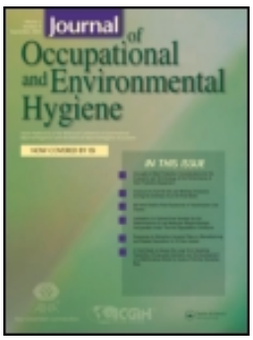

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Analysis of Portable Impactor Performance for Enumeration of Viable Bioaerosols

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Portable impactors are increasingly being used to estimate concentration of bioaerosols in residential and occupational environments; however, little data are available about their performance. This study investigated the overall performances of the SMA MicroPortable, BioCulture, Microflow, Microbiological Air Sampler (MAS-100), Millipore Air Tester, SAS Super 180, and RCS High Flow portable microbial samplers when collecting bacteria and fungi both indoors and outdoors. The performance of these samplers was compared with that of the BioStage impactor. The Button Aerosol Sampler equipped with gelatin filter was also included in the study. Results showed that the sampling environment can have a statistically significant effect on sampler performance, most likely due to the differences in airborne microorganism composition and/or their size distribution. Data analysis using analysis of variance showed that the relative performance of all samplers (except the RCS High Flow and MAS-100) was statistically different (lower) compared with the BioStage. The MAS-100 also had statistically higher performance compared with other portable samplers except the RCS High Flow. The Millipore Air Tester and the SMA had the lowest performances. The relative performance of the impactors was described using a multiple linear regression model ($R^2 = 0.83$); the effects of the samplers' cutoff sizes and jet-to-plate distances as predictor variables were statistically significant. The data presented in this study will help field professionals in selecting bioaerosol samplers. The developed empirical formula describing the overall performance of bioaerosol impactors can assist in sampler design.

Keywords bacteria, bioaerosol sampling, fungi, microbial samplers, portable impactors

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INTRODUCTION

Airborne biological particles commonly found indoors and outdoors may include bacteria, fungi, viruses, allergens, bacterial endotoxins, peptidoglycans, pollen, and other constituents.[1] Most of them range from 0.3 μm to 100 μm in diameter,[2] and particles smaller than 5 μm can remain in the airborne state for a longer time.[3]

Exposure to bioaerosols causes many health problems, including infectious diseases, acute toxic effects, allergies, and other impairments and has received considerable attention in recent years. Human tuberculosis caused by *Mycobacterium tuberculosis* is cited as the one of the most prevalent and deadly airborne bacterial infectious diseases worldwide.[4–6] In 1976, an outbreak of severe respiratory disease caused by Legionnaires disease bacterium occurred in Philadelphia, Pa., and it is estimated that 180 people became sick, and 29 of them died.[7,8]

Fungal species are often identified as the cause for allergic diseases,[9] headaches, eye irritation, epistaxis, nasal and sinus congestion, and cough.[10] Fungal species and their derivatives, such as mycotoxins, have also been identified as one of the causes for sick building syndrome (SBS).[10–13] *Aspergillus fumigatus* is one of the most ubiquitous airborne saprophytic fungi, capable of causing severe and sometimes fatal invasive aspergillosis.[14]

Industrial activities such as waste recycling, enzyme production, and waste composting often produce high level of bioaerosols, some of which are particularly harmful to human health.[1] High prevalence of respiratory symptoms and airway inflammation in these industries have been highlighted in literature.[15–19] In addition to occupational and environmental exposures, there is also a threat that microbial agents such as *Francisella tularensis*, *Yersinia pestis*, and *Bacillus anthracis* could be used during a bioterrorism attack.

Presence of bioaerosols in indoor and outdoor environments can be detected using a variety of available bioaerosol samplers. Portable (battery-powered) microbial samplers are becoming more popular for monitoring viable bioaerosols because of their ease of use, portability, and high sampling flow rates without the need for postcollection processing. Use of battery power allows the investigator to be mobile during sampling. A number of studies[20–23] have used the portable samplers for monitoring airborne microorganisms both indoors and outdoors. However, the performance characteristics of

newly introduced or modified portable samplers when sampling viable bioaerosols have not been widely investigated. Yao and Maineli[24–26] have investigated physical and biological performances as well as adherence to inhalation conventions of seven portable microbial samplers when collecting laboratory-aerosolized, nonbiological and biological particles. These laboratory studies showed that samplers' cutoff sizes and their ability to enumerate airborne microorganisms could vary substantially depending on sampler model.

As part of our continued effort to develop sensible bioaerosol sampling protocols, this study is focused on the performance of seven portable microbial impactors when sampling culturable bacteria and fungi in indoor and outdoor environments. The performance of portable samplers in both environments was compared with that of a single-stage, Andersen-type impactor and gelatin filter loaded into a button aerosol sampler. The information about the performance of portable samplers can be used by the field professionals and bioaerosol researchers when selecting a bioaerosol sampler for their needs.

MATERIALS AND METHODS

Microbial Samplers

The study investigated seven battery powered impactors for culturable aerosols: MAS-100 (EMD Chemicals, Inc., Gibbstown, N.J.) operating at 100 L/min with 50 mL agar; Microflow (Aquaria srl, Lacchiarella, Italy) operating at 120 L/min with 25 mL agar; BioCulture (A.P. Buck Inc., Orlando, Fla.) operating at 120 L/min with 30 mL agar; SMA MicroPortable (Veltek Associates, Inc., Phoenixville, Pa.) operating at 141.5 L/min with 25 mL agar; SAS Super 180 (Bioscience International, Inc., Rockville, Md.) operating at 180 L/min with 40 mL agar; Millipore Air Tester (Millipore Corp., Billerica, Mass.) operating at 140 L/min with manufacturer prefilled agar cassette (20 mL); and RCS High Flow (Biotest Diagnostics Corp., Denville, N.J.) operating at 100 L/min with manufacturer-supplied agar strip. Their complete physical collection characteristics including operating parameters were described in an earlier study.[23] The amount of agar used for each impactor was either that recommended by the manufacturer or that determined to yield lower cutoff sizes in an earlier study.[23] All investigated portable microbial impactors are designed to collect airborne culturable microorganisms directly onto agar collection medium without postcollection sample processing.

