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Emission of Microbial Aerosols from Polluted Waters in Densely Populated Regions

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EMISSION OF MICROBIAL AEROSOLS FROM POLLUTED WATERS IN DENSELY POPULATED REGIONS

By

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Principal Investigator

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ABSTRACT

The air surrounding three activated sludge tanks was sampled over a two year period for the emission of bacterial aerosols under a variety of climatic conditions and at varying distances upwind and downwind of the aerated tanks. All plants emitted species of enteric bacteria which are significant as index organisms and as frank pathogens. The emission pattern of these bacteria were influenced by distance from the plant and wind direction. Within the parameters of a plant, defined arbitrarily in this study by sampling sites less than 150 m upwind and less than 900 m downwind, distance from the source was the only reliable predictor of emissions, and no statistical significance was found in the differences between upwind and downwind samples at the same distances from the plant. Multiple regression analysis revealed no consistent influences of any environmental factor on emission rate, but relative humidity, wind speed, air temperature, and ozone levels showed some contribution on the bacterial count, while light intensity appeared to have little influence. The deposition and retention of enteric bacteria on foliage plants near aerated basins was used as an alternate sampling method, and it emphasized the potential hazard of these aerosols. This method confirmed the inability to predict the emission rate by climatic factors, but wind speed contributed directly to the counts, and there was a pronounced difference in the average counts of upwind and downwind samples.

The LD_{50} in mice was the same for aerosolized Klebsiella pneumoniae as for a strain of Klebsiella pneumoniae recovered from the sputum of a patient with pneumonia. Escherichia, Enterobacter and Klebsiella were recovered from the respiratory tract of mice forced to inhale air at a

sewage treatment plant when the respiratory organs were assayed immediately after exposure, but not when the assay followed a prolonged period of observation, during which there was also an absence of clinical symptoms. This investigation also included a pilot study of the numbers of viable cells in the colony forming units on plates exposed in an Andersen Sampler, and CFU was found to be an unreliable index of viable cell counts. The study concludes that bacterial aerosols are a hazard for residents living near package plants, and recommends adopting alternate methods of sewage treatment that would remove the emissions of numerous package plants from the environment of densely populated regions.

Descriptors: Enteric Virus*, Wastewater Treatment Plants*, Water Quality, Air Pollution*, Pathogenic Bacteria*, Aerosols

INTRODUCTION

The measurement of microbial emissions as aerosols from sewage treatment plants is a logical extension of the study of such facilities as potential sources of pollution. All sewage, by definition, contains noxious materials and any sewage with animal waste products will include microorganisms which should be contained within the treatment facility. Most studies of treatment plants have been concerned with the quality of effluents, which are the most visible products disseminated outside the containment of the facility. While the potential problem of aerosolization of dangerous pollutants was recognized as early as 1907[1], field studies designed to test those emissions have a more recent history, and there are relatively few reports amenable to precise estimates of the distribution of microorganisms as a function of the complex interdependent variables that govern the formation and dissemination of aerosols with viable organisms. The paucity of earlier studies reflects the relative difficulty in the technology required for precise quantification of microorganisms from aerosolized droplets.

The field studies which have been reported include: analysis of alternate sampling techniques; differential counts of total bacteria and indicator organisms at varying distances from point sources; comparisons of numbers of organisms emitted by different treatment processes; and factors which influence the formation and distribution of aerosol droplets containing viable microorganisms.[2-18] The results are difficult to compare because of variation in technique, experimental design, and analytical techniques, but certain conclusions can be made. Aerated wastewater is a source of bursting bubbles which emit droplets into the environment.

The composition of the droplets is a function of their origin: when the wastewater is composed of domestic wastes associated with human excrement, bacteria are dispersed in the aerosols, and these bacteria must be presumed to contain potential human pathogens. The literature contains a comprehensive survey of these studies.[19]

The full significance of these microbial aerosols is complicated by the absence of a documented health hazard based on incidence of disease attributable to these aerosols, and the uncertain status of the hazard based on theoretical principles of microbial pathogenicity. It is assumed that the potential pathogens in domestic wastes derive from fecal material. Most pathogens which exit the human body in feces require ingestion as an effective portal of entry, but the apparent probable contact with aerosols is by inhalation. Therefore, estimates of the health hazard depend in part on the probability that contaminated emissions will be ingested, and there is sparse information on the transfer of particles from the respiratory to the alimentary tracts. However, most field studies have shown that Klebsiella is a common isolate from aerosols which derive from domestic waste, and several species in this genus are associated with significant respiratory disease.

There are no extensive epidemiological studies of individuals exposed to these aerosols, and the limited analysis from health records has produced ambiguous conclusions.[19] The conspicuous gap in the available data reflects the difficulty of an accurate epidemiological survey for the types of diseases which are predictable by contact with these organisms. The clinical syndromes would be gastrointestinal or respiratory distress, symptoms which are shared by many diseases whose causative agent is rarely identified if the patient does not require hospitilization. Even if the

pathogen is identified, the source of airborne infections is difficult to trace. Thus, data obtained by an examination of hospital records or public health reports are of limited value in resolving this problem, and a survey based on direct questioning of susceptible populations is unreliable.

Jefferson County, Kentucky, has particular reason for concern about this potential health hazard, since most of that County's sewage treatment in suburban areas is decentralized in small treatment plants ("package plants"). There are approximately 400 decentralized facilities in Jefferson County, and every densely populated subdivision has at least one plant located within the development. Most of these small plants are in very close proximity to residences; some are as close as 1 meter from residential homes and apartments. Figures 1 and 2 demonstrate this vividly. These are photographs of a typical package plant in a subdivision in southwestern Jefferson County. Note the turbulence created by the agitator churning the wastes in the sludge tank (Figure 1), and the proximity of this sludge tank to residences (Figure 2).

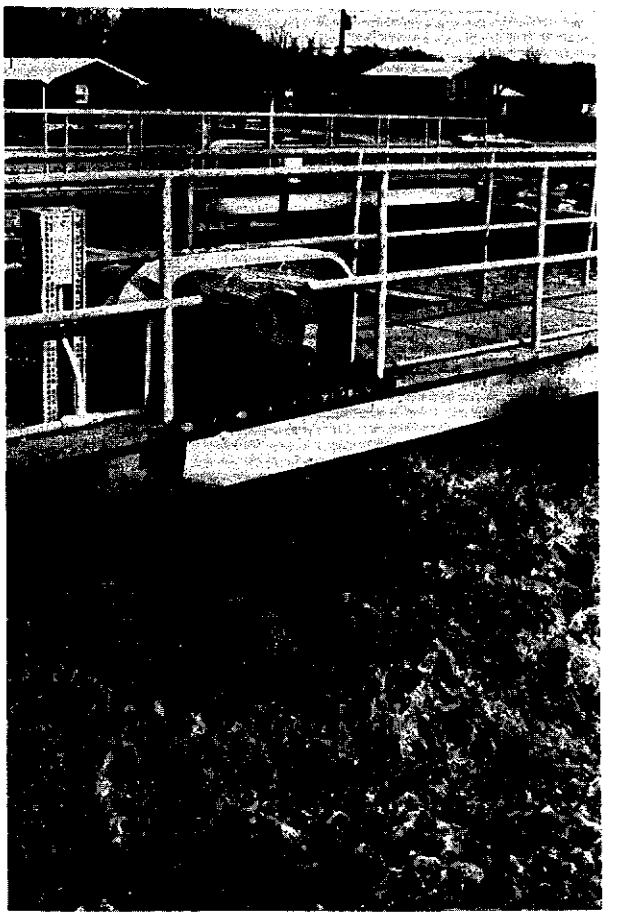
Most of these package plants rely on activated sludge processes with aeration provided by diffusers or mechanical aerators. Thus, this most densely populated county in Kentucky has a system of waste disposal which may insure the contact with microbial aerosols by large numbers of residents. By virtue of this decentralization, the problem of control is also magnified by comparison to communities which may institute effective controls by building barriers over a single, centralized facility.

The primary purpose of this study, which was initiated at the request of the Jefferson County Board of Health, was to determine an index of the actual distribution of microbial aerosols from package

treatment plants under a variety of environmental conditions. These data were considered essential for determining policies for the renovation and operation of existing plants, and for the design and location of future plants.

A corollary study of a decorative fountain was included as part of the original experimental design. A large recreational facility in downtown Louisville, known as the Belvedere, includes a series of fountains that splash into pools. Large stepping stones traverse these pools and form a pathway between scenic areas on the promenade. The Belvedere is used by thousands of visitors each year, and it is apparent that the water may become contaminated by the runoff from the stepping stones, by debris thrown into the water, and by the common practice of using the shallow pools for wading. The literature does not reveal field studies of aerosol droplets from such sources, even though similar decorative fountains are very common in urban parks and other recreational areas.

The purpose of this study was fulfilled by these experiments: (1) the determination of numbers of bacteria in airborne particles within the parameters of a sewage treatment plant under a variety of environmental conditions; (2) the determination of numbers of bacteria deposited on leaf surfaces at a sewage treatment plant; (3) the virulence of aerosolized bacteria by direct animal exposure; and (4) the estimate of the reliability of using colony forming units (CFU) on plates exposed in an Andersen Air Sampler as an index of numbers of airborne cells.



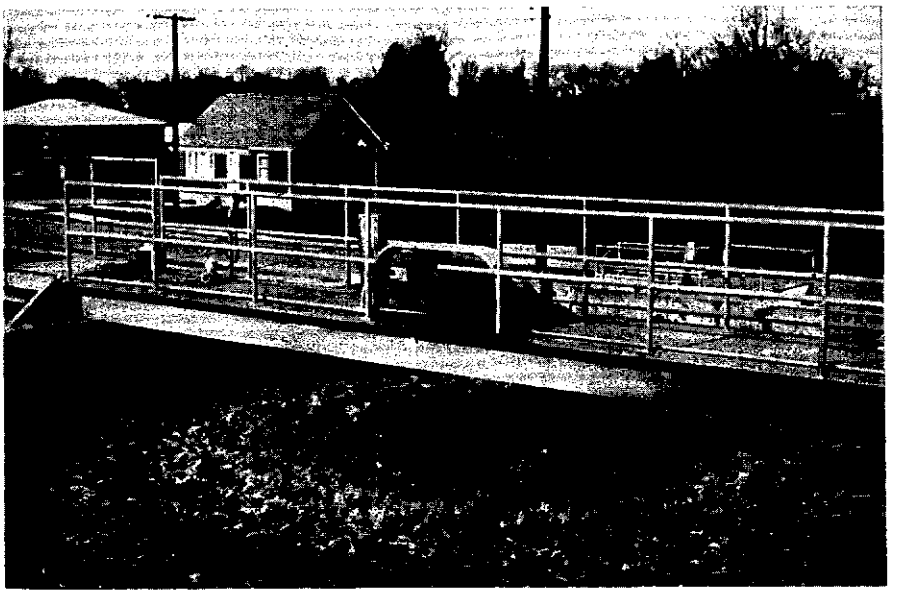


Figure 1

A Major Source of Microbial Aerosols

An activated sludge tank of a small package treatment plant; aeration in this plant is provided by mechanical agitators.

