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Background Bioaerosols and Aerosols at Two Sites in Northern Australia: Preliminary Measurements

R. Ian Tilley, Jim Ho
and Derek Eamus

DSTO-TR-1203

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Combatant Protection and Nutrition Branch
Aeronautical and Maritime Research Laboratory

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ABSTRACT

The biological aerosol content of the air has been measured at Darwin and RAAF Base Tindal in the Northern Territory of Australia. The number of culturable bacteria and fungal spores together with organic material caught in a spore trap are reported. The total airborne particulate matter in the size range 0.5 to 30 microns was also measured at Tindal in October 1998 and April 1999. The ability of an aerodynamic particle sizer to act as an early warning device for the presence of biological warfare agents in this environment is discussed. It was concluded that such a device could only be successfully used as a trigger in the event of a military release of a biological agent in an environment with a well characterised aerosol background.

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Background Bioaerosols and Aerosols at Two Sites in Northern Australia: Preliminary Measurements

Executive Summary

Detection of airborne aerosols of biological warfare (BW) agents must be accomplished against a background of naturally occurring bioaerosols. Knowledge of this natural background will allow an operator to optimise the ability of detection instrumentation to identify potentially harmful airborne microorganisms.

DSTO has recently commenced a program of work in the detection of BW agents that includes background bioaerosol characterisation. Consequently a series of measurements have been made of the bioaerosol content of the air at two sites of military interest, namely Darwin and RAAF Base Tindal in the Northern Territory.

Airborne bacteria, fungal spores and other biological material in the respirable size range of 1 to 10 microns were trapped and characterised. The nature and quantities of these airborne materials are reported.

Also the ability of a commercially available particle sizing instrument to act as an early warning device in the detection of BW agents has been assessed at RAAF Base Tindal. It was found that under favourable conditions such an instrument could have a role in BW agent detection and warning.

The work reported is part of an ongoing program to keep DSTO staff conversant with the techniques and methodologies that underpin effective defence against BW agents. This allows DSTO to provide accurate and timely advice to the ADF if and when they are required to operate in a BW environment.

Authors

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Ian has a Ph.D. in solution chemistry and has worked in the NBC area of DSTO for more than 20 years. His work has encompassed the material and physiological aspects of NBC protective clothing, the chemistry of sulfur mustard and the detection of chemical and biological warfare agents. His current interest is in bioaerosols.

Jim Ho
Defence Research Establishment Suffield, Canada

Jim has a Ph.D. from the University of Kentucky and has worked in the biological warfare (BW) agent detection field for nearly 20 years. Jim is an internationally recognised expert in the fields of biological aerosols and their detection. Jim was instrumental in developing and building the first fluorescence aerodynamic particle sizer (FLAPS) for real time detection of biological aerosols.

Derek Eamus
University of Technology, Sydney

Derek is a plant physiologist and ecophysiologist. From 1990 to 2000 he was a member of the CRC for Tropical Savannas and the Northern Territory University. He was recently appointed to the Chair of Environmental Sciences at the University of Technology, Sydney.

Contents

1. Introduction

Detection and early warning of the deliberate release of an aerosol of pathogenic microorganisms is an essential element of defence against biological warfare (BW) agents. Instruments have been developed which can monitor the air at a given site for the presence of generic bioaerosols which are not normally present in the background. These trigger devices or sentries can be programmed to alarm when a preset change in the aerosol content of the air occurs. Procedures for the identification of the specific BW agent present can then be initiated. This approach requires that the native or normal background bioaerosol content of the air at a given site be characterised. This report describes bioaerosol measurements, made at Darwin and RAAF Base Tindal in northern Australia, designed to provide preliminary information on the background bioaerosol content of the air at these sites.

2. Experimental

2.1 Measurement Sites

2.1.1 Darwin, Australia

Darwin is located on the northern coast of Australia at latitude 12.4° South and longitude 130.8° East. Two measurement sites were used on the Casuarina campus of the Northern Territory University (Casuarina is a seaside residential suburb of Darwin). The first site was used in March 1998 and was located 1 km west of the Science Faculty Building. The second site was located 200m east of the Science Faculty Building.

2.1.2 Katherine, Australia (RAAF Base Tindal)

Katherine is located about 300 km south of Darwin at latitude 14.5° South and longitude 132.3° East. The climate is tropical and the terrain is open country with a moderate cover of small trees and grasses. Measurements were made in October 1998 and April 1999 at RAAF Base Tindal which is about 17 km south of Katherine.

2.2 Instrumentation

2.2.1 Meteorology

In Darwin the wind speed and direction, air temperature and relative humidity (RH), and photosynthetically active solar radiation (PAR; 400-700 nm) were logged. Air temperature, RH and PAR were sampled every minute and 15-minute averages recorded on data loggers using an Environdata (Environdata, Queensland, Australia) weather station. Wind speed and direction were logged on a Campbell 21-X logger using a commercial wind direction/speed system. At Tindal in April 1998 wind speed

and direction, air temperature and RH were obtained from the Australian government Bureau of Meteorology weather station located on the air base. At Tindal in April 1999 the meteorological data were obtained from instruments included with the Canadian fluorescence aerodynamic particle sizer (FLAPS).

2.2.2 Aerosol/Bioaerosol Measuring Instruments

Counts and size distributions of airborne particles (0.5 to 30 microns aerodynamic diameter) were obtained using an aerodynamic particle sizer (APS 3310, TSI Inc., St. Paul, MN, USA).
Viable airborne microorganisms, mainly bacteria and fungal spores, were collected with a slit-to-agar (STA) biological air sampler (New Brunswick Scientific Co., New Jersey, USA). Air (35 L/min.) at constant velocity was drawn through a small slit into a chamber containing an agar plate mounted horizontally which was set to rotate once in 24 minutes.
A second bioaerosol sampler used was a recording volumetric spore trap (RVST, Burkard Manufacturing Co., UK). A glass slide coated in a suitable sticky medium and located inside a drum moved vertically past a narrow inlet slit over a period of 24 hours. Air passed through the slit at 10 L/min. The drum had an attached wind vane which ensured that the slit always faced the prevailing wind.
A fluorescence aerodynamic particle sizer (FLAPS) developed by Canadian defence scientists at the Defence Research Establishment Suffield was used at Tindal in April 1999. Air was passed at 350 L/min. into a pre-concentrator and fed at 1 L/min. to the FLAPS detector. Biological particles having aerodynamic diameters of 2.5 to 14.5 microns were detected by sensing the fluorescent light emitted by particles after excitation by a UV laser.

