A COMPARATIVE STUDY OF COLLECTION EFFICIENCY OF AIRBORNE FUNGAL MATTER USING ANDERSEN SINGLE-STAGE N6 IMPACTOR AND THE AIR-O-CELL CASSETTES

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ABSTRACT

In the United States, the Andersen single stage N6 impactor and Air-O-Cell cassettes are used for sampling and enumerating airborne fungal particles. The Andersen N6 sampler collects respirable fungal spores on an agar plate. The Air-O-Cell cassettes collect fungal matter including hyphal fragments, conidiophores, and spores, whether viable or not. The collection efficiency of airborne fungal matter using the Andersen sampler and Zefon Air-O-Cell cassettes is compared in this study. A total of 1,431 sets of samples collected between June 1997 and September 1998 are included. The correlation coefficient (r) between these two sampling methods was at 0.32 for total fungal matter and at 0.31 for *Cladosporium*. The correlation coefficients (*r*) between the total fungal and *Cladosporium* concentrations collected with Andersen samplers and those collected with Air-O-Cell cassettes were statistically significant at 0.77 and 0.59, respectively. Results of this study suggest that the data of airborne fungal populations derived from the Andersen samplers and the Air-O-Cell cassettes were not well correlated and could not be compared directly. Fungal levels, either total or *Cladosporium* alone, derived from the Air-O-Cell cassettes were consistently higher than those of the Andersen sampler. The Air-O-Cell samples also had a higher detection rate of *Stachybotrys* than the Andersen samples.

INTRODUCTION

The Andersen single-stage N6 impactor and Zefon Air-O-Cell cassettes have been widely used in the United States for evaluating airborne fungal levels in the indoor environment. The Andersen sampler is used for recovering respirable, culturable airborne fungal spores on suitable fungal media. The Air-O-Cell cassette is a combination of Burkard spore trap and filter cassette. The Air-O-Cell cassette collects all fungal and other airborne matter. The advantage of using the Andersen sampler is the capability for proper fungal identification. The advantage of using the Air-O-Cell cassettes is the recovery of total airborne fungal matter, including hyphae, conidiophores, and spores, whether viable, dormant, or non-viable.

This study compares the collection and recovery efficiency between the Andersen sampler and Air-O-Cell cassettes. *Cladosporium* and *Stachybotrys* are used as indicator organisms for comparisons. *Stachybotrys* spores are atypical in dry, clean buildings, while *Cladosporium* is one of the most common airborne fungi indoors and outdoors [1, 2].

METHODS

Field samples collected side-by-side using an Andersen single-stage N6 sampler on 2% malt extract agar (2% MEA) and Air-O-Cell cassettes were analyzed and compared. The Andersen sampler was calibrated at 28.3 liters per minute (lpm). Each Andersen sample was collected for either one minute for 28.3 liters (L), two minutes for a total of 56.6 L, or three minutes for a total of 84.9 L. Air-O-Cell cassettes were collected based on the same flow rate for 2-4 minutes. A total of 1431 sets of samples were collected, from buildings and outdoors, between June 1997 and September 1998.

