

Bioaerosols as contributors to poor air quality in Taichung City, Taiwan

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Abstract Bioaerosols are a type of suspended sediments that contribute to poor air quality in Taiwan. Bioaerosols include allergens such as: fungi, bacteria, actinomycetes, arthropods and protozoa, as well as microbial products such as mycotoxins, endotoxins and glucans. When allergens and microbial products are suspended in the air, local air quality will be influenced adversely. In addition, when the particle size is small enough to pass through the respiratory tract entering the human body, the health of the local population is also threatened. Therefore, the purpose of this study is to attempt to understand the concentration and types of bacteria and the bacteria numbers for various particle size ranges during a study period of June 2005 to February 2006 in Taichung City, Central Taiwan. The results indicate that the total average bacterial concentration by using NA medium incubated for 48 h were 8.0×10^2 , 1.4×10^3 ,

2.4×10^3 and 1.3×10^3 , 1.9×10^3 , 3.5×10^3 cfu/m³ for CMES, TRIPS and RFS sampling sites during the daytime and nighttime period of June 2005 to February 2006. Moreover, the total average bacterial concentration by using R₂A medium incubated for 48 h were 8.5×10^2 , 1.5×10^3 , 2.2×10^3 and 1.2×10^3 , 1.7×10^3 , 2.5×10^3 cfu/m³ for CMES, TRIPS and RFS sampling sites the daytime and nighttime during the same sampling period. The total average bacteria concentration was the same in either NA or R₂A medium for the same sampling times or sites. The total average bacterial concentration was higher in daytime period than that of nighttime period for CMES, TRIPS and RFS sampling sites. The high average bacterial concentration was found in the particle size range of 0.53–0.71 mm (average bioaerosol size was in the range of 2.1–4.7 µm) for each sampling site. Also, 20 kinds of bacteria exceeded levels for each sampling site and were classified as according to shape: rod, coccus and filamentous.

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Introduction

Bioaerosols are usually defined as aerosols or particulate matter of microbial, plant or animal origin and are often used synonymously with organic

dust. This category of suspended particulate matter includes pathogenic or non-pathogenic, live or dead bacteria and fungi, viruses, actinomycetes, arthropods, high molecular weight allergens, bacterial endotoxins, mycotoxins, peptidoglycans, glucans, pollen and plant fibres. (Lighthart 1997; Matthais-Maser et al. 2000; Hargreaves et al. 2003; Jones and Harrison 2004; Zollinger et al. 2006). Meteorological variables affect the initial release of these materials and their dispersal once airborne. Temperature and water availability also affect the size of the source and control the release of some actively released fungal spores (Jones and Harrison 2004).

Exposure to bioaerosols within the occupational environment are associated with a wide range of health effects, including infectious diseases, acute toxic effects, allergies and cancer (Douwes et al. 2003; Jo and Seo 2005; Lee and Jo 2006; Taha et al. 2006; Fang et al. 2007; Özkara et al. 2007; Fierer et al. 2008; Rusca et al. 2008). Moreover, the various types of material that are found in bioaerosols have a wide range of sizes (Reponen et al. 2001; Jones and Harrison 2004). When a particle size is small enough to pass through the respiratory tract and the human body, the health of the local population is threatened. The purpose of this study is to understand the concentration and types of bacteria during a study period between June 2005 and February 2006 in Taichung City, Central Taiwan.

Materials and methods

Sampler

An Andersen six-stage viable sampler was used for sample collection. The six-stage viable particle sampler is a multi-orifice, cascade impactor, which is normally used to measure the concentration and particle size distribution of aerobic bacteria and fungi in ambient air. Each stage contains 400 orifices. The jet orifice dimensions were 1.18, 0.91, 0.71, 0.53, 0.34 and 0.25 mm for particle sizes of 7.0 μm above, 4.7–7.0, 3.3–4.7, 2.1–3.3, 1.1–2.1 and 0.65–1.1 μm from first stage to sixth stage, respectively.

