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# Student absenteeism and the comparisons of two sampling procedures for culturable bioaerosol measurement in classrooms with and without upper room ultraviolet germicidal irradiation devices

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## Abstract

Upper room ultraviolet germicidal irradiance (UVGI) has been shown to reduce the concentration of bioaerosols in controlled chambers. However, there is a lack of experimental results on the reduction of bioaerosol concentrations by UVGI devices in actual uncontrolled buildings. This study was carried out in an American elementary school in the Midwest. Two sampling procedures were carried out in six selected classrooms with similar dimensions that were separated into two groups: (1) UVGI exposure group and (2) non-UVGI control group. Two-stage Tisch culturable impactors were utilized to collect airborne culturable bacteria and fungi. Monthly samples were collected during unoccupied period in sampling Procedure A and during close-to-occupied periods in sampling Procedure B. Student absenteeism data were collected. Nonparametric statistical methods were applied. Neither analysis of microorganisms nor student absenteeism showed a significant difference between the UVGI exposure and non-UVGI control groups in Procedure A. Analysis of the airborne culturable fine and total bacteria levels (1–8 µm) was significantly lower in the exposure classroom than those of the control classroom using Procedure B ( $P$  values < 0.05). The result indicates that collecting airborne bacteria close to occupied time could be more effective in evaluating the performance of upper room UVGI. In this case study, upper room UVGI can reduce culturable bioaerosols in a crowded environment like classrooms.

**Keywords:** Bioaerosols, Elementary school, Indoor air quality, Upper room UVGI

## Introduction

Associations between adverse health effects and airborne biological particles have previously been reported in a number of studies; these health effects have included

allergic sensitization to air microbes and nonspecific responses to biological indoor air pollution.<sup>1–3</sup> There is the potential that building characteristics, such as excessive dampness, combined with microbiological contaminants may reduce the attendance of students.<sup>4–6</sup>

Ultraviolet (UV-C) light has been verified in controlled chamber studies to disinfect the air of microbial organisms by reducing their reproduction.<sup>7–9</sup> When UV-C light is applied in buildings it is mainly used in two configurations: (1) in-duct ultraviolet germicidal irradiance (UVGI) and (2) upper room UVGI.<sup>7–9</sup> The upper room UVGI is installed on the wall or suspended from the ceilings. The objective of upper room UVGI is to disinfect airborne infectious agents in the upper part of the environments while maintaining a safe environment for those actively inhabiting the room. The UVGI lamp is shielded or louvered above a predetermined height to minimize the radiation exposure and maintain occupant safety in the lower part of the rooms.<sup>10</sup>

UV germicidal irradiation disinfects specific bioaerosols in laboratory studies with multiple research studies demonstrating that upper room air UVGI can remove or inactivate bacterial bioaerosols in both chamber and one-pass tests.<sup>11–16</sup> However, limited studies have been conducted with fungal spore challenges for upper room UVGI and air cleaners containing UV lamps, so as a result the impact of UVGI on many fungal spores is not clear.<sup>12,17</sup> The effectiveness of upper room UVGI systems has been shown to be affected by environmental parameters, such as room air-flow pattern, air mixing and many other factors.<sup>18</sup> These variables, which influence fungal and bacterial deactivation in the real-world environment, require greater studies in the real-world environment.

Several on-site evaluations of UVGI have been conducted during past few decades. One of the earliest on-site studies showed that a UVGI device was effective in controlling an epidemic of measles.<sup>19</sup> In another study, Menzies has found that UVGI was capable of leading to a 99% reduction of surface microbial contamination near the UVGI devices, but there was no significant decrease of the airborne microbial concentrations. There were significantly fewer work-related symptoms as well as respiratory and mucosal symptoms when using the UVGI device.<sup>20</sup> The on-site performance of upper room UVGI was also evaluated by transmission of tuberculosis (TB) to guinea pigs. The TB infection of the UVGI group was reduced to 9.5%, compared to 35% of the control group.<sup>21</sup> Each of these studies lend support to the use of upper room UVGI for bioaerosol reduction in real-world settings.

However, there remains a lack of field evaluations on the effectiveness of upper room UVGI in real-world settings. Therefore, this study is an initial step towards filling in this knowledge gap. The purpose of the study was to evaluate the performance of UVGI to reduce both bioaerosol concentrations and student absenteeism in a Midwestern US Elementary School.

## Materials and methods

### Location

Our study took place within a public elementary school located in the Midwestern United States that was within 1-h ground transportation to the laboratory. Six reading and math classrooms from two grades were selected for the collection of airborne culturable bacterial and fungal samples.

The sampling for Procedure A had all classrooms evaluated from September 2011 to May 2012. The original design of the experiment included three groups with two classrooms in each one: UVGI group, placebo group and control group. The placebo group has UV device but installed normal light bulbs. The control group had nothing installed in the rooms. These two groups had been merged together as one larger control group. Then in Procedure B, only two of the six rooms were sampled from October 2012 to January 2013. All six classrooms had floor areas between 82m<sup>2</sup> and 85.5m<sup>2</sup> as shown in Figure 1.

