A comparative study of qualitative sampling methods for the analysis of the indoor air moulds

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ABSTRACT

Accurate and informative sampling methods are very important in the evaluation of fungal exposure in indoor air quality (IAQ) investigations. We have investigated the relationship between indoor culturable airborne and dust-borne fungi and compared the performance of culturable and non-culturable air samplers—Reuter Centrifugal Air Sampler (RCS) and Zefon Air–O–Cell^{TM} (AOC). Two 12-h investigations were conducted and two dust samples were collected in each of the two typical office premises in Hong Kong in autumn and winter, 2001. Culturable samples were incubated on Dichloran Glycerol 18 and 2% Malt Extract Agar at 25°C. The results indicate more diverse and abundant fungal species were present in dust samples than in air in both seasons. Simpson's Diversity Index values in dust samples were 16–18% higher than that in air samples and also with much higher counts (>500 times) recorded. Our investigation also shows that higher fungal counts (25–110 times higher) but with similar species diversity (Orchiai Similarity Index 0.81–0.86) were collected by AOC, compared with RCS in both seasons. The dominant fungi collected in dust and air samples were *Penicillium* spp., *Aspergillus* spp. and *Cladosporium* spp., with increased dominance of *Cladosporium* spp. in winter. This study suggests that more informative IAQ investigations on fungi can be conducted by collecting culturable samples in dust and non-culturable samples in air.

INDEX TERMS

Airborne fungi; Air sampler; Dust-borne fungi; Office premises

INTRODUCTION

Exposure to airborne fungi has been proved to be the cause of allergenic, respiratory symptoms and diseases (Fischer and Dott, 2003). There are also many other diseases, such as chronic sinusitis, chronic bronchitis, asthma, humidifier fever and sick building syndrome, which are potentially caused by fungal exposure (Lacey and Dutkiewicz, 1994).

People spend about an average of 97% of their lives indoor (Platts-Mills, 1995). Therefore, accurate and informative sampling methods are very important in the evaluation of fungal exposure in indoor air quality (IAQ) investigations. When sampling, the most important factors affecting the collection and survival of microbes in the sampler were their resistibility, sampling time and flow rate (Macher and Willeke, 1992; Nevalainen *et al*., 1993; Li and Lin, 1999). The performance of the air sampler also depends on the physical collection efficiency as well as the stress imparted to the microorganisms (Li and Lin, 1999). In the present study, the performance of each sampling method, based on the counts and composition of fungi collected in the same environment, was compared.

Unlike other chemical air pollutants, there is no universal safety health standard for the threshold level of indoor fungal counts. In addition, there are only a few published studies on indoor fungi in Hong Kong (Wong, 1997; Law *et al.*, 2000).

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The objectives of this study were to compare (1) the performance of culturable and non-culturable air samplers—Reuter Centrifugal Air Sampler (RCS) and Zefon Air–O–Cell^{TM} (AOC); (2) the counts and composition of fungi between indoor air and dust samples. The seasonal changes of fungi in indoor air and dust samples in Hong Kong were also studied.

METHODS

Two 12-h investigations were conducted in two typical office premises at the City University of Hong Kong in autumn (September) and winter (December) in 2001. Site 1 is the General Office of the Department of Biology and Chemistry, and Site 2 is the Facilities Management Office. These two offices had no record of moisture or water damage and are under the control of separate Heating, Ventilation and Air Conditioning (HVAC) systems in two different buildings. The ages of the buildings of the two sites are also different—Site 1 was built about 12 years ago while Site 2 is about 3 years old. Air samples were taken every 3 h from 7:00 a.m. to 7:00 p.m. and one dust sample was taken in each site during each sampling. Air samples included the culturable and non-culturable samples, which were taken from the air inlet (air return), air outlet (air discharge) and desktop level (breathing zone). The corresponding outdoor air samples were also taken. A Zeal Whirling Hygrometer was used to measure the temperature and the relative humidity of the indoor and outdoor environments.

Air Sampling

The Biotest RCS Plus Air Sampler–Reuter Centrifugal Sampler was used for culturable air sampling (Biotest Company, 1996). Malt Extract Agar (MEA) with Rose Bengal (Biotest) was used as the sampling media. After sampling, the agar strips were incubated at 25ºC for 3–4 days without disturbance until they are ready for reading. The airborne fungi counts were calculated as CFU/m^3 and the colonies were isolated and cultivated for identification using 2% MEA.

