



Review Article

## Mini-review of the concentration variations found in the alfresco atmospheric bacterial populations\*

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### Abstract

Variations in the atmospheric surface layer's culturable, and to a lesser extent, total bacteria-associated atmospheric particle characteristics will be discussed in terms of (a) their temporal variation from 2 min resolution through diurnal to annual periods, (b) the effect of meteorological conditions on their abundance and size, (c) total to culturable bacterial ratio, and (d) the total number of bacteria per culturable particle (e) bacterial survival in droplet/particles, and (f) the general particle size distribution including aerodynamic Count Median Diameter (CMD).

Meteorological and topographic conditions that control total and culturable bacteria-associated atmospheric particle concentration will be presented in terms of (a) precipitation, (b) wind direction, (c) time of day, (d) sky conditions (i.e., cloudy, sunny, rain, etc.), (e) season, and (f) atmospheric inversion conditions.

Simulation models will be described that support hypotheses of diurnal and annual concentration cycles in the Earth's (and perhaps other planetary atmospheres) atmospheric surface layer.

### 1. Introduction

This mini-review is concerned with the most frequently observed culturable airborne bacteria (CAB) and to a lesser extent the total (TAB (= CAB + viable but not culturable (VBNC) + nonviable (i.e., dead)) airborne bacteria in the surface layer (*def.* bottom 10% of the boundary layer (Stull, 1988) of the alfresco (*def.* clean, outdoors) atmosphere. How they vary in terms of (1) their concentration in time, (2) their particle size distribution in time, (3) certain factors that cause these variations, and (4) models simulating these variations will be discussed.

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#### 1.1 Temporal concentration

For over 130 years variations in the quantity of CAB in the atmospheric surface layer have been observed (Pasteur, 1861) and associated with the time of year (Bovallius et al., 1978; Ganio et al., 1995; Miquel, 1883; Miquel and Bnoist, 1890; Vladavets and Mats, 1958; Tong and Lighthart, in review) and time of day (Figure 2; Lighthart and Shaffer, 1994; Miquel and Bnoist, 1890). Generally, findings show an annual summer-time and diurnal early morning and afternoon maxima, and winter and night-time minima, respectively. TAB also show the same annual and diurnal patterns but with mean concentrations approximately two orders of magnitude higher (Figure 3, Table 1). Dawn peak concentrations such as shown in Figure 2 were also observed in forested, urban, rural-agricultural areas, but not near ocean shores suggesting a general phenomenon particular to terrestrial but not marine environments (Lighthart and Shaf-

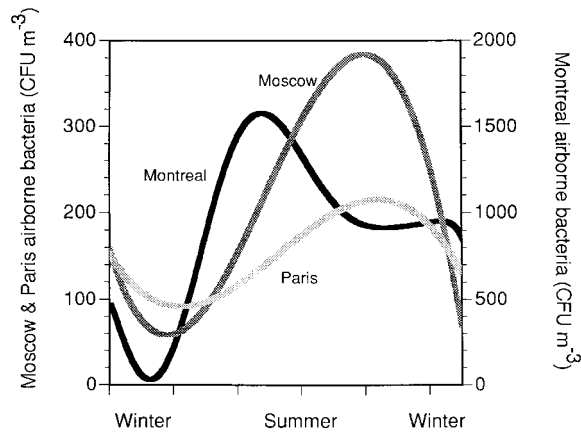


Figure 1. Diagram of the annual airborne culturable bacteria in the atmosphere of Paris (28, 29), Moscow (Vladavets and Mats, 1958), and Montreal (Kelly and Pady, 1954).

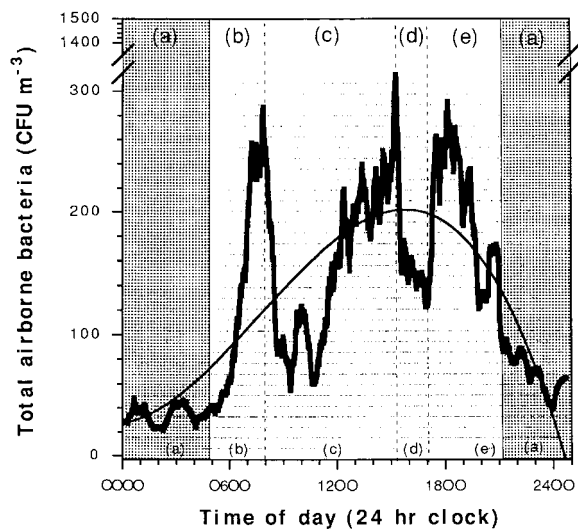


Figure 2. One hundred data point moving average of airborne culturable bacteria 1.5 m above ground level over a grass seed field in the mid-Willamette River Valley, Oregon (22): (a) calm night-time, (b) dawn fumigation/plant release peak, (c) accumulation below the inversion cap, (d) seabreeze intrusion trough, (e) seabreeze subsides and night-time inversion returns. Horizontal "lines" are 6000 data points (Lighthart and Shaffer, 1995a). Smoothed line is a polynomial regression fit of the data.

fer, 1994; Lighthart and Shaffer, 1997; Shaffer and Lighthart, 1997). Shorter term variations (e.g., 2 min) in CAB have been observed to vary greatly between intervals. A 1400% change between two succeeding 2 minute intervals has been observed for CAB (Figure 4; Lighthart and Shaffer, 1994).

