# Airborne Bacteria in the Atmospheric Surface Layer: Temporal Distribution above a Grass Seed Field

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Temporal airborne bacterial concentrations and meteorological conditions were measured above a grass seed field in the Willamette River Valley, near Corvallis, Oreg., in the summer of 1993. The concentration of airborne bacteria had a maximum of 1,368.5 CFU/m<sup>3</sup>, with a coefficient of variation of 90.5% and a mean of 121.3 CFU/m<sup>3</sup>. The lowest concentration of bacteria occurred during the predawn hours, with an average of 32.2 CFU/m<sup>3</sup>, while sunrise and early evening hours had the highest averages (164.7 and 158.1 CFU/m<sup>3</sup>, respectively). The concentrations of bacteria in the atmosphere varied greatly, with a maximum difference between two 2-min samples of 1,995 CFU/m<sup>3</sup>. The concentrations of bacteria in the atmosphere could be divided into five time periods during the day that were thought to be related to the local diurnal sea breeze and Pacific Coast monsoon weather conditions as follows: (i) the nighttime minimum concentration, i.e., 2300 to 0600 h; (ii) the sunrise peak concentration, i.e., 0600 to 0800 h; (iii) the midday accumulating concentration, i.e., 0800 to 1515 h; (iv) the late-afternoon sea breeze trough concentration, i.e., 1515 to 1700 h; and (v) the evening decrease to the nighttime minimum concentration, i.e., 1700 to 2300 h. The sunrise peak concentration (period ii) is thought to be a relatively general phenomenon dependent on ground heating by the sun, while the afternoon trough concentration is thought to be a relatively local phenomenon dependent on the afternoon sea breeze. Meteorological conditions are thought to be an important regulating influence on airborne bacterial concentrations in the outdoor atmosphere in the Willamette River Valley.

Microorganisms were first shown to be in the atmosphere in 1837, when they were found to cause fermentation and putrefaction of sterile material (28). Further experiments showed that they were universally found in the atmosphere of the investigator's laboratories (26, 31). Microorganisms were discovered in the surface air far out at sea (2, 4) when Charles Lindbergh found them at high altitude by holding open petri dishes outside his airplane window over the Atlantic Ocean in 1933 (21, 22) and from commercial aircraft over the Caribbean Sea (23). More recently, with an airborne sampler, the spatial distribution of outdoor bacteria was positively related to air masses (7, 8, 10), frontal and urban activity (9, 10), and altitude (9). Imshenetsky et al. (11), isolated nil to several thousand bacteria per m<sup>3</sup> at 60 to 70 km above the Earth's biosphere.

The annual distribution of bacteria in the atmosphere was measured in Moscow, Russia (32), and on the top of a 400 foot high building in Montreal, Canada (12). The Moscow measurements showed an increasing concentration that reached a plateau in spring between a winter minimum and a summer maximum. Vladavets and Marz attributed the spring plateau to washout of the bacteria in the atmosphere by the spring rains and attributed the summer bacterial maximum to hot, dry, dusty weather. A graph of the Montreal data showed that the annual bacterial distribution had minor and major minima in summer and winter, with maxima in spring and fall, and that the fungi had a winter minimum and summer maximum (16). It must be emphasized that all of these measurements had large concentration variations between sample times.

In the past, observation of the microorganisms in the outdoor atmosphere was motivated by curiosity, problems associated with airborne disease transmission, and the cycle of life on the planet. Today, there are in addition other practical problems regarding plant and animal disease dynamics and evaluation of present-day atmospheric microbial loads to determine the extent of microbial air pollution levels and to develop atmospheric microbial detection instrumentation in which natural background microorganisms might obscure specific anthropogenic agents, e.g., microbiological pest control agents and biological warfare agents.

The present report describes the changes (with a 2-min resolution) in the diurnal airborne bacterial concentration in the atmospheric surface layer (SL) over a grass seed field in the Willamette River Valley, Oreg., during three time periods: early, mid-, and late summer of 1993. The SL has been defined by Stull (30) as the bottom 10% of the atmospheric boundary layer. In the present case, it is the 10 m just above the ground. This report will provide documentation of the dynamic changes occurring in the atmospheric bacterial populations that are important for air pollution and defense considerations.