2.3 Methodology

2.3.1 Darwin

Measurements made in Darwin were preliminary in nature and were designed to familiarise research personnel with instrumentation and techniques and to develop methodologies for sampling bioaerosols in a tropical environment. Measurements were made with the STA and RVST. The STA was used 11 times over a one month period in March and April 1998. Times of collection varied between 0900h and 1600h. The RVST was used over three separate 24 hour periods in March 1998, generally starting at either 1000h or 1400h. Agar plates were stored in the dark at 37° in a temperature controlled cabinet for up to 7 days. Counts of bacterial and fungal colonies were made periodically during the 7 days.
Five to eight bacterial colonies were removed from 10 randomly selected agar plates and stained for gram positive and gram negative identification using standard tests. Bacteria were also identified as either rod or cocci.
RVST slides were stored in the dark at 4° prior to counting. Counts were made of the number of particles of ash, sand, salt crystals, pollen and spores.

2.3.2 RAAF Base Tindal, October 1998

Sampling was done in daylight hours over five consecutive days. Agar plates were exposed in the early morning, mid-morning, midday, mid-afternoon and late afternoon. The APS was used to collect one-minute counts while the STA sampler was operating. After incubation the bacterial and fungal colonies were counted and characterised as described above.

2.3.3 RAAF Base Tindal, April 1999

The STA, RVST, APS and FLAPS were operated continuously for one 48-hour period (12 noon, April 13 to 12 noon, April 15). Additional measurements using the RVST, APS and FLAPS were made as circumstances permitted. APS counts were recorded every 5-minutes during the period 1146h, April 13 to 2358h, April 17. STA plates were exposed for 24-minute periods with a total of 48 plates collected in a 24-hour period. After exposure, plates were incubated and bacteria and fungal colonies counted and characterised as described above.
The RVST sampled the air from 1600h on April 13 to 0923h on April 18 providing 6 slides. The latter were analysed using an Olympus BH2-UMA Metallurgical microscope (reflected light, 500x magnification) and numbers of pollen, spores and other material collected were determined. Hourly counts were made in the size ranges 2-5, 5-10, 10-15 and >15 microns for fungal spores and organic debris and 15 to >30 microns for pollens. In all cases the abundance of fungal palynomorphs was so great that counts were expressed as estimates rather than absolute numbers. Identification of pollens was restricted in most cases to families (Poaceae, Cyperaceae, Asteraceae, Acanthaceae, Myrtaceae) and genera (*Acacia, Pandanus*). Biogenic silica bodies, such as phytoliths and sponge spicules, which are of biological origin but are not organic, were not counted.
Measurements made using the FLAPS are the subject of a separate report by Dr. Jim Ho of the Defence Research Establishment Suffield, Canada.

3. Results

3.1 Darwin

3.1.1 Microclimate

Wind direction in the morning between midnight and midday tended to be either northerly (between 320 and 30 degrees) or east south easterly (between 100 and 140 degrees). In contrast the afternoon wind direction tended to be predominantly northerly (between 280 and 10 degrees). Wind speed was uniformly low (mostly below 2 m s^{-1} and never above 3 m s^{-1}). Air temperature mostly varied between 24° and 37° although occasional periods of very high temperature (>40°) were recorded. Relative humidity varied between 25%, typically in mid afternoon, and 98% at night.

3.1.2 Bacterial and Fungal Counts

Bacterial counts on the 11 plates exposed ranged from 21 to 81 with an average count of 45.8. Numbers of fungal colonies ranged from 10 to 47 with an average of 24.5. These values equate to an atmospheric loading of 58 m^{-3} for bacteria and 31 m^{-3} for fungal spores. Two of the 11 plates were exposed 3 hours after a significant rain event giving bacterial and fungal counts of 21 and 10 respectively. These values are approximately 50% lower than the mean of the other samples and are consistent with wash out or wet deposition of bacteria and fungal spores from the air.
A random sample of 62 bacterial colonies taken from 8 plates was found to consist of 33 Gram-positive rods (53%), 16 Gram-positive cocci (26%), 12 Gram-negative rods (19%) and 1 Gram-variable bacterium (2%). It can be seen that the ratio of Gram-positive to Gram-negative bacteria was approximately 4:1.

3.1.3 Spore and Pollen Counts

The distribution of spores and organic debris on the slides was markedly discontinuous. It was evident that diurnal changes in bioaerosol density occurred although this was not numerically assessed. Spore counts were extremely high, organic debris (plant and animal) counts reasonably high and pollen counts insignificant by comparison (see Table 1).

Table 1. Concentration of Airborne Particles Collected by the Burkard Spore Trap.

Material	Particles per m^3 of air		
	Slide 1	Slide 2	Slide 3
Spores, single celled			
<5 microns	6409	2700	8681
5-25 microns	389	204	576
>25 microns	161	23	416
two celled	50	347	547
three celled	19	142	758
four or more cells	31	33	416
Total spores	7059	3449	11394
Organic debris	1984	2587	2144
Pollen	155	68	44
Ash, sand, salt	373	1696	350

3.2 RAAF Base Tindal, October 1998

3.2.1 Weather

Consistent climatic conditions were experienced over the five days that measurements were made (see Table 2). Mean air temperature increased from 27.6° at 0700h (range 26.9 - 28.1°) to a maximum of 37.4° at 1600h (range 37.2 - 38.4°). Mean relative humidity dropped from 70% at 0700h (range 64 -75%) to 28% at 1500h (range 26 - 30%). Solar radiation was high and skies clear.

Table 2. Meteorological Conditions at Tindal during the Bioaerosol Sampling Period.