### Ventilation and environment parameters

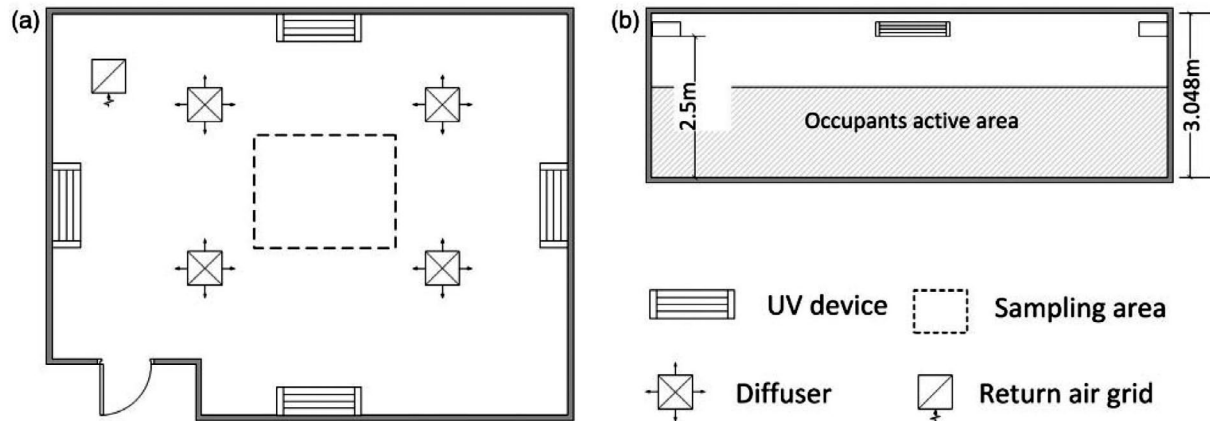
The ventilation rates were estimated in each sampling procedure. Every classroom had a separate heat pump ventilation system. The ventilation system for an occupied schedule runs from 6:00 a.m. to around 7:00 p.m. The filters installed in the heat pump unit are MERV 7. There were four air supply inlets and one air exhausting outlet in each classroom and there is no recirculation pathway from other classrooms. Flow rates at every inlet and outlet were measured three times during the test, and then averaged. The total supply air was the combination of all four inlets in each room. All measurements were completed with an Alnor balometer capture hood (TSI, USA). The designed fresh air for each classroom was the same. The supply and return air flow rates were verified to design parameters with two visits throughout the testing years. The data in each of the outlets were repeated three times. The results were compared to the design flow rates and show good agreement. Temperature and relative humidity (RH) were measured with an Omega OM-73 (OMEGA Engineering, INC, Stamford, Connecticut) temperature/ humidity data logger from the beginning to the end of each visit days.

### UVGI parameters

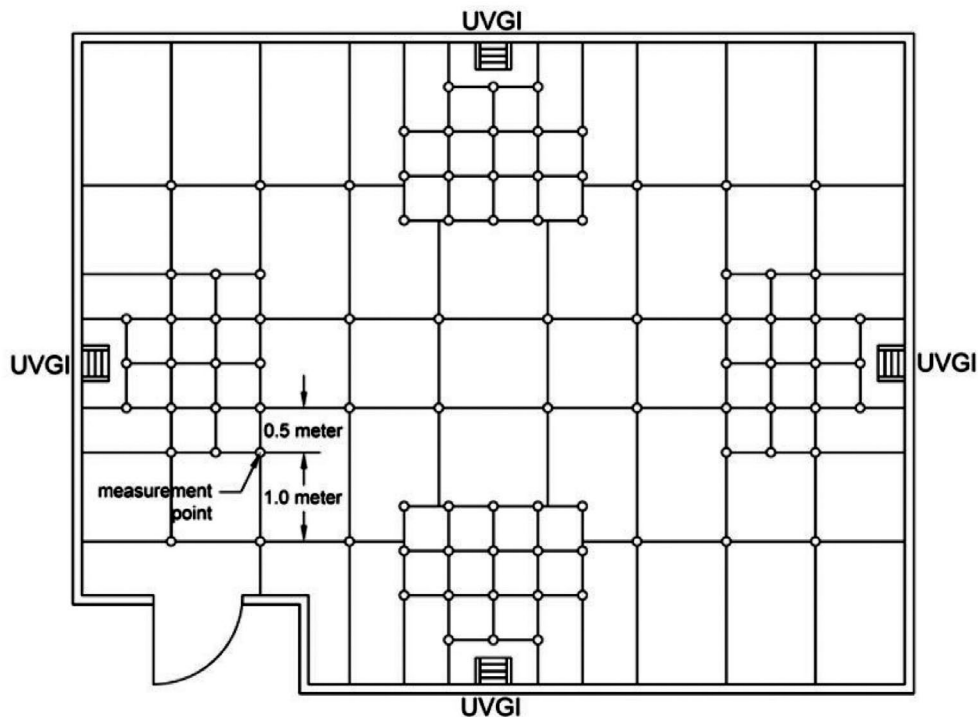
Upper room UVGI units were installed in two selected classrooms. In each classroom, four UVGI units (Lumalier WM-136, Lumalier Corporation, Memphis, USA), each with a 36W UV lamp, were installed on four walls as showed in Figure 1. The UVGI units were installed above 2.4 m in

height to keep the UV irradiance in lower area below the safety requirement for occupants,  $0.2 \mu\text{w}/\text{cm}^2$ .<sup>10</sup> The UV lamps ran continuously during both occupied and unoccupied times, and were replaced after 8000 running hours. A radiometer (Model IL 1700A with SED 240 detector, International Light Inc., Newburyport, MA) was used to measure the UV irradiance of upper room UVGI units in field. Similar method of testing the UV intensity in upper room

area of a chamber was applied.<sup>22</sup> The floor of the tested room was divided into a number of square grids 0.5 m of each side. In the area not directly facing the UV lamp, the square grids were increased to 1 m on each side. The locations of the UV irradiance measurement points are shown in Figure 2. The radiometer was attached to a tripod. The measurement plane of the sensor located at 2.4 m above the floor, which was the same height as the upper room



**Figure 1.** Floor plan and upper room UVGI installation of tested classrooms: (a) layout plan of the classroom and (b) section drawing of the classroom.