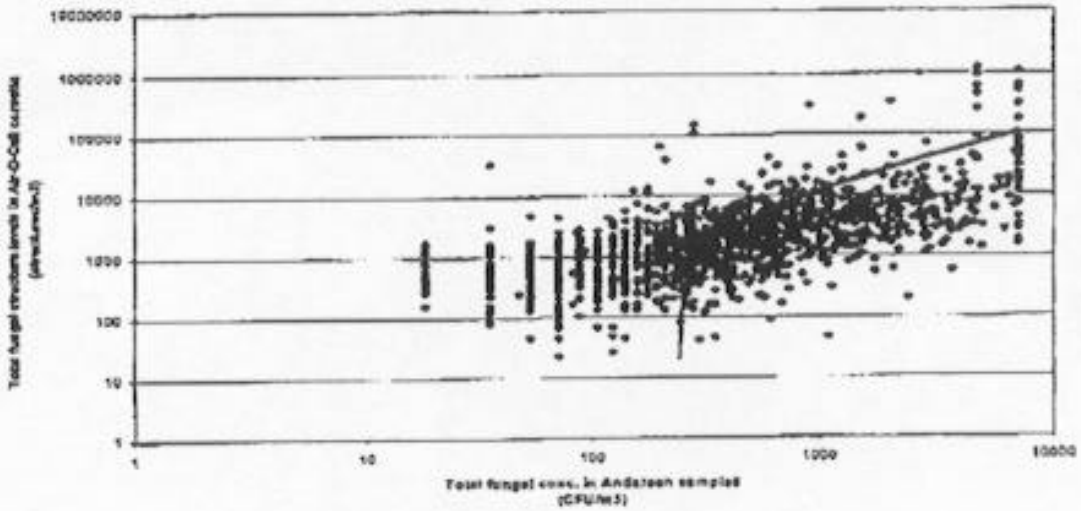
Andersen samples were incubated at 25 °C for seven to ten days. All fungal colonies were enumerated and identified, and total concentrations in colony-forming-units per cubic meter of air (CFU/m^3) were calculated. Air-O-Cell cassettes were mounted with biological dyes (cotton blue or acid fuchsin) and analyzed using the modified NIOSH 7400 fiber counting method. One quarter of the collection band on the slide was analyzed using an Olympus BH-2 compound microscope at 400X magnification. This magnification is capable of detecting the smallest fungal spores (approximately 2 µm). Individual spores, hyphal fragments and conidiophores were counted. Results were calculated and presented as fungal structures per cubic meter of air. Spores were presumptively identified when possible.

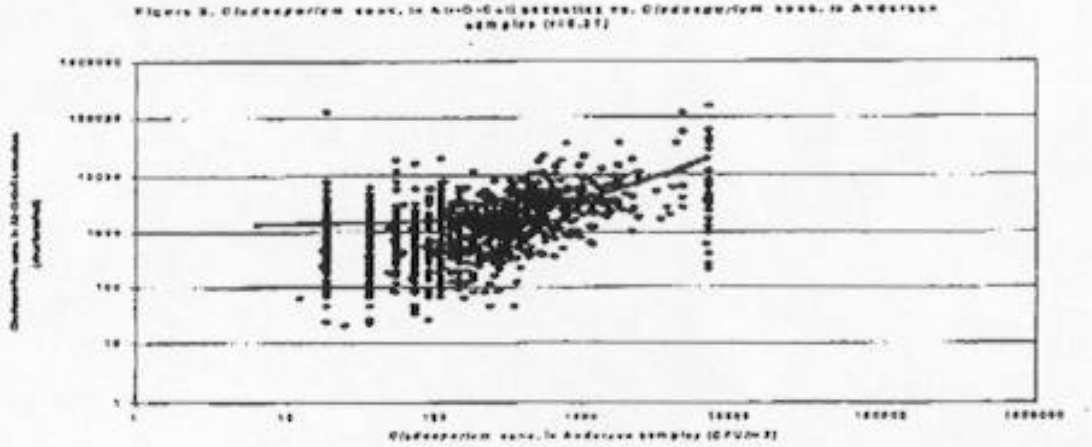
The data collected were subject to statistical correlation analysis.

RESULTS

The Andersen samples generally yielded lower fungal concentrations than Air-O-Cell cassettes. The correlation coefficient (r) between these two methods was at 0.32 for total fungal matter (Figure 1) and at 0.31 for *Cladosporium* (Figure 2).