Date	Time[A] (hours)	Air Temp. (°C)	Relative Humidity (%)	Windspeed (km/h)	Wind Direction (°)
07/10/98	1213	34.7	40	9	250
	1342	36.6	32	11	118
	1511	37.5	29	7	030
	1628	37.2	27	7	120
	1721	37.0	27	7	123
08/10/98	0816	28.7	72	15	350
	1110	33.9	41	15	270
	1344	35.9	29	11	090
	1528	37.1	27	13	345
	1713	36.8	27	11	300
	0813	29.7	58	13	260
09/10/98	1113	34.9	36	13	320
	1332	37.4	30	9	300
	1514	38.5	26	17	330
10/10/98	0823	28.4	64	11	280
	1015	30.7	53	19	256
	1353	35.6	29	11	250
	1601	36.9	26	13	264
11/10/98	0743	27.2	77	9	330
	1000	30.2	61	13	300
	1140	34.0	41	15	305
	1458	37.7	30	15	340

[A] 12 minutes after initial exposure of agar plates.

3.2.2 Bacterial and Fungal Counts

Table 3 shows the numbers of bacteria and fungal colonies found on the exposed agar plates. Random samples of 4 bacterial colonies taken from 19 of the 21 plates were found to contain 44 Gram-positive rods (57%), 23 Gram-positive cocci (30%) and 10

Gram-negative rods (13%). The ratio of Gram-positive to Gram-negative bacteria was approximately 7:1.

Table 3. Bacteria and Fungal Counts at Tindal, October 1998.

Date	Time[A] (hours)	Bacteria (no. per m^3)	Fungi (no. per m^3)
07/10/98	1213	73	58
	1342	68	78
	1511	53	62
	1628	109	92
	1721	48	49
08/10/98	0816	93	25
	1110	37	49
	1344	114	33
	1528	91	51
	1713	169	64
09/10/98	1113	64	38
	1332	51	33
	1514	63	25
10/10/98	0823	158	76
	1015	104	83
	1353	38	13
	1601	263	63
11/10/98	0743	54	28
	1000	43	45
	1140	45	52
	1458	13	20

[A] 12 minutes after initial exposure of agar plates.

3.2.3 Total Particle Counts and Particle Size Distribution

Table 4 summarises the airborne particle measurements made with the TSI Aerodynamic Particle Sizer. Counts can be converted to particles per cubic metre in the air by multiplying by 1000. This follows since 1 litre of air was sampled each 5 minutes and a cubic metre of air contains 1000 litres. Total particle concentrations ranged between 2.25 and 5.71 million particles per cubic metre of air with a mean of 2.95 million. Approximately 80% of the particles had an aerodynamic diameter less than 1 micron. Particles in the 1 to 2 micron size range comprised 17% of the total, those in the 2 to 5 micron size range 3% and particles greater than 5 microns less than 1%.

Table 4. Airborne Particles Measured by the TSI Aerodynamic Particle Sizer

Date	Time[A] (hours)	Number of particles in given size ranges (aerodynamic diameters in microns) in 1 litre of air					
		Total	<1	1.03-1.98	2.12-5.04	5.42-10.3	11.1-30.5
08/10/98	0816	4978	4009	818	127	20	4
	1110	3267	2516	616	100	27	8
	1344	2476	1868	508	77	15	8
	1528	2253	1736	423	70	17	7
	1713	2279	1774	404	81	15	5
09/10/98	0813	5707	4913	621	135	27	11
	1113	2772	2230	439	80	17	6
	1332	2454	1946	408	81	13	6
	1514	2477	1981	399	71	20	6
10/10/98	0823	3531	2820	562	125	18	6
	1015	3236	2439	628	141	22	6
	1353	2359	1839	412	84	20	4
	1601	2328	1828	391	85	18	6
11/10/98	0743	2690	2189	412	72	13	4
	1000	2538	1963	467	84	19	5
	1140	2408	1866	440	84	14	4
	1458	2246	1785	363	72	18	8

[A] 12 minutes after initial exposure of agar plates.

3.3 RAAF Base Tindal, April 1999

3.3.1 Weather

Climatic conditions showed little variation from day to day. Air temperature rose from a minimum of 21-22° at 0600h to 33-34° at 1500-1600h. Relative humidity fell from a maximum of 91% at 0630h to 50% at 1230h where it remained until 1800h and then increased during the night to its maximum at dawn. There was no air movement from 1830h to 0600h (overnight). Windspeed rose evenly with time to a maximum of 14-15 km/h at 1300h and dropped uniformly to zero at 1830h. Wind direction was 109-139 degrees. Solar radiation was high and skies clear.

3.3.2 Bacterial and Fungal Counts

Table 5 gives the concentrations of bacteria and fungi calculated from the counts obtained from the slit-to-agar sampler.

Table 5. Concentration of Bacteria and Fungi at Tindal, April 1999.

April 14			April 15		
Time[A] (hours)	Bacteria (no./m^3)	Fungi (no./m^3)	Time[A] (hours)	Bacteria (no./m^3)	Fungi (no./m^3)
1216	236	218	0012	162	135
1245	172	162	0042	177	115
1314	69	82	0142	141	109
1342	96	93	0212	240	149
1412	91	85	0242	149	100
1442	62	95	0312	80	82
1512	76	81	0412	111	56
1542	61	148	0442	114	63
1611	57	164	0519	128	51
1642	56	117	0547	213	93
1812	107	77	0615	117	51
1842	76	101	0644	130	104
1012	71	73	0712	149	87
1942	165	87	0742	81	111
2012	253	88	0812	128	91
2042	259	138	0842	92	74
2112	111	51	0912	134	71
2146	154	109	0942	96	128
2214	95	64	1012	44	53
2242	239	95	1042	194	131
2312	288	95	1112	321	117
2346	153	88	1142	457	77

[A] 12 minutes after initial exposure of agar plates.

Gram-positive bacteria generally outnumbered Gram-negative bacteria in an approximately 4:1 ratio. Cocci and rod bacteria were present in an approximately 4.5:1 ratio. Small numbers of yeast were also found to be present.