**Figure 2.** Distribution of the measurement points for the upper room UVGI.

UV field. The tripod was placed on the crossing of each square. Measurement in each spot was taken every 90° by rotating the tripod. The tripod was horizontally and vertically aligned through the process. The area-weighted averages of the UV irradiance at two UVGI classrooms were 25.7  $\mu\text{W}/\text{cm}^2$  and 26.3  $\mu\text{W}/\text{cm}^2$ . The values of UV irradiance were comparable to the results of other studies of upper room area UVGI and the installation fulfilled the recommendation of 30 W for each 19  $\text{m}^2$ .<sup>23</sup>

### **Occupants and absenteeism**

The third-grade class sizes were between 18 and 20 and the fourth grade class sizes were between 25 and 27. Students in each grade shared the same class schedules and had similar activity levels during the visiting days. The absenteeism rates due to the illness in all tested classrooms were recorded from September 2011 to May 2012 and November 2012 to January 2013. Information on absenteeism was collected from the school nurse. The absenteeism rate was counted as percentage of total students of the classes to compare between different classrooms. The unit was the percentage absent per day. Normally students would not move between the classrooms being tested. They will move to the special classroom for classes like music, art, computer and Physical Education (PE). All grades have special classes in the morning, but on different schedules. For fourth grade, students have one special class between two normal class sections in the morning. For third grade, the special class is after two normal class sections. This is also the reason we selected fourth grade for the Procedure B to collect bioaerosols three times per day. This study has been approved by the Institutional Review Board of the University of Nebraska–Lincoln.

### **Sampling procedures**

The sampling procedure would have been to collect samples while classrooms were occupied; however, the sampling pumps generate noise that would distract children and teachers. Two alternative methods were applied in our study (Table 1). Procedure A was to collect samples when classes were over for the day and all students had been dismissed. Procedure B was to sample immediately after the children had left the classrooms and while the school day was ongoing. Regardless of whether Procedure A or B was employed, the school was visited monthly. During Procedure A, 15-min samples were used to collect the maximum amount of bioaerosols with 5-min samples collected as an alternative if the 15-min samples were overgrown. Six classrooms were tested with two classrooms

**Table 1.** Comparison of sampling schedules in two procedures.

Class schedules	Sampling procedures	
	A	B
Class 08:20–09:35	—	—
Morning break	—	Indoor samples
Class 10:15–11:45	—	—
Lunch break	—	Indoor samples
Class 12:20–14:55	—	—
After class 15:00–18:00	Indoor samples	Indoor samples

The breaks last 40 min and all samples were collected within 10 min after students leaving classrooms.

in the UVGI exposure group and four classrooms in the non-UVGI control group. Duplicate samples were collected for both time periods at all sampling sites. From September 2011 to May 2012, all six classrooms were tested for airborne culturable bacteria and fungi with all sampling data converted to colony-forming units per cubic meter of air (CFU/ $\text{m}^3$ ).

In the sampling Procedure B, the sampling periods were set as 10 and 5 min, due to the limited time between class periods. Two tested classrooms from the previous six were evaluated from November 2012 to January 2013. Only airborne culturable bacterial analyses were conducted with fungal samples discontinued. One classroom with upper room UVGI (UVGI exposure group) and another without UVGI (non-UVGI control group) were utilized for the sampling evaluation. The students in the two rooms shared the same class schedules and similar activity in each class session. Samples of different sampling periods were collected in parallel in each classroom. And in each period, samplers were operated simultaneously in UVGI and non-UVGI control rooms.

### **Airborne culturable sampling**

Two-stage Tisch culturable impactors (Model TE-10-860) were utilized to collect the airborne bacterial and fungal organisms. The samplers included two stages that collect the fine (1–8  $\mu\text{m}$ ) and coarse size (>8  $\mu\text{m}$ ) distributions of the microorganisms. The airborne bacteria of coarse size was captured by the first stage of the sampler and represent the particles less likely to reach human lungs. The fine size captured by the second stage of the sampler represents the bioaerosols that could reach human lungs. Before each round of sampling, the impactor samplers were disinfected with 70% isopropyl alcohol.<sup>24</sup> Vacuum pumps



were calibrated before and after sampling to 1.698 m<sup>3</sup>/h with a tetraCal<sup>®</sup> Calibrator (BGI Incorporated, Waltham, MA).<sup>25</sup> Trypticase soy agar (TSA) was used to collect airborne bacteria and malt extract agar (MEA) was used for fungi collection. All agar plates were poured with 27 ml of agar per manufacturer instructions within a week of the sampling day.

### **Sample handling and analysis**

All samples were transported with icepacks to the laboratory within 12 h of collection. Bacterial samples were incubated at 37°C and counted after 24 and 48 h. Fungal samples were incubated at 25°C and counted on the fifth, seventh and ninth day. There is the possibility that more than one viable particle had penetrated through the same sampling hole and formed as one single colony. The observed numbers of colonies were adjusted for this phenomenon using the positive-hole correction table.<sup>26</sup> The CFU/m<sup>3</sup> was calculated for each sample. The numbers of culturable CFU/m<sup>3</sup> of bacteria for coarse (>8 µm) and fine (1–8 µm) particle size were obtained. Quality control was maintained with nonexposed plates of TSA and MEA plates taken to the sampling site during collection along with positive control plates within the laboratory.