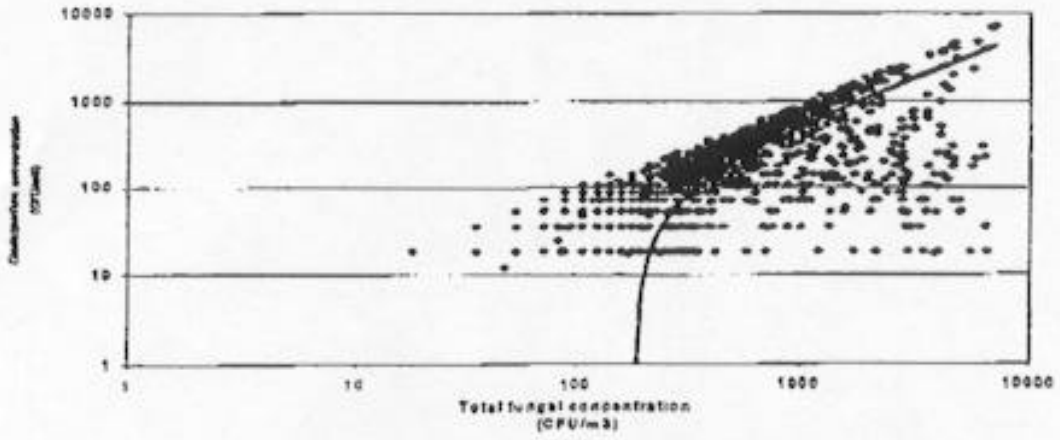
Figure 1. Total fungal structure levels in Air-O-Cell cassettes vs. total fungal concentrations in Andersen samples (r=0.32)



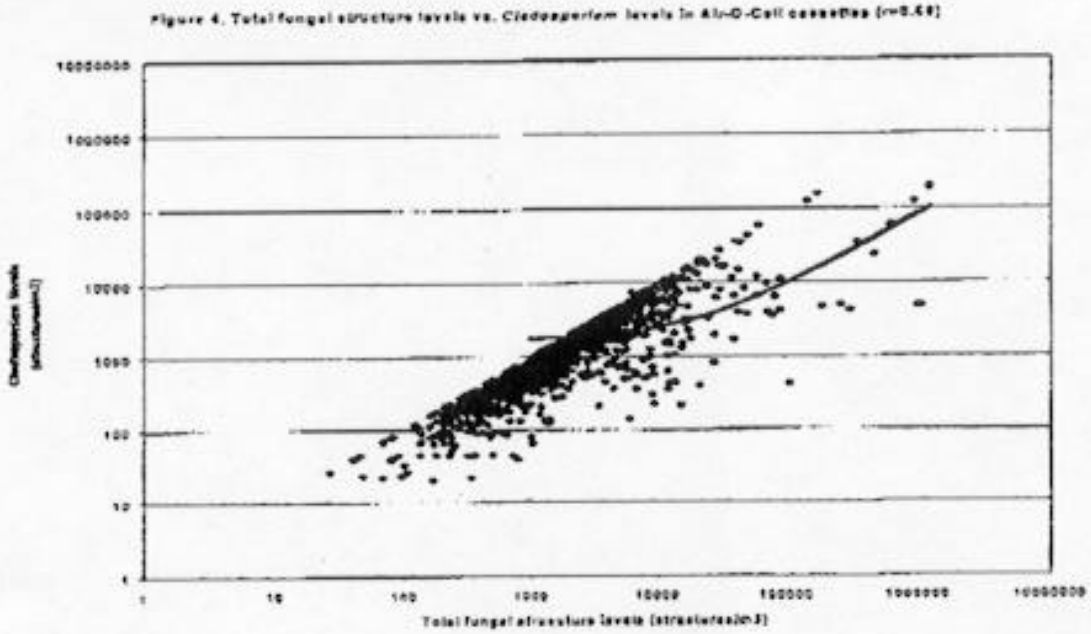


The correlation coefficient (r) between the total fungal and *Cladosporium* concentrations derived from Andersen air samples and those collected with Air-O-Cell cassettes were statistically significant at 0.77 and 0.59, respectively (Figures 3 and 4). *Cladosporium* was not detected in eight sets of Andersen and Air-O-Cell cassette samples. 1319 of 1431 samples (92.17 %) of the Air-O-Cell cassettes yielded higher fungal concentrations than the Andersen air samples. 1337 of 1423 Air-O-Cell samples (93.96 %) had a higher *Cladosporium* recovery rate than Andersen samples.

