourable to the growth of particular species. The most common reported genera are those living on leaves, including *Cladosporium*, *Alternaria*, *Epicoccum* and *Aureobasidium*, while soil-based species such as *Aspergillus* and *Penicillium* are relatively uncommon in outdoor air but are found at increased levels indoors (Hargreaves and Parappukaran 2000). Hargreaves et al. (2003) reported in a study of 14 domestic dwellings in suburban Brisbane, the most frequently isolated fungal genus was *Cladosporium* (> 50%), followed by, less frequently isolated *Curvularia*, *Alternaria*, *Fusarium* and *Penicillium*.

These historic results are somewhat in line with results obtained in the current study. The current work also showed four distinct high prevalence genera isolated from indoors, namely *Penicillium*, *Aspergillus*, *Cladosporium* and *Alternaria*. In contrast to Hargreaves and Parappukaran (2000) who reported lower levels of selected genera outdoors, the current study outdoor diversity results closely resembled indoor profiles (same top four dominant genera).

Air samples for viable fungi showing extremely low diversity may be indicative of indoor air quality problems, or a potential indoor source. This is particularly true if high levels of a single genus are cultured, as this may indicate water damage and the rapid growth of opportunistic fungi. During the course of this study, no fungal species specifically identified as toxic or of serious pathogenic concern were identified. However, the absence of pathogenic or toxigenic fungi is not necessarily an indicator of good indoor air quality. The overall spore load of the air must also be taken into consideration. The spores of many

common species (*Penicillium*, *Aspergillus*, *Cladosporium*) can act as allergens, and as such a high concentration in air may lead to occupants developing allergy based illnesses, rather than strictly mycotic diseases (fungal infections). Many fungal genera are known to cause allergies such as hayfever (CASANZ, 2002). Furthermore, *Alternaria* and *Aspergillus* are both known to be important triggers of asthma, particularly in drier, inland Australia.

4.4 Factors affecting indoor fungal levels

During the course of this work, additional samples were collected in warehouse and industrial spaces which were located adjacent to sampled offices. These spaces tended to be constructed with large open flooring areas, high ceilings, exposed overhead beams and pipe work and were primarily naturally ventilated by large open doors designed for access by vehicles.

Calculated fungal concentrations from these locations tended to be higher than indoor samples and lower than outdoor samples. Similarly, fungal diversity profiles from warehouse spaces demonstrated a higher diversity of identified fungal genera than indoor locations, but were generally not as diverse as true outdoor samples. These differences are likely a factor of building design as warehouses are often not provided with filtered or air-conditioned air, and exposed building structures and shelving fosters the accumulation of dust in inaccessible areas. However as these spaces are sheltered from the majority of prevailing wind and dust, fungal concentrations may be marginally lower than samples collected in outdoor environments.

Air conditioning systems can be an important means by which levels of fungi are increased indoors. It provides both a means whereby spores are dispersed while providing areas, such as moist ventilating ducts, where the fungi can grow. Systems which are poorly maintained or particularly where filters are not routinely inspected, cleaned or replaced provide points where fungal spores will accumulate and ultimately be circulated throughout buildings. In its own right this reduces HVAC system efficiency and increases operating cost and may be an indicator of general overall building condition and maintenance procedures.

In some instances, measurements of IAQ parameters may inform efforts to locate and remediate mould contaminated materials. However, no single parameter can inform the bioaerosol content of the air. Increased humidity may indicate water intrusion or damage, whilst increased temperature and CO₂ not only suggest inadequate ventilation, but provide

conditions conducive to mould growth. The results obtained in the current study for IAQ parameters showed similar trends to fungal concentrations (although not strong significant relationships), however, it is not recommended to use IAQ parameters as proxy indicators of airborne fungal load. Measurements of IAQ parameters provide information to the OHS assessor on indicators of thermal comfort, and fresh air provision (%RH, temperature, CO₂) and HVAC performance.