## MATERIALS AND METHODS

Airborne bacterial samples were taken and meteorological monitoring was performed from a 67-ha grass seed field situated in the middle (ca. 65 km wide) of the western edge of the Willamette River Valley ( $123^{\circ}17'38''W$ ,  $44^{\circ}32'6''N$ ). The valley lies between the Cascade Mountains (maximum elevation, 4,000 m), which are 50 km (mostly grass seed field covered) to the east, and the Coast Range (maximum elevation, 1,300 m), which is 15 km (mostly Douglas fir tree covered) to the west. The city of Corvallis is 5 km to the north northeast, and a house is 100 m to the north of the sample tower (Fig. 1). The air over the field was sampled on 15 days for 8 to 24 consecutive 1-h intervals during the following three periods: (i) while the grass was in the rapidly growing stage when the soil was still wet from previous winter's rains (14 to 15, 16, 17, and 18 June 1993), (ii) the mature swathed stage after the late summer combine harvest when the seed and soil were day (2, 3, 4, 5, 6, 7, 8, and 9 August 1993), and (iii) just after the first fall rainfall, when harvested grass seed stubble was still in place (28 October and 10 November 1993).

The bacteriological and meteorological methods are the same as those used to measure the bacterial flux of the chaparral at the Hanford Nuclear Reservation,

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FIG. 1. Map of Willamette River Valley study area.

Richland, Wash. (15). In brief, the bacteria were measured at 1.5 and 7.5 m above ground level (AGL) with a slit impact sampler (S-T-A Biological Air Sampler; New Brunswick Scientific Co., Inc., Edison, N.J.) run sequentially for 1-h periods during the course of the experiment. Colony counts were made of each  $12^{\circ}$  segment of the agar surface representing a 2-ft<sup>3</sup> (or 0.057-m<sup>3</sup>) sample volume in a 2-min time period. This resolves the great variation in the airborne bacterial concentration for more detailed interpretation of the data with respect to the ambient micrometeorological conditions. The exposed agar plates were incubated aerobically for 7 days at  $25^{\circ}$ C. (These are usual incubation conditions for many environmental samples under which drying of plates does not appear to be a problem.) The culture medium used was Luria Bertani agar (Difco Laboratories, Detroit, Mich.) amended with 200 µg of cycloheximide (Sigma Chemicals, St. Louis, Mo.) per ml. Although it is known that other media may yield higher rates of recovery of airborne bacteria, they have not been tested extensively enough (19), or they require impractical field manipulations (20).

Meteorological measurements were made at a 10-m height for solar radiation with a pyranometer (LI-COR, Inc., Lincoln, Nebr.), as well as of wind speed and wind direction (Met One, Inc., Grants Pass, Oreg.). Temperature was measured at 1.5 and 7.5 m AGL (Campbell Scientific, Inc., Logan, Utah).

A first approximation of an atmospheric stability parameter may be estimated by the difference between the potential temperatures at 1.5 and 7.5 m, i.e.,

Atmospheric stability 
$$\approx \Delta T/\Delta z \approx (273.126 \text{K}^\circ + \text{C}^\circ + \Gamma \times z)_{1.5 \text{ m}} - (273.126 \text{K}^\circ + \text{C}^\circ + \Gamma \times z)_{7.5 \text{ m}}$$

where the dry adiabatic lapse rate,  $\Gamma, \approx 0.01^{\circ} {\rm C/m}$  (1) and z is the height in meters.

Data were analyzed as running averages of the airborne bacterial concentrations, wind speeds, and wind directions in order to distinguish first-order features of the data. The numbers of points used for the running averages shown in the figures were arbitrarily selected to emphasize trends in the data.