The Andersen viable bioaerosol impactor (Graseby Andersen, Atlanta, Ga.) has been regarded as a sampler of choice for enumerating the viable airborne microorganisms[27] and has been recommended and used as a reference sampler in several bioaerosol studies.[28,29] In this study, the BioStage Impactor (SKC Inc., Eighty Four, Pa.), a single-stage Andersen N6-equivalent microbial sampler, was used as the reference sampler. The BioStage is a replicate of the last stage of the Andersen six-stage impactor, with a cutoff size of 0.65 μm[30] and used with 50 mL agar. In addition, a Button Aerosol Sampler (SKC Inc.) coupled with a gelatin filter was also included in the study to test the performance of gelatin filter when sampling culturable microorganisms in field environments. The Button Aerosol Sampler adheres to the American Conference of Governmental Industrial Hygienists (ACGIH®) inhalable particle convention,[31] whereas the gelatin filter provided high collection efficiency and nutrient rich environment for the microorganisms.[32]

Experimental Methods

Agar plates were prepared using trypticase soy agar (Becton, Dickson and Company, Sparks, Md.) for bacteria and Sabouraud dextrose Agar (Becton, Dickson) for fungi. Manufacturer-prepared agar strips and plates with the same types of agar as above were used for the RCS High Flow and Millipore Air Tester, respectively.

Air samples were simultaneously collected by all nine samplers in an outdoor environment near an office building and in a residential apartment. During the experiments, all samplers were placed about 0.3 m from each other and at a height of 1 m above the ground or floor. The sampling in each environment was repeated twice about 5 days apart. During each sampling day and for each environment, sampling of bacteria and fungi was repeated five times. The monitored microorganisms included all bacteria and fungi capable of growing on the respective collection media.

The tested portable impactors have sampling flow rates of 100 L/min or higher. The BioStage impactor has a standard sampling flow rate of 28.3 L/min. The recommended sampling flow rate for the Button Aerosol Sampler is 4 L/min; however, at this sampling flow rate, a sampling time of 30–60 min is needed to achieve a sample volume comparable to that of portable samplers. A long sampling time is likely to affect the culturability of the collected microorganisms because of the desiccation effect. Therefore, to decrease the needed sampling time, the sampling flow rate of the Button Aerosol Sampler with gelatin filter was increased to 10 L/min.

Hauck et al.[33] have shown that 10 L/min sampling flow rate for the Button Aerosol Sampler resulted in a uniform particle distribution on the filter. For seven portable microbial samplers and the BioStage impactor, the sampling time was limited to 5 min; the Button Aerosol Sampler with gelatin filter was operated for 15 min. The humidity level and temperature were measured by a Traceable Hydrometer (Fisher Scientific, Pittsburgh, Pa.). Additionally, in all environments, the particle concentration levels in different size ranges were simultaneously monitored for the entire sampling periods by an optical particle counter (OPC) (model 1.108; Grimm Technologies, Douglasville, Ga.).

After sampling, all impactor agar plates were directly incubated at room temperature for at least 48 hours for bacteria and at least 72 hours for fungi. The gelatin filter was removed

from the Button Aerosol Sampler and dissolved in 2 mL autoclaved Milli-Q water, and aliquots of 200 μL suspension were plated onto the agar plates in triplicate.

The culturable bacterial and fungal concentrations were determined using the following equation:

$$C_{CFU} = \frac{N_{CFU}}{Q \times t} \quad \textbf{(1)}$$

where C_{CFU} is the culturable microorganism concentration measured by a test sampler, gelatin filter, or the reference sampler BioStage impactor; N_{CFU} is the number of colony-forming units recovered by a sampler and corrected for coincidence;[34] Q is the sampling flow rate of the sampler; and t is the sampling time used for each sampler. For the gelatin filter, a factor of 10 was applied to account for the dilution of the sample during plating.

The portable sampler's ability to enumerate airborne microorganisms was compared with that of the BioStage impactor during the same repeat in the same environment. This performance comparison was quantified by the relative overall efficiency, R_{EFF}, which was calculated using the following equation:

$$R_{EFF} = C_{Test}/C_{BioStage} \quad \textbf{(2)}$$

where C_{Test} is the culturable microorganism concentration measured by the test samplers, including the Button Aerosol Sampler with gelatin filter, and $C_{BioStage}$ is the concentration measured by the BioStage impactor. Five R_{EFF} values were obtained for each sampler in each test environment when sampling bacteria and fungi. The results are presented as averages and standard deviations of those five repeats.

STATISTICAL ANALYSIS

Two-way analysis of variance (ANOVA) tests were performed to analyze the relative overall efficiency, R_{EFF}, as a function of sampler model for different sampling environments, bioaerosol species (bacteria and fungi), and sampling days. The Bonferroni multiple comparison test was used to compare samplers' performance relative to the BioStage impactor (reference) as well as to perform pairwise comparisons between the samplers. This test was chosen because it adjusts the observed significance level for multiple comparisons. In addition, single and multiple linear regression were applied to analyze the pooled R_{EFF} data as a function of two predictors (independent variables): sampler cutoff size and jet-to-plate distance. P-values less than 0.05 indicated statistical significance in all tests.

RESULTS AND DISCUSSION

Particle Size Distribution

The particle number and size distributions in the sampling environments were monitored using an OPC, which measured both biological and nonbiological particle fractions. The particle concentrations are normalized over the channel width of the OPC as shown in Figure 1. Particles of less than 0.5 μm had the highest number concentrations both for indoor and outdoor environments, typically ranging from 10^3 to about 10^5 particles per liter of air. For particles larger than 0.5 μm, their concentrations were relatively lower, ranging from 10^2 to 10^4 particles per liter. Higher particle concentrations and higher variability were observed for all size ranges in indoor environments compared with the outdoor environments. This particle concentration difference between indoor and outdoor environments could be attributed to the indoor activities that resuspend a substantial number of particles.[35] A small peak at approximately 2 μm observed in both environments is indicative of the presence of larger particles. In general, both indoor and outdoor environments had similar particles size distribution profiles.