4.5 Influence of sampling technique on fungal assessment

There exists some debate in regards to which sampling methodology collects and produces most accurate or representative results. Multiple methods are used to carry out indoor fungal assessments, and often the availability of equipment, the capability of the OHS professional and the location of the actual site sampled influence the method used. The suggested NIOSH standard (NIOSH, 1998) is viable active sampling, in which a known volume of air is impinged upon a nutrient agar plate (most commonly malt extract agar) and any fungal colonies which appear are counted and identified. This method is rapid to carry out and represents the majority of fungal species which occur in indoor environments.

Passive/settle plate sampling is technically more simple, requiring no equipment to be carried out and therefore easier to provide if equipment transportation/cost is a relevant factor. However this method may not collect as representative a sample as it requires air and spores of differing sizes to settle upon an agar plate surface in a defined manor. It has commonly been utilised for routine sampling of 'clean-room' surgical suites to enable staff to collect samples with little expertise required.

Direct spore collection (often referred to as Total Spore Count i.e. Viable and Non-viable) and identification may be used where both an air sampler/spore trap and the expertise to identify spores is available. This assessment method may provide a much broader range of results to the analyst, but may require that more time be spent interpreting the results obtained.

The use of any of these methods is often a function of availability, cost and lab specific practice. Any sampling carried out, regardless of the method used should always be interpreted along with other contextualizing information such as an occupant health/symptom survey, IAQ parameters, visual assessment for mould where appropriate etc.

5.0 Conclusions & Recommendations:

- When assessing fungal contamination of indoor air, both concentration (CFU/m³) and diversity play a role in identifying potential problems and evaluating the biological quality of the air. Low diversity and high concentrations may indicate a potential indoor source of contamination. High diversity and moderate/high concentration may not strictly be an indicator of a specific indoor issue depending on its relationship to outdoor results, with seasonal variations taken into account.
- Because dose-response data are not available for fungal spore exposure, indoor levels must be interpreted with respect to control environments, such as outdoor air and/or interiors with no complaints or symptoms. In general, indoor levels should be lower than those outdoors, and taxa should be similar indoors and out. In addition, taxa recovered indoors should be those commonly present in outdoor air at that season.
- International guidelines which suggest a fungal concentration of <500 CFU/m³ is a reasonable threshold for indoor environments, may be appropriate for South Australian conditions. This is subject to satisfying the criteria outlined above with respect to seasonal variation and relationship to outdoor levels.
- Use of baseline information relevant to the sample location (for example outcomes from the current study) for typical outdoor and indoor environments may also assist OHS professionals when assessing indoor air quality.
- Concurrent measurement of indoor air quality parameters (e.g. temperature, humidity, carbon dioxide) may inform efforts to locate and remediate mould contaminated materials. However, there is not sufficient evidence to suggest a single parameter can inform the bioaerosol content of the air.
- This study examined only non-complaint office environments. When conducting indoor air quality assessments in the workplace, where fungal contamination is suspected, OHS professionals should undertake an occupant survey of health symptoms. Survey responses together with air monitoring results may help inform recommendations while also identifying possible sensitive individuals requiring special consideration.
- On-site assessment of HVAC condition and maintenance will help inform the OHS assessor of potential sources of fungal contamination. The issue of fungal contamination in indoor air environments cannot be resolved by simple visual observation in the workplace because moulds can be inaccessible, and/or spores may come from a source

off-site. Furthermore, visual and/or olfactory assessment may or may not accurately indicate the true extent of the problem.

- When designing a monitoring strategy, inclusion of multiple sample replicates should be considered, where budgetary and time allowances are available. As a minimum, a sample of the indoor complaint area, an indoor non-complaint area and outdoors should be performed. Where multiple replicates of each are not performed, care should be taken in interpreting single sample results due to the natural variability of microbial load in the air.