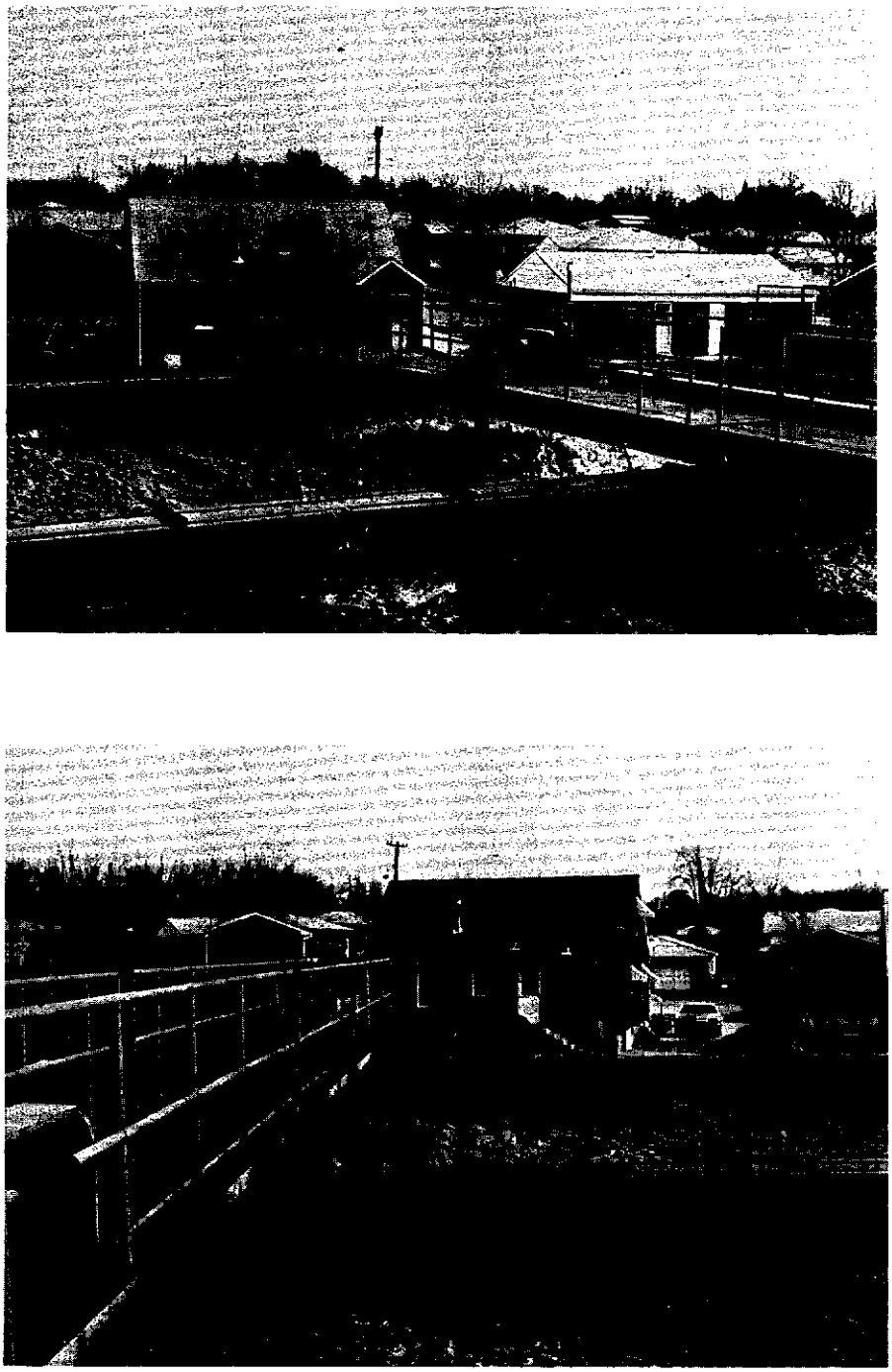


Figure 2

Proximity of Activated Sludge Tanks to Residences

Several residences in this subdivision are immediately adjacent to the activated sludge tank, and dozens of homes are well within the parameters demonstrated to be susceptible to elevated bacterial counts resulting from bacterial aerosols emitted from these sludge tanks.

MATERIALS AND METHODS

Treatment Plants Studied for Aerosol Emissions.

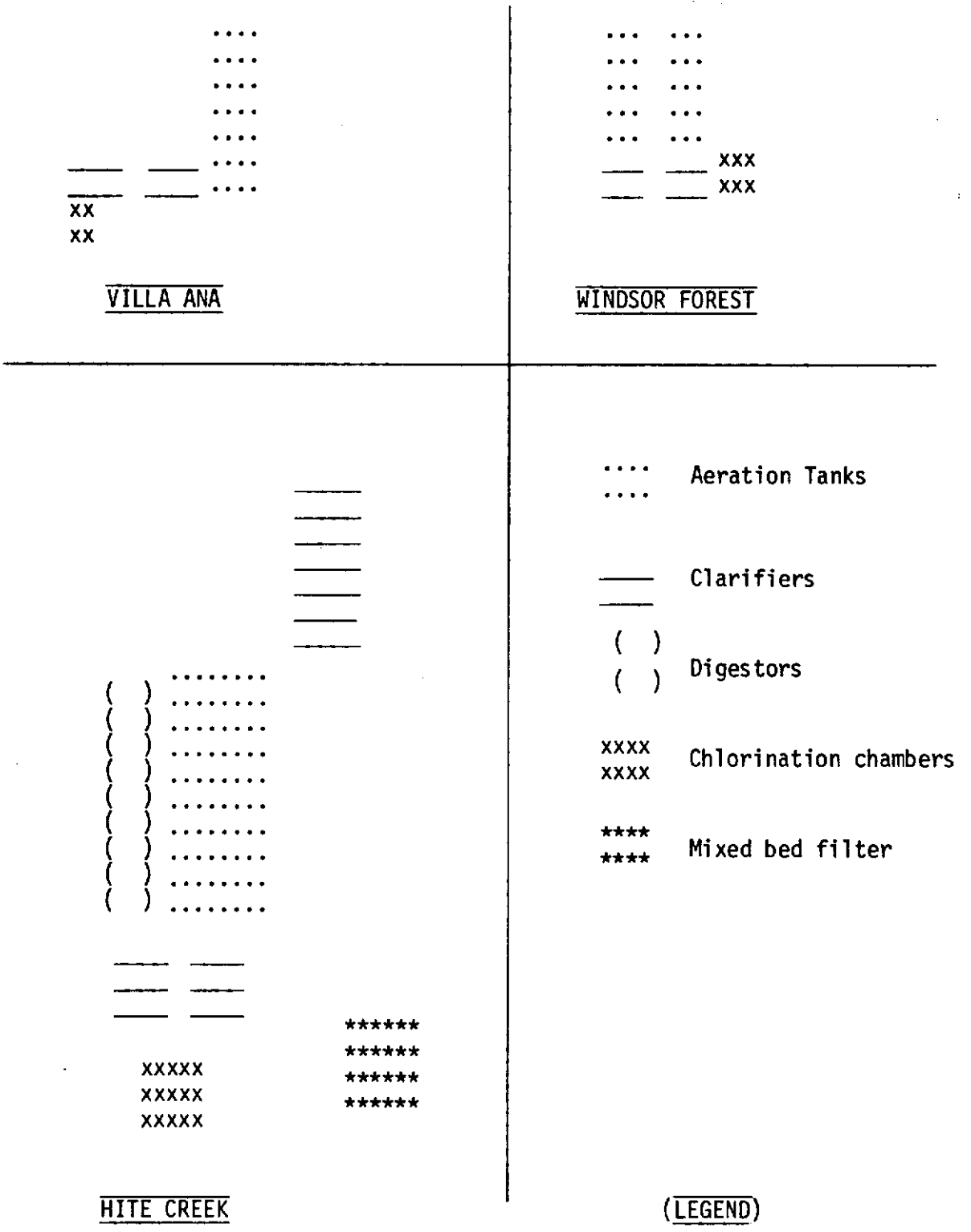
Air samples were collected from 3 treatment plants: Villa Ana, Windsor Forest, and Hite Creek. The first two plants are small facilities in southwestern Jefferson County. They are typical of the numerous package plants located in subdivisions close to residences. The influent is domestic waste from the subdivision. Hite Creek is a large modern facility, the largest sewage treatment plant outside of the centralized metropolitan treatment district. It treats a combination of domestic and industrial wastes, the latter primarily from a Ford Motor Company assembly plant.

The Villa Ana and Windsor Forest plants are designed similarly, with an activated sludge tank aerated by diffuse aeration. Hite Creek is a more elaborate structure, with digestors in addition to the sludge tank, and with a mixed bed filter, which treats the effluent from the clarifiers prior to chlorination. Since the mixed bed filter is enclosed, the most conspicuous difference between Hite Creek and the other two plants from the standpoint of apparent point sources of aerosols is the size of the Hite Creek sludge tank, and the vigorous mechanical aeration in these tanks compared to the diffused aeration in the smaller facilities. This vigorous aeration at Hite Creek is one of the reasons the plant was selected. Figure 3 simulates the design of these plants.

Villa Ana and Hite Creek are operated by the Metropolitan Sewer District. Windsor Forest is privately owned and operated, as are most

Figure 3

Design of Sewage Treatment Plants
Studied for Emission of Aerosols



of this County's package treatment plants. These two Metropolitan Sewer District - operated facilities were studied by this author in 1974-1976, and there are extensive data available on the effluent quality of these two plants.[20] While the Villa Ana plant has a higher quality effluent than many of the small package treatment plants, the quality is inferior to that of Hite Creek judged by turbidity, chlorine residual, pH, and coliform count.[20] The differences in the quality of the effluents -- particularly between Hite Creek and Villa Ana -- were additional reasons for selecting these plants for comparison of aerosol emission.

The sampling sites for this study on aerosolization were selected with reference to the aeration tanks, shown on Figure 3. Sampling stations at the Belvedere were selected with reference to the ponds that collected the water from the fountains.

Sampling Equipment.

The type of equipment used to study aerosols is critical with respect to the information provided and to the ability to compare published reports. The Andersen solid-medium impactor was used for this study, and it has been the preferred equipment for most related studies. The sampler draws air in by means of a vacuum pump which connects to an orifice on the device. The sampler fractionates aerosols by size, which is important because the ability of particles to penetrate the respiratory tract has been shown to depend upon particle size.[21-23] The Andersen sampler with 6 stages emulates the deposition in the respiratory tract by forcing air through critical orifices that separate airborne particles onto a series of stages that simulate the assumed contimuum of deposition from the nares, through the pharynx, into the bronchii and alveoli of the lung. The fractionation is based on the following assumed deposition size for

respiratory retention: stage 1, 7-11 μ, non-respirable; stage 2, 4.7 - 7 μ, penetration to pharynx; stage 3, 3.3 - 4.7 μ, penetration to primary bronchi; stages 4 and 5, 2.1 - 3.3 μ and 1.1 - 2.1 μ, penetration to secondary and terminal bronchi; stages 6 and 7, 0.65 - 1.1 μ, and 0.43 - 0.65 μ, penetration to lung alveoli.

The intermediary respirable particles have been considered interesting primarily to those studying the aerodynamics of particle fractionation, and most studies concerned with the health significance of microbial aerosols generalized the results into respirable vs non-respirable droplets.

Coincident with the beginning of this study, the Andersen 2000 Company manufactured a disposable, 2-staged sampler, which separated particles into respirable and non-respirable classes by an effective cut-off of 7 μ on the first stage. These samplers were considered more convenient and practical than the six stage device for this study, since multiple samples may be run simultaneously at small cost, and since the disposable samplers are presterilized. The author has recently corresponded with other workers using these 2-staged samplers, and there is now concern that they underestimate the counts. This author also found her counts low by comparison to others using the 6-staged sampler; however, the difference appeared to be relatively constant, and was not considered to interfere with the statistical tests that form most of the analysis of this study.

Culture Medium Used to Collect, Isolate, and Enumerate Bacteria from Aerosols.

All sampling included total bacterial counts, defined as units of

growth on Standard Plate Count Agar (Difco), and "enteric" counts, defined as the oxidase negative, Gram negative, facultative anaerobes classified in Groups I and II of the Family Enterobacteriaceae.[24] The term enteric count was used throughout this study to avoid perpetuating the confusion in terminology over the designation of organisms used as indicators of the bacterial contributions from treatment plants. The term "coliform" has been used most frequently in aerosol studies to describe the contribution from sewage, and as a generic term it suffices to describe the bacteria normally associated with fecal pollution. However, sanitary microbiologists traditionally distinguish between "fecal coliforms" and "non-fecal coliforms", and recent developments in the science of bacterial taxonomy and water microbiology have altered the original concept of these terms. The species Enterobacter aerogenes (formerly Aerobacter aerogenes) is now considered to contain 2 biotypes, distinguishable by the ability to produce gas from carbohydrate at 44.5°C, which permitted a distinction between fecal and non-fecal origin.[24] However, the species Escherichia coli is still considered by many to be the index organism of fecal pollution, and there are schemes for identifying E. coli which do not rely on differential growth characteristics at 44.5°C. Further, the genus Aerobacter itself is now discarded[24] and bacteria once placed in that genus are now assigned to either Klebsiella or Enterobacter. Concurrent with these changes, species of Klebsiella assumed more importance as an increasing number of reports indicated they are ubiquitous in water with fecal contamination; however, Klebsiella spp are not limited to fecal material. In summary, the original concepts of the terms fecal and non-fecal coliforms have been altered, the taxonomic relationships of bacteria in these groups is

still uncertain, and the choice indicator species is still a matter of debate. For these reasons, and the relative infrequency with which the genera in Groups I and II of the Enterobacteriaceae are isolated as part of the normal atmospheric microflora, in this study the indicators of contribution from the sewage treatment plants were considered to be all members of this Group, and are referred to collectively as the "enterics".

