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Bacterial Bioaerosol Concentrations in Public Restroom Environments

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ABSTRACT

The number concentrations of total culturable bacterial bioaerosols in seven public restroom environments were measured via a standard measurement method provided by the Ministry of Environment of the Republic of Korea. Outdoor bioaerosols were also measured to compare with data collected in public restrooms. The measurements were conducted for one year, with varying concentrations of airborne bacterial bioaerosols observed in public restrooms. The effect of the number of users on the concentration of bioaerosols in public restrooms was also analyzed. The experimental results provided useful and basic information for the study and development of control methods against bioaerosols in public restrooms.

Keywords: Bioaerosols; Restroom; Impactor; Air quality; Indoor air.

INTRODUCTION

The outbreak of the influenza A (H1N1) virus infection in 2009 has reminded us of the importance of monitoring and controlling airborne microorganisms (termed bioaerosols) in public facilities. The spread of bioaerosols has threatened public health in many countries, including the US, Mexico, and the Republic of Korea (ROK), as well as industries associated with traveling and transportation (ECDC, 2009) during this outbreak. The importance of research on these bioaerosols increased after the bioterrorism incidents that occurred in the US during 2001. However, practical measurements of the concentration of bioaerosols and the development of control methods against them remain inadequate (Yeo and Kim, 2002; Menetrez *et al*., 2007; Lee *et al*., 2008a; Lee *et al*., 2008b; Lee *et al*., 2011). Several studies on the measurements of bioaerosols in outdoor and indoor environments, including schools and health care facilities, were reported in 2007 and 2008 (Godwin and Batterman, 2007; Kim and Kim, 2007; Menetrez *et al*., 2007; Fang *et al*., 2008; Zuraimi and Than, 2008); however, during the influenza A H1N1 outbreaks in 2009, there were few published reports on the practical monitoring of airborne microorganisms in public facilities.

The bioterrorism incidents and the many appeals made regarding the hygiene problems associated with airborne microorganisms led the Ministry of Environment (ME) of ROK to recognize the importance of bioaerosols and regulate bioaerosol concentrations in public facilities since 2003. The regulatory parameter is the concentration of total airborne bacteria in indoor air environments, particularly in hospitals and health care facilities. The maximum allowable value of the total bacterial bioaerosol concentration is 800 colony forming units (CFU)/m^3 (Ministry of Environment, ROK, 2010). Compared with ROK, most other countries do not have any regulations for bioaerosols. The Environment Protection Agency (EPA) of the US does not have any clear concentration guidelines for bioaerosols. The World Health Organization (WHO) has suggested guidelines for the prevention of mold problems in indoor environments (WHO, 2009); however, they do not specify any appropriate level of bioaerosol concentration. Although ROK has regulations

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for bioaerosols, currently the available information on the concentration of bioaerosols in public environments is insufficient. We need more information regarding the bioaerosol concentration in not only health-related facilities but also in many public places, where numerous people are exposed to indoor air every day. These places include subway systems, shopping centers, hospitals, university lecture buildings, and public libraries. Also, this information on bioaerosol concentration can be useful to many countries where regulations on bioaerosols are being considered.

In this study, the concentrations of bacterial bioaerosols in a subway station, a shopping center, old and new lecture buildings, a student center, a hospital, and a library were measured. More specifically, the restroom environments in these locations were chosen because we suspected that the public restrooms were major potential sources of microorganisms, where people might be markedly exposed to bioaerosols if bioaerosol concentration was high. Also, we compared the concentrations of bioaerosols in public restrooms to nearby outdoor bioaerosol concentrations to identify the effect of outdoor seasonal variations on the bioaerosol concentrations in indoor restrooms. ROK has humid and hot weather conditions during the summer season and cold and dry weather during the winter season. These weather conditions result in environmental distinctions for bioaerosols; for example, good growth conditions for most bacteria in summer and poor survival conditions for bacteria in winter. Therefore, we conducted measurements during the summer and winter seasons which we then compared. We also counted the number of users and measured the concentration of bioaerosols in restrooms for an initial, stepwise investigation of the effect of humans on the concentration of bioaerosols in public facilities.