For Procedure A, the Mann–Whitney nonparametric test was used to compare the samples from UVGI and non-UVGI control classrooms. For Procedure B, the Friedman test was used to compare samples from different sampling (morning, noon and afternoon) times in each visiting. Then samples from the same visiting day were combined.<sup>27</sup> A Wilcoxon ranked sum test (nonparametric, dependent test) was used to compare the airborne bacteria concentrations between UVGI and non-UVGI control classrooms. The Mann–Whitney test was applied to compare the mean absenteeism between the UVGI exposure and non-UVGI control classrooms. Statistical Package for the Social Sciences version 21 (IBM Corporation, Armonk, New York, USA) was used to achieve all the statistical analysis.

## **Result**

### **Results of sampling Procedure A**

During Procedure A, a total 54 samples of airborne culturable bacteria were collected in nine months, among which 18 samples were from the UVGI exposure classrooms, and 36 from the non-UVGI control-rooms. The same number of fungal samples was collected. Table 2 presents the descriptive statistic summaries for the concentrations of airborne culturable bacteria during Procedure A.

The highest concentrations of airborne bacteria in the UVGI classrooms appeared in October with a mean of 99 CFU/m<sup>3</sup>, ranging from 95% confidence interval (CI) of 57 CFU/m<sup>3</sup> to 140 CFU/m<sup>3</sup>. For the non-UVGI classrooms, the month with the highest concentration was also October, with a mean value of 155 CFU/m<sup>3</sup> and ranges from 95% CI of 114 CFU/m<sup>3</sup> to 195 CFU/m<sup>3</sup>. No consistent statistical differences were found for coarse, fine or total bacteria between the UVGI and non-UVGI control classrooms in six out of nine tested months (*P* values > 0.05).

For the outdoors, the coarse bacteria ranged from 4 CFU/m<sup>3</sup> in February to 309 CFU/m<sup>3</sup> in October. The range of fine size bacteria was from 7 CFU/m<sup>3</sup> in February to 725 CFU/m<sup>3</sup> in October. The lowest bacterial concentrations were recovered during the winter with the highest concentrations in the autumn. Both particle sizes had a similar seasonal trend as shown in Figure 3.

For fungi, the highest level in UVGI classroom appeared in September with a mean of 154 CFU/m<sup>3</sup>, range from 95% CI of 111 CFU/m<sup>3</sup> to 196 CFU/m<sup>3</sup>. For the non-UVGI room, October had the highest concentration of airborne fungi, with a mean value of 194 CFU/m<sup>3</sup> and ranging from 95% CI of 128 CFU/m<sup>3</sup> to 260 CFU/m<sup>3</sup>. However, there was also no consistent trend between the UVGI and non-UVGI control classrooms throughout the sampling period using Procedure A (Figure 4). The outdoor concentration of coarse fungal organisms ranged from 7 CFU/m<sup>3</sup> in January to 483 CFU/m<sup>3</sup> in October. The highest concentration of fine fungi was recovered in autumn.

### **Results of sampling Procedure B**

Using sampling Procedure B, a total of 24 samples of airborne culturable bacteria were collected in three months, 12 from the UVGI exposure classroom and 12 from the non-UVGI control room. All samples were collected within 10 min of students leaving the classrooms. The highest concentrations of bioaerosols in the UVGI classroom appeared in October with a mean value of 152 CFU/m<sup>3</sup>, and ranged from 95% CI of 96 CFU/m<sup>3</sup> to 208 CFU/m<sup>3</sup> (Table 3). For the non-UVGI classroom, the highest concentration was in November, with a mean value of 357 CFU/m<sup>3</sup> and a 95% CI of 226 CFU/m<sup>3</sup> to 488 CFU/m<sup>3</sup>. The concentrations of coarse bacteria were lower than fine bacteria through the total sampling visits. The fine and total bacterial concentrations observed in UVGI classrooms were significantly lower than those for control classrooms (*P* values < 0.05). The *P* values for total bacteria from October to December were 0.008, 0.008 and 0.011, respectively. Similar trends existed in all the three sampling months. Figure 5 presents the difference between UVGI and non-UVGI

**Table 2.** Sampling Procedure A, descriptive statistics of total airborne culturable bacteria concentrations for UVGI and non-UVGI control classrooms from 2011 to 2012 (CFU/m<sup>3</sup>).

Month	<i>N</i> samples	<i>M</i> ± <i>SD</i>	Median	Range	95% CI
September					
UVGI	2	94±40	88	28–147	60–127
Non-UVGI	4	86±23	92	49–123	74–99
Outdoor	1	61±16	61	49–72	—
October					
UVGI	2	99±50	67	59–172	57–140
Non-UVGI	4	155±76	128	66–308	114–195
Outdoor	1	1033±156	1033	923–1143	—
November					
UVGI	2	58±28	55	28–103	35–81
Non-UVGI	4	63±45	41	27–171	39–87
Outdoor	1	189±62	189	145–232	—
December					
UVGI	2	52±30	50	20–108	27–77
Non-UVGI	4	29±20	27	0–68	19–40
Outdoor	1	40±57	40	0–80	—
January					
UVGI	2	33±17	29	12–60	19–47
Non-UVGI	4	42±30	33	7–104	26–58
Outdoor	1	21±10	21	14–28	—
February					
UVGI	2	72±29	66	37–114	48–96
Non-UVGI	4	47±41	39	0–155	25–69
Outdoor	1	11±5	11	7–14	—
March					
UVGI	2	51±24	50	19–87	31–71
Non-UVGI	4	41±16	38	22–88	32–49
Outdoor	1	170±21	170	155–184	—
April					
UVGI	2	49±9	48	36–60	42–57
Non-UVGI	4	39±17	42	9–81	30–49
Outdoor	1	183±15	183	172–193	—
May					
UVGI	2	95±32	94	44–140	69–122
Non-UVGI	4	65±56	44	0–222	36–95
Outdoor	1	43±31	43	21–65	—