The choice of medium for bacteriological assays of polluted waters is also in an uncertain status. Selective media are useful primarily for assessing the total microflora, which the selective medium designed to inhibit the growth of non-specific bacteria also inhibits the growth of some of the index organisms. This is a particular problem when the inoculum contains damaged cells, and there is evidence that aerosolized bacteria are injured.[25-28] A pilot study compared the yields of enteric bacteria using EMB, McConkey's, Endo, and mFC medium, all common media for isolating and identifying enterics. The latter is a relatively new medium designed to rescucitate damaged cells[29] and it appeared to give the best yields with the Andersen Sampler.

The API-20 system was used to identify the presumed enterics isolated on mFC medium. Identification was made on pure cultures isolated on Trypticase Soy Agar after picking from colonies on the plates from the sampler. At intervals, parallel sampling was done with SS medium (Difco) for the detection of Salmonella and Shigella.

Factors Influencing Dispersement of Microbial Aerosols.

Since it is accepted that aerated sewage facilities are a source of microbial aerosols, the important remaining questions involve the conditions

which favor the distribution of viable particles from that source. The survival of microorganisms is always a function of their environment, and most studies of microbial aerosols include the effects of the factors most likely to impact on microbial survival. Similarly, every air sample in this study was accompanied by a record of temperature, relative humidity, and light intensity, as factors common to bacterial survival, and wind speed, distance, and direction (upwind or downwind) from the point source as specific determinants for viability and dispersal of bacteria in aerosols. Ozone levels were considered since ozone is well known as a microbicide, and Jefferson County, Kentucky is increasingly under "ozone-alerts". Except for ozone levels, which were obtained from records of the Jefferson County Air Pollution Board, all measurements were made at the time the samples were collected. In addition, effluent samples were tested for total and fecal coliforms by the standard MPN technique.[30]

Samples were collected from late summer, 1976 to late summer, 1978, at intervals that permitted data collection during most seasons. The extreme cold of the 1977-78 winter made it impossible to collect samples because the agar froze and the equipment would not work reliably in the sub-zero weather. With the exception of that period, the data were collected over the variety of seasonal conditions prevalent in the two year period. The major variable controlled was distance from the aeration tanks.

Since this study assumed that aerosols are emitted from these point sources, no attempt was made to pair each sample with air collected at extreme distances from the source, although air was sampled at several intervals at a site removed from any treatment plant. Paired samples were collected at upwind sites to ascertain the effects of prevalent wind direction, and the dispersion of the aerosols by local turbulence.

Deposition and Retention of Microorganisms on Foliage.

The sampling devices and techniques described permit an analysis of the dispersion of microbial aerosols up to the efficiency of the system. Even if 100% efficiency obtained for the recovery of bacteria during the sampling, the significance of the results is a problem for the reasons discussed previously; i.e., the pathogenesis of most of the organisms is not associated with inhalation. A second theoretical problem in determining the health significance is the consideration of the life-span of these aerosolized organisms if they are deposited on surfaces that might serve as vectors.

An alternative sampling technique was used to determine the deposition of microorganisms in the environment upwind and downwind of aerated sewage tanks, and the life-span of enteric organisms deposited on surfaces in nature. This involved the assay of bacterial counts on foliage plants, which also has practical significance because gardens are maintained near these treatment facilities. Pepper plants and geraniums, maintained in clay pots, were placed at sites upwind and downwind of the prevailing winds at Windsor Forest. Control plants were maintained in the laboratory in environmental chambers. At intervals, leaves were removed, placed in sterile containers, and prepared for total and enteric bacterial counts from quantified macerated leaf preparations. Paired samples were collected for counts taken immediately after collection from the field compared to counts from leaves maintained in the laboratory for 24 to 48 hours after removal from the foliage plant.

Analysis of Virulence of Aerosolized Bacteria.

Reference has been made to the evidence that aerosolization

damages cells. This is a second reason to question the health significance of such organisms, since this damage may affect the virulence of the pathogens. This question has been addressed in only a few studies. Pereira and Benjaminson[18] inoculated presumed Mycobacterium recovered from the stack lumen of a sewage treatment plant into guinea pigs and obtained gross lesions and histopathology; Randall and Ledbetter[5] tested capsule production of aerosolized Klebsiella as a function of distance from the source, and found that fewer of the bacteria isolated at 20, 50, and 100 feet were capable of encapsulation than those recovered at the source.

We tested the pathogenicity of aerosolized bacteria according to the classical standard for virulence -- the LD_{50} (lethal dose, 50%) of the bacteria in susceptible animals. Klebsiella pneumoniae was used because it is so common in aerosols from treatment facilities, because it is relatively more sensitive than such organisms as Mycobacterium, and because the susceptibility of mice to K. pneumoniae makes it a convenient assay organism. K. pneumoniae isolated from plates in the respiratory range were prepared for inoculation by methods that would minimize the intervening effects of subculturing. A presumed K. pneumoniae colony was prepared so that the inoculations could be made as soon as the API strip verified the identification. Prior to the series of inoculations, the density of K. pneumoniae in suspension was determined turbidimetrically. The optical density was determined for an overnight culture at 10 minute intervals, at which times triplicate pour plates were prepared for viable cell counts. The numbers of cells corresponding to optical density

was calculated by linear regression so that optical density could be used as a rapid method for enumerating bacteria immediately prior to injection. Twenty three samples were used to construct the regression equation.

Strain CFW mice, maintained on food and water ad libitum were weighed and them injected intraperitoneally (ip) with 0.25 ml of the cells suspended in varying densities in physiological, non-pyrogenic saline. The mice were observed daily for 7 days, and any animal dying during that period was autopsied, and organs with gross pathology (usually abscesses) were macerated in saline, and placed on mFC agar. The bacteria recovered were inoculated into API strips, and the API profile was compared to the original inoculum. These LD-50 tests were performed over a 3 month period, using samples collected from March, 1978, to June, 1978. A total of 121 mice were tested.

The LD_{50} was determined by a precise calculation of dose/gr wt of mouse, using a modified Reed-Muench method (shown in detail in the Results section). After the LD_{50} for the aerosolized bacteria was established, it was compared to the LD_{50} for a strain of K. pneumoniae isolated from the sputum of a hospitalized human with pneumonia. These bacteria were enumerated and prepared for ip inoculation into CFW mice by the same methods described above, and injections were made using doses numerically identical to the LD_{50} dose which had been determined for the aerosolized bacteria. The assumption was that if 50% of the mice inoculated with the known pathogen died within 7 days, the virulence of the aerosolized bacteria was approximately the same as the known

pathogen; if more than 50% of the mice died after injection with the known pathogen, the aerosolized bacteria should be considered less virulent.

There are theoretical problems with this method of testing virulence. Bacteria cannot be inoculated until they have grown on the culture medium in the Sampler, and subcultured for identification and preparation of the inoculum. There would therefore be a selection for those aerosolized bacteria capable of growth on the medium, which would bias the sample if those cells were not a predominant portion of the aerosolized population. In addition, the cells obtained after growth on the medium would have the opportunity to recover from a transitory damaged status. Therefore, a second method was employed to assay virulence, by the forced inhalation of air by mice at a sewage treatment plant. In June, 1978, mice were placed into respirators designed from bottles which would force contaminated air into the chamber by connecting the respirator to a vacuum source. The respirators were placed on catwalks which spanned the aeration basins, and the mice were exposed to 15 cu ft of air. Ten animals were tested on 10 sampling runs for one series of experiments in which the animals were caged after exposure, and observed for clinical symptoms for 2 weeks. After this time, they were sacrificed and the liver, lungs, spleen, and diaghragm were examined for gross pathology and prepared for plating on mFC agar. Six additional animals were tested for a second experiment, in which a portable laboratory was moved to the field, and the animals were sacrificed immediately after removal from the respirator. Their entire respiratory tract, from the pharyngeal-tracheal junction to the lungs,

was incubated in Trypticase Soy Broth at 37°C. After 24 hours, the broth suspensions were inoculated on mFC medium, and colonies were then isolated for identification by the API method.

Number of Viable Cells in Aerosolized Droplets.

Virtually all studies report the data obtained from aerosol samplers as colony forming units (CFU) per unit air sampled based upon the numbers of colonies that form when the plates are incubated. For very large counts, conversion factors are used on the CFU, but those factors are based upon probabilities of the numbers of droplets entering the orifice. The use of CFU as an index of cell number is common in bacteriology, but its validity depends upon the probability that a single cell gave rise to the colony. The use of CFU in aerosol studies would imply either that each colony derived from a single colony-forming unit, or that the numbers of CFU/droplet was constant. Andersen's major paper reviewing the properties of the Andersen Sampler estimated the numbers of cells as a function of particle size based on microscopic examination of Bacillus subtilis spore-laden aerosols in the six-staged sampler.[31] The estimates showed an increasing density of cells per droplet as droplet size increased.

There is good reason to assume that there might be many cells in one droplet. The size of many bacteria are much smaller than the droplets collected; e.g., Escherichia cells are rods measuring 1.1 - 1.5 μm to 2.0 - 6.0 μm.[24] Further, as bubbles emerge toward the air-water interface, there is a "scavenger effect" which concentrates the numbers of cells in the bursting bubbles. Blanchard and Syzdek[11] found a concentration

of bacteria in droplets from 10 to 1000 times their density at the origin of the droplets.

Although some investigators have noted that there might be more than one cell per droplet[11] and the techniques for studying the numbers of cells per droplet were mentioned by Andersen[31], the field studies at sewage treatment plants have reported counts as CFU from plates without assessing the numbers of cells which gave rise to the colony.

The actual number of cells in droplets is an important consideration when one contemplates the health significance of aerosols, since the numbers of pathogens in a dose have a profound effect upon the fate of the exposure, and every pathogen has some minimum infective dose. Furthermore, the numbers of CFU are used as the major index of the magnitude of contribution from the point source. Hickey and Reist[19] point out that the diminishing numbers of bacteria with distance from the source must be interpreted with care, because the numbers might reflect the rapid decay rate of aerosolized bacteria. If there are multiple bacteria per droplet, it must be assumed that a rapid death rate could effect the survivors per droplet, so that the numbers of viable cells would not be constant under varying sampling conditions.

A pilot study was performed as part of this investigation to determine if it is legitimate to assume consistency by using CFU. Two methods were tested, both beginning with the simultaneous collection of equal volumes of air at one site at distances varying from 3 m to 18 m of the aeration tanks. One set of plates was incubated without further manipulation for total CFU. The paired plate was prepared for total