# RESULTS

The diurnal distribution of bacteria in the atmosphere at the Willamette River Valley site may be divided into five time periods as follows (Fig. 2) (Tables 1 and 2): (i) the nighttime minimum concentration (i.e., 2300 to 0600 h), (ii) the sunrise peak concentration (i.e., 0600 to 0800 h), (iii) the midday accumulating concentration (i.e., 0800 to 1515 h), (iv) the late-afternoon sea breeze trough concentration (i.e., 1515 to 1700 h), and (v) the evening decrease to the nighttime minimum concentration (i.e., 1700 to 2300 h). The bacterial concentrations, atmospheric stabilities, wind speeds, and wind directions



Time of day (24 hr clock)

FIG. 2. Airborne bacterial counts 1.5 m AGL in a grass seed field in the Willamette River Valley, Corvallis, Oreg., for all or parts of 15 time-coincident days during the summer of 1993. Solid dark line shows 100-point running averages. Multiple datum points may appear as lines in the figure because of transformation of quantized colony counts. a through e, periods i to v as described in the text.

during these periods are indicated in Fig. 2, 3, 4, and 5, respectively.

Table 1 shows the airborne bacterial range, mean, standard deviation, and coefficient of variation for each of the time periods for the 15 sampling days. Concentrations ranged from a high of 1,368.5 (coefficient of variation of 90.5%) to a low of  $\leq$ 17.7 CFU/m<sup>3</sup>, with an overall average of 121.3 CFU/m<sup>3</sup>. The predawn hours had the smallest number of airborne bacteria, with a maximum of 353.2 CFU/m<sup>3</sup> and an average concentration of 32.2 CFU/m<sup>3</sup>. The late-afternoon and early-evening hours had the highest number, at 1,368.5 CFU/m<sup>3</sup>, with an average of 158.1 CFU/m<sup>3</sup>. This is a range for the maximum and average concentrations of 194.0 and 491%, respectively, over a 24-h period.

The description of the airborne bacterial concentration (as indicated by the 100-point running average in Fig. 2) as it relates to meteorological conditions shows that the nighttime low concentration (period i) is accompanied by light winds from the north northwest (Fig. 5) and that the atmosphere is very stable (Fig. 3), resulting in a generally nonturbulent condition. At sunrise (period ii), there is a sharp rise in the airborne bacterial concentration, with an average increase of over 500% in less than 2 h (Fig. 2). Accompanying the increase,

TABLE 1. Airborne bacterial concentration statistics for the five time intervals at the grass seed field in Willamette River Valley during the summer of 1993

Time of day (24-h clock)						
	Maximum	Minimum	Average	SD	CV (%) <sup>2</sup>	n
2300-0600	706.3	<17.7	64.4	51.0	79.2	751
0600-0800	1,977.6	<17.7	329.4	299.5	90.9	308
0800-1515	1,483.2	<17.7	273.4	216.5	79.2	1,144
1515-1700	1,907.0	<17.7	316.1	201.2	63.6	306
1700-2300	2,736.9	<17.7	301.7	279.3	92.6	8,341
All day	2,736.9	<17.7	242.5	219.4	90.5	3,360

<sup>a</sup> CV, coefficient of variation.

TABLE 2. General descriptions of parameters for the five time periods described in the text

Parameter	Description for the following times of day (24-h clock):					
	2300-0600	0600-0800	0800-1515	1515-1700	1700-2300	
Bacterial concn	Low	Concn peak forming	Peak reduction; accumulation	Concn trough	Reduction to low	
Atmospheric stability	Highly stable	Going from stable to unstable	Unstable	Temporarily stable and then unstable to stable	Stable	
Wind speed	Low (0–2 m/s)	Sharp peak increase at sunrise to 4 m/s	Slowly increasing from $1 \rightarrow 3$ m/s	Sharp peak (8 m/s)	Decreasing from 2→1 m/s	
Wind direction	Northeast north northwest	West	South	West southwest (onshore breeze)	Northeast north north- west	