Modulation of diurnal and annual terrestrial patterns of CAB have been attributed to meteorological

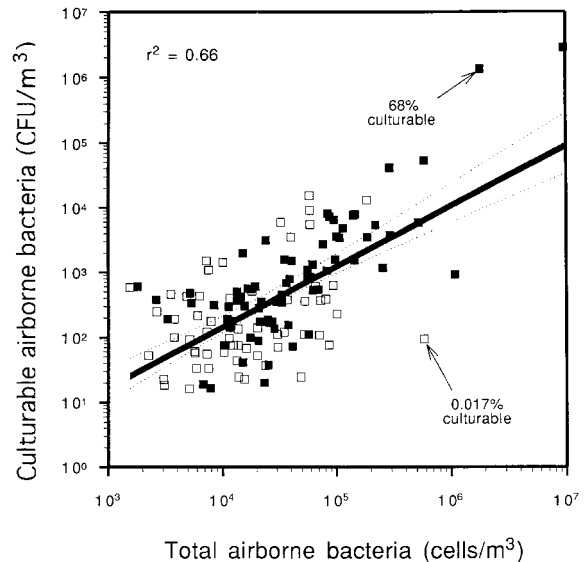


Figure 3. Linear regression of total and culturable airborne bacteria measured in 1997 at two locations (open and filled squares) in the mid-Willamette River Valley, Oregon.

and topographic conditions. Meteorological conditions that contribute are: wind direction (Miquel, 1883; Lighthart and Shaffer, 1994; Tong and Lighthart, in press), rain events where CAB concentrations were inversely related (Lighthart and Shaffer, 1994; Tong and Lighthart, in press), solar radiation where higher TAB and CAB concentrations were observed on clear days and lower on cloudy days (e.g., Table 2; Tong and Lighthart, 1998), higher CAB during frontal events (Fulton, 1965c) and proximity to cities have been observed (Fulton, 1965a; Shaffer and Lighthart, 1997) suggesting a "bacteria island" effect, reduced CAB concentrations on traversing the ocean by land air masses (Fulton, 1965b), agricultural activities that were estimated at one location to contribute 40% of the CAB in an air mass (detailed explanation in Lighthart (1984)) (Table 1). Topographic features also modulate CAB concentrations, e.g., mountain gaps allowing off-shore seabreeze intrusion into near-shore valleys (Lighthart and Shaffer, 1994).

It has been hypothesized (Lighthart, 1998) that the near coincidence of the solar radiation cycles and temporal atmospheric bacterial distribution patterns indicate that the bacterial distribution in the alfresco atmosphere is a function of the diurnal and annual solar cycles (Figures 5a, b). A maximum concentration of bacteria is found in reference to the solar zenith as a standing wave in the rotating coordinate system of

Table 1. Means of total (TAB; cells m<sup>-3</sup>) and total culturable (CAB; CFU m<sup>-3</sup>) atmospheric bacteria during 10 clear summer day and one harvest day in the Willamette River Valley, Oregon in 1996

Time of day	TAB		CAB		(CAB/TAB)*100	
	NO harvest	Harvest	NO harvest	Harvest	NO harvest	Harvest
0600	20704	25135	94	188	0.45	0.75
0800	17812	33517	100	345	0.56	1.03
1000	21167	75579	160	2751	0.76	3.64
1200	35751	9590315	564	2848836	1.58	29.71
1400	50590	1823914	1143	1238996	2.26	67.93
1600	54143	527154	1895	5852	3.50	1.11
1800	45803	592404	1002	52953	2.19	8.94
2000	69203	294420	1196	41227	1.73	14.00
Means	39396.6	1620304.8	769.3	523893.5	1.63	15.89
Mean Harvest/No harvest ratio		41		681		