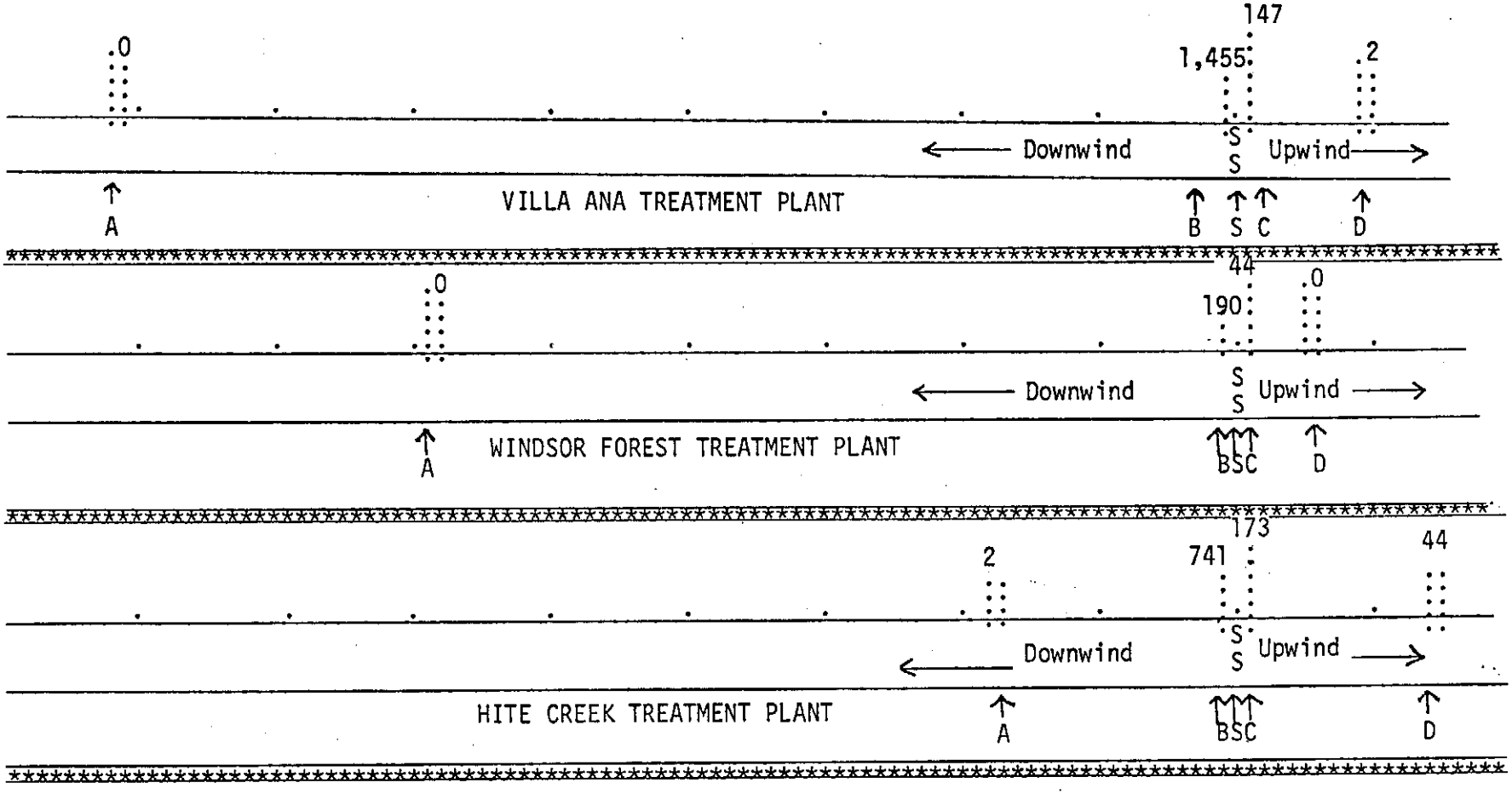
counts in two ways: for one, the surface was washed with sterile medium, and serial dilutions were used to inoculate plates for viable cell counts; for the other, 0.1 ml sterile medium was pipetted onto the plate, and the plate was spread with a sterile bent glass rod while the dish was rotated on a turntable. The second method was determined to be the more accurate and convenient, and was used to determine the data on cell counts per droplet reported in the Results. For this portion of the study, the 6-staged Andersen Sampler was used, since that is the equipment that has been used by most investigators.

RESULTS

Aerosolized Bacteria Recovered by the Andersen Sampler.

The total bacteria and enteric bacteria recovered from the samplers at the treatment plants confirmed in general that bacteria originating from aerated sludge basins are dispersed as airborne particles. Extreme variation was found in both the total numbers and the enteric bacteria, including great diversity in the numbers of bacteria that may be considered as "background"; i.e., away from any site considered as a probable point source of microbial aerosols. Samples taken at least 10 miles from any type of treatment facility yielded counts of total bacteria from 5/ cu m to more than 3,000/ cu m, and the apparent reasons for the differences included such factors as distance from a road and proximity to foliage (counts were lower downwind of areas with heavy foliage, presumably because the organisms are trapped on the plants).

An index of the ranges of enteric bacteria recovered is shown in Figure 4. The highest enteric count was 1,455/ cu m at 3 m downwind of an aerated tank. This may be compared to the highest number of enterics recovered upwind, which was 173/ cu m at 3 m. This fits the general pattern of a much higher recovery of enteric bacteria downwind of these tanks. However, since this study was designed to assay conditions affecting dispersal, and assumed that bacterial aerosols are emitted from these tanks, the upwind sites were not selected as background controls, but to determine the extent to which turbulence might



A = count at farthest distance tested, enteric bacteria, downwind
B = maximum count, enteric bacteria, downwind
S = source (aerated sludge basin)
C = maximum count, enteric bacteria, upwind
D = count at farthest distance tested, enteric bacteria, upwind

·————· = 100 meters

General Pattern of Enteric Bacterial Recovery from Air Samples

Figure 4

distribute the aerosols "upwind". The major species of enteric bacteria isolated, and their relative frequency, are shown in Table 1. The total bacterial counts also varied greatly, but they were consistently higher than enteric counts. The particles were distributed approximately equally between respirable and non-respirable, based on the fractionation available from the two-staged sampler.

An index of the differences between the three plants with respect to the dispersion of airborne bacteria is found in the percentages of samples with enteric bacteria. A total of 54 samples at Hite Creek, 35 at Windsor Forest, and 38 at Villa Ana were collected within 183 m downwind of the tanks. Using a total of 5 enterics/ cu m as an arbitrary standard, 67% of the Hite Creek samples were positive, compared to 43% of the Windsor Forest and 37% of the Villa Ana samples. This may be compared to the quality of the effluent: At Windsor Forest, 76% of the grab samples taken while the air samples were collected yielded total and fecal coliforms > 100/ml effluent; at Villa Ana the comparable tests yielded 80% total coliforms and 57% fecal coliforms; at Hite Creek these counts were 31% total coliforms and 13% fecal coliforms.

Analysis of the Factors Influencing Emission of Bacterial Aerosols.

The statistical relationships between the environmental factors and bacterial counts were tested by multiple regression. This is a standard biometric technique based on a linear regression model. The method is useful for analyzing the effects of 2 or more independent variables on a dependent variable. The test assesses the influence of

Table 1

Relative Frequency of Enteric Isolates from Aerosol Samples

SPECIES	Percentage of Identified Isolates			
	HITE CREEK	WINDSOR FOREST	VILLA ANA	TOTAL
Enterobacter agglomerans	35	18	29	29
Enterobacter cloacae	13	38	27	25
Klebsiella pneumoniae	17	22	13	18
Citrobacter freundii	16	6	21	15
Escherichia coli	12	10	4	9
Serratia liquefaciens	4	0	2	2
Enterobacter aerogenes	0	6	0	< 2
Klebsiella ozonae	2	0	2	< 2
Shigella flexnerii	0	0	2	< 1
Citrobacter diversum	1	0	0	< 1

variation of the predictor variables on the predicted variables using the method of least squares. The multiple correlation coefficient (R) indicates the proportion of the variance of the predicted variable which is explained by a linear relationship between predictor variables (X) and predicted variable (Y); in this case the environmental factors (X) and bacterial counts (Y). The regressions were performed separately for the total and enteric counts, using the combined counts from the two sampler stages. The significance of R^2 was determined by the F test, using the 95% confidence level. The magnitude of t-test values for each predictor variable was used to assess the significance of its contribution to the overall regression. The validity of a linear model was tested by scatter plots of the standard residuals vs the predicted Y.

The predictor variables were: Direction (upwind or downwind of the aeration tank; here a discrete variable assuming the value of 0 for upwind and 1 for downwind was used); relative humidity (%); air temperature (^{o}C); wind speed (mph); light intensity (ft. candles); distance from source (meters); water temperature (^{o}C); and ozone levels (ppm). The predicted variables were: total bacteria (CFU on plate count medium); and enteric bacteria (CFU on mFC medium, identified as enterics).

The regression coefficients, F-values, and t-values are shown in Table 2 for enteric counts and Table 3 for total counts for each plant, which was analyzed separately. For total counts, R^2 was significant only for Hite Creek, and distance from the source was the only factor which contributed significantly to the regression. The order of magnitude at that plant for variables not significant was: wind speed ≈ direction >

Table 2

Environmental Influences on the

Dispersal of Bacterial Aerosols

I. Enteric Counts

PLANT	R^2	Fs(df)	prob(Fs)	variable*	t	prob.(t)
VILLA ANA	0.419	3.959(8,44)	<0.002	Dir.	1.56	n.s.
				R.H.	3.27	p< 0.01
				A.T.	1.97	n.s.
				W.S.	2.45	p< 0.02
				L.I.	1.68	n.s.
				Dis.	2.90	p< 0.01
				W.T.	1.91	n.s.
				Oz.	2.04	n.s.
HITE CREEK	0.336	3.1621(8,50)	<0.005	Dir.	1.52	n.s.
				R.H.	0.49	n.s.
				A.T.	1.40	n.s.
				W.S.	0.19	n.s.
				L.I.	1.43	n.s
				Dis.	3.14	0.001<p<0.01
				W.T.	0.75	n.s.
				Oz.	1.46	n.s.
WINDSOR FOREST	0.178	0.758(8,28)	0.5<p<0.75	Dir.	1.04	n.s.
				R.H.	0.37	n.s.
				A.T.	1.17	n.s.
				W.S.	0.17	n.s.
				L.I.	0.55	n.s.
				Dis.	1.19	n.s.
				W.T.	1.32	n.s.
				Oz.	0.50	n.s.

Dir = Upwind or downwind; R.H. = Relative humidity (%); A.T. = Air temp.(^{o}C); W.S. = wind speed (mph); L.I. = Light intensity (ft. candles); Dis. = distance from source (meters); W.T. = Water temperature (^{o}C); Oz. = Ozone levels.

Table 3

Environmental Influences on the Dispersal of Bacterial Aerosols II. Total Counts

PLANT	R^2	Fs(df)	prob (Fs)	Variable*	t	prob (t)
VILLA ANA	0.118	0.7305(8,44)	0.5	Dir.	0.80	n.s.
				R.H.	0.15	n.s.
				A.T.	0.35	n.s.
				W.S.	1.07	n.s.
				L.I.	1.32	n.s.
				Dis.	1.47	n.s.
				W.T.	0.75	n.s.
				Oz.	0.56	n.s.
HITE CREEK	0.320	2.942(8,50)	0.01	Dir.	1.51	n.s.
				R.H.	1.04	n.s.
				A.T.	1.21	n.s.
				W.S.	1.52	n.s.
				L.I.	0.05	n.s.
				Dis.	3.59	0.005<p<0.001
				W.T.	1.04	n.s.
				Oz.	1.38	n.s.
WINDSOR FOREST	0.126	0.5061(8,28)	0.8	Dir.	0.37	n.s.
				R.H.	0.82	n.s.
				A.T.	0.77	n.s.
				W.S.	0.67	n.s.
				L.I.	0.80	n.s.
				Dis.	0.64	n.s.
				W.T.	0.35	n.s.
				Oz.	0.74	n.s.

* Dir = upwind or downwind; R.H. = relative humidity; A.T. = air temperature; W.S. = wind speed; L.I. = light intensity; Dis = distance from source; W.T.= water temperature; Oz. = ozone levels

ozone > air temperature > water temperature = relative humidity > light intensity.

For enteric counts, R^2 was significant for Villa Ana and Hite Creek, but not for Windsor Forest. At Villa Ana the variables which contributed significantly to the regression were distance, wind speed, and relative humidity. At Hite Creek distance was the only significant contributing factor. Of the remaining non-significant variables, ozone came the closest to significance.

When the standard residuals were plotted against the predicted Y, there appeared to be a slight deviation from a linear model in 5 of 6 cases. This was manifest in the form of a triangular shape in the scatter plots (for example, see Figure 5 for enteric counts at Villa Ana). The shape of these plots indicated greater variability in the samples collected at the higher values of Y. To correct for this, the transformation $Y = \frac{1}{X + 1}$ was used on the counts. The regression analysis was redone on the transformed data. The results produced scatter plots compatible with a linear model, (see Figure 6 for plot of data shown in Figure 5 after transformation), and the transformation did not change dramatically the regression patterns. For example, at Hite Creek, enteric counts, the transformed data yielded an $R^2 = 0.410$, $Fs = 4.344$, $p < 0.001$, compared to untransformed data, $R^2 = 0.336$, $Fs = 3.162$, $p \approx 0.005$. Therefore, the results from the untransformed data were used, and a linear regression model was assumed to be valid.

The Effects of Direction of Wind.