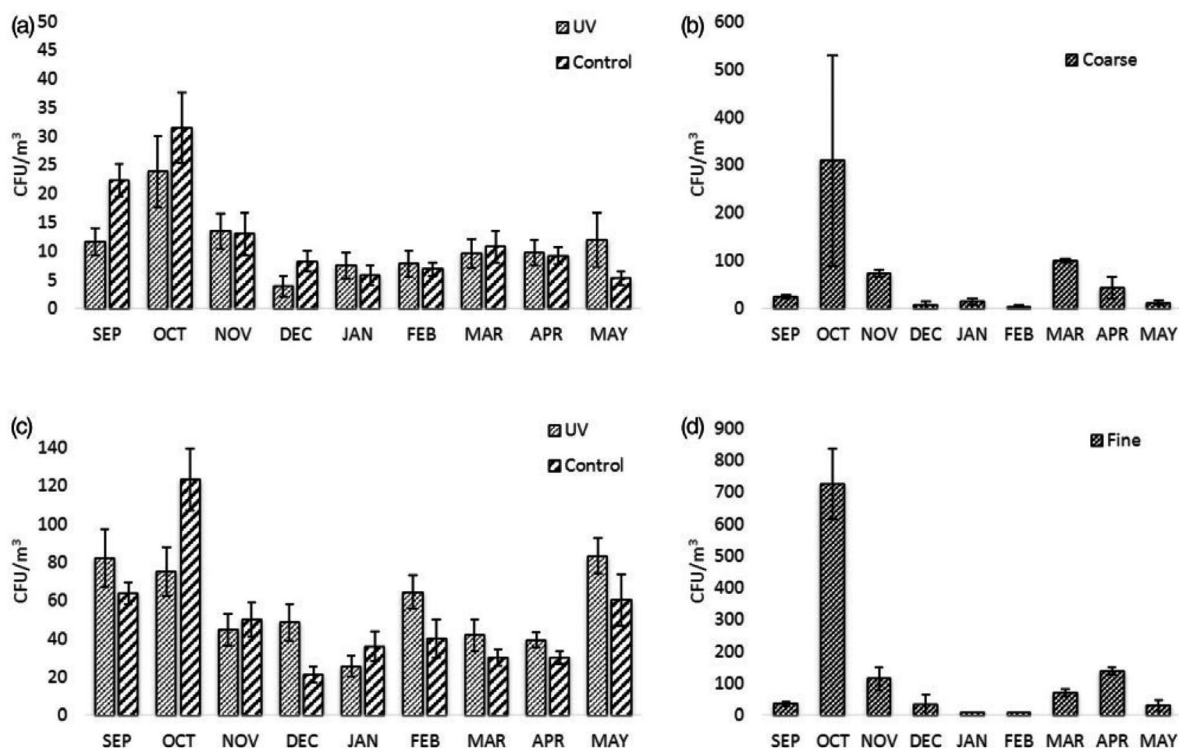
CI, confidence interval; UVGI, ultraviolet germicidal irradiance.

classrooms. However, for coarse bacterial, there were no significant statistical differences between UVGI and control classrooms. The outdoor level of airborne bacteria shows that the highest level appeared in October during the fall season. The lowest concentration was in January.

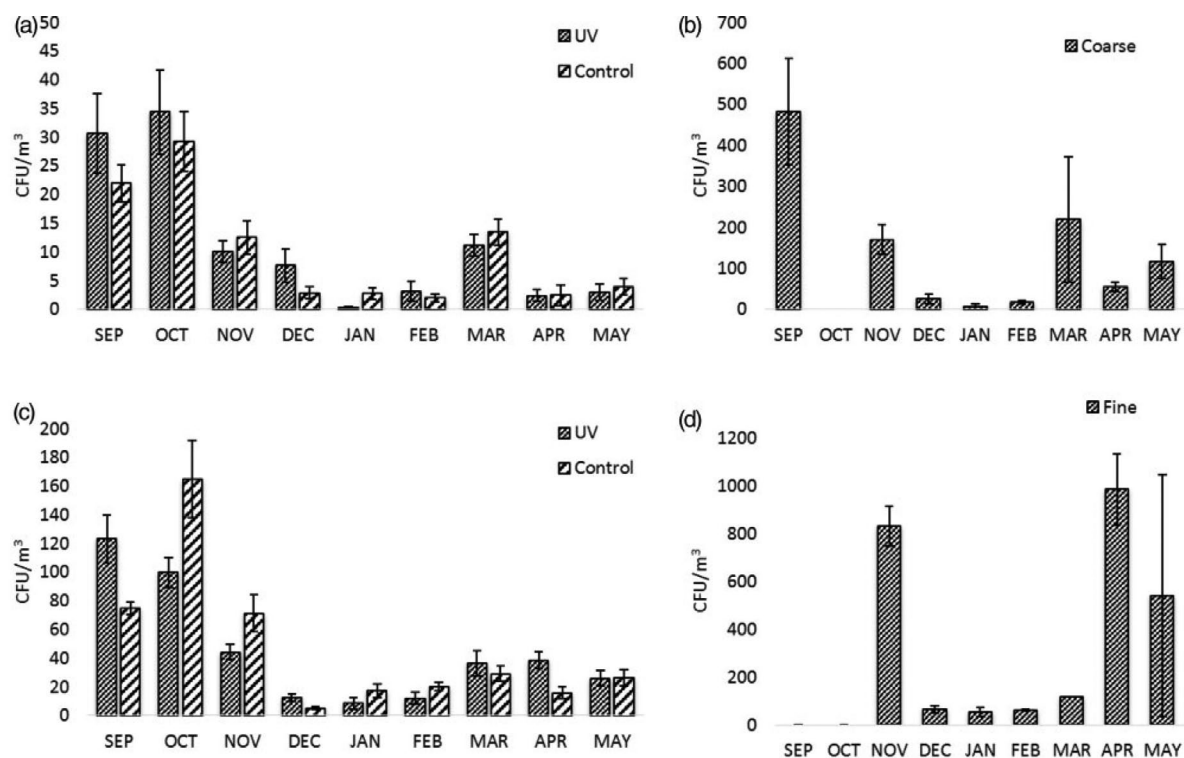
The outdoor airborne bacteria and fungi concentrations in Procedure A were at the highest levels in autumn season. It has been demonstrated that the outdoor levels of airborne fungal organisms in the Midwest are significantly higher than indoor levels in facilities without indoor