Sampling of Bacteria

Figure 2 shows the relative overall efficiency of the microbial samplers when sampling indoor bacteria during 2

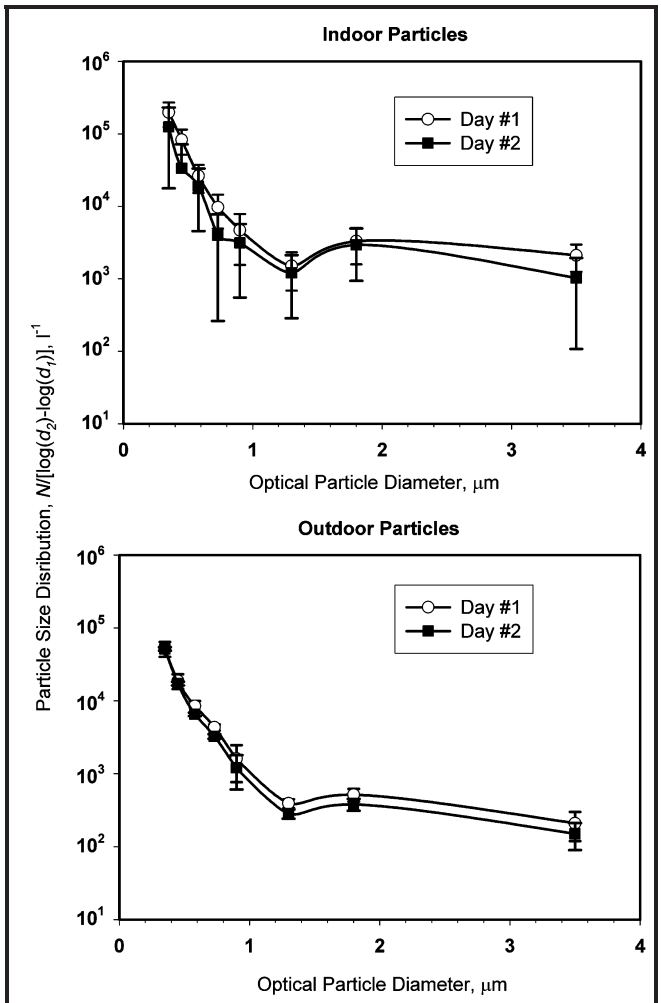

FIGURE 1. Particle size distributions in indoor and outdoor environments during the entire sampling periods.

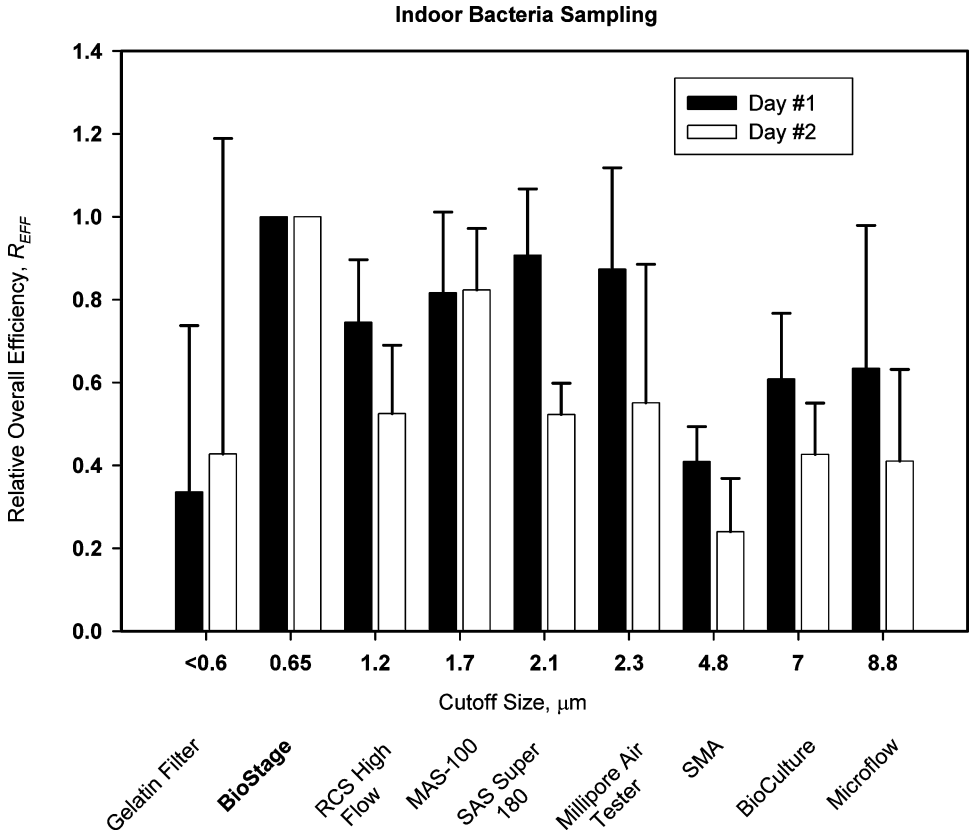

FIGURE 2. Overall performance of microbial samplers relative to BioStage impactor when sampling indoor bacteria; data represent averages of five repeats and error bars stand for one standard deviation.

separate days. The y-axis represents the R_{EFF} value for each sampler, and x-axis indicates the tested samplers and their respective cutoff sizes. Cutoff size indicates aerodynamic size of the particles at which 50% collection efficiency is achieved. These values were determined in earlier research.[24] The gelatin filter does not have a cutoff size *per se*, but a physical collection efficiency of about 100% when sampling *Pseudomonas fluorescens* (aerodynamic size 0.6 μm) was observed.[25] Therefore, its cutoff size as <0.6 μm is indicated.