Since wind direction did not appear as a significant influence

--- NOTE ANALYSIS OF RESIDUALS

--- PLOT C21 VS C22

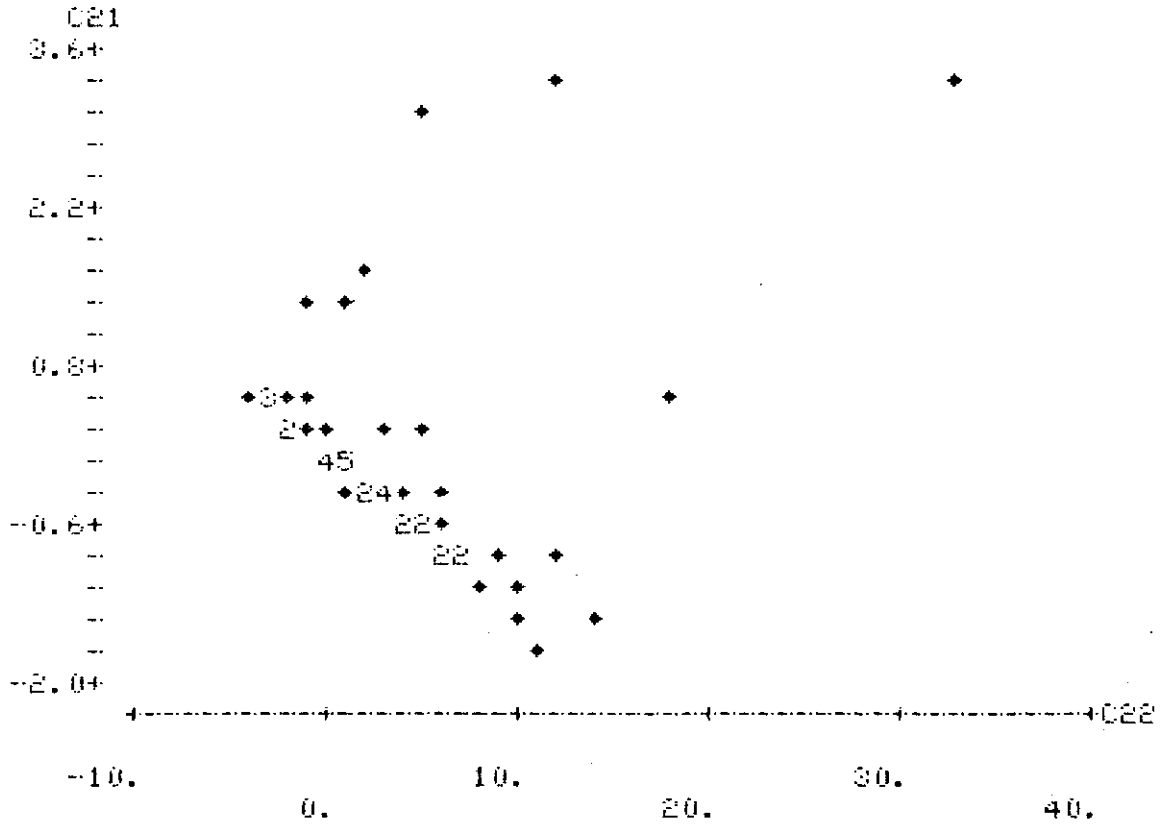


Figure 5

Plot of Standardized Residuals of Predictor Variables vs Predicted Variable

Villa Ana Plant, Enteric Counts (C22)

Residuals (C21)

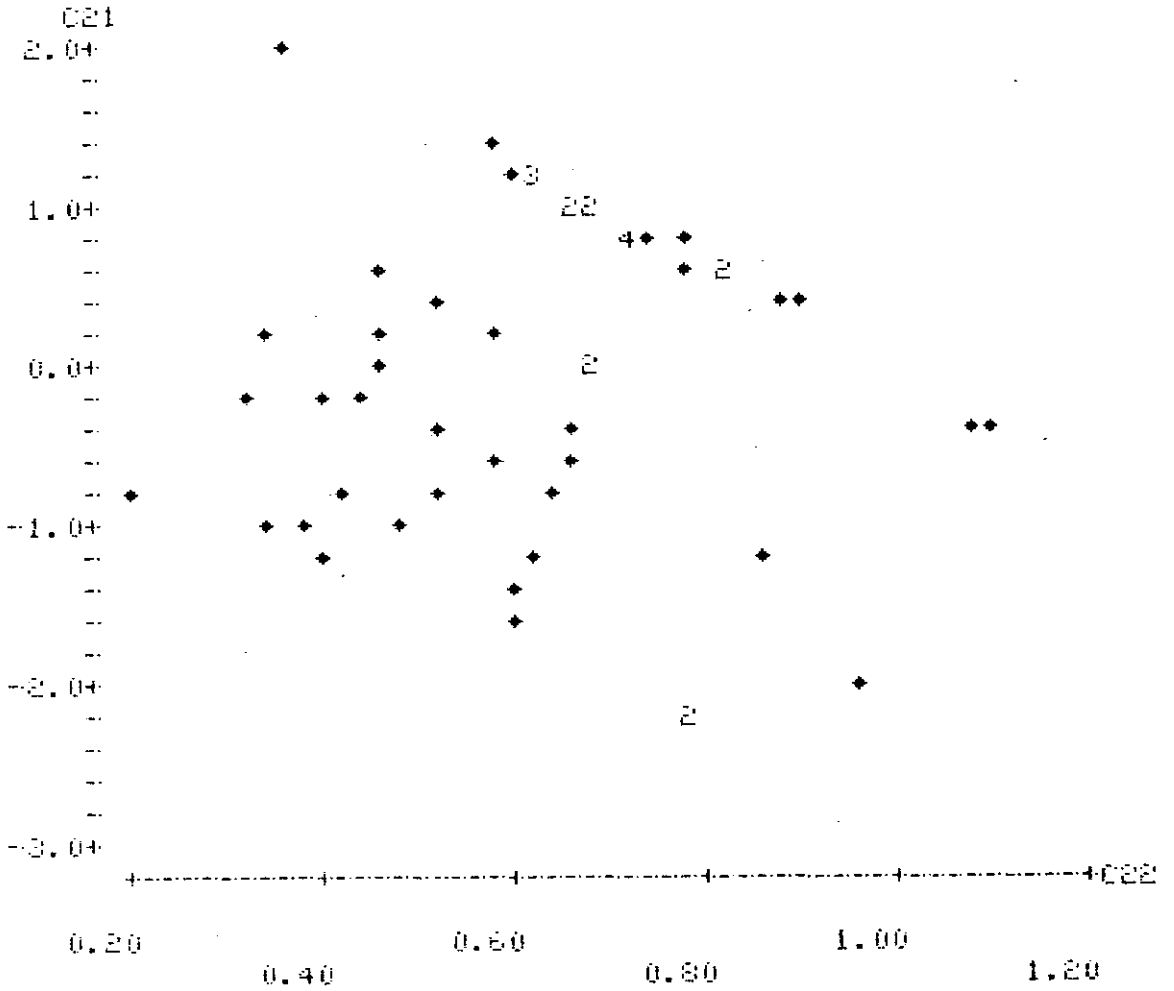


Figure 6

Plot of Standardized Residuals of Predictor Variables vs Predicted Variable, transformed $Y = \frac{1}{X + 1}$

Villa Ana Plant, Enteric Counts, Transformation of Data Plotted in Figure 5

when all predictor variables were combined for multiple regression, and since most workers have assigned significance to this factor even when samples were taken within the parameters of a treatment plant, separate tests were done for 29 paired samples taken at 6 m, 9 m, 15 m, 23 m, 46 m, 92 m, and 138 m upwind and downwind of the plant during the 2 year study, without respect to the values of the other independent variables, and with the data for all three plants combined.

The means of the total counts for the combined upwind data was 106 and for the downwind it was 108 -- obviously no difference could be shown by statistical analysis of the differences between means. Although there was a large difference in the means for the enteric counts ($\bar{X}$ = 3 upwind, $\bar{X}$ = 70 downwind), the t-test for the difference between these means also was not significant ($p < 0.15$); however, there was extreme variance in the downwind data (the counts ranged from 0/ cu m to 1,323/ cu m). Inspection of the enteric counts for the paired upwind and downwind samples does show that 62% of the downwind samples were positive for enteric bacteria, compared to 41% positive samples upwind, with a maximum upwind recovery of 44/ cu m, compared to the 1,323/ cu m downwind maximum count. A nonparametric test, the Wilcoxon Signed Rank Test for paired data, was also done on the enteric count data, but it also was not significant at the 95% level of confidence.

Therefore, within a range of 138 m upwind and downwind of the aeration basins, distance was the only factor that consistently affected the counts of enteric bacteria at a level of statistical significance. However, other variables show varying degrees of influence. Relative humidity and wind speed affected the regression significantly in one

data set, and ozone showed some tendency toward an effect in several sets. There was also a suggestion that air temperature and water temperature affected the enteric counts. Since all facilities were outdoors, the water temperature would be a function of air temperature. The one variable which consistently appeared to have little influence was light intensity.

Aerosolized Bacteria at the Belvedere Fountain.

The data for the samples collected at the Belvedere fountain are shown in Table 4. There were difficulties collecting numerous samples over a wide variety of conditions because the fountains are shut down regularly for the winter months, and because the fountains were turned off for long periods of time for repairs during the span of this study. The data in Table 4 are summarized for the characteristics of the air and water samples obtained:

1. Enteric bacteria are dispersed in airborne droplets as far as 23 m from the fountain spray (one sample at the Belvedere yielded the highest count of enteric bacteria of any sample collected during this two year study).
2. Coliforms (as defined in the MPN test used[30]) were present in the grab samples from the pools on 71% of the days tested and enteric airborne bacteria were recovered on each of these days;
3. On samples paired for air temperature, relative humidity, light intensity, and water temperature, distance from the fountain affected the numbers

Table 4

Enteric Bacteria Isolated

from Air and Water Samples at the Belvedere

	Date	A.T.*	R.H.*	Dis.*	W.S.*	L.I.*	W.T.*	Airborne Enteric*	Waterborne Total*	Waterborne Fecal*
I.	(9-21-76	24	46	2	5	5,000	19	59	490	0
	(9-21-76	24	46	3	3	5,000	19	15	**	**
	6-4-78	19	95	3	3	NA	17	TNTC	2,800	2,800
	5-31-78	21	94	5	3	NA	19	438	5,400	0
II.	(9-17-76	24	52	9	4	26	20	6	7,000	0
	(9-17-76	24	61	9	7	69	20	3	**	**
	(9-17-76	26	90	9	4	270	20	0	**	**
	5-23-78	22	90	9	0	NA	18	41	0	0
	9-11-77	19	60	9	4	190	16	153	9,200	9,200
III.	(8-16-77	27	90	18	9	2,000	24	347	0	0
	(8-16-77	27	90	23	9	2,000	24	118	**	**

Notes:

* A.T. = Air Temperature (^{o}C)
R.H. = Relative humidity (%)
Dis. = Distance (meters)
W.S. = Wind speed (mph)
L.I. = Light Intensity (ft. candles)
W.T. = Water Temperature (^{o}C)
Airborne Enteric = CFU enteric bacteria
Waterborne Total = total coliform/100 ml
Waterborne Fecal = Fecal coliform/100 ml

** Grab samples for coliforms taken only once when more than one air sample was taken on one day.

NA = not available; TNTC = Too numerous to count.

I,III were samples taken on one day to detect differences in counts by distance, II, samples taken on one day to detect differences in counts by light intensity.

of enteric bacteria recovered; e.g., at 18 m there were 347 bacteria/ cu m, and at 23 m there were 118 bacteria/ cu m.

4. There were four species of enteric bacteria of particular interest isolated at the Belvedere: Enterobacter agglomerans and Escherichia coli, which are common indicators of fecal pollution; Klebsiella pneumoniae, considered by many to be the best index organism of fecal pollution in aerosolized droplets; and Shigella flexnerii, which is one of the frank pathogens in the Enterobacteriaceae.

Virulence of Aerosolized Bacteria.

The range of dosage/ gr wt and the number of animals who died in the experiment on the LD_{50} of aerosolized Klebsiella pneumoniae is shown in Table 5 as part of the display of the dosages given to each of the 121 animals. These data show that the deaths were clustered at the heaviest dosage/weight. Each of the 28 animals which died exhibited abscesses on the internal organs, and those organs yielded K. pneumoniae of the identical API biotype as the inoculum.