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APPENDIX A

Workplace feedback report template

DISCIPLINE OF PUBLIC HEALTH
SCHOOL OF POPULATION HEALTH
FACULTY OF HEALTH SCIENCES

OCCUPATIONAL HYGIENE LABORATORY

28 Anderson Street, Thebarton, South Australia 5031
TELEPHONE +61 8 8303 4957 FACSIMILE +61 8 8303 4955
Web: www.adelaide.edu.au/oeH
Email: oeH@adelaide.edu.au



TO:

Feedback from indoor air quality monitoring

Understanding and managing occupational health risks from fungal contamination in indoor environments.

Purpose

Indoor air monitoring was conducted on, at, as part of participation in a research project. The aim of the project is to obtain baseline profiles of indoor airborne fungi/mould in typical South Australian workplaces. The work is being conducted by the University of Adelaide and has the support of the SA Government.

Methods

Air monitoring for fungi/mould was carried out using a BioStage® single-stage viable cascade impactor containing media plates into which the sampled air was impinged. Samples were obtained from several locations in the building as well as from outdoors for reference. Samples were assessed in the laboratory for enumeration. Results are expressed as colony forming units (CFU) per cubic meter of air (m³). Indicators of general indoor air quality were also monitored (e.g. temperature and humidity) using a TSI Q-Track Indoor Air Quality Meter Model 8551.

Results and interpretation

As can be seen from the results in Table 1 on the day of monitoring:

- Temperature, relative humidity, carbon monoxide and carbon dioxide concentrations were all [above/below] their respective occupational exposure or indoor air quality guideline limits. Results indicate ...[e.g.] reasonable fresh air provision and do not appear to be significant issues.

Table 1: Instantaneous air monitoring results for temperature, relative humidity, carbon dioxide and carbon monoxide.

Locations	Temp. °C	Relative Humidity %	Carbon dioxide (ppm)	Carbon monoxide (ppm)
...				
...				
...				
Suggested Indoor Air Quality Guidelines/Limits	20-24	30-60	1000*	9
SafeWork Australia Exposure Standard (8 hour time weighted average)**	-	-	5000	30

*1000 ppm as a surrogate indicator of sufficient outside air provision.

** Exposure Standards represent airborne concentrations of individual chemical substances, which according to current knowledge, should neither impair the health of, nor cause undue discomfort to nearly all workers.

Table 2: Fungal/mould cultures from air monitoring.

Sample location	Concentration (CFU/m ³)
....	
....	
Outdoor air reference samples (20 meters from entrance)	
Outdoor air reference samples (20 meters from entrance)	
Suggested Indoor Air Quality Guidelines/Limits	See explanation below

There are still currently no recommended national exposure standards for airborne fungi in indoor environments. A guideline concentration of **1000 CFU/m³** has been used as a generic guide although this really does not take into account the specific nature and toxicity of moulds/fungi. Fungal spores can be readily found in the environment both indoors and outdoors and their concentrations vary with the season, climate, time of day etc, thus care is needed in the interpretation of results.

Indoor air concentrations of fungal spores (in mechanically ventilated interiors) should in general be **below half that of the outdoors**, and indoor levels below 100 CFU/m³ **should not be of concern**. Outdoor aerosol may routinely exceed 1000 CFU/m³ and may average much higher in the summer months.

Table 2 results;

- Table 2 results for viable airborne fungi/mould indicates ... indoor concentrations, with respect to outdoor reference airborne concentrations and guideline levels outlined above, and are not indicative of a viable mould issue.

Thank you for participating in the research. Information gathered may help to inform local guidelines on indoor microbial air quality under Australian conditions. Please feel free to contact me should you require any further information or clarification on these issues.

Kind regards,

A handwritten signature in black ink, appearing to read 'Sharyn Gaskin', with a stylized flourish at the end.

Dr. Sharyn Gaskin
BAppSc(ENVH), BSc(Hons), PhD
Occupational Hygienist