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Split fungal spore-trap samples for use in building inspection

Abstract

Building inspections for mould still commonly use spore trap air sampling for non-viable mould. A flaw with non-viable air sampling of buildings for mould is that the samples only capture a short duration 'snapshot' of the mould content from a single time point. To help mitigate this issue, we propose and explore the use of a different sampling strategy in which each sample is collected using a single spore trap cassette with two sampling events separated by a time gap. Preliminary data for samples taken in this fashion showed promise as the split fungal spore trap samples showed more mould types observed and fewer types missed as compared to standard sampling methods. This sampling strategy could provide building inspectors with more representative data without the requirement of spending more resources on air sampling cassettes.

Keywords: building inspection, mould inspection, mould assessment, mould sampling, fungal analysis, non-viable, spore-trap, air-O-cell, allergenco-D, gross fungi, gross fungal, building inspection

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Abbreviations: ASTM, American society for testing and materials; FS, fungal structures

Introduction

The majority of building inspection sampling for fungi is conducted through sampling for gross fungi (both viable and non-viable) by means of direct microscopy. Sampling for gross fungi remains an incredibly useful tool for building inspectors in obtaining data about fungal contamination in premises in a timely fashion. Other methodology for assessing samples for fungi include gene sequencing-based technology to identify fungi; and culturing of fungi on petri dishes for subsequent analysis. Each of these techniques has benefits and drawbacks. DNA sequencing of fungi can identify fungi to the species level, but requires specialised equipment and as a result incurs a higher cost of analysis, as well as a timeframe of at least a day for analysis.[1,2] Culturing of fungi can also identify to the species level in many cases, however requires an incubation period, sometimes weeks long. Additionally, culturing identifies only viable fungi – and of those only ones which grow on the agar mediums used at the temperatures used for incubation.[3] Direct microscopy of gross fungi is cost effective and can be conducted very quickly, however cannot identify fungi to the species level – only to genera. Despite this limitation, data of fungal genera are useful to building inspectors in isolating fungal issues in premises.

A drawback which applies to fungal sampling in any of the methodologies outlined above is that the air sampling only catches a very short timeframe of the microbial content of the sampled location.[4-7] Typically, sampling times are less than 10 minutes, and it is always a concern that the 'snapshot' of the microbial content has missed mould genera. The potential of spore trap samples to miss genera has been noted in several studies.[5,8-11] While there are options available to sample constantly over a long period of time (such as a Buchard sampler),[12] these methods are impractical, and the equipment cumbersome – more suited to fixed location sampling. Here we suggest using the typical spore trap cassettes used widely in building inspection in 'split samples.' 'Split sampling would involve using one cassette in two or more instances separated by a time interval – such as one at 9:00, and one at 11:00. Sampling in this manner would likely improve the chances that a short duration sample would not miss genera, while maintaining low cost. It would also help address issues such as changing air currents affecting sample content, and different spore release characteristics by time separating samples.

It should be noted that splitting samples is not a novel concept. In methamphetamine testing use of 'pooled' samples is a common practice.[13] For 'pooled' (or composite) samples many samples are collected and then combined into one sample to obtain a sample which is a better overall representation of the site. Some guidance for mould sampling suggests that two outdoor reference samples be taken per site, at times as close to the sampling of the indoor environment as possible.[2,14] Yang et al have indicated that ideally multiple samples taken over several days should be collected.[1]

Methods

Outdoor air samples were collected for use in this preliminary experiment as a starting point as they provide varied composition of fungi.[7,15] Spore trap samples were collected using a Zefon Bio-pump, using Air-O-Cell media at 15 litres per minute for each sample as recommended by the manufacturer. Samples were collected on the grassy area outside the IECL premises in Queensland, Australia during fine weather across multiple days. Relevant environmental data from the site is listed in supplementary data (Table 4).