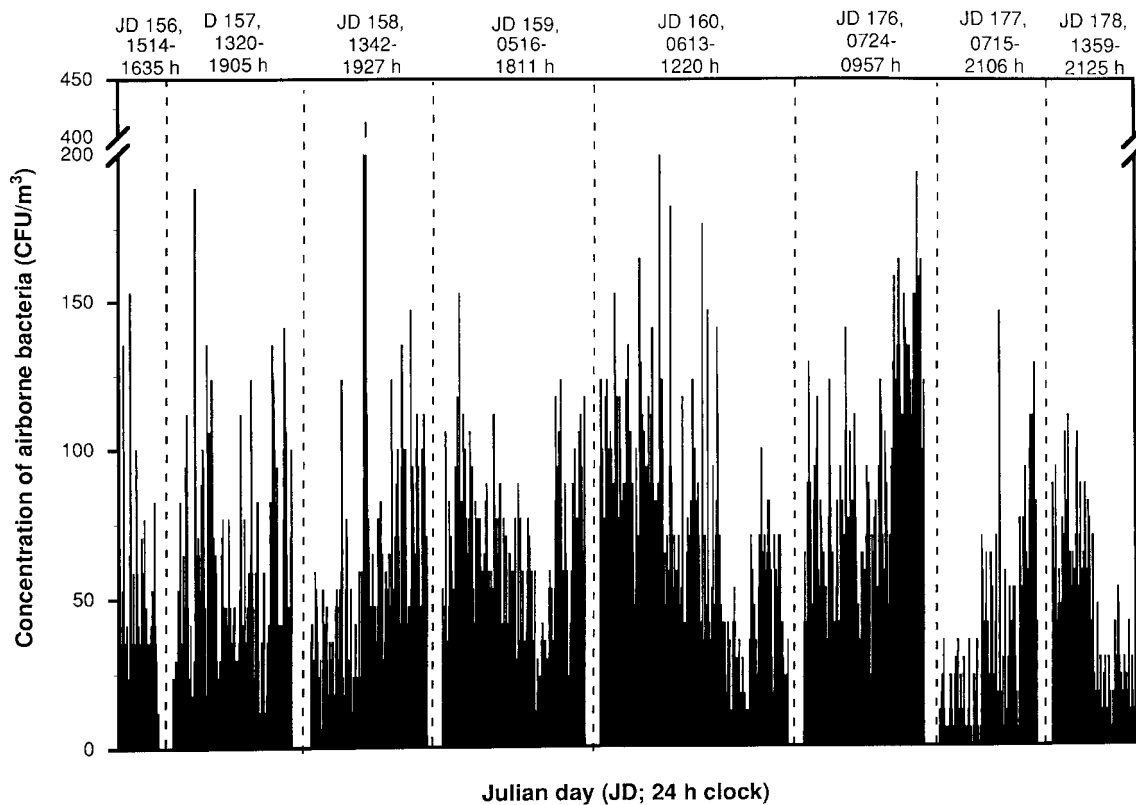


Figure 4. Two minute resolution of total culturable airborne bacteria (CAB) at the Hanford Nuclear Reservation, WA in the summer of 1995 (Lighthart and Shaffer, 1995b).

Table 2. Tabulation of the means (log transformed and back transformed for presentation) with Tukey-Kramer (HSD)<sup>1</sup> comparisons symbols for Total (TAB; cells/m<sup>3</sup>), Total Culturable (CAB; CFU/m<sup>3</sup>) and Particulate Culturable (PCB; CFU/m<sup>3</sup>) bacteria, and their ratios for the yearly seasons, sky and rain conditions, and wind directions at the Willamette River Valley observation sites in 1997

Factors	Total bacteria				CAB	PCB	TAB/PCB	CAB/TAB <sup>2</sup>	TCB/PCB	TAB/PCB	CMD
	N	Cell/m <sup>3</sup>	Rods	Cocci							
<i>Season</i>											
Spring	28	20813.4 AB	6037.7 B	14775.7 AB	121.5 B	74.8 B	267.4 A	0.96 B	1.65 B	267.4 A	3.82 A
Summer	39	32949.9 A	22685.5 A	10264.3 ABC	2595.8 A	294.0 A	184.1 A	9.48 A	6.22 A	184.1 A	4.14 A
Fall	34	12724.9 B	11131.8 AB	1593.1 CD	706.8 B	206.8 AB	125.2 A	10.16 A	3.90 AB	125.2 A	3.95 A
Winter	27	4997.2 B	2710.6 B	2286.6 BCD	241.0 B	153.5 B	101.0 A	4.38 AB	1.42 B	101.0 A	3.71 A
<i>Rain</i>											
no rain	##	23743.1 A	15481.5 A	8261.6 A	1310.2 A	163.7 A	193.3 A	7.92 A	4.31 A	193.3 A	4.32 A
rain	25	6795.3 B	3463.2 B	3332.2 A	299.2 A	315.0 B	72.9 A	4.87 A	1.11 B	72.9 A	2.33 B
<i>Sky</i>											
clear	50	35265.6 A	22846.5 A	12419.2 A	2163.7 A	221.6 A	218.2 A	7.17 A	5.78 A	218.2 A	4.48 A
cloudy	56	9083.1 B	6427.1 B	2656.0 B	416.9 B	180.3 A	129.4 A	11.47 A	2.43 B	129.4 A	3.49 BC
fog	4	5310.6 AB	4606.8 AB	703.8 AB	183.1 AB	67.5 A	87.9 A	0.67 A	3.21 AB	87.9 A	4.55 C
partly clear	11	12376.8 AB	3609.0 AB	8767.9 AB	75.0 AB	70.0 A	169.7 A	0.92 A	1.08 AB	169.7 A	3.37 ABC
partly cloudy	8	13442.1 AB	8726.2 AB	4715.9 AB	1026.4 AB	342.5 A	155.2 A	7.00 A	2.84 B	155.2 A	4.13 BC
<i>Wind direction</i>											
north	21	41863.7 A	30522.9 A	11340.8 A	2859.0 A	295.8 A	174.8 A	8.07 A	6.33 A	174.8 A	4.00 A
east	17	28711.9 AB	19951.8 AB	8760.0 A	1248.0 AB	189.4 A	176.3 A	6.25 A	4.17 AB	176.3 A	4.50 A
south	61	13440.1 B	7677.0 B	5763.1 A	730.2 B	203.4 A	163.3 A	6.54 A	2.68 B	163.3 A	3.80 A
west	17	14499.9 B	7614.3 B	6885.7 A	688.8 B	113.9 A	138.4 A	13.16 A	4.58 AB	138.4 A	3.80 A