Figure 3. Total fungal concentration vs *Cladosporium* conc. in Andersen samples (r=0.77)



Stachybotrys-like spores were detected in 105 Air-O-Cell samples, while *Stachybotrys chartarum* was identified in 13 Andersen samples. Seven sample sets detected both *Stachybotrys*-like spores and *Stachybotrys chartarum* on both Air-O-Cell and Andersen samples.



DISCUSSION

Based on the correlation coefficient calculated from Andersen samples and Air-O-Cell cassettes using total fungal spores and *Cladosporium* for comparisons, the data did not show a good correlation. Results of this study suggest that it is not practical to use the results from one of these two samplers to estimate or predict the yield of another sampler.

The Air-O-Cell cassettes provided a better assessment of airborne fungal matter and yielded higher fungal types and concentrations than the Andersen samples. In this study, fungal concentrations, either total fungal spores or *Cladosporium* alone, derived from Air-O-Cell cassettes were consistently higher than those of Andersen samples. There are several reasons. In Air-O-Cell cassettes, fungal structures (hyphal fragments and conidiophores) and individual spores, whether viable, culturable or not, were counted, while only culturable fungal spores germinated and grew into colonies on agar plates. Spores of different genera in culture may also be grouped and identified together. Spore counts of *Cladosporium* may include spores of *Cladosporium* spp. and other similar species, such as *Hormoconis resinae* and *Fulvia fulva*.

Medium selection is another factor influencing the total fungal concentrations recovered from Andersen samples. Xerophilic fungi were likely to be under detected on the 2% MEA medium. Fast growing fungi, such as *Aspergillus* and *Penicillium*, are known to interfere with growth of *Stachybotrys chartarum* [3, 4]. In addition, each spore cluster deposited on an Andersen sample may develop and grow into one single colony. The Andersen sampler has also an upper quantitation limit of 400 colonies for each plate. These factors significantly affect the results derived from these two samplers.

The Andersen sampler has a 50% cut-size (the size at which 50% of the particles collected and 50% of the particles penetrate the samples) of 0.65 μm [5]. The particle shape and flow rate greatly affect the collection efficiency of the Air-O-Cell cassette [6]. At the flow rate of 30 lpm, the cut-size value of the Air-O-Cell cassette was 1.8 ± 4% μm. The difference is insignificant because few fungal spores are smaller than 2 μm. The study [6] also showed that the spiny fungal spores bounced more than the spherical smooth particles.

Cladosporium was commonly detected in both Andersen samples and Air-O-Cell cassettes. It is used as one of the indicator fungi for comparison. A good correlation between *Cladosporium* concentrations and total fungal levels was detected from both methods. In some samples, species of *Aspergillus* and *Penicillium* or *Aspergillus/Penicillium*-like spores were the dominants that were indicated as the outliers in Figure 3 and 4. A further analysis of these data points showed that the dominant types, either *Cladosporium* or *Aspergillus/Penicillium* group, selected from each sample had a good correlation with the total fungal level detected in each sample ($r = 0.95$).

Predictably, Air-O-Cell samples yielded higher fungal levels than Andersen samples on 2% MEA. Air-O-Cell samples also had a higher detection rate of *Stachybotrys*-like spores. However, identification of fungal spores in Air-O-Cell samples is presumptive at best. Fungal colonies growing on 2% MEA facilitate proper identification of fungi to genus or species. The higher frequency of detecting *Stachybotrys*-like spores from Air-O-Cell cassettes than from Andersen samplers was expected because *Stachybotrys* spores may not grow into colonies depending on their viability, the culture media and the presence of other interfering fungi [3, 4]. Gravesen et al. [3] indicated that only a small percentage of the conidia of *Stachybotrys chartarum* are viable in cultures.

Results from total spore counts are a better assessment of airborne fungal matter because a significant portion of airborne fungal structures was either non-culturable on the medium used or non-viable. Results of this study suggest that both a culturable method, such as collection with an Andersen sampler, and total spore counts, using the Air-O-Cell cassette or other spore trap samplers, are complementary in the assessment of airborne fungal populations. Air-O-Cell samples provides a better chance of detecting *Stachybotrys* than Andersen samples.

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