To collect the most comparable samples possible, three Bio-pumps (Zefon) were used simultaneously (Figure 1). Sampling times of 2 and 5 minutes were selected as the equipment can only be set to 1-minute intervals, and typically samples are collected for totals of 5 or 10 minutes. The first split sample (2x2) is collected onto a single Air-O-Cell cassette for 2 minutes at the start and an additional 2 minutes at the end of the 2-hour interval. The second split sample (2x5) is collected onto a single Air-O-Cell cassette for 5 minutes at the start and an additional 5 minutes at the end of the 2-hour interval. A 5-minute control sample was also collected at the start (CS – control start) and another control sample collected at the end of the 2 hours (CE – control end). Using 3 Bio-pumps together enables the 2x2, 2x5 and control samples to all be taken together in simultaneous runs.

Table 1 shows the sampling strategy used. Six separate experiments were conducted (N = 6). The use of six experimental replicates was selected in order to balance analysis costs while providing enough data points to perform statistical analyses. The combined count used in Tables 2 and 3 are calculated by amalgamating all the mould count data from the samples (CS, 2x2, 5x2 and CE) with the total sampling duration (typically 24 minutes). CS and CE control samples were also grouped together and used in some analyses. Individual sample data is available in supplementary data (Table 3). Data on statistics for each sample is reported in supplementary data (Table 5).

Table 1 Sampling strategy for the experiment

Table 1 sampling durations	Control start sample (CS)	Split sample 2 minutes x 2 (2x2)	Split sample 5 minutes x2 (2x2)	Control end sample (CE)
0h (start point)	5 min	2 min	5 min	-
2h (end point)	-	2 min	5 min	5 min
Total sampled time	5 min	4 min	10 min	5 min

Table 2 Comparative data from the experiment for the types of mould observed and missed, and how the counts compare to the combined count for each sample. Data from individual samples are shown in the appendix in Table 3

Sample	Total number of mould types in sample	Number of mould types missed	Total FS/m³ distance from the combined count	Number of mould types outside of ±500 FS/m³ from the combined count
(1) CS	12	3	840	1
(1) 2x2	10	7	5160	2
(1) 5x2	13	4	1627	1
(1) CE	10	6	34	0
(2) CS	9	7	827	1
(2) 2x2	7	8	1400	0
(2) 5x2	15	1	5627	5
(2) CE	11	5	402	1
(4) CS	7	4	110	0
(4) 2x2	9	2	187	0
(4) 5x2	10	0	1493	1
(4) CE	7	3	312	0
(5) CS	6	4	5	0
(5) 2x2	5	5	542	0
(5) 5x2	9	1	1915	1
(5) CE	5	5	350	0
(6) CS	12	2	2144	1
(6) 2x2	10	4	1389	1
(6) 5x2	10	4	3450	3
(6) CE	8	6	1773	1
(7) CS	8	6	752	0
(7) 2x2	10	4	16	0
(7) 5x2	11	3	1827	1
(7) CE	9	5	214	0
(Avg.) CS	9	4.33	779.67	0.5
(Avg.) 2x2	8.5	5	1449	0.5
(Avg.) 5x2	11.33* (p=0.0478)	2.17* (p=0.01564)	2656.5* (p=0.00323)	2* (p=0.0058)
(Avg.) CE	8.33	5	514.17	0.33
(Avg.) Both CS and CE	8.67	4.67	646.92	0.42

N=6, *indicates statistical significance (p value shown).

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Figure 1 Sampling setup for collection of 3 samples simultaneously using 3 Bio-pump air samplers.

Samples were then analysed through microscopy using Nikon Eclipse Ci microscopes at 400x magnification. Samples were analysed in traverses as outlined in ASTM D7391,[16] however stopping rules were ignored in order to obtain a full profile of the data. Laboratory analysis of samples was conducted prior to staff receiving explanation of the sample labels in an effort to minimise observer bias.