Experimental data shown in Figure 2 indicate that for both sampling days the mean values of the relative overall efficiency, R_{EFF}, for test samplers including gelatin filter were lower than unity, i.e., they recovered lower concentrations of culturable bacteria compared with the BioStage impactor. The airborne culturable microorganism concentrations measured by the BioStage impactor were 185 CFU/m^3 and 84 CFU/m^3 for Days 1 and 2, respectively.

Among all the test samplers, the RCS High Flow, MAS-100, SAS Super 180, and Millipore Air Tester had the higher mean values compared with other samplers. The mean R_{EFF} of the gelatin filter and SMA sampler were the lowest among the test samplers during both days of sampling. Because our primary goal was to analyze performances of the portable impactors and compare them with the BioStage, the results of the gelatin filter were excluded from most of the statistical analyses involving portable impactors. Additionally, high variability of the gelatin filter's R_{EFF} values due to its high detection limit was observed in most of the experiments, and it would have affected the results of statistical analysis. The gelatin filter's performance was separately compared with the BioStage.

Detailed results from statistical analysis of portable sampler performance, including multiple pairwise comparisons using Bonferroni statistics with and without the BioStage impactor as a reference, are presented in Table I. Only the main findings are described here and later in text. As seen in the table, the effects of sampler model and sampling day were statistically significant ($p < 0.0001$). When data from both days were pooled, only the MAS-100 was not statistically different from the BioStage, while the R_{EFF} values of other impactors were below unity. The R_{EFF} value of the gelatin filter was statistically below unity for both days ($p = 0.004$).

The statistically significant effect of sampling day indicates that indoor bacterial composition and/or their size distribution might have changed from Day 1 to Day 2. In general, a similar trend of the relative overall efficiencies of portable samplers relative to the BioStage impactor was observed during Days 1 and 2.

Figure 3 shows the relative overall efficiencies of microbial samplers when sampling outdoor bacteria during 2 separate days. Among the portable impactors, the RCS High Flow and

TABLE I. Performance of Portable Microbial Impactors

	Two-Way ANOVA for Sampling Day and Sampler	Statistical Significance of Multiple Pairwise Bonferroni Comparisons Relative to the BioStage Impactor[A] for Day 1 and Day 2 Separately		Statistical Significance of Multiple Pairwise Bonferroni Comparisons Relative to the BioStage Impactor[A] when Data from Day 1 and Day 2 are Pooled	Statistical Significance of Multiple Pairwise Bonferroni Comparisons of the Pooled Portable Impactor Data (BioStage not included)
Bacteria					
Indoors					
Sampling day	$p < 0.0001$	Day 1	SMA, BioCulture, and Microflow different (lower)	All samplers different (lower), except MAS-100	SMA different (lower) compared with the SAS Super 180, the Millipore Air Tester, and the MAS-100
Sampler	$p < 0.0001$	Day 2	All samplers different (lower), except MAS-100		
Outdoors					
Sampling day	$p = 0.73$	Day 1	None different	Millipore Air Tester (lower)	MAS-100 different (higher) compared with the Millipore Air Tester
Sampler	$p = 0.006$	Day 2	None different		
Fungi					
Indoors					
Sampling day	$p = 0.65$	Day 1	Millipore Air Tester and SMA different (lower)	All samplers different (lower), except MAS-100 and Microflow	The SMA different (lower) compared with the MAS-100; Microflow different (higher) compared with the Millipore Air Tester and the SMA
Sampler	$p = 0.006$	Day 2	Millipore Air Tester and the SMA different (lower)		
Outdoors					
Sampling day	$p = 0.024$	Day 1	None different	Millipore and SMA (lower)	RCS High Flow different (higher) compared with the Millipore Air tester and the SMA;
Sampler	$p = 0.0001$	Day 2	Millipore Air Tester, SMA, and Microflow different (lower)		MAS-100 different (higher) compared with the SMA

Note: Only statistically significant relationships are presented ($p < 0.05$ indicated statistical significance).
[A]BioStage impactor used as reference sampler.