<sup>1</sup>Honestly Significant Difference (HSD) @ 95% C.I. (Kramer, C.Y. 1956. Extension of multiple range tests to group means with unequal number of replications. Biometrics. 12:309-310.)

<sup>2</sup>as percentage of TAB

the moving earth. Conditions of weather, topography, source strength, and human activities (e.g., microbial air Pollution (MAP) as indicated in Lighthart (1984)) are thought to modulate the cyclic patterns of atmospheric bacterial loadings as indicated in the preceding paragraph.

### 1.2 Variation in the quality of al fresco atmospheric bacteria

The quality (i.e., genera) of airborne bacteria is a function of the source environment and time of year (Shaffer and Lighthart, 1997). The predominant categories of CAB found at 4 divers locations in Oregon (i.e., Douglas Fir forest, onshore ocean seabreeze, urban location and rural grass seed farm) were largely Gram (+) (i.e., 80.4 ± 5.9%) with 39.6 ± 7.0% in the genus *Bacillus* in the terrestrial environments and only 12.3% in the onshore ocean breeze samples. Gram (-) bacteria made up 19.6 ± 5.9% of the populations with the onshore ocean breeze containing the greatest proportion, i.e., 27.7%. The percentage of the two Gram groups changed with time of day, and at the rural site, with crop maturity.

### 1.3 Particle size variation

Airborne bacterial size and shape vary depending on their source as shown in Figure 6a from a liquid (i.e., an agricultural sprayer) or Figure 6b a powder source of *Bacillus subtilis* var. *niger* spores, and Figure 6c a natural source, probably water. Variations in the terrestrial CAB particle size distributions (PSD) for diurnal and annual cycles have been observed (Tong and Lighthart 1999, in press). Generally, the diurnal PSD has smaller sized particles at night and larger sizes during the day (e.g, Figure 7a, b). There was a very large proportion of ≥ 7.0 μm aerodynamic diameter particles (≈80%) associated with the sunrise peak (Figure 7a, b; 0600–0800 h) and mid-sizes (≈60%) at the time of the onshore breeze trough (Figure 7a, b; 1400 h). During the annual cycle the PSD had a major peak in the summer of mostly larger sized particles (80–90% > 2.1 μm aerodynamic diameter), and several smaller autumn and winter-time peaks (≈70% < 2.1 μm aerodynamic diameter) (Figure 8a, b). The winter and autumn minimal concentrations were often associated with marked PSD distribution