air quality issues associated with fungal growth.<sup>28</sup> This corresponds to the findings in our study. For the relation between indoor and outdoor concentrations, it was found that the highest levels were from the same month and season for both airborne bacteria and fungi.

The absentee rates for UVGI and non-UVGI classrooms were not statistically different (Figure 6). The absentee rates for rooms when Procedures A (*P* value = 0.37) and B (*P* value = 0.69) were conducted were not statistically different.



**Figure 3.** Sampling Procedure A mean and standard deviation of airborne bacteria concentration for UVGI exposure and non-UVGI control, (a) indoor coarse bacteria, (b) outdoor coarse bacteria, (c) indoor fine bacteria and (d) outdoor fine bacteria.



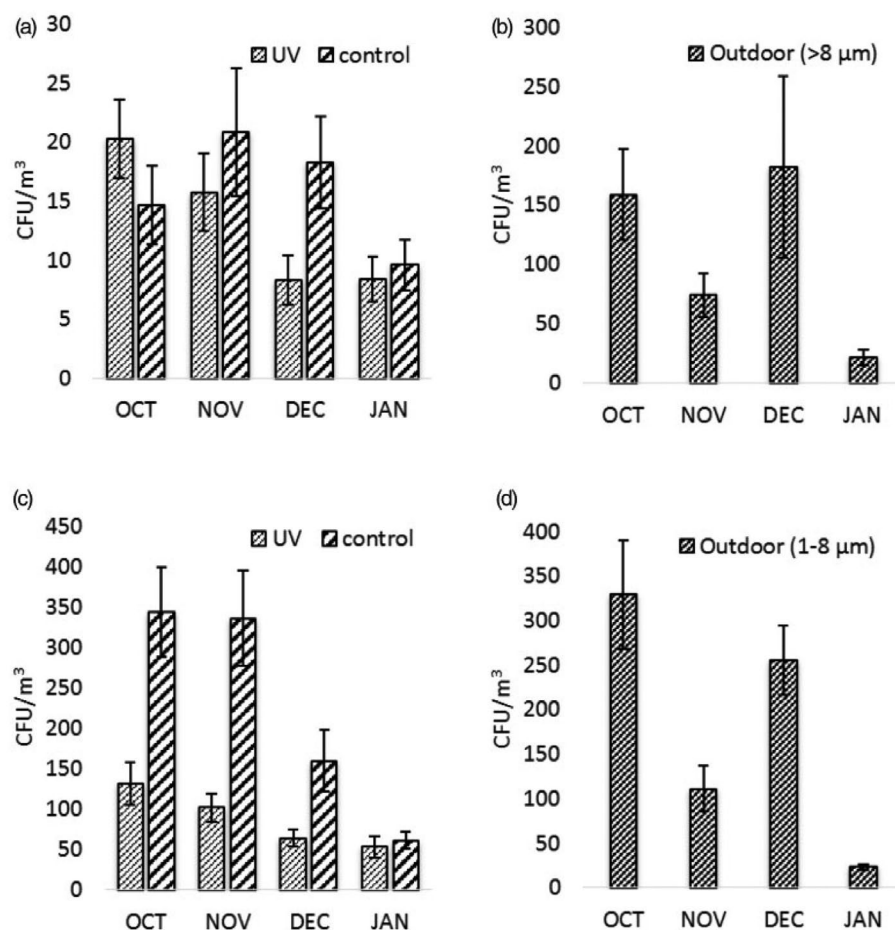
**Figure 4.** Sampling Procedure A mean and standard deviation of airborne fungi concentration for UVGI exposure and non-UVGI control, (a) indoor coarse fungi, (b) outdoor coarse fungi, (c) indoor fine fungi level and (d) outdoor fine fungi.



**Table 3.** Sampling Procedure B descriptive statistics of total airborne culturable bacteria concentrations for UVGI and control classrooms from 2012 to 2013 (CFU/m<sup>3</sup>).

Month	<i>N</i> samples	<i>M</i> ± <i>SD</i>	Median	Range	95% CI
October					
UVGI	3	152±102	122	58–436	96–208
Non-UVGI	3	358±218	321	55–762	238–479
Outdoor	3	488±241	519	131–813	303–673
November					
UVGI	3	118±68	107	43–304	80–156
Non-UVGI	3	357±237	321	59–849	226–488
Outdoor	3	185±100	201	78–387	108–262
December					
UVGI	3	72±45	59	14–164	48–97
Non-UVGI	3	178±156	109	22–534	92–264
Outdoor	3	438±335	282	226–1288	180–695
January					
UVGI	3	62±58	33	7–182	29–94
Non-UVGI	3	71±43	65	11–132	47–95
Outdoor	3	45±26	36	21–88	25–64

CI, confidence interval; UVGI, ultraviolet germicidal irradiance.