Medium

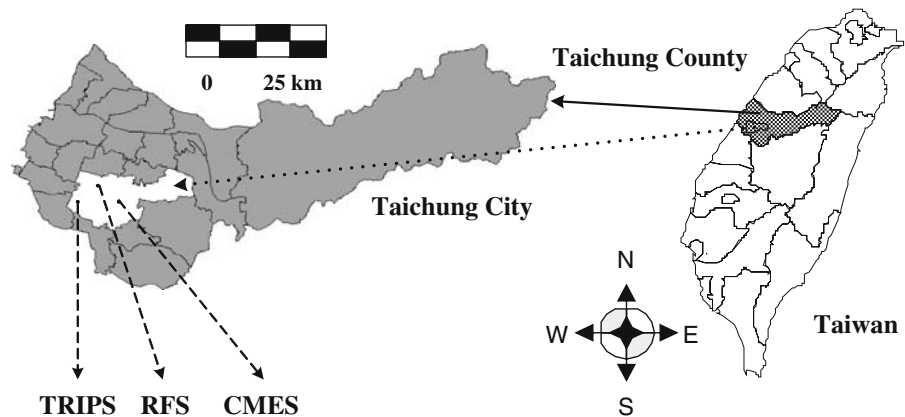
The media employed in this study were nutrient agar (NA) and R₂A. NA is used for the cultivation of bacteria and for the enumeration of organisms in air, water, sewage, faeces and other materials, as it is a general purpose culture medium used for the growth of a large variety of non-fastidious microbial species. R₂A is a medium with low nutrient content and used for the cultivation of heterotrophic bacteria. Collection plates are prepared by aseptically pipetting 27 ml of sterile NA (R₂A; 45–50°C) into each of the sampling dishes. The composition of NA included: 3 g/L beef extract, 5 g/L peptone and 15 g/L agar. The composition of R₂A included: 0.5 g/L yeast extract, 0.5 g/L proteose peptone, 0.5 g/L casein hydrolysate, 0.5 g/L glucose, 0.5 g/L soluble starch, 0.3 g/L sodium pyruvate, 0.3 g/L di-potassium hydrogen phosphate, 0.05 g/L magnesium sulfate and 12 g/L agar.

Sampling site

Figure 1 shows the sampling sites for this study. The sampling sites include Chung-Ming Elementary Sampling Site (CMES), Taichung Refuse Incineration Plant Sampling Site (TRIPS) and Rice field sampling site (RFS). CMES is located on the middle of Taichung City, on the corner of Taichung Harbor Road and Chung-Ming Nan Road on the side of Chung-Ming elementary. Taichung Harbor Road and Chung-Ming Nan Road have a traffic density of 10,000 vehicles per hour during the sampling period. TRIPS is located on the west side of Taichung City. It is near the entrance of the Taichung Refuse Incineration Plant and on the side of Wen-Shen Golf Course. Garbage trucks often pass by the front of the site at a density of 80 vehicles per hour. RFS is located on the northwest side of Taichung City. It is near a rice field, and the traffic density is very low (about 120 vehicles per hour).

Sampling process

Samples were taken during the period of 30 June, 2005 to 11 February, 2006. The sampling times were 8–12 A.M. and 6–10 P.M. The total number of

Fig. 1 The bioaerosols sampling sites

sampling days was 17. The height of the all sampling sites is approximately 1.5 m above ground level. The average humidity and temperature at the sampling sites during the period of June 2005 to February 2006 are shown in Table 1.

Each collection plate, with the cover removed, is inserted on each stage of the sampling instrument. After starting the pump, the air to be sampled enters the inlet cone and cascades through the succeeding orifice stages with successively higher orifice velocities from stages 1 to 6. Successively smaller particles are inertially impacted onto the NA (R_2A) collection surfaces. Viable particles are retained on the agar plates, and the exhaust air is carried through the vacuum hose to the vacuum source. The flow rate is 28.3 L/min and the sampling time is 3 min. After the sampling is complete, the NA (R_2A) collection plates are removed from the sampling instrument, and the cover is replaced on each Petri dish. All agar plates are inverted to prevent condensation drip then placed in an incubator at 35°C for 12–60 h. After incubation, the number of colonies on each plate is counted using a standard bacterial colony counter. The average number of viable particles (aerobic bacteria) per unit volume of air is calculated using

the following formula: the average concentration of bacteria (cfu/m^3) = number of colony (cfu) $\times$ 1000 (l/m^3) / [28.3 (l/min) $\times$ 3 min]

To measure the bacterial numbers for various particle size ranges, the average bacterial concentration was calculated in terms of $dn/d \log dp$ (where n is the average bacterial concentration at 48 h; and p is the particle size).

The preparation of experiment, sampling and the calculation of the total bacterial number were all conducted with caution. And the procedure for all the researchers is needed to be aseptic with 70% ethanol. Besides, this aseptic procedure was carried out three times prior to each sampling period.