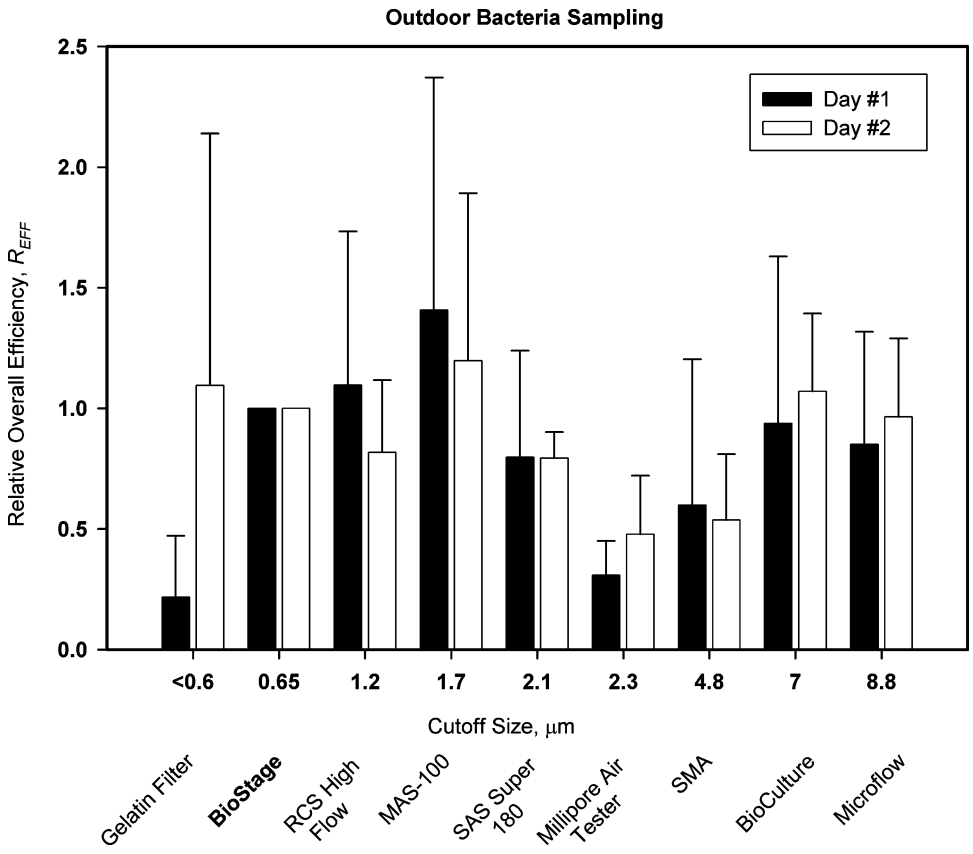

FIGURE 3. Overall performance of microbial samplers relative to BioStage impactor when sampling outdoor bacteria; data represent averages of five repeats and error bars stand for one standard deviation.

the MAS-100 had the highest mean R_{EFF} values during Day 1, while MAS-100 and BioCulture had the highest mean values during Day 2. In fact, the mean R_{EFF} values of the MAS-100 were above unity for both days, i.e., it enumerated higher average microorganism concentration compared with the BioStage. Outdoor bacteria concentrations as measured by the BioStage impactor were 155 CFU/m^{3}and 114 CFU/m^3 for Days 1 and 2, respectively. For both days, the Millipore Air Tester and the SMA sampler had the lowest mean R_{EFF} values among the portable impactors. During Day 2, the gelatin filter also had the R_{EFF} value above unity. Visually, a similar trend of the relative overall efficiency of portable samplers was observed for Days 1 and 2.

Results from statistical analysis presented in Table I indicate that there was a statistically significant sampler effect ($p = 0.006$) but not sampling day effect ($p = 0.73$). When analyzed separately for each day, there was a statistically significant difference in the R_{EFF} values among samplers for Day 2 ($p = 0.033$), but not for Day 1 ($p = 0.19$). Analysis of portable sampler data from both days (pooled) indicate that only the Millipore Air Tester was statistically different (lower) compared with BioStage. Multiple pairwise comparisons of the portable sampler data (BioStage impactor not included) showed that MAS-100 was different (higher) compared with the Millipore Air Tester. The R_{EFF} value of gelatin filter was different from unity during Day 1 ($p = 0.0012$) but not during Day 2 ($p = 0.58$).

In general, the relative overall efficiency of the Millipore Air Tester decreased when sampling bacteria outdoors compared with indoor sampling, while the R_{EFF} values for the rest of the portable samplers increased for outdoor sampling compared with indoor sampling.

Different from indoor sampling, when sampling outdoors there was no statistically significant sampling day effect. This result indicates that the outdoor bacterial composition and/or size distribution remained relatively constant during 2 days of sampling.

BioCulture and Microflow have cutoff sizes of 7.0 μm and 8.8 μm, respectively, which are higher compared with other samplers.[24] These samplers also have relatively lower overall performances compared with other portable samplers when sampling laboratory-aerosolized bacteria.[25] In contrast, in the outdoor environment, the R_{EFF} values of these two samplers were not different from samplers with lower cut-off sizes. It is likely that in a field environment, bacterial species are attached to larger particles or are present as agglomerates, for which BioCulture and Microflow have sufficiently high physical collection efficiencies. In addition, their

collection efficiencies begin to increase for particles larger than 3 μm.[24]

Shaffer and Lighthart[36] have shown that most of the bacteria present in natural environments are 3 μm or larger. This large size of bacterial species substantially increases the efficiency of their collection by most of the samplers tested. For bacteria sampling, the Millipore Air Tester and the SMA had lower mean R_{EFF} values than could be predicted by their cutoff sizes. This phenomenon is discussed further in the next section.

Gelatin filter was observed to enumerate some culturable outdoor and indoor bacteria, indicating that certain hardy bacterial species resistant to desiccation stress were present in the environments. High variability of the filter efficiency was observed throughout this study, which was likely due to low concentration of desiccation-resistant microorganisms in the environments. In addition, low sampling flow rate and the need to dissolve the filter in 2 mL of liquid prior to cultivation have increased the detection limit and uncertainty of the results.

The average concentration of culturable bacteria measured by the BioStage in both environments and during both dates ranged from 84 CFU/m^{3}to 155 CFU/m^3. These concentrations constitute a very small fraction of all airborne particles of 0.5 μm and larger as shown in Figure 1.

Sampling of Fungi

Figure 4 shows the relative overall efficiencies of the microbial samplers when sampling fungal species indoors during 2 days. During both days, all the portable samplers had average overall efficiencies below unity, i.e., they measured lower fungal concentrations compared with the BioStage. The average fungi concentration was relatively low and ranged from 68 CFU/m^3 for Day 1 to 41 CFU/m^3 for Day 2 as measured by the BioStage. Among the portable samplers, the RCS High Flow and MAS-100 had the highest mean R_{EFF} values during Day 1, while the MAS-100 and the Microflow had the highest R_{EFF} values during Day 2. The Millipore Air Tester and the SMA had the lowest R_{EFF} values during both days. Similar to the data analysis with bacteria, the investigators concentrated the statistical analysis of the portable samples and their performance relative to the BioStage, and analyzed the performance of gelatin filter separately.