3.3.3 Spore and Pollen Counts

Figure 1 shows the hourly total counts of particles of biological origin caught in the Burkard spore trap over the period 1600h on April 13, 1999 to 0930h on April 18, 1999. The hourly total particle count varied between 112 and 738 with a mean of 377 which equates to an atmospheric loading of 628 particles per cubic metre of air. Counts from the start of sampling (1605h on April 13) to 2305h on April 15 averaged 441 while those from 5 minutes past midnight on April 16 to the end of sampling (0923h on April 18) averaged 316. The difference in the two means is significant (Student 't' test, p=0.975). No diurnal cycle was evident although counts at night tended to be slightly higher than daytime counts. The difference was not significant.

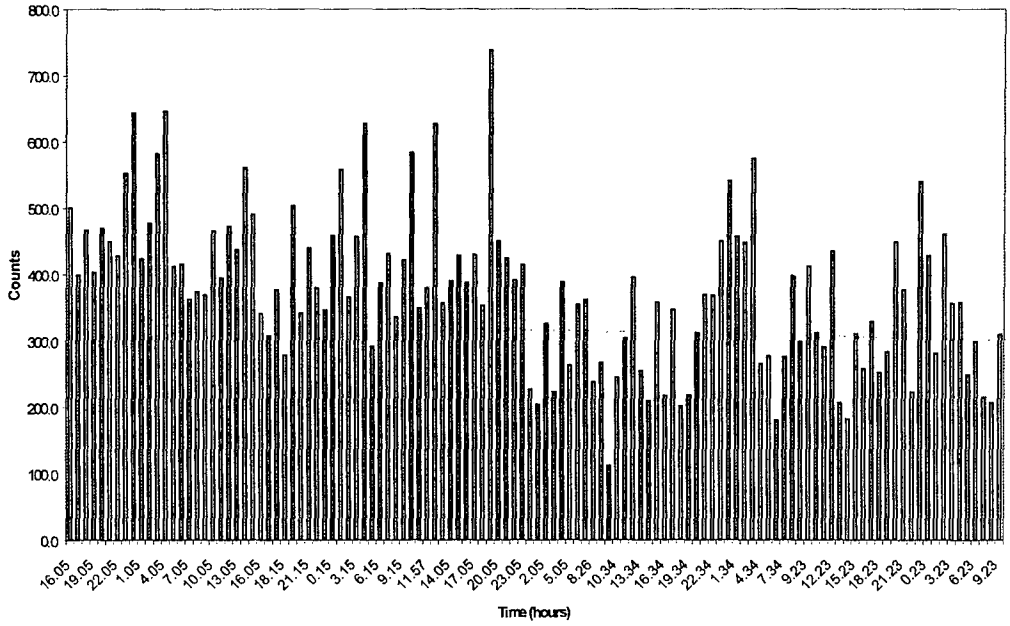

Figure 1. Burkard Spore Trap Total Hourly Counts

The vast majority of particles counted were fungal spores (92.4%) in the following size ranges: 2-5 microns (29.8%), 5-10 microns (49.5%), 10-15 microns (9.8%) and >15 microns (3.3%). Insects and insect remains comprised 6% of the catch and organic debris 1.3%. Over the entire sampling period only 126 pollen particles were caught out of a total of 45,500 particles. Figure 2 shows the hourly counts of fungi in the four size ranges given above. The two distinct periods previously discerned for total counts and occurring either side of 2305h on April 15 can readily be seen in Figure 2. Mean hourly counts of fungi before and after 2305h on April 15 are as follows: 2-5 microns (70 increasing to 146), 5-10 microns (272 decreasing to 142), 10-15 microns (57 decreasing to 24) and >15 microns (32 decreasing to zero).