METHODS

The bioaerosol concentrations were measured following the regulation set out by the Ministry of Environment, ROK, based on the monitoring of the total airborne culturable bacteria using impaction methods and incubation. However, the regulation does not cover the enumeration of non-culturable bioaerosols, species identification, or detail analyses such as gram-staining or pathogenic characteristic tests, as these types of microbial studies incur heavy costs and entail complex techniques, which are not adequate as an initial step for legal regulation. In line with the regulation of the Ministry of Environment, ROK, the Bioculture (Buck Bio-culture, Model B30120, A.P. Buck Inc., Orlando, FL, USA) was used to sample bioaerosols, which incorporates an impactor-type sampler for collecting airborne microorganisms. The data obtained from the Bioculture were compared with those from Anderson impactors (Anderson impactor, Z-A6, Zefon, USA) (Lee *et al*., 2008a) used in other environmental monitoring studies (data not shown in this study); both data sets were found to be practically the same with some correction via a manual (Buck Bio-culture, Model B30120, A.P. Buck Inc., Orlando, FL, USA) of the Bioculture. Airborne microorganisms were accelerated by passing them through the nozzles of the Bioculture. Sub-micrometer sized particles passed through the Bioculture device; however, bacterial aerosol particles were deposited onto the culture plates due to their inertia in the airflow, which was driven by a vacuum pump through the 400 nozzles of the Bioculture device. The sampling flow rate was 100 liters per minute, and the sampling time was between two to five minutes per sample, depending on the bioaerosol concentration, with at least three replicates performed for all monitoring conditions. We used nutrient agar (beef extract 0.3%, peptone 0.5%, and agar 1.5%; Difco; 20 mL agar) plates (Hwang *et al*., 2010; Lee *et al*., 2010) in the Bioculture to sample and enumerate the total airborne bacterial bioaerosols. The sampled bacterial bioaerosols were incubated at 37°C for 24 hours, the number of colonies was enumerated and the concentration of culturable bioaerosols in the air environment was calculated and expressed in units of CFU/m^3. We conducted all the measurements in the afternoon of the measurement days. At least three replications were conducted under the same experimental conditions, and the data were analyzed using a statistical tool (Excel, Microsoft).

RESULTS AND DISCUSSION

Bacterial Bioaerosols in Seven Public Restrooms

Table 1 shows the representative data of the bacterial bioaerosol concentrations measured from summer and winter seasons in each restroom inside the seven public facilities. We measured the concentrations roughly bimonthly over a year. Table 1 shows representative data from summer and winter in the seven restrooms. Hundreds of CFU/m^3 of bacterial bioaerosols were common in the seven public restrooms. As shown in Table 1, the bacterial bioaerosol concentrations in the restrooms of the hospital lobby were 350 ± 43 CFU/m^3 and 360 ± 44 CFU/m^3 during winter and summer, respectively, which were comparable with previous data for bacterial bioaerosols in hospitals, i.e. 404 ± 211 CFU/m^3 (Kim and Kim, 2007) in consideration of the large standard deviation. The concentrations of bioaerosols were significantly smaller during winter than summer at five locations, with statistically significant differences (t test p-values: 0.04, 0.01, 0.01, 0.04, and 0.02 < 0.05 for restrooms in a lecture building (old), student center, lecture building (new), library, and subway station, respectively), but with a statistically insignificant difference at one location (t test p-value: 0.1 > 0.05 for the restroom at the shopping center); the hospital lobby was the exception for seasonal significant variation in bioaerosol concentration. This inferred that the hospital closely maintained its indoor air conditions artificially via air conditioning systems, which was the reason for the similar concentration of bacterial bioaerosols inside hospitals during both winter and summer. The temperature and relative humidity in the hospital restrooms were 27.3 ± 0.1°C and 46.9 ± 0.1%, respectively, in summer and 20.9 ± 0.1°C and 41 ± 0.5%, respectively, in winter. In summer, the concentrations of bioaerosols in the restrooms of 1) the student center, 2) the library, and 3) the subway station were relatively higher than those in the other locations, with mostly statistically significant differences (based on 9 out of 12 t-test p-values as shown in Table 2). In winter,

Table 1. The number concentrations of bacterial bioaerosols in seven public restrooms at the given locations. Representative sampling dates were September 1, 2009 and February 1, 2010 for 9 measurement campaigns at fixed locations in one year, with at least three replications per measurement. The temperature and relative humidity are indicated with the bioaerosol number concentrations.