**Figure 5.** Procedure B mean and standard deviation of airborne bacteria concentration for UVGI exposure and non-UVGI control, (a) indoor coarse bacteria, (b) outdoor coarse bacteria, (c) indoor fine bacteria level and (d) outdoor fine bacteria.

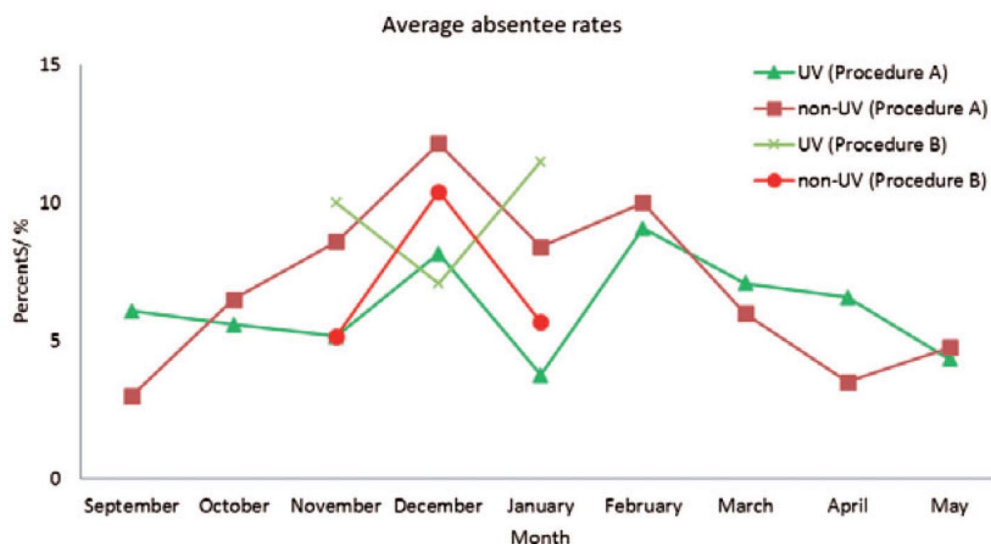


Figure 6. Average absenteeism rates for UVGI and non-UVGI control classrooms in both sampling Procedures A and B.

## Discussion

During sampling Procedure A, the concentrations of airborne bacteria were below 160 CFU/m<sup>3</sup>. This may be a result of the collection period that was after the occupied time and the main source of airborne culturable bacteria was humans. In sampling Procedure B, consistent statistical significant differences between two groups of classrooms were found when the concentrations were closer to 400 CFU/m<sup>3</sup>. When the concentrations of airborne bacteria were 160 CFU/m<sup>3</sup> or lower, there was no consistent statistical significant difference between UVGI and non-UVGI control groups. This may indicate that collecting airborne bacteria close to or within the occupied time maybe more effective than collecting samples at the end of the day in evaluating the performance of upper room UVGI.

The concentration of airborne bacteria was comparable to other previous studies (conducted with similar methods) in similar environments. The concentration of total airborne culturable bacteria during working hours vary from a range of 200 CFU/m<sup>3</sup> to 500 CFU/m<sup>3</sup> in other environments during the occupied time, such as domestic and office.<sup>29</sup> Using a one-stage Anderson sampler and TSA agar, the concentrations of bacteria, which ranged from 24 CFU/m<sup>3</sup> to 1447 CFU/m<sup>3</sup> were monitored in two elementary schools.<sup>30</sup> Comparing these studies, the level of airborne culturable bacteria in our result was similar.

Comparing the two sampling procedures, results and conclusions that were drawn from the statistical tests were significantly different. The major difference between the two procedures was the time we choose to collect samples. In sampling Procedure B, airborne bacteria concentrations differences between the UVGI exposure and

non-UVGI control classrooms were observed. In this procedure, two tested classrooms shared the same class schedule, which suggested that the students' activity levels were similar. All the samples were collected very close to the occupied conditions. It has been found that the occupants' activity may cause the resuspension of biological particles and an increase in particle concentration during the occupied time.<sup>31,32</sup> This may explain why we observed higher concentrations with procedure B. In sampling Procedure A, the sampling process lasted for 3–4 h to complete the measurement of six classrooms in each visit. This indicated that the concentration of airborne bacteria could have reached a steady state under the constant ventilation and unoccupied condition. In addition, the airborne bacteria released by occupants before the sampling period, could be reduced by ventilation dilution and natural death. The results of the measurement support this hypothesis.

In this study, the environmental parameters and the operating conditions for the ventilation system throughout these two sampling procedures were relatively consistent as shown in Tables 4 and 5. The average indoor temperature and RH were similar for both sampling Procedures A and B (Table 4). The indoor temperatures were controlled within the range of 20–25°C during the entire test periods.

Since there are individual heat pump ventilation systems in each classroom, the chance of cross contamination by a centralized recirculation pathway was eliminated. All these individual ventilation systems shared the same outdoor air intake. The outdoor environmental factors, such as seasonal effect or outdoor levels of airborne microorganisms, were considered the same for all classrooms in both UVGI and non-UVGI control groups.