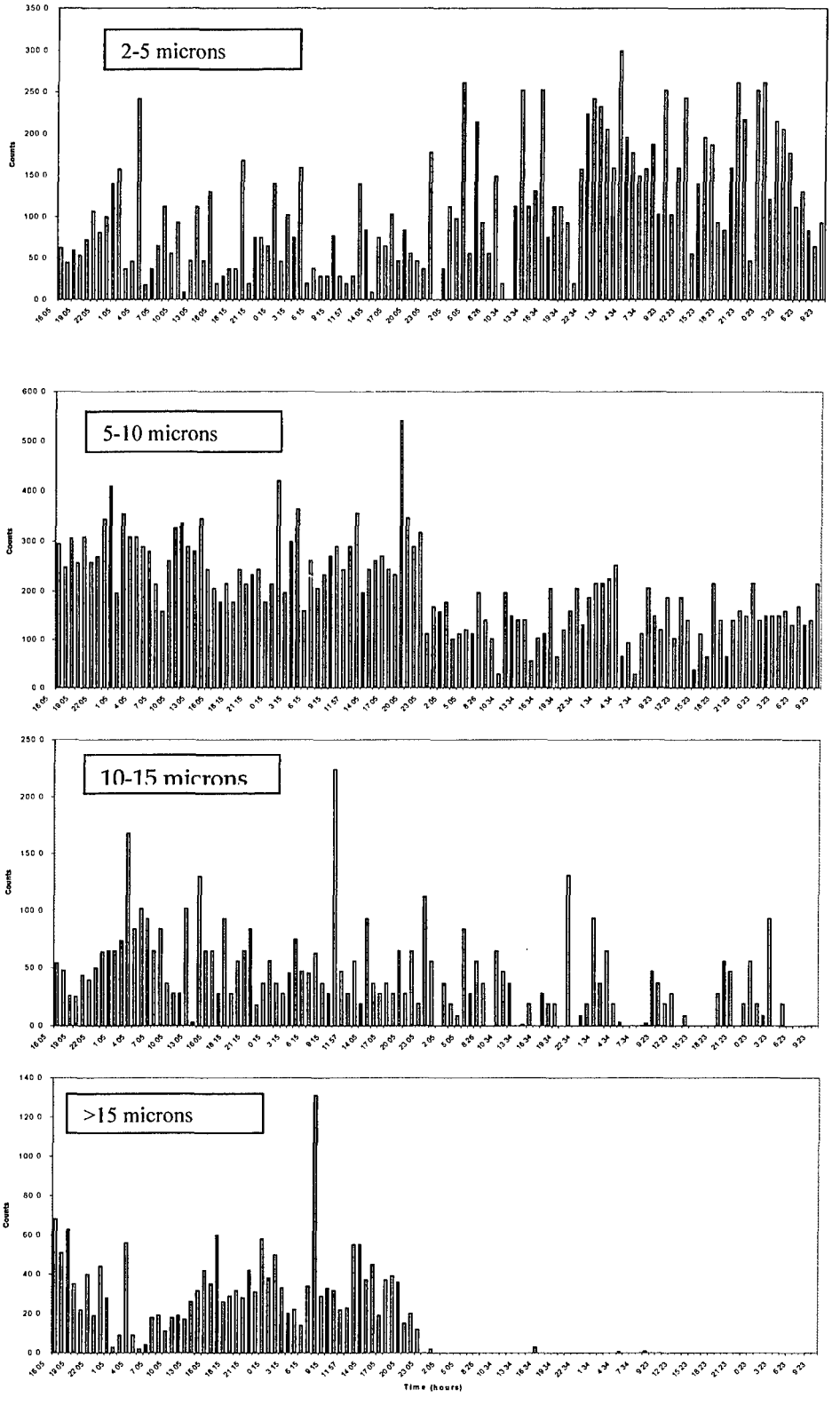

Figure 2. *Mean Hourly Counts of Fungi (Burkard Spore Trap, 1605h April 13 to 0923h April 18, 1999)*

3.3.4 Total Particle Counts and Particle Size Distribution

Measurements made with the TSI Aerodynamic Particle Sizer commenced at 1146h on Tuesday April 13 and ended at 0015h on Saturday April 17. The average particle count for a 5-minute sampling time over this period was 7778 which equates to an atmospheric loading of 1.5556×10^6 particles/m^3 of air. The average number composition (mean ± standard deviation) of the sampled particles was (i) particles having an aerodynamic diameter of less than 1 micron, 67.7 ± 2.8%, (ii) 1-2 microns, 27.4 ± 2.4%, (iii) 2-5 microns, 4.0 ± 0.7%, (iv) 5-10 microns, 0.6 ± 0.2%, and (v) 10-15 microns, 0.3 ± 0.1%. Figure 3 shows the five-minute total particle counts for the sampling period and Figures 4 and 5 give the number of particles in the size ranges <1, 1-2, 2-5, 5-10 and 10-15 microns.

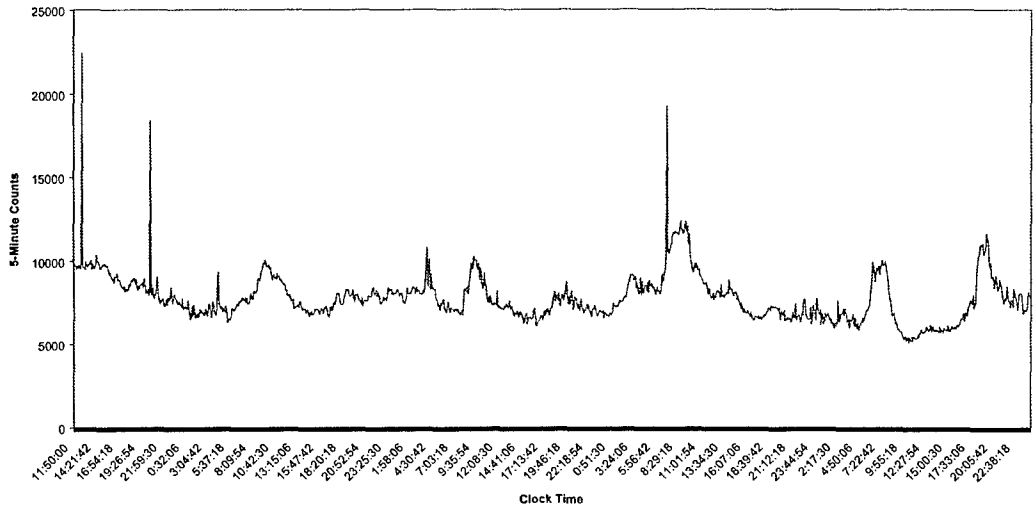

Figure 3. Total Particles Counted at 5-Minute Intervals during the Sampling Period at RAAF Base Tindal (13-16 April 1999).

A feature of the results in Figure 3 is the three higher than normal particle totals which appear as spikes on the particle count line at 1248h and 2032h on Tuesday April 13 and 0707h on Thursday 15 April. The increased count for the first spike is due solely to an increase in the number of particles in the submicron size range (see Figure 4). The increased count for the other two spikes has resulted from an increase across all size ranges. An increase in submicron particles also occurred around 1930h on Friday. Submicron particles are known to be produced by internal combustion engines and it is likely that vehicles are the source of these particles in these instances. In the latter case there was an outdoor film showing for staff and families on the base, and the arrival of the film audience in their cars coincides with the increase in submicron particles.
Further analysis of Figures 3,4 and 5 shows increases in all particle sizes occurring around 0800h to 1000h on each day. This increase coincides with activities associated

with the arrival of staff and the start-up of machinery at the beginning of the working day.