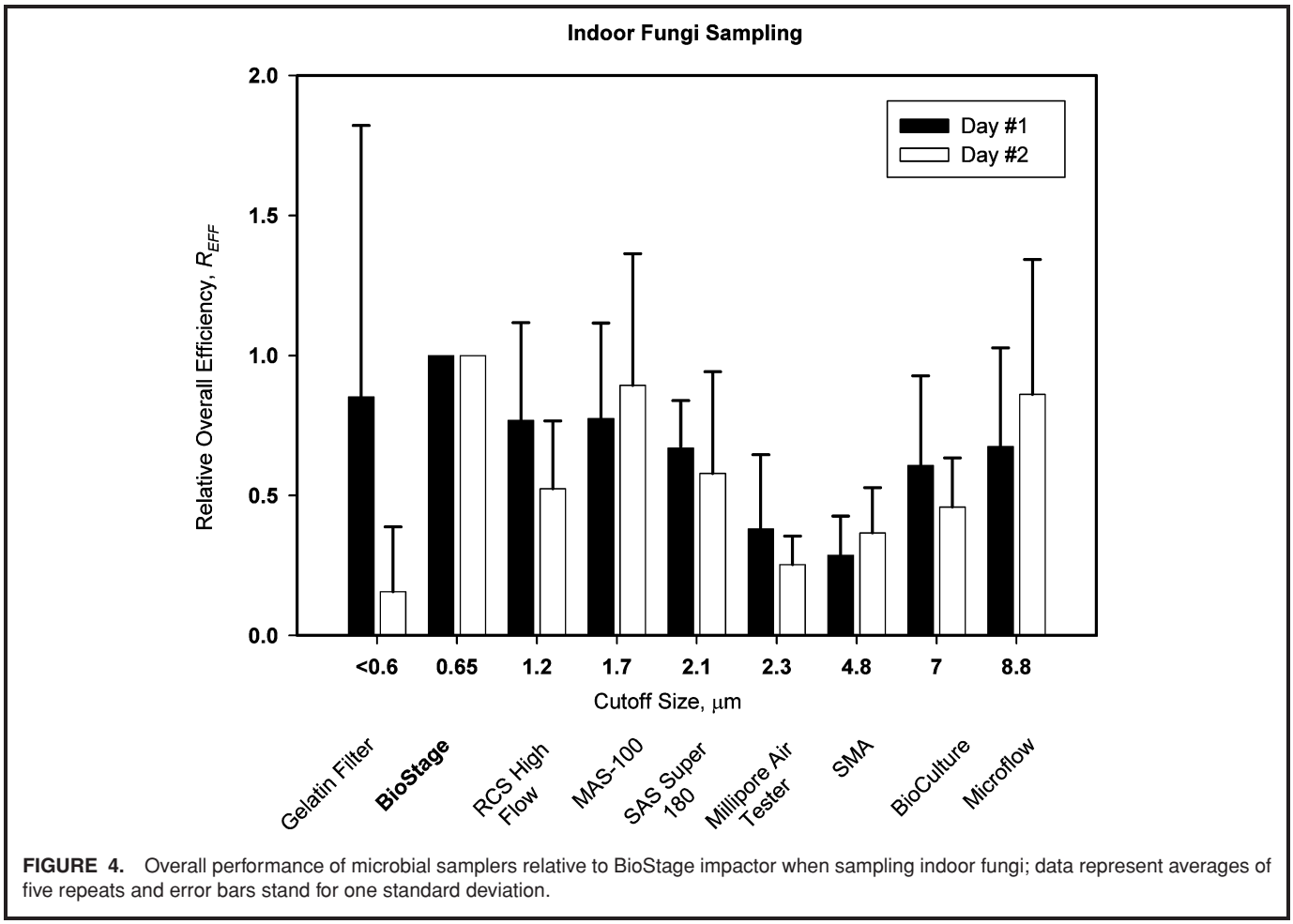

FIGURE 4. Overall performance of microbial samplers relative to BioStage impactor when sampling indoor fungi; data represent averages of five repeats and error bars stand for one standard deviation.

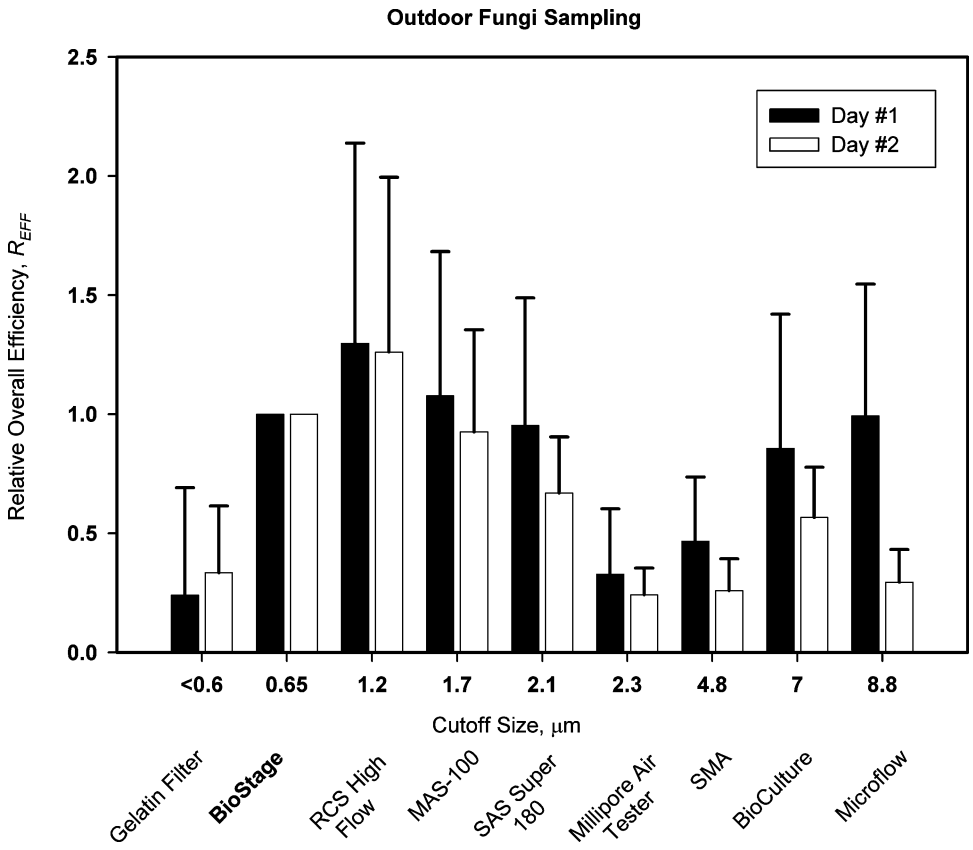

FIGURE 5. Overall performance of microbial samplers relative to BioStage impactor when sampling outdoor fungi; data represent averages of five repeats and error bars stand for one standard deviation.