Results and discussion

CMES

Table 2 shows the average concentration of bacteria for daytime and nighttime sampling periods at the CMES sampling site during June 2005 to February 2006 by using NA and R_2A medium. While the unit area bacterial number for different incubated time at various particles size ranges is

Table 1 The average humidity and temperature at the sampling sites during the period of June 2005 to February 2006

Data item	2005							2006	
	June	July	August	September	October	November	December	January	February
Temperature (°C)	27.5	28.8	27.9	28.2	25.7	23.3	16.8	17.7	18.3
Humidity (%)	80	76	82	77	73	73	72	80	78

Table 2 Average concentration of bacteria for daytime and night-time periods at CMES sampling site in Taichung City, Central Taiwan during June 2005 to February 2006 by using NA and R₂A media

Range of particle sizes (α m)	Incubation time (h)	Average bacterial concentration (cfu/m ³)			
		NA medium		R ₂ A medium	
		Daytime	Nighttime	Daytime	Nighttime
0.65–1.1	12	13	12	12	7
	24	57	46	40	64
	36	82	90	71	106
	48	128	176	141	133
	60	146	200	185	135
1.1–2.1	12	11	32	4	19
	24	44	92	65	70
	36	92	197	114	245
	48	152	264	170	325
	60	212	363	234	424
2.1–3.3	12	6	40	8	35
	24	83	211	68	103
	36	160	389	154	285
	48	229	502	236	393
	60	309	581	315	524
3.3–4.7	12	14	40	7	25
	24	58	87	61	68
	36	111	154	159	138
	48	193	191	213	171
	60	234	240	258	230
4.7–7.0	12	5	34	4	24
	24	33	114	25	70
	36	63	178	59	130
	48	95	195	87	149
	60	190	232	111	188

also presented, the results show that the average bacterial concentration increased as the incubated time increased for each particle size ranges. This result is independent of sampling times and medium types.

Table 3 shows the unit particle size average for bacterial concentrations at 48 h incubation time during daytime and nighttime periods at the CMES sampling site during June 2005 to February

2006 by using NA and R₂A media. When the medium was NA, the results reflected that the unit particle size average bacterial concentrations was 560, 541, 1,167, 1,257, 549 and 770, 940, 2,557, 1,244, 1,127 cfu/m³/μm during daytime and nighttime periods for particle sizes of 0.65–1.1, 1.1–2.1, 2.1–3.3, 3.3–4.7 and 4.7–7.0 μm, respectively. When R₂A medium was used, the results showed that the unit particle size average bacterial

Table 3 The unit particle size average bacterial concentrations at 48-h incubation time for daytime and nighttime periods at CMES sampling site in Taichung City, Central Taiwan during June 2005 to February 2006 by using NA and R₂A media

Range of particle sizes (α m)	The unit particle size average bacterial concentrations (cfu/m ³ / α m)			
	NA medium		R ₂ A medium	
	Daytime	Nighttime	Daytime	Nighttime
0.65–1.1	560	770	617	582
1.1–2.1	541	940	605	1,157
2.1–3.3	1,167	2,557	1,202	2,002
3.3–4.7	1,257	1,244	1,387	1,113
4.7–7.0	549	1,127	503	861

Table 4 Average concentration of bacteria for daytime and night-time periods at TRIPS sampling site in Taichung City, Central Taiwan during June 2005 to February 2006 by using NA and R₂A media

Range of particle sizes (α m)	Incubation time (h)	Average bacterial concentration (cfu/m ³)			
		NA medium		R ₂ A medium	
		Daytime	Nighttime	Daytime	Nighttime
0.65–1.1	12	5	28	8	5
	24	43	80	44	149
	36	86	162	83	174
	48	110	205	115	196
	60	142	243	133	220
1.1–2.1	12	10	18	20	23
	24	148	128	211	100
	36	230	415	319	280
	48	329	552	424	381
	60	446	674	524	508
2.1–3.3	12	27	35	36	32
	24	137	178	160	126
	36	239	555	277	586
	48	378	657	380	717
	60	555	762	517	862
3.3–4.7	12	17	25	29	19
	24	114	81	140	67
	36	239	244	218	198
	48	348	283	297	241
	60	380	353	337	316
4.7–7.0	12	10	30	14	31
	24	81	87	95	69
	36	170	165	194	143
	48	244	199	247	197
	60	288	226	296	264

concentrations was 617, 605, 1,202, 1,387, 503 and 582, 1,157, 2,002, 1,113, 861 cfu/m³/μm during daytime and nighttime periods for particle sizes of 0.65–1.1, 1.1–2.1, 2.1–3.3, 3.3–4.7 and 4.7–7.0 μm, respectively. The results indicate that the particle sizes of 3.3–4.7 and 2.1–3.3 μm have the largest bacteria number when compared with the rest of the other particle size ranges for either daytime or nighttime sampling period, respectively.