**Table 4.** Temperature and relative humidity rates for UVGI and non-UVGI control classrooms.

	Procedure A		Procedure B	
	Temperature /°C	Relative humidity/%	Temperature /°C	Relative humidity/%
September	23.1 (±1.4)	55.6 (±7.8)	—	—
October	23.0 (±0.7)	45.7 (±3.9)	22.7 (±0.6)	34.6 (±2.2)
November	22.0 (±0.9)	27.5 (±0.7)	22.5 (±0.8)	29.4 (±2.9)
December	20.6 (±1.1)	23.8 (±2.2)	22.6 (±0.8)	25.2 (±2.3)
January	19.9 (±2.3)	30.5 (±5.3)	22.3 (±0.8)	24.1 (±1.5)
February	21.8 (±0.5)	28.5 (±1.1)	—	—
March	22.1 (±0.8)	25.3 (±3.9)	—	—
April	23.1 (±0.8)	41.8 (±2.8)	—	—
May	22.6 (±0.6)	35.8 (±1.0)	—	—

**Table 5.** Summaries of the test conditions for the selected classrooms for the sampling Procedures A and B.

	Number of occupants	UVGI or non-UVGI	Bioaerosols type	Air flow rate (supply/ return, cfm)	Recommended outdoor air (cfm)
Procedure A					
Room 1	22	Yes	Bacteria/fungi	1024/735	220
Room 2	20	No	Bacteria/fungi	969/742	200
Room 3	19	No	Bacteria/fungi	1259/888	190
Room 4	23	No	Bacteria/fungi	985/800	230
Room 5	27	Yes	Bacteria/fungi	1004/713	270
Room 6	22	No	Bacteria/fungi	1013/767	220
Procedure B					
Room 1	28	Yes	Bacteria	993/812	280
Room 2	27	No	Bacteria	947/803	270

UVGI, ultraviolet germicidal irradiance.

Table 4 shows that the RH has a range of 23.8–46.7% during the tested months, except for September 2011. Previous studies have found that the UV efficiency could be adversely affected by high RH, especially when higher than 50%.<sup>18</sup> In our study, since all measurements were carried out at the RH level under 50%, we assumed the UV efficiency should be consistent and comparable among the results. The ventilation conditions and air temperature could also influence the UV efficiency adequately.<sup>33</sup> The ventilation rates were tested during both sampling procedures. Though the outdoor air rates were not directly measured during two sampling procedures, the recommended values by the American Society of Heating, Refrigerating and Air Conditioning Engineers (ASHRAE) standard 62.1 for elementary classrooms based on number of occupants are listed in Table

5.<sup>34</sup> In addition, the results from the two measurements agreed with the design parameters of the ventilation systems. The similar ranges of environmental factors like RH, temperature and ventilation rates in the UVGI exposure and non-UVGI control classrooms suggested that their influences on the performance of upper room UVGI units were consistent. However, the variation of RH throughout the two sampling procedures may have influenced the bioaerosol samples reflected as seasonal effects. The RH in both sampling procedures had a positively correlation with bioaerosol concentrations. This corresponds to the lowest level of both airborne bacteria and fungi collected from the classrooms. A possible explanation for the low concentration under low RH (15–20%) conditions is that the low moisture may have caused genetic damage.<sup>35</sup>

For the absenteeism rate, though we did not find statistical difference between the two groups in both sampling procedures, it still could be an effective indicator reflecting the health condition of the students. Available review has found that absenteeism has a positive relationship with severity of the disease in both schools and offices.<sup>4</sup> Researchers in recent years kept drawing similar conclusions, such as the increase of the absenteeism rate during an influenza pandemic in Iranian schools,<sup>36</sup> and an intervention to control both the rates of absenteeism and respiratory illnesses in a single elementary school system.<sup>37</sup>

There are several limitations of this study. Culturable organisms are only a fraction of all airborne organisms. The existence of viable but nonculturable bacteria was not explored as part of our study, but should be considered.<sup>38</sup> The sampling results may not provide a complete picture of the airborne bacteria, especially for sampling Procedure A. The reduction rate of bioaerosols due to the infiltration and other unique environmental conditions of each classroom was not fully explored in this study. More measurement of ventilation may provide more information about these factors, which is one of the limitations of our study. For example, though the mechanical ventilation rates of the two classrooms in Procedure B were consistent, the infiltration of the two tested rooms might be different. During the measurements, doors were opened frequently, which could introduce infiltration effects from the corridor and surrounding environments. Since the occupants and their activities were considered as one of the main sources of the indoor airborne bacteria, the difference in occupant numbers in the classrooms may result in variations, especially if absenteeism was unequal in those rooms. Absenteeism rates due to any kind of illnesses were reported to the school nurse. Therefore, the numbers were not limited to respiratory disease or other airborne infectious diseases. Furthermore, the sample sizes were limited and all samples were collected from one single school. The samples were collected near the occupied time, but still not within the occupied time, which could have altered the concentrations. Alternative methods, such as real-time bioaerosol monitors, could be applied to obtain data from actual occupied time in the next phase of the research.

## Conclusion

Both airborne bacteria and fungi collected during unoccupied time periods did not show significant statistical difference between UVGI and non-UVGI classrooms. The samples collected right after occupants evacuated the rooms showed that fine size airborne bacteria from the

UVGI exposure classroom was significantly lower than those from the non-UVGI classroom when microorganism concentrations were above a certain level. Though the absenteeism rates were obtained in both sampling procedures, no statistical differences were found between UVGI and non-UVGI classrooms. Our results indicate that collecting airborne bacteria close to or within the occupied time may be more effective in evaluating the performance of upper room UVGI. Further research, such as developing a method to evaluate UVGI in the field by collecting samples during occupied time, will be valuable to assess the performance of UVGI on airborne microorganisms.

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