Results from statistical analysis presented in Table I indicate that there was a statistically significant sampler effect ($p = 0.006$) but not sampling day effect ($p = 0.65$). When the R_{EFF} data from both sampling days were pooled together, only the MAS-100 and the Microflow were not statistically different from the BioStage, while performance of other portable samplers was lower compared with the BioStage. The R_{EFF} of gelatin filter was below unity for Day 2 ($p = 0.0006$) but not for Day 1 ($p = 0.37$); however, due to variability of the data, during Day 1 it had a coefficient of variation $>100\%$.

As indicated by the statistical analysis, the effect of the sampling day was not statistically significant on the portable sampler performance. This suggests that indoor fungi composition and/or size distribution remained relatively constant in contrast to indoor bacteria.

Figure 5 shows the relative overall efficiencies of the microbial samplers when sampling outdoor fungi species during 2 separate days. During both days, the RCS High Flow and the MAS-100 had the highest mean R_{EFF} values. In fact, the values were close to or above unity, i.e., the average fungi concentrations measured by these samplers were similar or higher than those measured by the BioStage impactor. Average airborne fungi concentrations measured by the BioStage were slightly higher compared with indoor environment: 85 CFU/m^{3}and 94 CFU/m^3 for Day 1 and Day 2, respectively.

According to the statistical analysis data presented in Table I, there was a statistically significant effect on the relative performance of the portable samplers by days ($p = 0.024$) and by sampler model ($p = 0.0001$). Multiple pairwise comparisons using Bonferroni statistics with data from both sampling days pooled together indicate that only Millipore and SMA were statistically different (lower) compared with the BioStage. Multiple pairwise comparisons of the pooled portable sampler data (BioStage not included) indicated that the RCS High Flow was statistically different (higher) compared with the Millipore Air tester and the SMA, while MAS-100 was different (higher) compared with the SMA. All other pairwise comparisons were not statistically different. The R_{EFF} value of the gelatin filter was below unity for both days ($p = 0.003$). Statistically significant sampling day effect suggests that the outdoor fungi composition and/or size distribution was different between the 2 sampling days.

The maximum concentration of culturable fungi in both indoor and outdoor environments as measured by the BioStage was close to 100 CFU/m^3. This concentration is much lower than the concentration of airborne particles larger than 2 μm in size (Figure 1). Data from both bacterial and fungal sampling

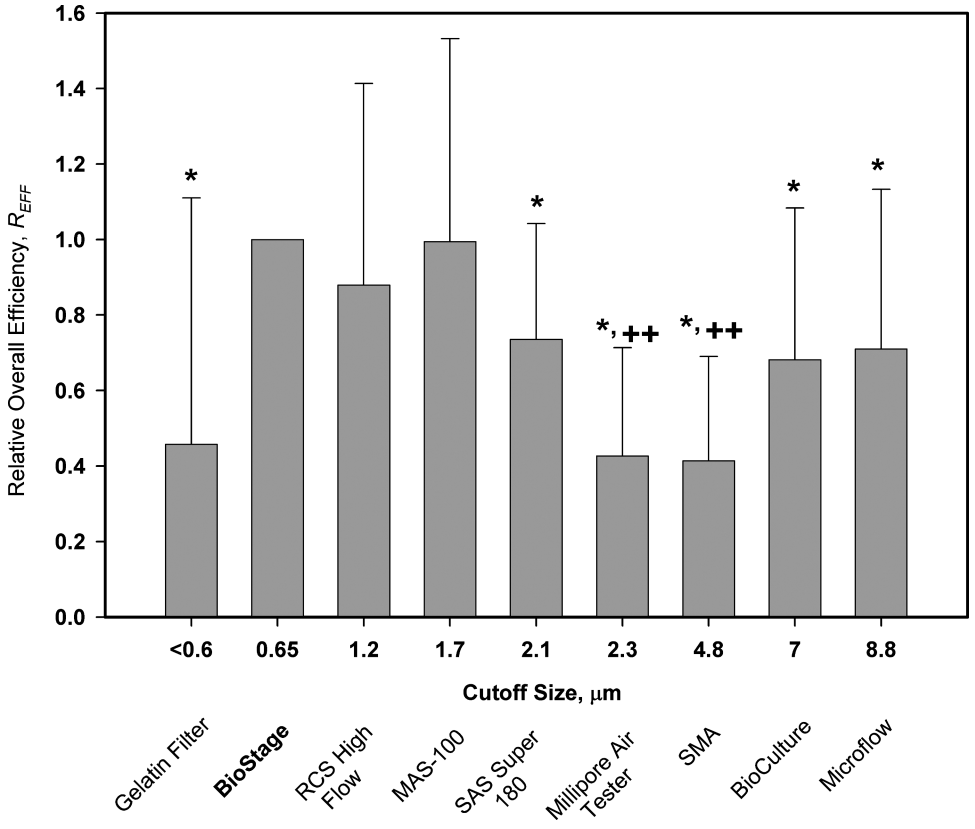

FIGURE 6. Relative overall efficiency of the bioaerosol samplers when collecting bacteria and fungi in indoor and outdoor environments; *indicates samplers that were different statistically different from the BioStage; ++ indicates samplers that were statistically different from other portable samplers.

indicated that culturable particles constituted only a small fraction of all airborne particles during our measurements.