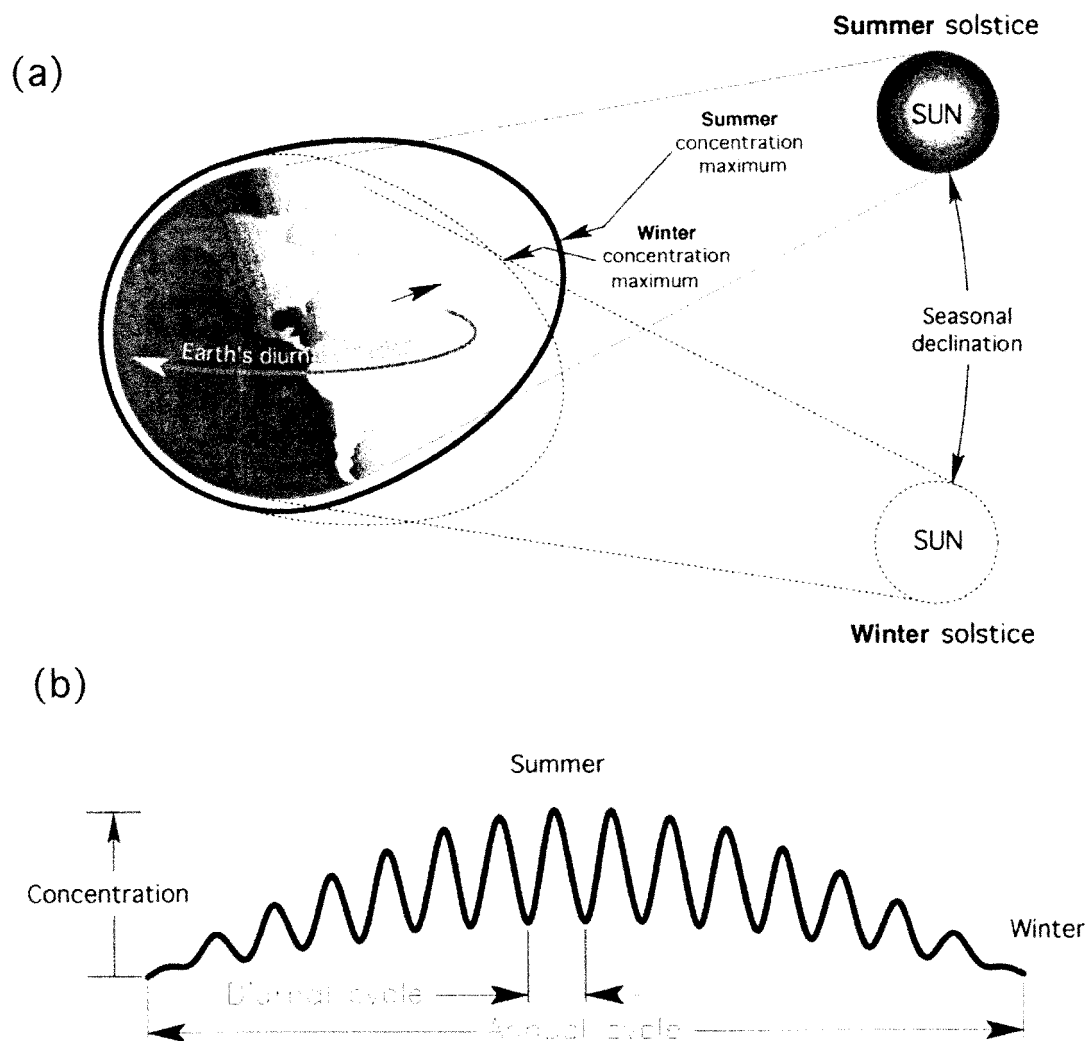


Figure 5. Diagrammatic representation of the theoretical alfresco airborne bacteria concentration at the Earth's solar zenith at (a) above atmospheric surface layer and (b) on the Earth's surface (Lighthart, 1998).

changes frequently associated with the rainy winter season or events.

#### 1.4 Some causative variation factors

Variations in the numbers of CAB has been well documented for temperature, relative humidity, and oxygen (Cox, 1987; Cox and Wathes, 1995; Lighthart and Mohr, 1994). Other factors that contribute to their survival are: (a) survival increases directly with agglomerated bacterial particle size (Figure 9 lower right;()), (b) droplet surface available for evaporation (Figure 9 upper left; Lighthart and Shaffer, 1997), and (c) solute composition such as presence of tre-

halose that replaces water molecules between the cell's rehydration sensitive lipid bilayer membrane (Figure 9 upper right; Leslie et al., 1995), and exposure to solar radiation (Figure 9 lower left; Tong and Lighthart, 1997b).

#### 1.5 Models of outdoor bacteria in the alfresco atmosphere

In Lighthart and Frisch (1976), and later in Peterson and Lighthart (1977) bacterial death rate and settling factors were included in Pasquill's (Pasquill, 1962) Gaussian plume model. Dynamic environmental factors of solar radiation, and particularly