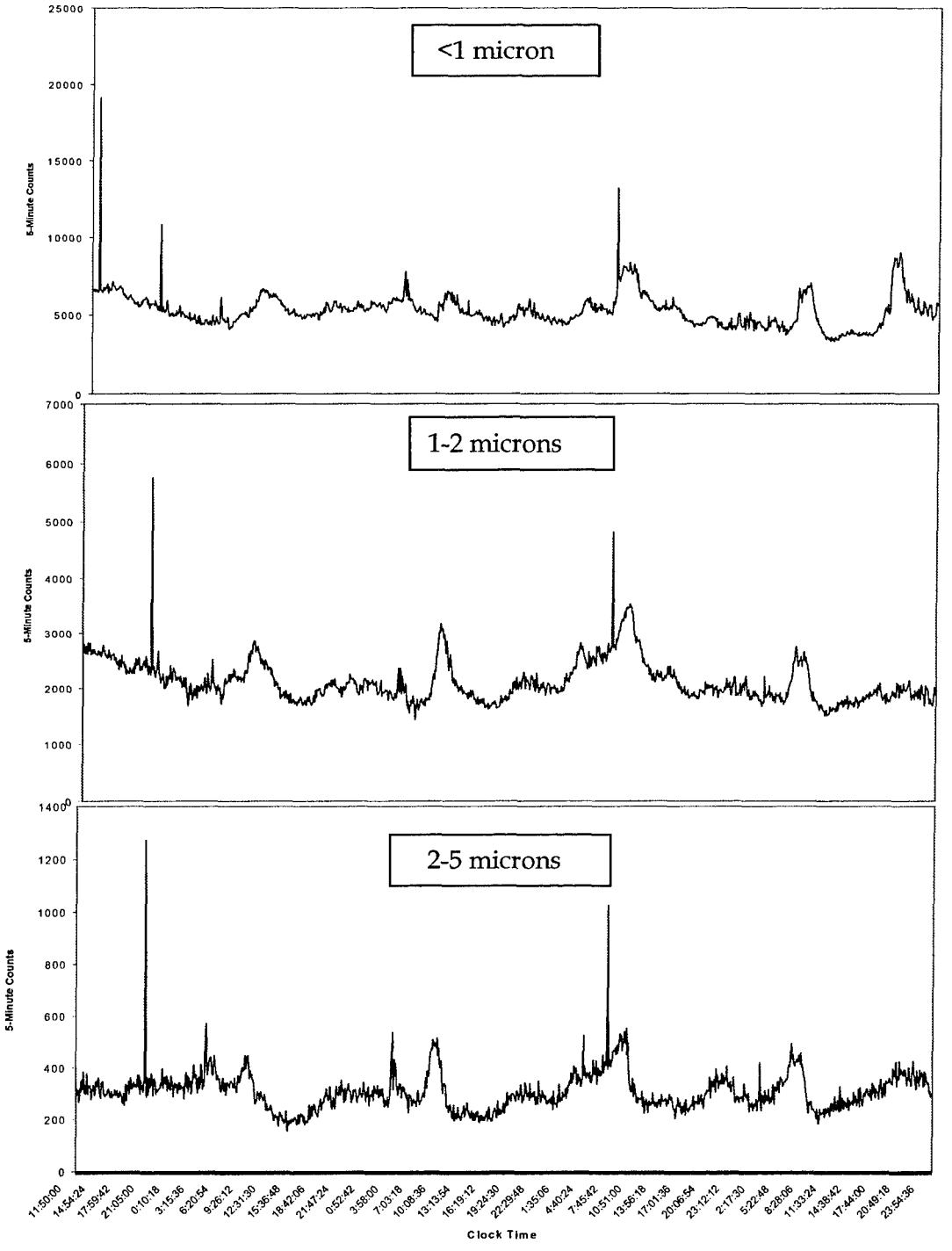

Figure 4. 5-Minute Counts of Particles (<1, 1-2 and 2-5 microns) at RAAF Base Tindal (13-17 April 1999).

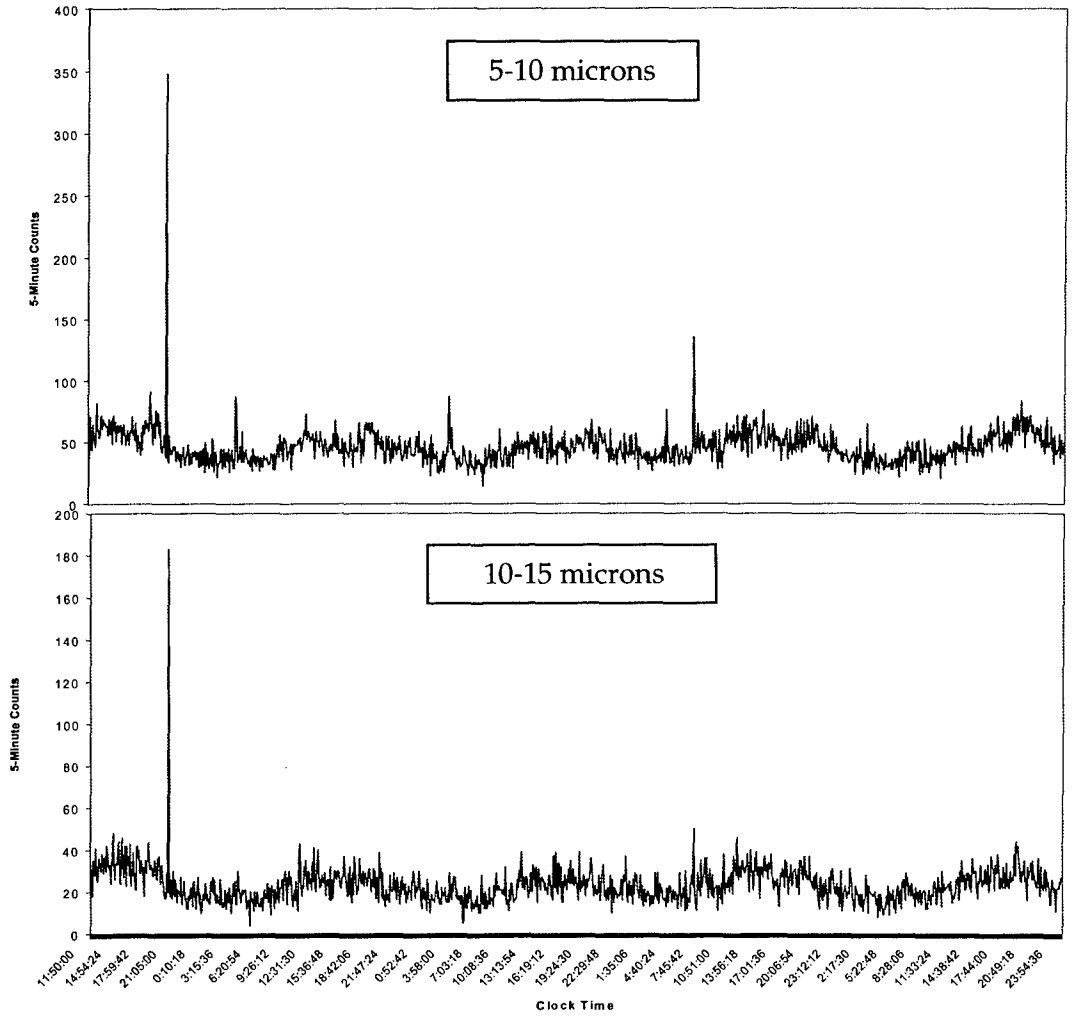

Figure 5. 5-Minute Counts of Particles (5-10 and 10-15 microns) at RAAF Base Tindal (13-17 April 1999).