General Description of Portable Impactor Performance

Because one of the main goals of this study was to compare the portable impactor performance in different environments when sampling different species, for the final analysis all the R_{EFF} data as a function of sampler model was analyzed; performance was compared with the BioStage. In this case, each sampler had a data set of 40 measurements. The mean R_{EFF} values and standard deviations for each sampler are presented in Figure 6. The MAS-100 had the mean R_{EFF} value equal to a unity, the highest among the portable impactors. The mean R_{EFF} value of the RCS High Flow was approximately 0.9, while the SAS Super 180, the BioCulture, and the Microflow had mean R_{EFF} values of approximately 0.7. The Millipore Air Tester and the SMA samplers had the lowest R_{EFF} values among the portable impactors, approximately 0.4. For gelatin filter the R_{EFF} value was slightly higher: 0.46.

Multiple pairwise comparisons with Bonferroni statistics against BioStage as a reference showed that among the portable impactors only the RCS High Flow and the MAS-100 were not statistically different from the BioStage, while the R_{EFF} values of all other samplers were below unity. Gelatin filter was also statistically different (lower) compared with the BioStage ($p < 0.0001$). Multiple pairwise comparisons of the pooled portable sampler data (BioStage not included) indicated that MAS-100 was statistically different (higher) compared with all portable samplers except the RCS High Flow. The two samplers showing the lowest R_{EFF} values, SMA and Millipore Air Tester, were statistically different compared with all other portable samplers. The rest of the pairwise comparisons were not statistically different. When gelatin filter was compared with the portable samplers, it was statistically different (lower) compared with the RCS High Flow, SAS Super 180, and MAS-100.

The concentration of airborne microorganisms determined by the gelatin filter was approximately 46% of the concentration determined by the BioStage (Figure 6). Although the gelatin filter provides a more favorable environment for the microorganisms compared with the regular filters, the desiccation effect is still substantial and only hardier species are enumerated. The used collection time of 15 min also might have played a role in the lower recovery by the gelatin filter.

The data in Figure 6 also indicate a substantial variability of the R_{EFF} for each test sampler (coefficients of variation >50%). This variability could be reflective of inhomogeneous microorganism distribution in the air. In addition, the measurements were performed during early spring and the relatively

low culturable microorganism concentrations as indicated by the BioStage (100–200 CFU/m^3) are likely to have contributed to the variability of the R_{EFF} values. On the other hand, the statistical differences among the samplers elucidated even from relatively low airborne microorganism concentrations are likely to be valid when microorganisms are present in higher concentrations.

Among the portable samplers, the RCS High Flow and the MAS-100 had the lowest cutoff sizes among the portable impactors, yielding best performances. Thus, their high relative performance could be attributed to efficient particle collection. The high relative performance of the BioStage could also be attributed to its low cutoff size, i.e., ability to collect even single bacteria. The BioCulture and the Microflow had the largest cutoff sizes (7.0 μm and 8.8 μm), but their R_{EFF} values were close to those of the SAS Super 180, which had a cutoff size of 2.1 μm. The BioCulture and the Microflow have rather low jet velocities and their collection efficiency curves begin to incline at 3 μm.[24] Willeke et al.[37] have suggested that high impaction velocity might negatively affect the microorganism survival during sampling. Thus, these two samplers were able to effectively collect biological particles attached to larger particles, while low jet velocity provided minimal mechanical damage to the particles.

The two samplers with the lowest R_{EFF} (the Millipore Air Tester and the SMA) do not have the largest cutoff sizes but feature the largest jet-to-plate distances among the tested impactors.[24] The authors speculate that impaction jets dissipate due to large jet-to-plate distances and desiccate a greater fraction of the agar surface thus providing an additional stress to the collected microorganisms. The role of the jet-to-plate distance on the performance of the portable impactors when collecting fungi was demonstrated in the authors' previous research.[25]

In an attempt to generalize the bioaerosol impactor performance (including the BioStage) investigators performed multiple linear regression analysis of the average R_{EFF} values using cutoff size and jet-to-plate distance as predictor variables. The analysis yielded the following model with correlation coefficient $R^2 = 0.83$ ($p = 0.012$):

$$R_{EFF} = 1.24 - 0.12S - 0.042d_{50} \quad \textbf{(3)}$$

where the S (in mm) is the jet-to-plate distance and the d_{50} (in μm) is the cutoff size. The p-value for each variable indicated statistical significance: 0.007 and 0.03 for S and d_{50}, respectively. The single linear regression with predictor variables S and d_{50} separately yielded R^2 values of 0.53 ($p = 0.04$) and 0.16 ($p = 0.32$), respectively. Thus, it is evident that the inclusion of both predictor variables yielded the best linear regression result. Inclusion of other design parameters, such as jet velocity or dimensionless S/W (jet diameter over jet-to-plate distance), into the regression model was not statistically significant.

In addition, humidity and temperature might affect the ability of a sampler to maintain culturability of the collected microorganisms, but these parameters remained relatively constant during our experiments and, thus, were not included in the regression analysis. Equation 3 implies that the highest achievable R_{EFF} value would be 1.24. This limiting value is produced by the limited number of samplers included in the model. Data from additional bioaerosol impactors, including those that have better performance than the BioStage or its equivalents, would provide a refinement to this empirical formula. Nonetheless, Eq. 3 could be used as an approximation in predicting overall performance of the portable microbial impactors when sampling in indoor and outdoor environments.

CONCLUSIONS

This study has shown that different portable microbial samplers yielded different airborne microorganism concentrations in indoor and outdoor environments. Impactor performance in a particular environment could vary from day to day, which was likely due to the different airborne microorganism composition and/or size distribution. Because the overall profile of the portable samplers' performance remained consistent in different environments and over separate sampling days, the data presented in this study are expected to assist field professionals and bioaerosol researchers in selecting bioaerosol samplers for their needs. The developed empirical formula to predict overall sampler performance might aid in designing more efficient bioaerosol samplers.

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