there is a sharp increase in the wind speed to 4 m/s from the west (Fig. 4), and, most importantly, the atmospheric stability becomes rapidly unstable (Fig. 3) because of the solar heating of the ground and overlying air. This appears to be a very usual phenomenon, because it occurred every time measurements were made during the early predawn to dawn hours (Table 3). During this time, it is presumed that a deepening mixed layer forms with a rising inversion layer (6). The summer inversion in the Willamette River Valley reaches its approximate daily maximum of 1,500 m at about 1600 h (25). At the beginning of the midday period (period iii), the airborne bacterial concentration decreases sharply (Fig. 2), presumably because of an intrusion of clean air from the inversion core (1), although increasing solar radiation, lower relative humidity, and higher temperatures during this time interval may play a role in the decrease in viable bacteria. Subsequent to the decrease, there is a slow increase in the airborne bacterial concentration (Fig. 2) under conditions of an unstable (Fig. 3), slowly increasing wind speed field from the south (Fig. 4 and 5). In mid- to late afternoon (period iv), the airborne bacterial concentration shows a sharp concurrent decrease (Fig. 2) and upward shift in the wind speed (8 m/s) from the west (Fig. 4 and 5), which is the direction of the afternoon onshore sea breezes from the Pacific Ocean (27). During the onshore breeze condition, the cool ocean air produces a temporary weakly stable atmosphere, which is dissipated as the onshore winds diminish. In the evening, the condition approaches those found in the predawn period (period i), in which the airborne bacterial concentration decreases (Fig. 2) under the influence of a stable atmosphere (Fig. 3) and light winds from the north northwest (Fig. 4 and 5).

# DISCUSSION

The temporal quantitative distribution of airborne bacteria (the qualitative distribution will be the subject of another communication) in the SL above a grass seed field in the mid-Willamette River Valley during the summer appears to be closely related to meteorological conditions that are influenced by a combination of both topographically generated winds and sea, land, and inland breezes (1, 5, 24, 27, 30). The explanation of the atmospheric bacterial concentration dynamics begins with the period prior to dawn (i.e., period i [Fig. 2]), by which time the Willamette River Valley has been fully invaded by the microbiologically clean Pacific Coast monsoon air (27, 29) coming off the Pacific Ocean during the night. The winds are from the north northwest or nil. The air filling the valley probably enters through low gaps in the Coast Mountain Range, primarily at the Columbia River (generating northerly winds at the sampling site) or west of Salem or Corvallis, Oreg. (generating westerly winds at the sampling site) (18, 27). At this time, the concentration of airborne bacteria in the SL is at its diurnal minimum. At sunrise (Fig. 3), there is a concurrent



# Time of day (24 hr clock)

FIG. 3. One hundred point running average of the atmospheric stability (thick line) and solar radiation (narrow line) in a grass seed field in the Willamette River Valley for all or parts of 15 days during the summer of 1993.



FIG. 4. Wind speed at 7.5 m AGL in a grass seed field in the Willamette

FIG. 4. Wind speed at 7.5 m AGL in a grass seed field in the Willamette River Valley for all or parts of 15 time-coincident days during the summer of 1993. The solid dark line shows 5-point running averages.



FIG. 5. Wind directions 7.5 m AGL in a grass seed field in the Willamette River Valley for all or parts of 15 time-coincident days during the summer of 1993. Solid dark line shows 30-point running averages.

increase in the winds (Fig. 4) and airborne bacterial concentration (period ii [Fig. 2]). Presumably, these winds are convective and result in the release and/or redistribution of bacteria on or near the ground and vegetation, causing an abrupt change in the airborne bacterial concentration. The convective winds are thought to be due to heating of the valley ground and eastern slopes of the Coast Range at sunrise, initiating an upslope anabatic air flow. After the initial convective upward airflow, subsidence of the overlying relatively particle-free central valley residual or inversion core air would replace it (1, 30). The clean replacement air could explain the decreasing concentration of airborne bacteria on the backside of the sunrise peak.