Location	Bioaerosol concentration (CFU/m^3) in summer (Sep. 2009).	Bioaerosol concentration (CFU/m^3) in winter (Feb. 2010)
Lecture building (old)	353 ± 90 (25.8 ± 0.1°C, 50.3 ± 0.2%)	113 ± 65 (13.9 ± 0.8°C, 31.2 ± 0.2%)
Student center	690 ± 85 (25.9 ± 0.1°C, 53.4 ± 1.0%)	130 ± 35 (9.7 ± 0.1°C, 44.6 ± 0.6%)
Lecture building (new)	403 ± 71 (27.6 ± 0.5°C, 38.1 ± 3.0%)	46 ± 15 (9.7 ± 0.1°C, 51.6 ± 0.3%)
Library	707 ± 216 (26.5 ± 0.2°C, 47.2 ± 1.5%)	310 ± 89 (13.8 ± 0.1°C, 44.9 ± 0.1%)
Hospital lobby	350 ± 43 (27.3 ± 0.1°C, 46.9 ± 0.1%)	360 ± 44 (20.9 ± 0.1°C, 41.1 ± 0.5%)
Subway station	770 ± 191 (27.2 ± 0.1°C, 50.0 ± 0.5%)	243 ± 83 (12.4 ± 0.1°C, 66.6 ± 0.6%)
Shopping center	287 ± 21 (25.7 ± 0.1°C, 49.0 ± 0.4%)	207 ± 55 (17.0 ± 0.3°C, 42.3 ± 0.7%)

the concentrations of bioaerosols in the restrooms of 1) the hospital lobby and 2) the library were higher than those in the other locations with mostly statistically significant differences (based on 7 out of 10 t-test p-values as shown in Table 3). The inner environment of the library was kept quiet and warm in winter; therefore, ventilation was limited during winter, which was inferred as the reason for the high bacterial bioaerosols concentration in the library restroom during winter.

Restroom Bacterial Bioaerosols, Outdoor Bioaerosols, and Number of Users

Table 4 shows the bioaerosol concentrations in the indoor restrooms and nearby outdoor environments. In five cases (all cases during summer, and the lecture building (new) and hospital lobby during winter), the concentrations of bacterial bioaerosols in the restrooms were clearly significantly higher, sometimes 20 times on an arithmetic scale, than those in nearby outdoor environments, with statistically significant differences (all t-test p values less than 0.05). In the case of the restroom in the lecture building (old) during winter, the concentration of bacterial bioaerosols was larger than that in nearby outdoor environments, with statistically insignificant differences (t-test p value 0.08 > 0.05). Therefore, overall, the air quality in public restrooms was established to be poorer than that in outdoor environments (although the concentration values were less than the Korean regulation limit of 800 CFU/m^3); many improvements will be required in the air quality of public restrooms in terms of the concentration of bacterial bioaerosols.

Table 2. T-test p-values between bioaerosol concentrations at seven restrooms during summer.

Location	Location	t-test p-values
Student center	Lecture building (old)	0.01 < 0.05
	Lecture building (new)	0.001 < 0.05
	Hospital lobby	0.02 < 0.05
	Shopping center	0.01 < 0.05
Library	Lecture building (old)	0.06
	Lecture building (new)	0.04 < 0.05
	Hospital lobby	0.06
	Shopping center	0.03 < 0.05
Subway station	Lecture building (old)	0.03 < 0.05
	Lecture building (new)	0.06
	Hospital lobby	0.03 < 0.05
	Shopping center	0.03 < 0.05

Table 3. T-test p-values between bioaerosol concentrations at seven restrooms during winter.

Location	Location	t-test p-values
Hospital lobby	Lecture building (old)	0.01 < 0.05
	Student center	0.01 < 0.05
	Lecture building (new)	0.005 < 0.05
	Subway station	0.09
	Shopping center	0.003 < 0.05
Library	Lecture building (old)	0.04 < 0.05
	Student center	0.02 < 0.05
	Lecture building (new)	0.01 < 0.05
	Subway station	0.16
	Shopping center	0.16

Another detail shown in Table 4 is that the outdoor bacterial bioaerosol concentrations during winter were smaller than those during summer, with statistically significant differences for the outdoor environments of the lecture building (new) and hospital lobby (t-test p values were 0.03 and 0.04 for the respective outdoor environments), but a statistically insignificant difference for the outdoor environment of the lecture building (old) (t-test p values: 0.11 > 0.05). The experimental results from the quantitative bacteria bioaerosol measurements confirmed that there were more bacteria bioaerosols in outdoor environments during summer than winter, implying that summer was superior to winter for the growth of environmental bacteria (Nielsen *et al*., 1997, 2000).

Fig.1 shows the relationship between the numbers of users and the numbers of bacterial bioaerosols in public restrooms. Due to the large seasonal variations in the concentration of bioaerosols, our focus was on the measurement data collected in February. Five measurement campaigns were conducted during February 2010. For this analysis, our focus was on the data collected from three restrooms, viz., those in the library, hospital, and shopping center, as these public buildings had strong inside air-conditioning systems; therefore, the effects of the outside environments would be weaker than in the

Table 4. The number concentration of bacterial bioaerosols in three restrooms and nearby outdoor locations.