TRIPS

The average concentration of bacteria for daytime and nighttime periods at TRIPS sampling site in Taichung City, Central Taiwan during June 2005 to February 2006 by using NA and R₂A medium is shown in Table 4. Also shown is that the average bacterial concentration increased as the incubated time increased for each particle size range.

Table 5 The unit particle size average bacterial concentrations at 48 incubation time for daytime and night-time periods at TRIPS sampling site in Taichung City, Central Taiwan during June 2005 to February 2006 by using NA and R₂A media

Range of particle sizes (α m)	The unit particle size average bacterial concentrations (cfu/m ³ / α m)			
	NA medium		R ₂ A medium	
	Daytime	Nighttime	Daytime	Nighttime
0.65–1.1	481	897	503	858
1.1–2.1	1,172	1,966	1,510	1,357
2.1–3.3	1,926	3,347	1,936	3,653
3.3–4.7	2,266	1,843	1,934	1,569
4.7–7.0	1,410	1,150	1,428	1,139

Table 6 Average concentration of bacteria for daytime and nighttime periods at RFS sampling site in Taichung City, Central Taiwan during June 2005 to February 2006 by using NA and R₂A media

Range of particle sizes (α m)	Incubation time (h)	Average bacterial concentration (cfu/m ³)			
		NA medium		R ₂ A medium	
		Daytime	Nighttime	Daytime	Nighttime
0.65–1.1	12	35	96	44	55
	24	95	248	204	132
	36	164	362	302	179
	48	200	455	367	207
	60	219	518	409	226
1.1–2.1	12	24	82	45	71
	24	371	310	218	229
	36	728	672	299	479
	48	930	860	434	558
	60	1,007	966	518	653
2.1–3.3	12	92	98	30	105
	24	208	349	165	366
	36	503	875	258	658
	48	675	994	364	749
	60	979	1,130	448	837
3.3–4.7	12	35	118	45	97
	24	158	237	342	305
	36	262	483	428	426
	48	324	655	719	504
	60	360	702	774	643
4.7–7.0	12	26	104	33	129
	24	104	237	148	286
	36	171	411	215	392
	48	251	504	290	450
	60	305	687	335	534

The unit particle size average bacterial concentrations at 48 h incubation time for daytime and nighttime periods at TRIPS sampling during June 2005 to February 2006 by using NA and R₂A medium is shown in Table 5. It indicates that the particle sizes of 3.3–4.7 and 2.1–3.3 μ m have the largest bacteria number when compared with the rest of the other particle size ranges for either daytime or nighttime sampling periods, respectively.

RFS

Table 6 shows the average concentration of bacteria for daytime and nighttime periods at RFS sampling site in Taichung City, Central Taiwan during June 2005 to February 2006 by using NA and R₂A media. It shows the unit area bacterial number for different incubated time at various particles size ranges. The results also show that the average

Table 7 The unit particle size average bacterial concentrations at 48 incubation time for daytime and nighttime periods at RFS sampling site in Taichung City, Central Taiwan during June 2005 to February 2006 by using NA and R₂A media

Range of particle sizes (α m)	The unit particle size average bacterial concentrations (cfu/m ³ / α m)			
	NA medium		R ₂ A medium	
	Daytime	Nighttime	Daytime	Nighttime
0.65–1.1	875	1,991	1,606	906
1.1–2.1	3,312	3,062	1,545	1,987
2.1–3.3	3,439	5,064	1,854	3,816
3.3–4.7	2,110	4,265	4,681	3,282
4.7–7.0	1,451	2,913	1,676	2,601

Table 8 The average kinds of bacteria at three sampling sites from the shape, size and colour of the colony for daytime and nighttime periods

Sampling site	The average bacteria kind			
	NA medium		R ₂ A medium	
	Daytime	Nighttime	Daytime	Nighttime
CMES	27	25	25	20
TRIPS	22	20	29	25
RFS	22	20	22	20

bacterial concentration increased as the incubated time increased for each particle size range and that this result is independent of sampling times and medium types.