The specific source(s) of the bacteria forming the initial peak is unknown but could be the result of several processes. According to the first process, bacterium-containing particles settle from the overlying atmosphere during the night, slowing with increasing drag on the particles as they pass through the cooler and therefore increasingly denser air layer near the ground. This process would cause an accumulation of particles in the denser air near the ground. Perhaps the coolness and moisture contribute to the survival of bacteria remaining in the air. At sunrise, particles that are still aloft could be convectively mixed into a relatively shallow surface layer, resulting in what is the initial peak increase in the airborne bacterial concentra-

 TABLE 3. Prevailing conditions at sunrise in the Willamette Valley

 experimental site as indicated for the five dates shown

Date (1993)	Т	ime of day	Bacterial	Solar	
	Sunrise (h)	Sunrise peak (h)	Lag (h)	peak (CFU/m <sup>3</sup> )	radiation (kW/m <sup>2</sup> )
17 June	0500 <sup>a</sup>	0730	1.5	400	0.225
18 June	0510	0710	2.0	680	0.160
5 August	0750	0900	1.2	210	0.065
6 August <sup>b</sup>	0550	0800	2.2	1,900	0.020
8 August	0600	0630	0.5	860	0.060

<sup>a</sup> Pyranometer reading, >0.0 kW/m<sup>2</sup>.

<sup>b</sup> Cloudy sunrise.

tion. A second process that could contribute to the sunrise peak may be convectively generated winds sweeping readily dislodged bacterium-containing particles from plant leaf and soil microlayer surfaces. Wind sweeping is probably a usual mechanism contributing to the flux of bacteria into the atmosphere (e.g., see references 13a, 15, and 17). A third mechanism has been suggested to be the flight of very small insects originating on plant surfaces (31a). Finally, the change in electrostatic charges of plants at sunrise (13) may contribute to the release of epiphytic bacteria into the atmosphere.

As the day proceeds (period iii [Fig. 2]), the residual inversion core air is probably replaced by solar ground-heated air moving upward at approximately 1 m/s (6), forming an inversion depth in the valley of approximately 1,000 m. Airborne bacteria generated by the flux from vegetation, soil, and/or anthropogenic sources are trapped below the inversion layer and accumulate with time during the day. Another explanation could be that as the wind speeds increase as the midday proceeds, there is a resultant increase in the liberation of cells from sources. Of course, there is a dynamic equilibrium between forces contributing to the loss and gain of viable cells in the atmosphere. Losses may be due to solar radiation, temperature, relative humidity, gaseous pollutants (14), deposition, and escape through the top of the inversion layer. From midmorning to early afternoon, the airborne bacterial concentration increases as the trapped particles accumulate below the inversion layer.

In midafternoon (period iv [Fig. 2]), there is a distinct intrusion of clean ocean air (29) into the valley from the west (27). This is probably replacement air intruding across the valley for large-scale strong thermal convection, forming cumulus and cumulonimbus clouds (3) on the west side of the Cascade Mountains. The intrusion is seen as cool, moist, relatively high-velocity winds from the west forming a temporary stable vertical atmospheric condition (Fig. 3) and low airborne bacterial concentrations (Fig. 2). The low concentration of airborne bacteria in the intruding air mass may be maintained, even though there may be a continual flux from the vegetation and soil (e.g., see reference 15) in the fetch. The mechanism for this may be the relatively high-speed, clean ocean winds that dilute any bacterial flux.

In late afternoon, the ocean air intrusion subsides, and the unstable atmospheric conditions return (Fig. 3), with the resultant processes of high airborne bacterial accumulation leading to high concentrations (period v [Fig. 2]). As the solar radiation decreases, the atmospheric instability further decreases until just before nightfall, when a stable nocturnal layer develops (Fig. 4), with a concomitant return of low airborne bacterial concentrations (Fig. 2). Perhaps the reasons for the decreased concentration are the lower wind speeds from the north (period i [Fig. 2]) and increasing amounts of bacteriologically clean ocean air entering the valley through the Coast Range and Columbia River gaps (27).

The overall conclusion that can be made from these observations is that because of the close relationship among sunrise, convective inversion, and sea breeze processes, the observed airborne bacterial concentration changes are greatly influenced by the local meteorological conditions in the Willamette River Valley.

In the future, with a better understanding of the bacterial sources (and particle characteristics) that contribute to the atmospheric load, air masses may be identified and tracked on the basis of their bacterial load. This would be very feasible with the current development of remote sensors able to detect and identify airborne microorganisms.

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