Statistical analysis

Mann-Whitney U tests were performed using Statistics Kingdom online software (Statistics Kingdom) and Boxplots were generated using GraphPad Prism 3 (GraphPad Software Inc., San Diego, CA, USA).[17] Separate experiments were performed N number of times as listed in each figure and table.

Results

Analysis of the split spore trap samples (2x2 and 5x2) through comparison to control samples (CS and CE) collected simultaneously at each point is shown in Table 2. It should be noted that samples from experiment 3 were unusable due to a fault in the sampling medium cassette for sample CE (see appendix data table) which failed to capture any mould.

Our data indicated that split 2-minute samples (2x2) did not statistically differ from the control samples (CE and CS) (Table 2). Data from the experiment also showed that the split 5-minute samples (5x2) were statistically more likely to observe more different types of mould in samples ($p = 0.0478$) (Table 2, Figure 2A). The data also indicated that split 5-minute samples (5x2) missed fewer mould types comparatively than the other samples taken simultaneously (CE, CS and 2x2) ($p = 0.01564$) (Table 2, Figure 2B). Furthermore, the (5x2) samples showed a statistically higher total FS/m³ distance from the combined count ($p = 0.00323$) (Table 2, Figure 2C). Another notable finding was that Cladosporium varied largely between the 2-hour time gap used in the experiment. Cladosporium were more likely to be observed in the (5x2) split samples as compared to the other samples (CS, CE, 2x2). For mould types showing large differences to the combined counts, Cladosporium was by far the most common type. Additionally, (5x2) samples appeared to be more likely to overestimate mould genera as compared to the combined counts which include the data from all samples (CE, CS, 2x2 and 5x2). It should also be highlighted that the control samples (CE and CS) did not differ significantly in the metrics for the total types of mould in the samples or the number of mould types missed. Finally, 4 minutes of total sampling time from the split 2-minute sample (2x2) did not show statistically different results as compared to the control samples (CE and CS). The number of mould types which showed counts further than 500 FS/m³ from the combined counts were also tested and found to be statistically significant($p=0.0058$) (Table 2, Figure 2D).

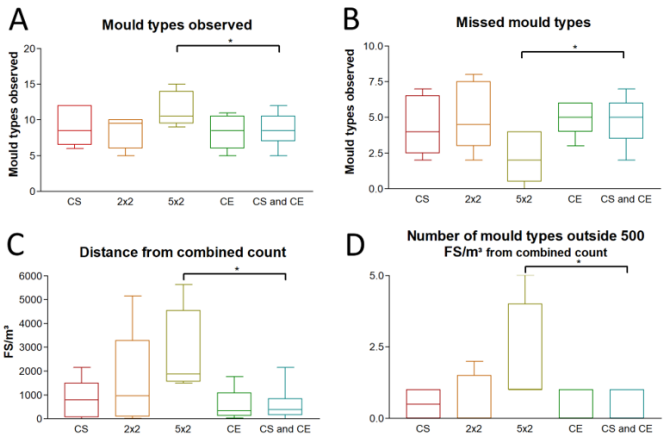

Figure 2 Box and whisker plots of tested metrics from Table 2.

A. Shows the number of categories of mould observed in sample groups. B. Shows the number of mould categories missed in groups as compared to the combined count of the replicates. C. Shows the distance in FS/m³ from the replicates combined count for the groups. D. Shows the number of mould categories per group in which the count was more than 500 FS/m³ from the replicates combined count. N=6.

Discussion

These findings demonstrate the high variability of mould levels with time. Additionally, use of a split sampling strategy allows inspectors to obtain more reliable data without additional cost. However, the trade-off is that inspectors would have to spend more time sampling on site, and the added potential of mislabelling or confusing samples. The data also demonstrates that 2-minute samples are more likely to miss mould genera as compared to a longer sampling duration.