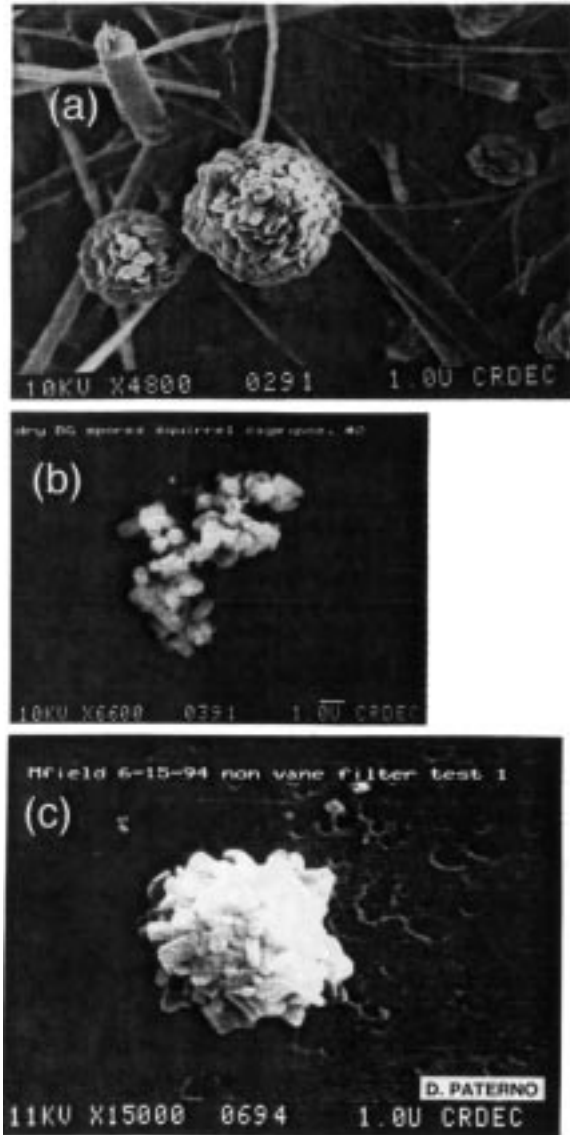


Figure 6. Electron micrographs of *Bacillus subtilis* var. *Niger* spores ("BG") disseminated as a (a) powder or (b) an aqueous slurry, and (c) a natural particle found in the terrestrial near shore ocean atmosphere that appears similar to (b). (Photos courtesy of D. Paterno, ERDEC, US Army).

wind speed effecting airborne bacterial and viral concentrations, were used in the Gaussian model with meteorological forcing functions of relative humidity, temperature, and solar radiation (Lighthart and Mohr, 1987). A droplet dispersion model has been described (Lighthart and Kim, 1989) to follow the dissipation pattern of genetically engineered bacteria dispersed from plants. Validation of this model was

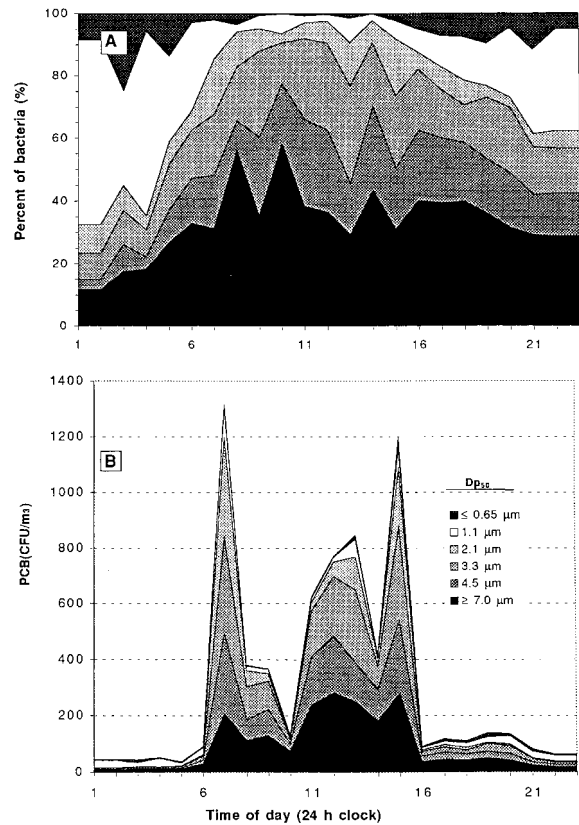


Figure 7. Diurnal atmospheric particulate culturable bacteria (PCB) (a) percentage size and (b) size distributions at a location in the mid-Willamette River Valley, Oregon in 1997.

done showing the model predicted the dispersion pattern of multiple sources reasonably well (Ganio et al., 1995).

A statistical death rate model of airborne bacteria using laboratory derived data for temperature, relative humidity and Gram reaction was prepared in 1989 by Lighthart (1989), and natural atmospheric bacterial communities for solar radiation by Tong and Lighthart (Tong and Lighthart, 1997a; Tong and Lighthart, 1997b; Tong and Lighthart, 1998).