The calculation of the LD_{50} is shown in Table 6. The data were combined arbitrarily into 6 groups according to the range of dose. Based on a linear model of dose - effect, one assumes that any animal dying at a lower dose would have died at any higher dose. Therefore, the percentage case fatality is calculated as cumulative deaths/survivors in ascending order of dose/weight (Table 6.B.). The LD_{50} was then calculated by the method of linear regression. The regression equation is shown in Table 6.C, and is plotted in Figure 7. The LD_{50}, determined by interpolation from the regression line, was 14,661,317 cells/gr wt.

Table 5

Dosage Rates and Deaths of CFW Mice

For Determination of Virulence

of Aerosolized Bacteria

Log of number of cells/ gr wt of mouse -- * death within 7 days

6.04	6.37	6.74	7.04*
6.07	6.37	6.80	7.06
6.12	6.37	6.84*	7.06
6.13	6.37	6.84*	7.08*
6.14	6.38	6.84	7.09
6.14	6.38	6.86	7.09*
6.17	6.39	6.89	7.10*
6.17	6.39	6.89	7.11*
6.18	6.40	6.90	7.12
6.20	6.40	6.90	7.12
6.20	6.41	6.93	7.13
6.20	6.42	6.93	7.13*
6.21	6.43	6.94	7.16*
6.22	6.43	6.94	7.18
6.23	6.43	6.95	7.18*
6.23	6.43	6.95	7.19*
6.24	6.44	6.96	7.20
6.24	6.44	6.96	7.20*
6.24	6.44	6.97	7.20*
6.26	6.45	6.98	7.22
6.26	6.45	6.98*	7.22*
6.26	6.48	6.99*	7.24
6.27	6.49*	6.99	7.24*
6.27	6.49	7.00*	7.28*
6.30	6.49	7.00	7.44*
6.32	6.52	7.00	7.47*
6.33	6.62	7.00	7.47*
6.34	6.63	7.02	7.49*
6.36	6.70	7.03*	7.51*
6.36	6.70	7.04	7.51*
			7.52*

Table 6

Determination of Case Fatalities

as a Function of Bacterial Dose

A. Assignment of Dosage Groups

GROUP	RANGE(log.no.)	AVG. GROUP DOSE	N	DEATHS	SURVIVORS
A	6.04 - 6.63	2,235,513	58	1	57
B	6.70 - 6.99	7,988,957	25	4	21
C	7.00 - 7.16	11,798,884	20	9	11
D	7.18 - 7.28	16,391,686	11	7	4
E	7.44 - 7.47	28,855,490	3	3	0
F	7.49 - 7.52	32,158,449	4	4	0

B. Cumulative Case Fatalaties

GROUP	CUMULATIVE DEATHS	CUMULATIVE SURVIVORS	PERCENT FATALITIES
A	1	93	1.1
B	5	36	12.2
C	14	15	48.3
D	21	4	84.0
E	24	0	100.0
F	28	0	100.00

C. Linear Regression Analysis

(Y = MX + B, where M = slope, B = Y intercept)

M = 251,339, B = 2,094,346;

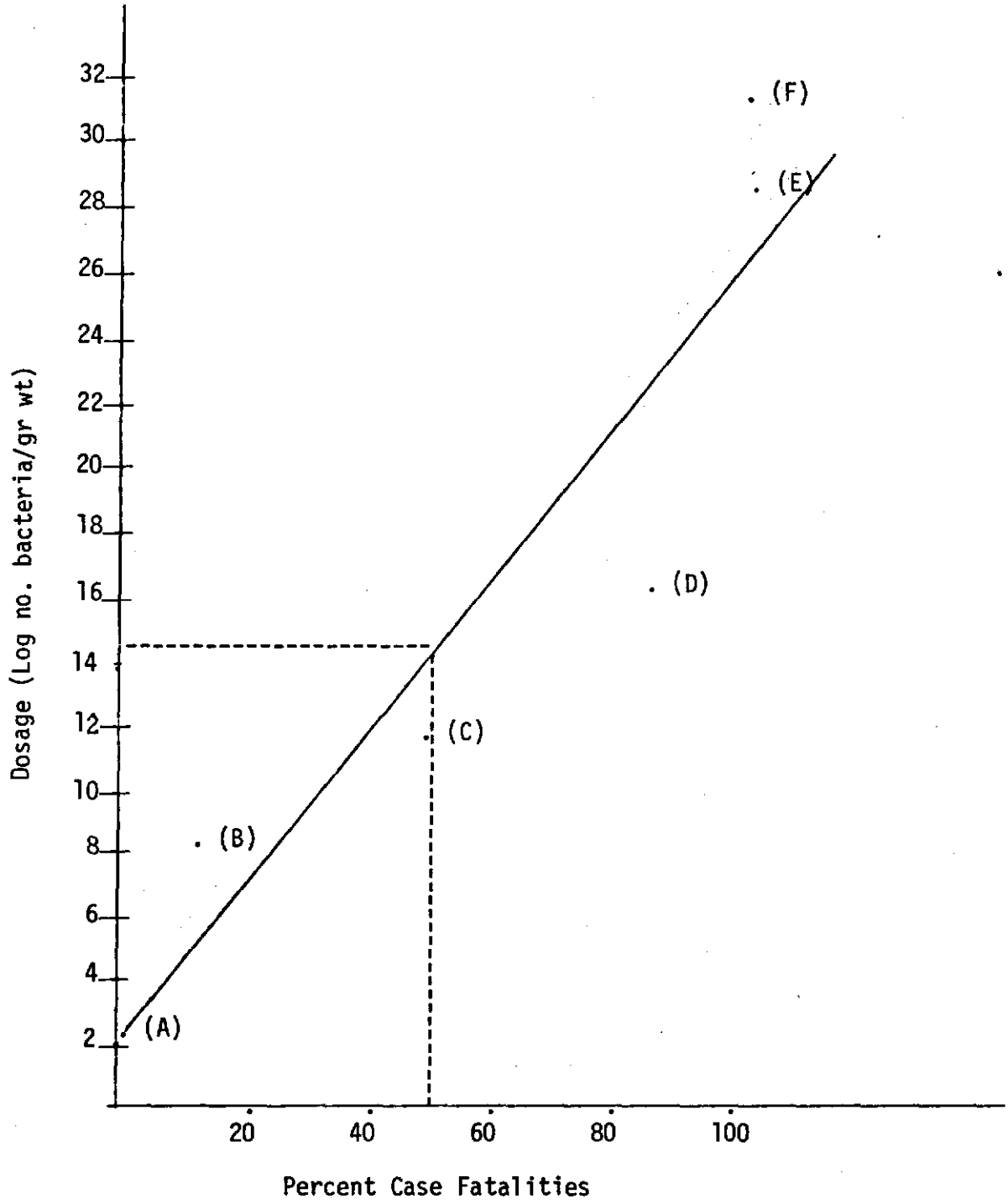
When X = 50, Y = 14,661,317 = $10^{7.16}$

D. LD_{50} for Aerosolized Klebsiella pneumoniae bacteria in CFW mice:

14,661,317 cells/ gr wt

Figure 7

Linear Regression Plot for LD_{50}
Of Aerosolized Klebsiella pneumoniae



The LD_{50} determined for the aerosolized K. pneumoniae was then used to inoculate the CFW mice with the strain of K. pneumoniae recovered from a hospitalized human patient. Within 7 days, 45% of these mice died. All dead animals yielded K. pneumoniae with an API profile matching the inoculum of the virulent strain.

The ten animals exposed to forced inhalation of air at a sewage treatment plant, and then observed for two weeks, showed no clinical symptoms, and autopsy revealed negative gross pathology. The cultures of macerated liver, lungs, spleen, and diaphragm were negative for enteric organisms.

In the case of the animals sacrificed immediately after forced inhalation of air at the sewage treatment plant, all the 24 hr cultures of the respiratory organs in Trypticase Soy Broth were positive for bacterial growth. Three of these broth cultures, from three different animals, subsequently yielded enteric bacteria on subculture. These enterics were identified as Escherichia coli, Enterobacter agglomerans, and K. pneumoniae.

Deposition and Retention of Enteric Bacteria on Foliage.

The total and enteric counts on foliage upwind and downwind of the aeration basin are shown in Table 7 and the counts for the paired samples tested at 0 and 48 hours are shown in Table 8.

The following analyses were done on these data: (1) Multiple regression to test the relationships between predictors relative humidity, air temperature, wind speed, and distance, on total and enteric counts; (2) the numbers of total and enteric counts on foliage maintained at the sewage treatment plant were compared to those on control plants maintained

Table 7

Total and Enteric Bacterial Counts

On Foliage Exposed to Bacterial Aerosols

Date	R.H.	A.T.	W.S.	Dis.	T.C. STP	E.C. STP	T.C. Con	E.C. Con
A. UPWIND SAMPLES								
5-5-78	66	13	17	12	630	---	630	---
5-5-78	66	13	17	12	130	---	33	---
5-20-78	60	27	15	12	1,000	400	100	47
5-20-78	60	27	15	12	200	33	97	40
5-27-78	42	30	7	12	330	100	100	33
5-27-78	42	30	7	12	1,300	33	67	0
7-14-78	68	29	9	12	1,300	330	0	0
7-14-78	68	29	9	12	1,700	0	0	0
7-16-78	51	28	11	12	0	0	0	0
7-16-78	51	28	11	12	670	670	0	0
7-18-78	43	29	8	12	330	0	130	33
7-18-78	43	29	8	12	1,300	1,300	130	0
7-31-78	67	25	5	12	14,000	10,000	170	0
7-31-78	67	25	5	12	5,000	2,700	67	0
8-12-78	79	23	4	12	9,700	1,300	0	0
8-12-78	79	23	4	12	4,700	1,300	0	0
8-14-78	61	29	5	12	28,000	20,000	470	33
8-14-78	61	29	5	12	3,300	330	100	0
B. DOWNWIND SAMPLES								
5-5-78	66	13	17	6	1,000	2,300	630	---
5-5-78	66	13	17	3.5	1,900	2,600	630	---
5-20-78	60	27	15	6	8,000	3,300	100	47
5-20-78	60	27	15	6	19,000	3,900	97	40
5-20-78	60	27	15	3.5	20,000	5,000	97	40
5-27-78	42	30	7	6	3,300	100	67	0
5-27-78	42	30	7	3.5	13,000	2,500	67	0
7-14-78	68	29	9	6	3,700	1,300	---	---
7-14-78	68	29	9	3.5	7,000	4,700	---	---
7-16-78	51	28	11	6	6,300	3,000	---	---
7-16-78	51	28	11	3.5	17,000	6,000	---	---
7-18-78	43	29	8	6	30,000	17,000	130	0
7-18-78	43	29	8	3.5	13,000	100	130	0
7-31-78	67	25	5	6	53,000	50,000	67	0
7-31-78	67	25	5	3.5	62,000	58,000	67	0
8-12-78	79	23	4	6	37,000	27,000	---	---
8-12-78	79	23	4	3.5	40,000	23,000	---	---
8-14-78	61	29	5	6	4,300	58,000	100	0
8-14-78	61	29	5	3.5	50,000	83,000	100	0

R.H. = relative humidity (%); A.T. = Air temperature (°C); W.S. = Wind speed (mph);
Dis. = distance (meters); T.C. = Total counts; E.C. = enteric counts;
STP = sewage treatment plant sites; Con = controls maintained in laboratory

Table 8

Retention of Bacteria on Foliage

Exposed to Bacterial Aerosols

A. BACTERIAL COUNTS (cells/ cm^2 of leaf tissue)

Date	Total Bacteria 0 hrs	Total Bacteria 48 hrs	Enteric Bacteria 0 hrs	Enteric Bacteria 48 hrs
5-20-78*	1,000	0	400	0
5-20-78*	200	0	33	0
5-20-78	19,000	1,000	3,900	900
5-20-78	20,000	2,700	5,000	3,000
5-27-78	13,000	3,200	2,500	1,100
8-14-78	4,300	670	58,000	0
8-14-78	50,000	8,300	83,000	0
7-16-78	6,300	3,000	3,000	1,300
7-16-78	17,000	13,000	6,000	3,300

B. STATISTICAL ANALYSIS (Wilcoxon Signed Ranks Test)

Variable	$\bar{X}$	S.D.	Ts	Critical Ts at .05	prob.
Total @ 0	13,270	14,985)	7.0	10-11	$0.01 < p < 0.025$
Total @ 48	5,620	8,639)			
Enteric @ 0	17,981	30,485)	0	8-9	$p = < 0.0039$
Enteric @ 48	1,066	1,292)			

*upwind samples; all others are downwind samples

indoors by the Mann-Whitney test (non parametric) and a 2 sample t-test (parametric) which allows for unequal variance; (3) the differences between samples tested at 0 and 48 hrs were analyzed by the Wilcoxon Signed Ranks test.