Our experiment aimed to explore a different sampling strategy which could provide outdoor reference data which is more representative of the composition of outdoor air without additional cost by using sampling medium in an unconventional manner. One of the largest problems cited for gross fungal analysis is that the samples only capture a 'snapshot' of the mould composition at the time of testing. This strategy attempts to mitigate this issue by combining two such 'snapshots.' The effects of environmental conditions such as temperature, relative humidity and wind on fungal composition of the samples were not explored in this study however this data is listed in supplementary data (Table 4).

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There was a failure of the sampling cassette used for the (CE) sample of replicate 3. In order to assess if this could have affected the results, a sensitivity analysis was performed. For this analysis, the statistical tests were performed under two scenarios: one on all the data including the faulty replicate, and one with the faulty replicate excluded (Supplementary Data Table 5). The paired results from both scenarios yielded the same conclusions on statistical significance and whether the value is within the 95% confidence interval for all the metrics tested. This analysis provides evidence that the compromised replicate did not fundamentally alter the study's outcomes.

It should be highlighted that the control samples (CE and CS) did not differ significantly in the metrics for the total types of mould in the samples or the number of mould types missed. This finding is somewhat surprising as it is widely known that mould composition of outdoor air samples can vary widely with even as short a gap between samples as 2 hours.[18-20] However, we are not directly comparing the composition of the samples, but rather how many types of mould are observed and how many types of mould are missed in comparison to the combined total mould composition for each set of samples (24 minutes total sampling time).

Our data indicated that split 2-minute samples (2x2) did not statistically differ from the control samples (CE and CS) in the total mould types observed or the number of types missed. It is widely known that mould content in air samples can fluctuate rapidly.[18-20] It was theorised that separating the sample with a 2-hour time gap would collect a more representative sample as it would combine two separate small sampling duration samples and miss fewer genera of mould as compared to a single 5-minute sample. However, as there were no statistically significant differences in the metrics tested in the 2-minute split sample these data do not support this theory. Conversely, as 4 minutes of total sampling time from the split 2-minute sample (2x2) did not show statistically different results as compared to the control samples (CE and CS) this could indicate that the shorter total sampling duration of split sampling are comparable to a longer sampling taken at a single time point.

Data from the experiment also showed that the split 5-minute samples were statistically more likely to observe more different types of mould in samples (Figure 2A). Group (5x2) showed an average of 11.33 for the total number of mould types in the sample, which is approximately 30% more types than were observed than the combined average of the controls, and were outside of the 95% confidence interval (8.29-10.48) (Supplementary data Table 5). The data also indicated that split 5-minute samples (5x2) missed fewer mould types comparatively than the other samples taken simultaneously (CE, CS and 2x2) (Figure 2B). Group (5x2) showed an average of 2.17 for the number of mould types missed in the sample, which is approximately 50% less types than were observed than the combined average of the controls, and were outside of the 95% confidence interval (2.86-4.8) (Supplementary data Table 5). In contrast to the split 2-minute samples the split 5-minute samples appeared to provide a more complete picture of the mould content at the site. It should be noted that the longer sampling duration of 10 minutes may have played a role in the higher number of genera observed and fewer genera missed as compared to the combined counts. Future experiments should explore whether a single 10-minute sample or a split 5-minute sample provides more representative data.

These points taken together highlight one of two possibilities: either collecting for longer durations will reduce the amount of mould genera which are missed from short sampling durations; or taking samples separately with a time interval between sampling will reduce the amount of mould genera which are missed; or a combination thereof. It is worth noting that sampling for longer durations is not always practical or achievable. Longer sampling durations can lead to overloaded samples which cannot be read[16,21-23] and consideration should be given to what is an appropriate sampling duration for any particular instance. Longer sampling durations also compound that amount of time to sample a site across multiple samples.