Using the trajectory pattern of a polydispersed bacterial aerosol it was shown that the downwind deposition pattern started with the largest droplet/particle sizes deposited near the source and ending with the smallest sizes deposited farthest from the source (Lighthart et al., 1991). The trajectory of the geometrically represented pattern of CAB containing dust plume emitted by agricultural combines in a relatively closed valley was used to estimate their contribution

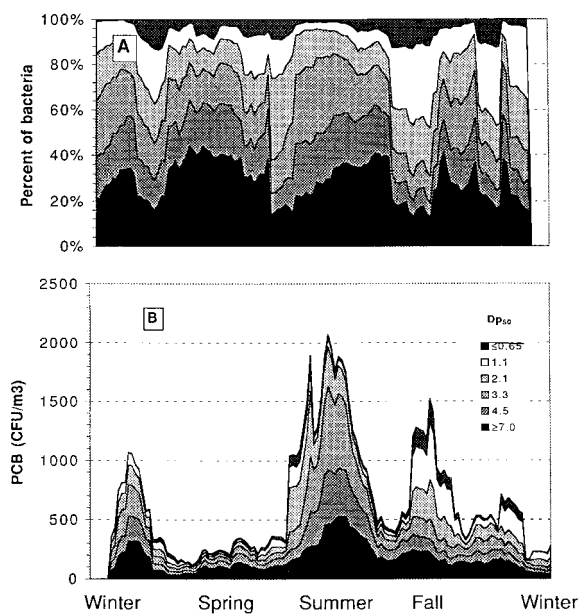


Figure 8. Annual atmospheric particulate culturable bacteria (PCB) (a) percentage size and (b) size distributions at a location in the mid-Willamette River Valley, Oregon in 1997.

to the atmospheric loading (Lighthart, 1984). The contribution was estimated to be approximately 40%.

Finally, a dynamic model driven by a set of time functions of the culturable bacterial flux from ground sources, mixed layer inversion depth, death rates due to solar radiation and for two bacterial populations (i.e., night-time and day-time) was prepared (Figure 10; Lighthart and Kirilenko, 1998) and found to compare favorably with Lighthart and Shaffer's observed data (1994). The method to determine the flux of bacteria from ground sources into the atmosphere was demonstrated by Lighthart and Shaffer (1994). The model estimated both CAB and TAB.

### 1.6 Some atmospheric factors affecting airborne bacterial survival

Until recently quantitative observations of airborne bacteria have used classical culture methods. Thus laboratory measurements have shown the deleterious effects of low relative humidity (see Leslie et al., 1995) for the molecular mechanism) and high temperature (Lighthart and Shaffer, 1995b). The effects of the gaseous components in the atmosphere oxygen (Cox and Wathes, 1995), nitrogen (Cox and Wathes, 1995), carbon monoxide (Lighthart, 1973), and sulfur dioxide (Lighthart et al., 1971) have also been investigated

in the laboratory. The ambient atmosphere has been shown to be highly toxic to airborne bacteria (May, Druett and Packman, 1968). The toxicant is thought to be an ozone olefin reaction product. Solar radiation has been shown to be damaging to natural populations of CAB (Tong and Lighthart, 1997b, 1998). However, the pigmented CAB fraction were shown to be less sensitive to solar radiation (Tong and Lighthart, 1997a). The outgrowth of airborne bacteria on culture media has been shown to be medium dependant in that over a 100% increase in outgrowth was observed with addition of catalase to the medium (Marthi et al., 1991.). Catalase was hypothesized to compensate and therefore protect against the inactivation of essential protective cellular peroxidases damaged during the dehydration and/or rehydration processes while airborne.

CAB in larger airborne droplet/particles were shown to be more resistant to the damaging effects of aerial exposure (Lighthart, 1998). Also droplet/particles containing a smaller ratio of sensitive vegetative cells compared to resistant spores also survive aerial exposure better (Lighthart and Frisch, 1976). It is thought that reduced desiccation rates in these circumstances were responsible for better airborne survival.

Recently, methods to study the total bacterial content (i.e., viable and nonviable) in the atmosphere have appeared (e.g., Lighthart and Tong, 1998). These measurements have indicated that only approximately 1% of the alfresco atmospheric bacteria are CAB the rest are either non-viable (i.e., dead) or not culturable on the medium used. This does not mean that "dead" bacteria (i.e., not culturable on any medium) cannot, in some cases, repair themselves (Dimmick, 1960) and be cultured, i.e., returned to life. The range of the culturable portion of the population was from a low of 0.017% to a high of 75%. The high percent was found near ground level above a grass seed field in the spring (Lighthart and Tong, unpublished).