The Zefon Air–O–Cell^{TM} was used for non-culturable air sampling (Zefon International, 2000). After sampling, the sticky strip on the piece of thin glass was stained with lacto-phenol cotton blue and placed onto another clean and labelled slide. The slide was examined under the light microscope at 400× magnification. The airborne fungi count was calculated as counts/m^3 according to the Zefon manual, and the identification of fungal species was based on the morphological characteristics of each spore using standard keys (Dillon *et al*., 1996).

Dust Sampling

A household 1100 W vacuum cleaner installed with a piece of sterilized fine mesh cloth was used for dust sampling and its microbial content was analysed (Dillon *et al.*, 1996). A serial dilution of the dust sample was prepared and each dilution was spread on two different sampling media—2% MEA and Dichloran Glycerol 18 Agar (DG 18)—and then incubated at 25ºC. These selective media were used for selecting the saprotrophic and xerophilic saprotrophic fungi, respectively. The fungal counts in dust were calculated as CFU/g dust and the colonies were isolated and cultivated for identification using 2% MEA and DG 18.

Data Analysis

The fungal counts, the identity of different genera and species, fungal diversity (Simpson's Diversity Index) and similarity (Orchiai Similarity Index) of the samples were calculated for comparing the performances of different sampling methods.

RESULTS

Relative Humidity and Temperature in Indoor and Outdoor Environments

The average relative humidity and temperature recorded at Sites 1 and 2 are presented in Table 1. In general, the indoor relative humidity was similar to that of the outdoor readings,

while the indoor temperatures were always lower than outdoor in autumn but were similar in winter. The average relative humidity increased and the temperature decreased from autumn to winter.

Table 1 Average[a] relative humidity (%) and temperatures (ºC) recorded at Sites 1 and 2

		Site 1		Site 2	
		Sept	Dec	Sept	Dec
Average relative humidity (%)	In	67.8 ±5.3	70.4 ± 2.3	60 ± 2.3	62 ± 4
	Out	62.5 ± 5.8	69.2 ±11.9	56 ±10.1	68 ± 2.3
Average temperature (ºC)	In	23.5 ±0.4	20.1 ±1.39	22.5 ± 0.6	21 ± 0.8
	Out	28.5 ±0.96	20.1 ±3.15	29.5 ±1.2	19 ± 1.5

[a]n = 10.
[b]In—Indoor, Out—Outdoor.

Table 2 Average[a] fungal count collected by different sampling methods

		Site 1		Site 2	
		Sept	Dec	Sept	Dec
Air samples					
Culturable (CFU/m^3)	In[b]	34	5	23	8
	Out	131	73	111	76
	I/O[c]	0.43	0.12	0.99	0.08
Non-culturable (CFU/m^3)	In	418	478	1260	880
	Out	3483	1219	6220	3980
	I/O	0.12	0.24	0.2	0.22
Dust Samples (CFU/g)		6007	11892	7368	30556

[a]n = 5 for culturable and non-culturable air samples; n = 1 for dust samples.
[b]In—Indoor, Out—Outdoor.
[c]I/O—Indoor/Outdoor ratio.

Comparison of Counts and Composition of Fungi Collected by Different Sampling Methods

The fungal counts, indoor/outdoor ratio, number of different genera, diversity and similarity index of fungal propagule in air and dust samples are shown in Tables 2, 3 and 4, respectively.

Table 3 Simpson's Diversity Index[a]

	Site 1		Site 2	
	Sept	Dec	Sept	Dec
C[b]	0.64 (4)[c]	0.78 (5)	0.67 (6)	0.38 (2)
N	0.51 (4)	0.76 (6)	0.15 (2)	0.40 (2)
D	**0.82 (6)**[d]	**0.94 (7)**	**0.83 (7)**	**0.89 (7)**

If the value is 1, the diversity is the highest or the composition of species collected by the two methods were completely equal.
[a]Formula = $1 - \sum P_i$
P_i = proportion of total sample belonging to ith species.
[b]C—Culturable air samples; N—Non-culturable air samples; D—Dust samples.
[c](X)—Number of genera identified.
[d]Bold type—the highest value within the same column.

Table 4 Orchiai Similarity Index[a]

	Site 1		Site 2	
	Sept	Dec	Sept	Dec
C&N	**0.81**	**0.86**	**0.5**	**0.5**
C&D	0.33	0.23	0.29	0.29
N&D	0.2	0.5	0.33	0.33

[a]Orchiai Similarity Index = Co-occurrences/Geometric mean of occurrences over two samples.