Comparing the levels of mould detected in the tested groups showed that the (5x2) group had higher total mould levels and had more types of mould which differed in levels as compared to the combined count (Table 2, Figure 2 C and D). The total FS/m³ distance from the combined count of 2657 was approximately four times higher in the (5x2) sample as compared to the combined average of the controls, and outside of the 95% confidence interval (610-2024) (Table 2, Figure 2C, supplementary data Table 5). The number of mould types which were further than 500FS/m³ from the combined counts was found to be statistically significant for the (5x2) sample (p=0.0058) (Figure 2D). The (5x2) sample showed higher levels than the other groups with a value of 2 as compared to the combined average of the controls of 0.42, which is nearly five times higher, and outside of the 95% CI (0.32-1.57). A possible explanation for this finding is that conducting two separate sampling events increases the likelihood of observing a cluster or chain of mould in the sample. It is well known that mould often forms clusters or chains which are detected during sampling.[1,24,25] Cladosporium and Aspergillus/Penicillium like spores were detected in multiple replicates for the (5x2) group both of which are often observed in chains.[1,24] This finding requires further investigation to increase the sample size and increase the robusticity of the data.

Another notable finding was that Cladosporium varied largely between the 2-hour time gap used in the experiment. Cladosporium were more likely to be observed in the (5x2) split samples as compared to the other samples (CS, CE, 2x2). Changes in the composition of air samples over time have been noted in the literature.[26-28] Cladosporium levels were noted to be affected by changes in relative humidity in short time periods of several hours.[28] Additionally, (5x2) samples appeared to be more likely to overestimate mould genera as compared to the combined counts which include the data from all samples (CE, CS, 2x2 and 5x2). A possible explanation for this phenomenon could be that a split sample is far less likely to miss mould genera only present at one of the two time points tested.

The use of a split 5-minute sample has been demonstrated here to provide more representative data in several metrics such as being less likely to miss mould types than a single 5-minute outdoor sample. This type of sample could be used instead of taking two 5-minute samples at the start and end of inspections as outdoor references which could save on analysis fees. A cost-effective middle ground is achievable through this strategy between taking a single sample which only represents a short snapshot of the mould content and multiple samples taken as a more representative data set. The reduced chance of missing mould genera seen in this preliminary experiment in outdoor samples could potentially be applied to indoor samples, and should be explored in future experiments. Use of this technique does require more on-site time both in the collection of samples and the delay between collection of samples. Extra caution with handling of samples is also needed to avoid issues of mixing up samples or mislabelling samples.

Owing to cost and time limitations as a result of this study being conducted in full by a small company, large sample sizes were unfeasible. Use of a small sample size does limit the robusticity of the

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data set, with the principal issue being a limited ability to resolve false negatives. Data from this small sample size is also prone to outlier vulnerability in which single atypical events can alter the entire dataset from a single outlier. As this preliminary experiment only used one outdoor sampling location the results cannot be extrapolated for use with indoor samples, and a separate experiment should be conducted. Additionally, a single outdoor sampling location may be affected by conditions in the local environment, and as such may not provide an accurate representation of wider outdoor sampling. This study also does not address environmental covariate analysis as including even one or two variables (e.g. temperature and relative humidity) would extend the scope of the study considerably in order to correlate mould content of samples with conditions during testing. Instead, the article attempts to provide proof of concept of split-sampling by comparing a few key metrics of the sampling used.

Conclusion

Our preliminary findings suggest that using split 5-minute samples is a useful strategy to provide more representative outdoor data without adding additional cost. Use of this method reduces the chance of missing fungal genera collected in outdoor reference samples and provides inspectors an avenue to increase the reliability of their sampling in a cost-effective manner.

Future directions

The preliminary experiment did not compare continuous 10-minute samples with split 5-minute samples. An important future experiment should be conducted comparing 10-minute samples from a single sampling as compared to split 5-minute samples taken with a 2-hour time gap in order to determine how this sampling strategy affects results in a more comparable manner, and should also employ multiple sampling locations. Use of split samples taken indoors should also be explored with consideration of use scenarios for the spaces (e.g. undisturbed, typical use, during works, etc).

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Conflicts of interest

The authors declare that there are no conflicts of interest.

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