The counts of particles in the 5 to 15 micron size-range, while relatively low, exhibit a diurnal variation with the highest counts occurring around 1600h and the lowest at 0500h (see Figure 5).

4. Discussion

This study was designed to (i) establish an appropriate level of expertise in background aerosol monitoring in DSTO and (ii) obtain preliminary data on the background aerosol content and composition at sites of military interest in northern Australia.

4.1 Instrumentation and Methodology

Solar radiation load and temperature are uniformly high in the Northern Territory. The drying effect of the air being drawn over agar in the STA sampler is large. The STA sampling system suffered because the agar tended to "sweat" excessively initially and then dehydrate if left in the Perspex sample housing . Agar plates that were too thin tended to split rapidly, possibly because the force of the air being drawn through the slit was too strong for the low mechanical strength of the agar and possibly due to dehydration of the agar. This splitting of the agar can be a problem if agar plates are poured that are too thin. There is also an upper limit to agar thickness set by the maximum allowable distance between the slit and the agar surface. These problems were easily overcome by shielding the Perspex housing with aluminium foil and by ensuring that the plate was not left unattended for more than 30 minutes. Another problem which occurred with some of the glass petrie dishes containing the agar was poor seating in the STA caused by irregular shaped dishes. In some cases the dish contacted the cover bonnet of the STA and did not rotate freely. This problem can be avoided if plastic dishes are used.

The most common use of the STA is within buildings such as laboratories, hospitals and offices. Such locations usually have a very low ambient wind speed which allows entrapment of a significant fraction of bioaerosols. However in the field wind speeds can be considerable. It is possible that under some conditions the suction generated by the STA may be insufficient to remove entrained spores from the airstream.

A problem with the RVST was similar to that experienced with the STA, namely, too high a temperature within the sampler. When temperatures were high the sticky material on the surface of the slide liquefied and slumped down the vertically-mounted slide. However a composition was found that was not adversely affected by the temperatures found in the Northern Territory.

RVST slides exposed at Tindal in April 1999 had an abundance of fungal palynomorphs so great that direct counting of each specimen required a significant time commitment. To complete the analysis within budget, fungal palynomorphs in the size ranges 2-5 μm, 5-10 μm and 10-15 μm were expressed as estimates rather than absolute numbers. Estimates (E_a) were calculated according to the following equation:

$$E_a = (A_a/F_c)F_T$$

where A_a is the actual abundance counted from a given number of view fields, F_c is the number of view fields from which the actual abundance was measured, and F_T is the total number of view fields in the transect. While this is a useful means of obtaining a standard measure of abundance it **does not** provide an accurate measure of abundance. Consequently all estimates of abundance should be expressed with a standard deviation error range. Slides exposed in Darwin were assessed using similar methodology.

A feature of all impact methods of bioaerosol sampling is that capture of entrained particles is not 100%. Furthermore, the STA which counts bacterial and fungal colonies on an agar plate will only measure those spores that are viable, and will not measure the presence of non-viable organisms. Related to this is the fact that some bioaerosols are alive and capable of growing in a suitable environment. If the environment in the STA chamber is not suitable for growth, viable but not-growing organisms will not be counted. Many fungal and bacterial spores do not immediately or readily grow on standard agar media. Therefore the counts obtained in the STA represent an underestimate of the total viable bacterial and fungal spore concentration in the air, and that even this larger value is an underestimate of the total (viable plus non-viable plus bioaerosols not caught by the agar) present in the air. A recent report by Tong and Lighthart[2] found that the geometric mean of total airborne bacteria concentration in the air above a grass-seed field in Oregon, USA, was about 27 to 222 times greater than that of culturable airborne bacteria.

The Canadian Fluorescence Aerodynamic Particle Sizer (FLAPS) had to be operated in the shade due to an inability to maintain the UV laser below 31°C when the instrument was located in the full sun. Above 31°C the laser is programmed to shut down. Continuous operation in the shade was achieved by increasing the cooling effect of the existing heat-exchanger fans by running cold water across the fan outlet housing.

4.2 Characteristics of the Background Bioaerosols

Airborne bacterial counts obtained in this work are comparable to previously reported values obtained by culture methods.[3,4,5,6] Average airborne bacteria concentrations measured in this work were 58 particles per m^3 (range 27-102) at Darwin, 83 (13-263) at Tindal in October 1998 and 146 (44-457) at Tindal in April 1999. Comparable average concentrations found by other investigators include 99 bacteria per m^3 (range 2-3400) at a rural site in Sweden[3], 63 (0-560) at a coastal site in Sweden[3], 113 at a coastal site in Oregon USA[4], 54 (0-412) at an isolated desert site in the USA[5] and 146 (4-1640) in a Washington, D. C. suburban area.[6]

Greater numbers of fungal spores were caught in the Burkard trap at Darwin than at Tindal. This can be explained by the prevalence of lush vegetation and wetter conditions in the gardens and parks of suburban Darwin which facilitate the growth of fungi and ferns (producers of large quantities of spores). The drier and more sparsely vegetated environment of Tindal is less conducive to fungal growth. The number of fungal colonies growing in the slit-to-agar sampler at both sites was much less than the number of particles caught in the Burkard spore trap. The reasons for this are the same as those discussed above for bacteria.

Very few pollen particles were found at both sites. Pollen abundance in the air is dependent on the time of year that sampling occurs. Consequently it is reasonable to infer that the low counts indicated that sampling occurred at times when pollen production by plants was low. The sampling times in this study coincided with the

dormant period for pollinating plants where the previous growing season had ended and the new season for plant growth had not yet begun.