As shown in Table 2, different fungal counts were obtained by different sampling methods. In general, in the culturable air and dust samples, higher fungal counts were observed in the dust samples. The fungal counts obtained in non-culturable air samples were also higher than that in the culturable air samples. It is also observed that fungal counts in both the culturable and non-culturable air samples decreased from autumn to winter (30–85% decreased). However, the fungal counts in the dust samples increased from autumn to winter (90–300% increased). In addition, the I/O ratios obtained in both sites were below 1, implying that the environment had relatively good indoor air quality.

Over 80% of all the fungi isolated have been identified. In the air samples, six genera of fungi were isolated, namely, *Penicillium*, *Aspergillus*, *Cladosporium*, *Alternaria, Curvularia* and *Sympodiella*. In dust samples, these six genera were also recorded in addition to *Eurotium*, *Rhizopus* and yeasts. No yeast was found in the air samples in both indoor and outdoor environment. In the outdoor air samples, apart from the genera mentioned above, *Aureobasidum* (5.3%) and *Nigrospora* (2.3%) were also recorded in very low abundance. The abundance (%) of the dominant fungi, namely *Penicillium*, *Aspergillus* and *Cladosporium*, in indoor air and dust samples collected in autumn and winter are presented in Figures 1 and 2.

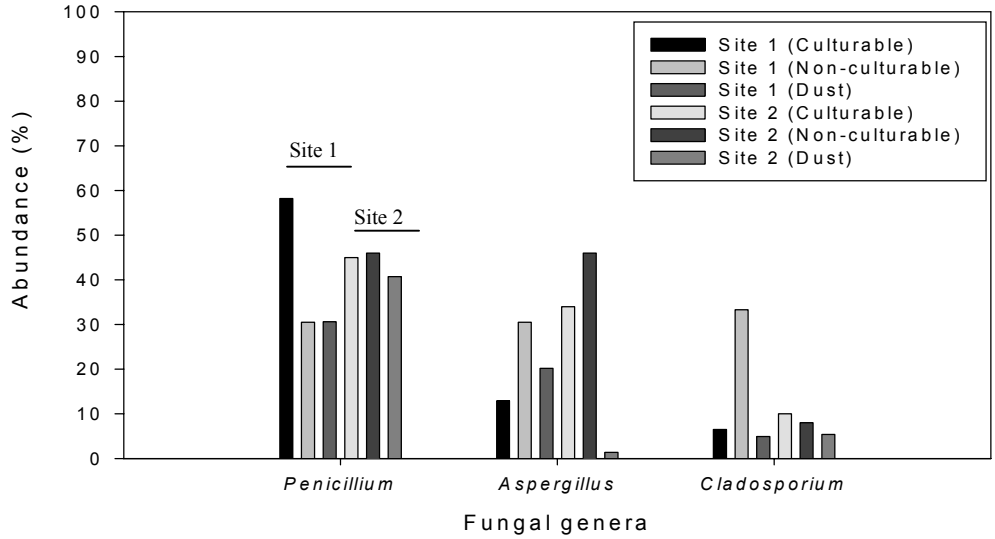
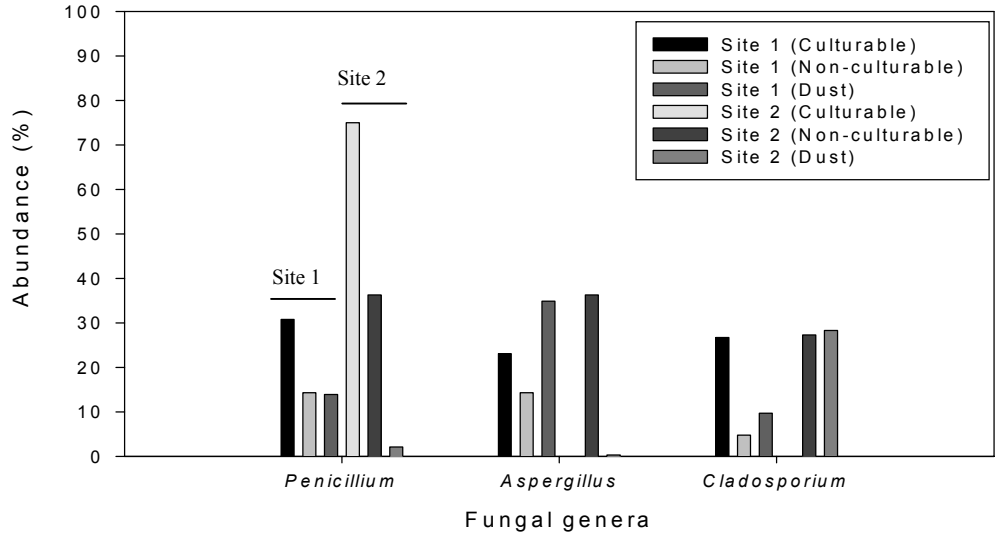


Figure 1 Abundance (%)* of dominant fungi in indoor air and dust samples collected in autumn (September 2001).