## 2. Conclusions

A. Airborne bacterial quantity and quality vary with time of day, year and location. The flux and therefore the quantity in the air are thought to be associated with solar heating processes that somehow causes the release of bacteria from plant and soil surfaces into the atmosphere. The bacterial

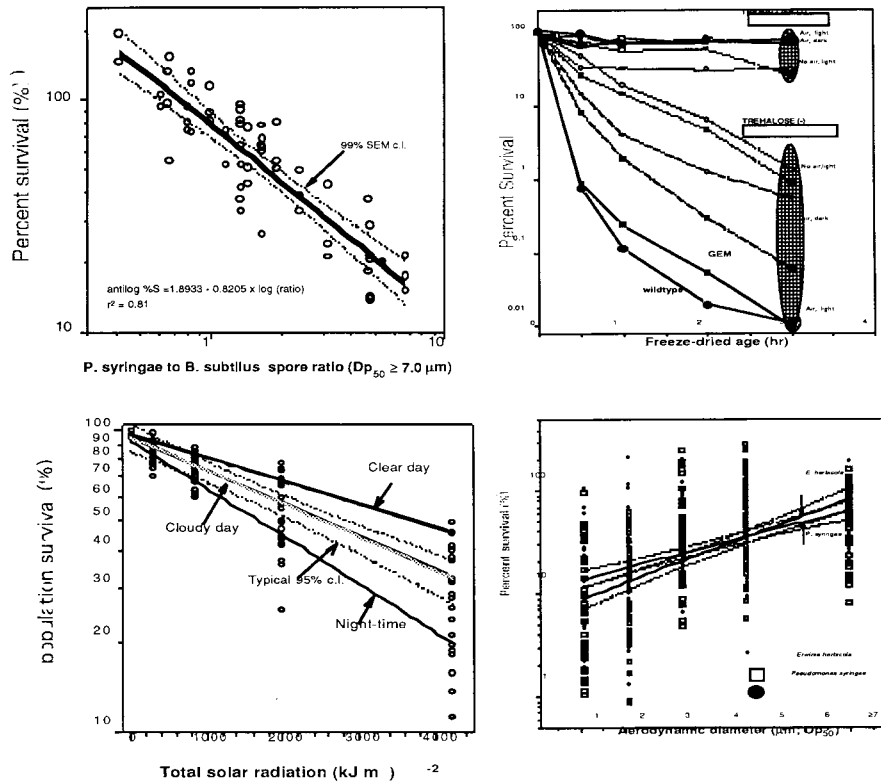


Figure 9. Bacterial survival as a function of particle content (upper left; Lighthart and Shaffer, 1997); Protection of freeze dried bacteria (*E. Coli*: GEM and wildtype) in trehalose to ambient air and incandescent light (upper right); Effect of solar radiation on natural populations of airborne bacteria collected on clear and cloudy days, and night (lower left); Survival of *Erwinia herbicola* and *Pseudomonas syringae* as a function of aerodynamic particle size (lower right)(24).

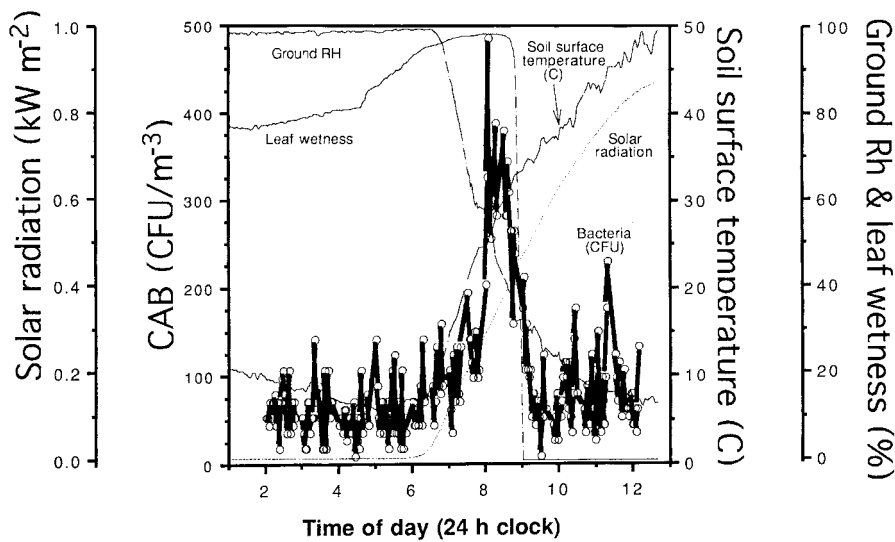


Figure 10. Graph showing the relationship of culturable airborne bacteria (CAB) 1m above ground level and solar radiation, soil surface temperature, near ground level relative humidity (RH), and leaf wetness probe (%) (unpublished data).



concentration has been observed to vary between 2 2-minute periods by as much as 1400%.

- B. Forcing functions of topographic (e.g., mountain gap allowing seabreeze intrusion into interior valleys) and meteorological conditions modulate rates of release and airborne survival of the bacteria (e.g., temperature, relative humidity and solar radiation).
- C. The particle size distribution varies annually and diurnally.
- D. The culturable atmospheric bacteria makeup, on the average,  $\approx 1\%$  of the total airborne bacteria (which has been measured at  $190,000 \text{ cells/m}^{-3}$ ) and have a range of about 0.01 to 75%.

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