Mean total particle counts obtained from the APS at Tindal in October 1998 and April 1999 were 2.95 x 10^6 and 1.54 x 10^6 particles per cubic metre of air. These quantities are within the range expected for a rural site[7]. The difference in particle numbers found at the two Tindal sites may be due to one or many factors including time of year, weather and proximity to local sources of airborne particles. The October 1998 site was closer to areas of human activity such as workshops, offices and vehicle traffic which might be expected to result in higher levels of airborne particulate matter. The size distribution of airborne particles at each site was slightly different in the submicron to 2 micron range. Although both sites had 95-96% of particles in the above size range the October 1998 readings showed that 79% of particles were less than one micron diameter compared to 68% for the April 1999 measurements. The percentages of particles in the 2-5 micron size range were 3.2 (range 2.4 - 4.4) and 4 (range 3.0 - 7.1) respectively.

4.3 The Aerodynamic Particle Sizer as an Early-Warning or Trigger Device

The potential for using an APS as an early-warning or trigger device in a biological detection system can be considered by using the five days' data accumulated at Tindal in April 1999. The 2 to 5 micron fraction of airborne particles could be monitored for "unnatural" or "unusual" increases in quantity or percentage of the total. This size range is chosen for the following reasons: (i) it has been reported[8] that 4-6 micron particles are ideal for deposition in the lungs, (ii) bacteria need a particle size of at least 5 microns to remain viable in the air[8], and (iii) by selecting a size range of greater than 2 microns, 95-96% of airborne particles need not be considered.

During the five days of APS operation the average number of 2-5μ particles counted in five minutes was 309 (standard deviation 75, range 154 -1274) and the average total number of particles was 7780 (standard deviation 1400, range 5108 - 22455) giving an average percentage contribution of 3.98 for the 2-5μ particles. If the 2-5μ particle concentration increased by 50 particles per litre of air (PLA) all but one of the individual 5-minute readings of percentage concentration of 2-5μ particles (%2-5μ) would be above the mean background value of 3.98%, i.e., one false negative. This reading was that represented by the first spike in the sub-micron count shown in Figure 4 and is not a genuine false negative. Also if a new average 2-5μ percentage value is calculated for the addition of 50 PLA none of the background values exceed it, i.e., no false positives. To put this detection sensitivity into context a dispersion model was used to provide some indication of particle concentrations of anthrax spores after a line release under conditions described previously[9]. At a point 3 kilometres from a 2km line release of 50kg of anthrax spores the concentration of particles increases to 8100 PLA in 80 seconds. This would easily be detected by an APS operating in an area with a native background similar to that found at Tindal in this study. Also the warning

time could be reduced to seconds if a pre-concentrator was fitted to the inlet of the APS.

The above calculations are rudimentary and only give an indication of the level of "foreign" aerosols which might be detected. For example it is assumed above that a "foreign" aerosol would only contain 2-5 micron particles; this is unlikely. This does however point to the need to know the composition of man-made aerosols intended for military use. It has also been shown[10] that particle sizing alone will cause unacceptable false alarms in some environments. The use of a particle sizing instrument which can discriminate between biological and non-biological particles (e.g., FLAPS) provides an acceptable trigger device for the detection of biological aerosols.

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6. References

1. Hirst, J. M. (1952). "An Automatic Volumetric Spore Trap." *Ann. Appl. Biol.* **39**, 257.
2. Tong, Yongyi and Lighthart, B. (1999). "Diurnal Distribution of Total and Culturable Atmospheric Bacteria at a Rural Site." *Aerosol Sci. and Technol.* **30**, 246-254.
3. Bovallius, A., Bucht, B., Roffey, R. and Anas, P. (1978). "Three-Year Investigation of the Natural Airborne Bacterial Flora at Four Localities in Sweden." *Appl. Environ. Microbiol.* **35**(5), 847-852.
4. Shaffer, B. T. and Lighthart, B. (1997). "Survey of Airborne Bacteria at Four Diverse Locations in Oregon: Urban, Rural, Forest, and Coastal." *Microbial Ecol.* **34**, 167-177.
5. Lighthart, B. and Shaffer, B. T. (1995). "Viable Bacterial Aerosol Particle Size Distributions in the Midsummer Atmosphere at an Isolated Location in the High Desert Chapparal." *Aerobiologia* **11**, 19-25.
6. Jones, B. L. and Cookson, J. T. (1983). "Natural Atmospheric Microbial Conditions in a Typical Suburban Area." *Appl. Environ. Microbiol.* **45**(3), 919-934.
7. Green, H. L. and Lane, W. R. (1957). *Particulate Clouds: Dusts, Smokes and Mists.* p. 352, E. & F. Spon Ltd., London.
8. Stopa, P. J. (1998). Some Considerations Regarding the Sampling of Biological Aerosols. in *Proceedings of the NSWC Information Exchange on Bio-Aerosol Sample Collection.* Naval Surface Warfare Center, Dahlgren, VA, compiled by Southern Research Institute, Birmingham, Alabama, USA.
9. WHO Group of Consultants (1970). Health Aspects of Chemical and Biological Weapons. World Health Organisation, Geneva, Switzerland.
10. Ho, J., Spence, M. and Hairston, P. (1999). "Measurement of Biological Aerosol with a Fluorescent Aerodynamic Particle Sizer (FLAPS): Correlation of Optical Data with Biological Data." *Aerobiologia* **15**, 281-291.

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The biological aerosol content of the air has been measured at Darwin and RAAF Base Tindal in the Northern Territory of Australia. The number of culturable bacteria and fungal spores together with organic material caught in a spore trap are reported. The total airborne particulate matter in the size range 0.5 to 30 microns was also measured at Tindal in October 1998 and April 1999. The ability of an aerodynamic particle sizer to act as an early warning device for the presence of biological warfare agents in this environment is discussed. It was concluded that such a device could only be successfully used as a trigger in the event of a military release of a biological agent in an environment with a well characterised aerosol background.

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