* Abundance (%) – no. of occurrence of a particular species / total no. of occurrences in one site X 100

Figure 2 Abundance (%)* of dominant fungi in indoor air and dust samples collected in winter (December 2001)

As shown in Table 3, diversity and the number of genera of fungi in dust was the highest, followed by culturable and non-culturable air samples. The fungal composition collected by culturable and non-culturable air samplings were similar, as presented in Table 4.

In general, *Penicillium* was the most dominant fungi in both air and dust samples in autumn. In winter, the dominance of *Cladosporium* increased in both air and dust samples. In addition, the abundance of xerophilic saprotrophic fungi at both sites also increased from autumn to winter (data not shown).

DISCUSSION

In order to compare the performance of each sampling method, several criteria were used; namely, fungal counts, number of different genera, diversity and similarity index. In the culturable air and dust samples, higher counts were obtained in dust (Table 2). Although the dominant fungi collected by all the methods used were similar, the diversity of fungi in dust was highest (Table 3). The microbial richness in dust is probably due to the nutrients present in these samples (Korpi *et al.*, 1997) and the physical protection offered by the dust particles to the fungal propagules. In the air samples, the fungal counts in non-culturable air samples were at least 10 times higher than that in the culturable air samples. Lappalainen *et al.* (2001) suggested that only 1–25% of the airborne fungal spores are culturable. Moreover, the incubation conditions in the laboratory may not be suitable for germination and growth of all the airborne fungi collected. In addition, different fungal propagules may have different survival ability in different samplers due to their various morphological features.

The diversity of fungal species in culturable and non-culturable air samples was similar, and a higher correlation was recorded between these two samples (Table 4); these two samples were collected in the same environment. Although the Orchiai Similarity Index for (C & D) and (N & D) are low, the indices showed that the fungi in dust samples are more correlated to the culturable samples as both samples require culturing to obtain results. This agrees with the study conducted by Korpi *et al.* (1997).

In this study, it is found that the dominant fungi in air were *Penicillium, Aspergillus* and *Cladosporium*, which were similar to those recorded from the dust samples, though additional genera were found in the latter. This composition of fungi in air and dust agreed with the previous studies in Hong Kong (Wong, 1997; Law *et al.*, 2000) as well as those in different parts of the world, e.g. Taiwan (Li and Kuo, 1994) and Japan (Takahashi, 1997). The indoor and outdoor airborne fungal counts in autumn were higher than that in winter. Li and Kuo (1994) reported that the fungal counts in summer were approximately 20 times higher than those in winter in Taiwan, which is also similar in many other countries, e.g. Finland (Reponen *et al.*, 1989) and United States (Shelton *et al.*, 2002)**.** According to Wong (1997), the optimum temperature and water activity ranges for growth (25–30ºC; 0.829–0.963) and germination (25–35ºC; 0.829–0.976) for these three dominant groups of fungi fall within the range of climatic conditions in Hong Kong (Table 1). This explains their dominance in both indoor and outdoor samples.

Although the relative humidity in winter was higher than that in autumn, the lower temperature in winter probably restricted the fungal growth. The increased dominance of *Cladosporium* in winter (>2 times) can be explained by its lower optimum growth and germination temperature (20–25ºC) (Wong, 1997; Takahashi, 1997); *Penicillium* and *Aspergillus* had a higher optimum temperature range for growth and germination (25–30°C) (Wong, 1997).

CONCLUSIONS

This study suggests that more informative IAQ investigations related to the presence of fungi can be conducted by collecting culturable samples in dust and non-culturable samples in air. The dominant fungi collected in dust and air samples in Hong Kong were *Penicillium, Aspergillus* and *Cladosporium*, which is similar to findings in other temperate and subtropical

regions. *Penicillium* was more dominant in summer while *Cladosporium* was more dominant in winter.

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