Table 7 shows the unit particle size average bacterial concentrations at 48 h incubation time for daytime and nighttime periods at RFS sampling site in Taichung City, Central Taiwan during June 2005 to February 2006 by using NA and R₂A media. The table indicates that the particle sizes of 3.3–4.7 and 2.1–3.3 μm have the largest bacterial numbers when compared with the rest of the other particle size ranges for either daytime or nighttime sampling periods, respectively.

Shape and type of strain at the three sampling sites

Table 8 shows the bacterial types according to shape, size and colour of the colony for daytime and nighttime periods at the three sampling sites. Also shown is that the average bacterial types at three sampling sites were over 20. In addition, the colonies were purified, stained and observed by microscopy, concluding that rod-shaped, filamentous and coccal-shaped bacteria existed at all three sampling sites (Fig. 2).

Discussion and summary

Figure 3 shows the total average concentration of bacteria for daytime and nighttime periods at three sampling sites in Taichung City, Central Taiwan during June 2005 to February 2006 by using NA and R₂A media. Total average bacterial concentration is the sum of the average bacterial concentration for each particle size ranges. According to Fig. 3, the results indicate that the total average bacterial concentration increased as the incubated time increased. This result is independent of sampling site, sampling time and medium type. Compared to the rest of the other sampling sites, the result revealed that the RFS has the highest bacterial concentration (when the medium was NA, the total average bacterial concentrations at 48-h incubation time during daytime and nighttime periods were 8.0×10^2 , 1.4×10^3 , 2.4×10^3 and 1.3×10^3 , 1.9×10^3 , 3.5×10^3 cfu/m³ for sampling sites of CMES, TRIPS and RFS, respectively. When the medium was R₂A, the total average bacterial concentrations at 48-h incubation time during daytime and nighttime periods were 8.5×10^2 , 1.5×10^3 , 2.2×10^3 and 1.2×10^3 , 1.7×10^3 , 2.5×10^3 cfu/m³ for sampling sites of CMES, TRIPS and RFS, respectively). The

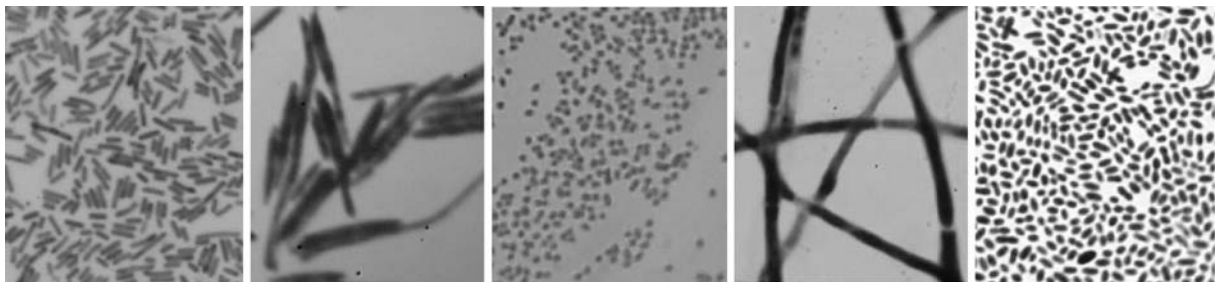
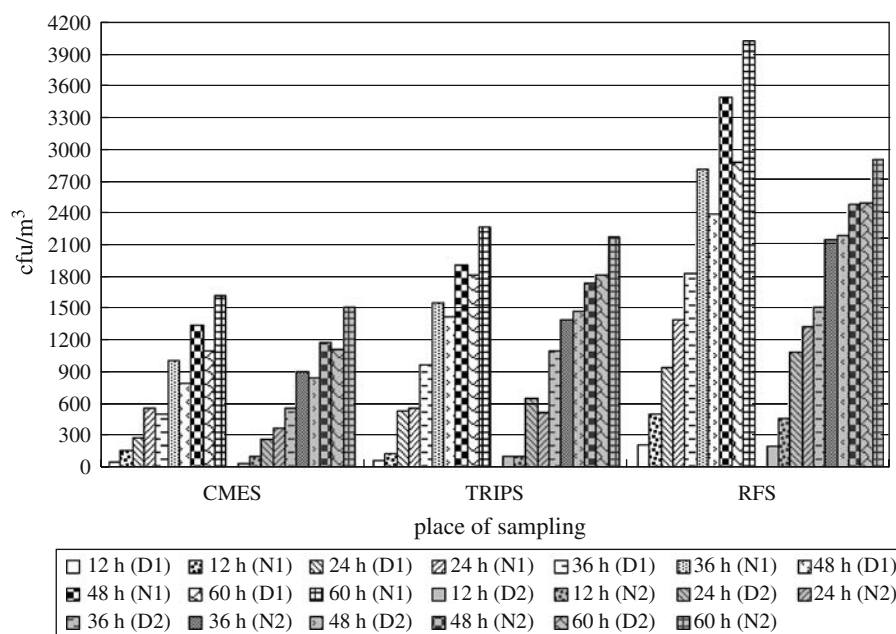
**Fig. 2** Microscopy picture of bacteria coming from three sampling sites in Taichung City, Central Taiwan during June 2005 to February 2006 (1,000 $\times$)