1. Multiple Regression Analysis of Influence of Environmental Factors.-- The descriptive statistics, t-values, regression coefficients, and Fs values for total and enteric upwind samples are shown in Table 9. These values for the downwind samples are in Table 10. For upwind samples, there was no significant relationship of environmental factors on either total or enteric counts. Wind speed was the only variable that showed any apparent influence on the regression. For downwind samples, the regression coefficients also were not significant at the 95% level, but they were very close to significance ($0.05 < p < 0.10$). In these samples also, wind speed was the one factor which appeared to influence the counts. There was no indication that a linear model was inappropriate.

2. Comparisons between Exposed and non-Exposed Foliage.-- The differences between the total and enteric counts for foliage downwind of the aeration basin at the sewage treatment plant site and for foliage maintained indoors was highly significant. The essential information for the statistical analyses is in Table 11. The differences for the foliage maintained upwind were significant for the total counts, but not for the enteric counts, tested by parametric and non-parametric statistics. There was a large difference between the central tendencies for these enteric counts, but the variation in the counts at the sewage treatment plant was extremely large.

Table 9

Effect of Environmental Factors

On Contamination of Foliage by Bacterial Aerosols:Upwind

Variable*	Descriptive Statistics: Mean	Descriptive Statistics: Standard Deviation	Regression Analysis: t	Regression Analysis: R^2	Regression Analysis: Fs(df)	Regression Analysis: Fs prob
A. Upwind, Total Counts (n=18)						
R.H.	59.7	11.9	0.77			
A.T.	25.9	5.17	0.19			
W.S.	9.0	4.42	1.76	0.314	2.139 (3,14)	$0.1<p<0.25$
Dis.	12.2	0	--			
T.C.	4,088.					
B. Upwind, Enteric Counts (n=18)						
R.H.	58.9	12.4	0.61			
A.T.	27.5	2.31	0.60			
W.S.	8.0	3.54	1.31	0.166	0.7986 (3,12	$0.5<p<0.75$
Dis.	12.2	0	--			
E.C.	2,406.	5,295.				

* R.H. = relative humidity (%)
A.T. = air temperature (^{o}C)
W.S. = wind speed (mph)
Dis. = distance (mph)
T.C. = total count (CFU)
E.C. = enteric count (CFU)

Table 10

Effect of Environmental Factors

On Contamination of Foliage by Bacterial Aerosols: Downwind

Variable*	Descriptive Statistics: Mean	Descriptive Statistics: Standard Deviation	Regression Analysis: t	Regression Analysis: R^2	Regression Analysis: Fs(df)	Regression Analysis: Fs prob
A. Downwind, Total Counts (n=19)						
R.H.	59.7	11.6	0.92			
A.T.	25.9	5.0	0.33			
W.S.	9.3	4.5	2.41	0.456	2.930 (4,14)	0.05<p<0.10
Dis.	4.9	1.2	0.91			
T.C.	20,500.	19,186.				
B. Downwind, Enteric Counts (n=19)						
R.H.	59.7	11.6	0.82			
A.T.	25.9	5.0	0.33			
W.S.	9.3	4.5	2.56	0.447	2.826 (4,14)	0.05<p<0.10
Dis.	4.9	1.2	0.18			
E.C.	18,463.	25,084.				

R.H. = relative humidity (%)
A.T. = air temperature (^{o}C)
W.S. = wind speed (MPH)
Dis. = distance (meters)
T.C. = total counts (CFU)
E.C. = enteric counts (CFU)

Table 11

Bacterial Contamination of Foliage

Exposed to Bacterial Aerosols

SAMPLE	Descriptive Statistics			Statistical Differences between Samples			
				T-test		Mann-Whitney	
	Mean	Standard Deviation	Median	T	prob	W	prob.
A. Downwind							
Total Count, STP	20,500	19,186	13,000	4.617	0.0001	437	0.0001
Total Count, Control	176	203	100				
Enteric Count, STP	18,463	25,084	4,700	3.201	0.005	247	0.007
Enteric Count, Control	42	4	40				
B. Upwind							
Total Count, STP	4,088	7,035	1,300	2.359	0.03	364	0.0003
Total Count, Control	174	182	100				
Enteric Count, STP	2,406	5,295	365	1.794	0.09	208	0.07
Enteric Count, Control	31	16	33				

3. Comparisons of Counts at 0 and 48 hrs after exposure.--

The total and enteric counts on leaves tested immediately after removal from the sewage treatment plant site were significantly higher than the counts on leaves from the same foliage plant, which leaves were retained in the laboratory for 48 hrs before testing. The significance was particularly large for the enteric counts. The descriptive statistics and values of the Wilcoxon Signed Rank Test are shown in Table 8.

Numbers of Cells in Aerosol Droplets.

The data for the comparisons between CFU on plates recovered from the second stage of the Andersen sampler and incubated with spreading, or after spreading, is shown in Table 12. Counts are for 0.5 cu m air. It is unnecessary to analyse the data statistically to detect that the CFU of the spread plates is consistently higher than the unspread plates. Multiple regression was done on the data to determine if the ratios of spread to unspread could be predicted as a function of the variables relative humidity, air temperature, wind speed, and distance. The regression of enteric counts on these variables yielded: $R^2 = 0.567$, $Fs = 3.27(df\ 4,10)$, $0.05 < p < 0.10$. The only factor which appeared to have an influence on this regression, which coefficient was close to significance, was wind speed ($t = 2.26$) The regression was significant for the total counts on these variables: $R^2 = 0.497$, $Fs = 3.707(df\ 4,15)$, $0.025 < p < 0.05$. The only variable which influenced this regression significantly was relative humidity, but the effect was not profound ($t = 2.18$, $0.04 < p < 0.05$).

When the ratios of spread to unspread plates for total counts are ordered in descending order, from the highest (22.17:1) to the lowest (1:1, excluding the 2 cases when spread was less than unspread), and com-

Table 12

Comparison of Enumeration by
Colony Counts and Viable Cell Counts

A. Bacterial Counts of Paired Samples

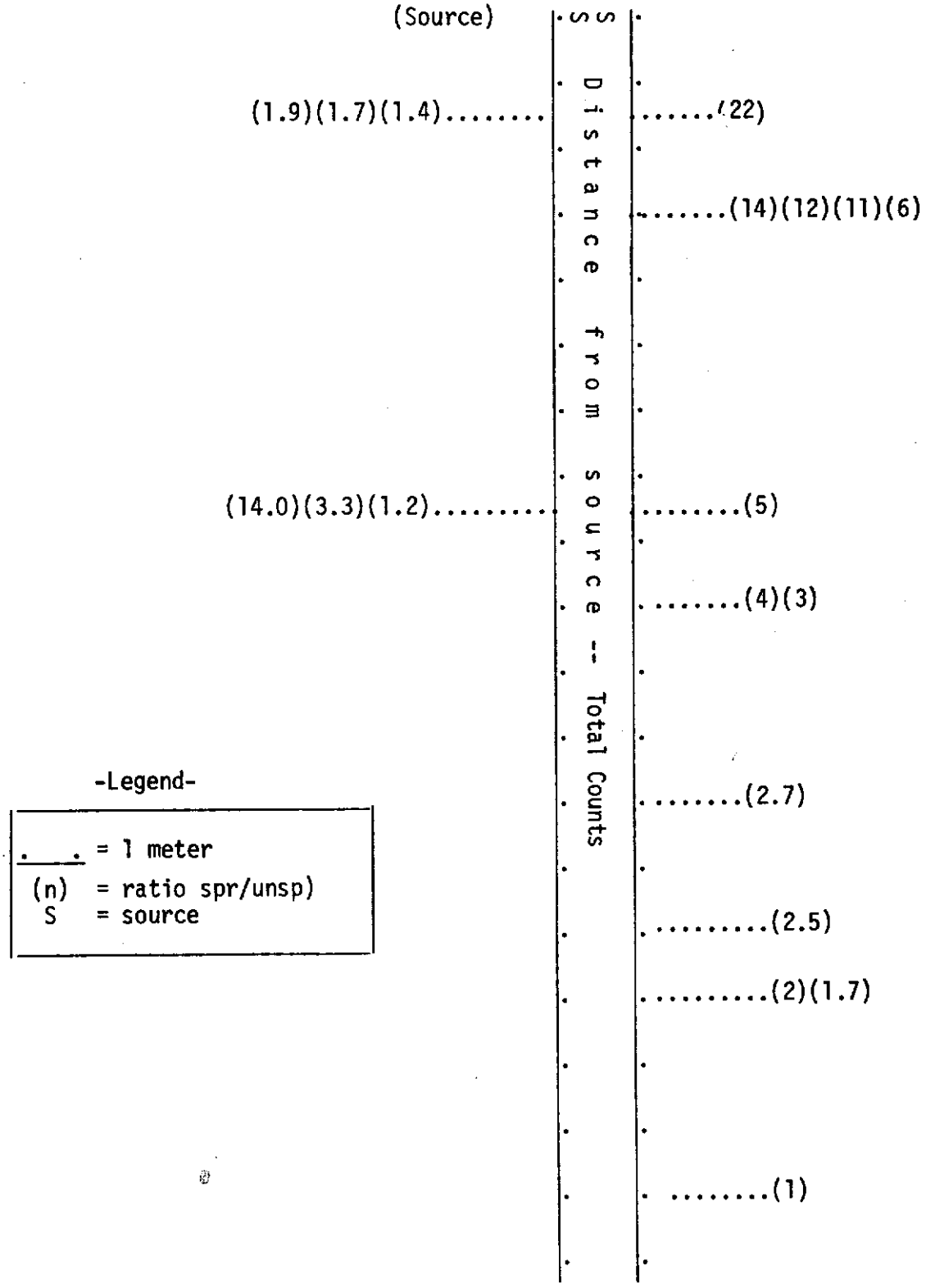
Date	R.H.	A.T.	W.S.	Dis.	Total Counts			Enteric Counts		
					Uns.	Spr.	Ratio	Uns.	Spr.	Ratio
4-30-78	65	18	9	3	123	1,400	11.38	--	--	--
5-6-78	60	16	6	3	240	3,500	14.58	--	--	--
5-8-78	93	20	9	3	210	2,600	12.38	61	400	25.00
5-20-78	60	27	15	9	2	9	4.50	--	--	--
5-27-78	42	30	7	12	21	57	2.71	8	40	5.00
5-16-78	78	16	4	9	15	12	0.80	3	16	5.33
7-14-78	68	29	9	9	4	12	3.00	3	3	1.00
7-14-78	68	29	9	18	2	2	1.00	2	5	2.50
7-18-78	43	29	8	1.5	35	776	22.17	13	24	1.85
7-18-78	43	29	8	3	11	66	6.00	13	3	0.23
7-18-78	43	29	8	7.5	1	14	14.00	--	--	--
7-31-78	67	25	5	1.5	38	66	1.74	63	220	3.49
7-31-78	67	25	5	7.5	13	70	5.38	25	130	5.20
7-31-78	67	25	5	14	2	5	2.50	1	1	1.00
8-12-78	79	23	4	1.5	17	24	1.41	4	10	2.50
8-12-78	79	23	4	7.5	10	12	1.20	3	8	2.67
8-12-78	79	23	4	15	8	14	1.75	--	--	--
8-14-78	61	29	5	1.5	18	34	1.94	43	180	4.19
8-14-78	61	29	5	7.5	3	10	3.33	13	80	6.15
8-14-78	61	29	5	15	5	10	2.00	2	3	1.50
B. Descriptive Statistics										
Mean (total)	64.2	25.15	6.7	7.7	-	-	5.69	-	-	-
Mean (enteric)	65.13	26.0	6.13	7.7	-	-	-	-	-	4.64
St.Dev. (total)	13.8	4.6	2.7	5.46	-	-	5.95	-	-	-
St.Dev. (enteric)	14.4	4.11	1.96	5.73	-	-	-	-	-	5.89

Notes: R.H. = relative humidity (%); A.T. = air temperature (C); W.S. = wind speed (mph); Dis. = distance (meters)

Total Counts = Counts on plate count agar; Enteric Counts = Counts from mFC agar; uns. = plate count from CFU following incubation directly after exposure in sampler; spr. = plate count after spreading plate immediately after exposure in sampler

pared to distance, a pattern emerges which does suggest that distance is inversely related to the ratio. This is depicted in Figure 8. Note that the highest ratio (22:1) was obtained at the closest distance (1.5 m) and the lowest (1:1) was obtained at the farthest site (18 m), and most of the data follow this progression. It is also interesting that the anomolous data were obtained at two points: the closest (1.5 m) and the middle distance (7.5 m). This pattern of an inverse relationship was also indicated in the enteric bacterial counts, but the relationship was considerably more tenuous. The highest ratio (25:1) was obtained at a relatively close distance (3 m) and the lowest (1:1) at a relatively far point (14 m). Most of the lower ratios were at the far distances except for the same anomaly noted in the total counts; that is, the most dramatic variation from the progression occurred at the closest distance, 1.5 m. In the case of the enteric plates, the data obtained at this close distance failed to show an inverse relationship between distance and ratio.