Location	Bioaerosol concentration (CFU/m^3) in summer (Sep. 2009).	Bioaerosol concentration (CFU/m^3) in winter (Feb. 2010)
Restroom, Lecture building (old)	353 ± 90 (25.8 ± 0.1°C, 50.3 ± 0.2%)	113 ± 65 (13.9 ± 0.8°C, 31.2 ± 0.2%)
Outdoor, Lecture building (old)	83 ± 50 (23.9 ± 0.3°C, 49.3 ± 0.3%)	27 ± 23 (4.8 ± 0.6°C, 55.9 ± 1.7%)
Restroom, Lecture building (new)	403 ± 71 (27.6 ± 0.5°C, 38.1 ± 3.0%)	46 ± 15 (9.7 ± 0.1°C, 51.6 ± 0.3%)
Outdoor, Lecture building (new)	130 ± 60 (32.6 ± 2.0°C, 27.7 ± 2.3%)	13 ± 6 (7.4 ± 1.0°C, 43.2 ± 0.9%)
Restroom, Hospital lobby	350 ± 43 (27.3 ± 0.1°C, 46.9 ± 0.1%)	360 ± 44 (20.9 ± 0.1°C, 41.1 ± 0.5%)
Outdoor, Hospital lobby	80 ± 36 (26.4 ± 0.4°C, 42.2 ± 0.5%)	17 ± 12 (6.1 ± 0.5°C, 55.0 ± 0.5%)

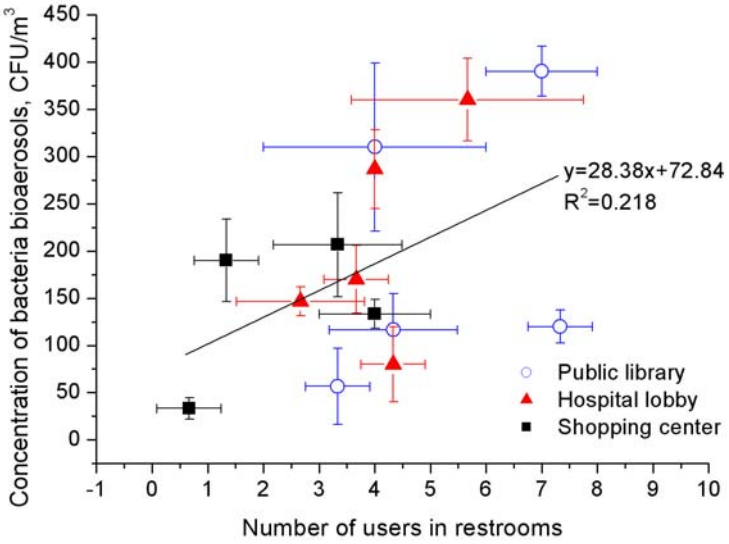

Fig. 1. Number of users during measuring experiments and the concentration of bacterial bioaerosols in public restrooms.

other locations when conducting the winter measurements. Also, there was some variation in the actual numbers of users in these locations compared to the other locations during the measurement campaigns. As shown in Fig. 1, restrooms with more users tended to have high concentrations of bacterial bioaerosols ($y = 28.38x + 72.84$; $R^2 = 0.218$). The human activities inside restrooms, such as washing and excretion, were estimated to produce airborne bacterial bioaerosols inside public restrooms. However, the relationship was not strictly linear and depended on the location of the public restroom. Here, the maximum number of users was less than 10 persons while conducting the measurements, which was a limitation of this study. The measurements in very large scale public restrooms (although these are rare) and at various time points over one day will be future research topics. Although reasonable experimental conditions for incubation were chosen as confirmed in our previous investigations (Hwang *et al*., 2010; Lee *et al*., 2010), the types of bio-samplers, agars, and incubation conditions may affect the bioaerosols concentration data, which are also other possible parameters requiring further research.

CONCLUSIONS

The bacterial bioaerosol concentrations were measured in seven public restrooms. Seasonal variations were observed in both restroom and outdoor bioaerosols concentrations and there were more bioaerosols in summer than in winter seasons. The number of humans affected the increment of the concentrations of bacterial bioaerosol in the restrooms; however, the relationship was not clear. As there is a strong demand for people to use safe public restrooms, the information from the study constitutes an initial step in the monitoring and controlling of bacterial bioaerosols in restrooms that can contribute to the improvement of the hygiene and public health in public places. More extensive measurements over several years with various parameters will be required to ascertain the practical, cumulative concentrations of bioaerosols in public places.

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DISCLAIMER

Reference to any companies or specific commercial products does not necessarily constitute or imply their endorsement, recommendation, or favoring by the Konkuk

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