Fig. 3 Total average concentration of bacteria for daytime and night-time periods at three sampling sites in Taichung City, Central Taiwan during June 2005 to February 2006 by using NA and R₂A medium (*D* daytime; *N* nighttime; 1 NA medium for using; 2 R₂A medium for using)



postulated reason here is that this sampling site is near the rice ground. The high concentration of bacteria was tended to be founded in straw. In addition, the straw reaping and burning were usually caused the high concentration of bacteria in the atmospheric environment. The above-mentioned factors lead to the high total average bacterial concentration at the RFS sampling site. However, the high concentration of bacteria found at the TRIPS sampling site was caused by garbage trucks passing by during the sampling period, landfill size, incinerator and the nearby golf course. Although a high concentration (10^4 cfu/m³) of bacteria was found at the RFS sampling site, this value did not exceed 10^4 cfu/m³ regulated in 1986 by American Conference of Governmental Industrial Hygienist (Lin et al. 2004). Thus, it will likely not impact the surrounding local ecosystem.

Figure 3 also indicates that the total average bacterial concentration was the same in either NA or R₂A media for the same sampling times or sites. In addition, it also shows that the total average bacterial concentration was higher in daytime period than that of nighttime period for CMES, TRIPS and RFS sampling sites. The proposed reason still needs further studies to verify the cause of this finding.

Tables 3, 5 and 7 indicate that high average bacterial concentrations were found in the particle size range of 0.53–0.71 μ m (average bioaerosol size was in the range of 2.1–4.7 μ m). In general, aerosols which are greater than 3 μ m are easily deposited in bronchial passages, while aerosols which are less than 3 μ m in size are easily deposited in pulmonary alveola (Heyder et al. 1986). Though the average bacterial concentration obtained at three sampling sites may have no significant impact to local ecosystem, the influence on lung disease patients still needs further study.

Conclusions

The total average bacterial concentration were 8.0×10^2 , 1.4×10^3 and 2.4×10^3 cfu/m³ by using NA medium incubated for 48 h at sampling sites of CMES, TRIPS and RFS during the daytime period of June 2005 to February 2006.

The total average bacterial concentration were 1.3×10^3 , 1.9×10^3 and 3.5×10^3 cfu/m³ by using NA medium incubated for 48 h at sampling sites of CMES, TRIPS and RFS during the nighttime period of June 2005 to February 2006.

The total average bacterial concentration were 8.5×10^2 , 1.5×10^3 and 2.2×10^3 cfu/m³ by using R₂A medium incubated for 48 h at sampling sites of CMES, TRIPS and RFS during the daytime period of June 2005 to February 2006.

The total average bacterial concentration were 1.2×10^3 , 1.7×10^3 and 2.5×10^3 cfu/m³ by using R₂A medium incubated for 48 h at sampling sites of CMES, TRIPS and RFS during the nighttime period of June 2005 to February 2006.

The total average bacteria concentration was the same in either NA or R₂A media for the sampling times or sites.

The total average bacterial concentration was higher in daytime period than that of nighttime period for CMES, TRIPS and RFS sampling sites.

The high average bacterial concentration was found in the particle size range of 0.53–0.71 μ m (average bioaerosol size was in the range of 2.1–4.7 μ m) for each sampling site. Moreover, there exceeded 20 different kinds of bacteria for each sampling site and the bacterial types were rod, coccid and filamentous.

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