Figure 8
Relationship of Ratio Cells/Droplet
and Distance from Source



DISCUSSION AND CONCLUSIONS

The primary purpose of this study was to analyze factors which might reveal the potential health hazard created by aerosols emitted from the numerous package treatment plants which are located in densely populated areas in Jefferson County, Kentucky. This study followed sufficient field testing of aerosols from sewage treatment plants by others to assume that bacteria are emitted from any treatment facility which creates airborne droplets. Therefore this study was intended to provide information associated with certain characteristics of bacterial aerosols that have not been studied extensively, including the quantitative measurement of virulence of aerosolized bacteria, and the potential hazard created by ingestion of these bacteria deposited on edible food products. In addition, the design permitted an analysis of the influence of environmental factors for comparison with other reports, in order to determine if there is a model which would permit predicting emission patterns as a function of major environmental influences. Such patterns have not been discerned clearly in previous studies. The one facet of the study related primarily to the science of aerosolization was the determination of the number of bacteria in each droplet.

The numbers of bacteria recovered by the Andersen sampler in this study tended to be lower than many of the reports in the literature. The plastic two-staged sampler apparently yields lower counts than the six-staged sampler. However, the numbers of CFU and the pattern of

variation were within the range of other studies, and we assumed there was a constant difference between this equipment and that used for other studies. One must assume that the efficiency of recovery is the same for each type of sampler, so the absolute values should not have affected any of the analyses in this study.

A major conclusion of this study is that it is not possible to construct a useful model for predicting emission rates from sewage treatment facilities as a function of any variable other than distance from the source. Certain factors which theoretically might affect bacterial survival, such as light intensity, showed no influence on the counts. Other factors, as relative humidity and air temperature, showed some influence, as did ozone levels. The inability to demonstrate statistical significance to factors other than distance does not mean that these factors do not influence the emission patterns. If it were possible to hold every variable constant and manipulate them individually, it is highly probable that significant relationships could be found. For example, in a recent study of coliforms emitted from wastewater effluent sprays used for irrigation, Teltsch and Katznelsen[32] studied the effects of relative humidity and solar irradiation by methods which reduced variation from other factors. Samplers were placed at equal distances from the source; the experiment was carried out over one 10 hr period; seeded bacteria with a selective genetic marker were used for the assays; only samples with the highest counts at each interval were considered representative; runs in which mean wind direction changed were discarded. Under these conditions, there was a high correlation between bacterial counts and the relative humidity (positive correlation) and solar irradiation (negative correlation). However, under non-standardized

conditions, there is extreme variation in the bacterial counts and essentially non-linear fluctuations in the predictor variables as sets of factors influencing each count. Therefore, it is compelling to conclude that the control of aerosol emissions in the typical plant, subject to the diverse environmental influences of the outdoors, must be based entirely on the consideration of distance from susceptible human populations if there is no intervening barrier.

This relationship of counts to distance held true also with respect to direction from the source when direction was included as one variable in a multiple regression. It is emphasized again that upwind distances were not selected as controls; rather, the upwind sites were within the plant parameter at distances that might be subject to countervailing currents which could create "mini-downwind" sites opposed to the apparent prevailing wind. There is little question that greater numbers of bacteria are dispersed consistently according to the prevailing downwind. This was shown even more dramatically on the bacterial samples collected on foliage plants. However, while the variations in the counts were primarily responsible for obscuring the difference between upwind and downwind samples collected in the Andersen Samplers, the frequency with which enteric bacteria were isolated upwind made it apparent that the emission of hazardous aerosols must be assumed to occur in all areas surrounding a sewage treatment plant.

The study on the deposition and retention of enteric bacteria on foliage plants contributes in several ways to the study of the health significance from bacterial aerosols. The method itself suggests an interesting alternative to the techniques commonly used for detecting bacteria near a sewage treatment plant, and for studying their half-life.

This investigation points up a hazard that has not been considered in most discussions of bacterial aerosols -- the ingestion of enteric bacteria on edible products grown in home gardens near a sewage treatment plant, or on such products in commercial agricultural lots near treatment plants. This danger has been considered for crops and soil contaminated by effluent sprays used in irrigation.

Knittel et al[33] showed that clinical isolates of Klebsiella pneumoniae can not only survive, but proliferate, on lettuce leaves maintained at room temperature at a growth rate comparable to K. pneumoniae recovered from environmental sources. Based on the data in this study, if one consumed an average size salad made of raw lettuce leaves eaten immediately after picking, approximately 20 million enteric bacteria might be consumed. Since most of the pathogens emitted from a sewage treatment plant would be associated with fecal contaminants, and since most of these bacteria require ingestion as an effective portal of entry, this could be a greater hazard than the inhalation of these bacteria. This also has implications for community health problems. Selden et al[34] showed that Klebsiella ingested from environmental sources may colonize the intestine, producing reservoirs for nosocomial infections. We did an antibiotic sensitivity profile on 2 isolates of K. pneumoniae isolates, which showed them sensitive to most of the antibiotics useful for Gram negative bacterial infections. However, it is typical for isolates unassociated with a hospital environment to exhibit sensitivity to most of the antibiotics which are ineffective against strains recovered from nosocomial infections in hospitals. The antibiotic sensitivity does not appear to affect the virulence, and the kinetics of mutation toward drug resistance suggests the resistance is obtained rapidly by plasmid transfer.

The particle count/CFU suggest that a more detailed analysis should be done on this characteristic of aerosolized droplets, and this investigator is carrying out such studies. If the data of this investigation are confirmed, studies which relied on CFU should be re-analyzed to determine if the same conclusions would hold if the actual numbers of bacteria/droplet were considered. The initial study of the question suggests that the variation in the cells/droplet might make it extremely difficult to rely on any constant for converting CFU to numbers of bacteria, although there is a strong suggestion that distance might be one reliable predictor.

Blanchard and Syzdek[11] studied the numbers of bacteria in droplets which were produced by air forced through a seeded water sample. The droplets were collected on an inverted Petri dish held just above the surface of the water. This method is not comparable to that used in this study, the major difference being the lack of environmental stress on the bacteria recovered so close to the origin, and the consistency of the environmental parameters. However, it is interesting that they found a large concentration of bacteria in bubbles, which concentration peaked at 1000 times for drop sizes of 70 μm, and then declined steadily toward unity for drops of 80 μm to 140 μm.

It is also interesting that the majority of the CFU as used in the standard method appear to be composed of pure cultures. We had little difficulty with consistent isolation of single species picked from the emergent colonies, and other reports do not note the difficulties that would be found identifying species if the colonies frequently contained mixed cultures. The plates that were spread after collection did not appear to contain a greater number of species than the paired unspread plates. This lends credence to the assumption

that selected bacteria are accumulated in bursting bubbles, probably as a function of their surface charge. Woodcock[35,36] has shown the differential accumulation of particles in droplets as a function of vertical distribution, another factor which biases the kinds of particles recovered in emerging droplets. However, another explanation of the occurrence of one species is that that species might become predominant under the conditions of growth provided after collection.

The experiments on virulence of the aerosolized bacteria should contribute toward conclusions on the health hazard of such bacteria. The results of the LD_{50} calculations are a clear demonstration that the factors determining virulence are not permanently altered in the aerosolized cells. Knittel et al[34] studied the virulence of Klebsiella pneumoniae after cells were passed through numerous generations on simulated environmental substrates. They found no loss of virulence in a bovine mastitis strain passed through 290 generations; while human isolates eventually showed a decreased virulence, the LD_{50} was not increased until after 100 transfers on the simulated natural substrate.

One must speculate on the importance of the rescusitation necessarily provided for the cells during the preparation for inoculating the experimental animals. It is reasonable to assume that the substrate provided by the respiratory canal of humans would also provide recuperation for cells, and that the countervailing antibacterial forces (ciliary movement, bacteriocidal secretions, phagocytic activity, etc.) would have no greater significance for aerosolized than non-aerosolized cells, unless the kinetics of the host immune responses compared to the bacterial resuscitation responses were unfavorable for bacterial survival.

The three experiments performed on mice to test virulence: LD_{50},

forced inhalation followed by immediate culture, and forced inhalation followed by observation for clinical symptoms, may be linked as follows: aerosol emissions from sewage treatment plants contain viable pathogenic bacteria genetically capable of metabolic activities correlated with virulence which are comparable to the virulence of a known pathogenic strain; these bacteria are inhaled, and some portion of the population is disseminated distal to the nares, where they may be isolated for short periods after contact; inhalation of these bacteria will not be associated necessarily with clinical disease, and they may not be recovered from the host after a prolonged period from the contact. The latter fact is certainly not exceptional for aerosolized pathogens, and all humans are in frequent contact with pathogens which do not become established because of the efficient immune reactions of normal hosts. This author therefore concludes that microbial aerosols generated from sources with pathogens do add to human contact with potentially hazardous bacteria. This would place the significance of bacterial aerosols in the same status as most hazardous abiotic and biotic pollutants in the air and water. That is, one can prove experimentally that contact with many pollutants may cause disease, as in the case of many carcinogenic abiotic organic contaminants, but it has been rare to prove that those contaminants in fact have raised significantly the rate of any disease. Most of the regulations controlling the emission of contaminants is based on this presumed hazard, and there is therefore no apparent reason to exclude the significance of microbial aerosols because the epidemiological evidence is lacking to prove that they have caused disease.

This study also confirms an assumption which is intuitively obvious; that is, there is an inevitable dilemma in determining controls which will protect the public from the hazards of poor quality effluent and from the contaminated air from the same treatment facility. The quality of the effluent in plants which use aerobic processes will depend in part on the degree of aeration, but the more vigorous the aeration, the greater is the airborne hazard. This was shown in the comparisons of the Hite Creek plant with the Villa Ana and Windsor Forest plants. Hite Creek has more vigorous aeration, a higher quality effluent, and a higher rate of bacterial aerosol emissions, than the other 2 plants.

The results of the studies on the Belvedere emphasize the increased burden of airborne pathogens produced by aerosols from contaminated water, and suggest the importance of extending the consideration of aerosol controls to facilities other than sewage treatment plants. In fact, such sources as decorative fountains are reasier to control than sewage treatment plants with aerobic processes. In the latter, the process of treatment requires the presence of bacteria and of vigorous aeration; in the former there is no necessary reason that these waters must be contaminated, and ordinances prohibiting wading would effectively block the source of hazardous bacteria. During the course of this study, these data helped persuade the Louisville Board of Alderman to pass an ordinance against wading in all such pools in the City of Louisville, which includes the Belvedere.

RECOMMENDATIONS

The conclusions of this study, and the recommendations based on those conclusions, are summarized:

A. Conclusions

1. Small package treatment plants with aerobic processes emit bacterial-laden aerosols which remain airborne for distances that encompass occupancy by residents in many of the locations with these plants in Jefferson County, Kentucky. Therefore, the location of these plants in densely populated regions increases the probability of human contact with pathogenic organisms.
2. In addition to the dangers of inhaling aerosolized bacteria, or direct contact by touching contaminated fomites, the bacteria dispersed from the treatment plants might be ingested on garden products grown near a sewage treatment plant.
3. The only reliable predictors of emission rate from a sewage treatment plant are wind direction and distance from source; however, at close distances to the aerated basins it is unsafe to assume the absence of bacterial aerosols upwind. Therefore, in cases where plants are built in close proximity to residential areas, the safest standard for protection of the residents is distance

from the plant regardless of the prevailing winds.

4. There is great variability of aerosol emission rate, which is not readily correlated with such climatic conditions as temperature, relative humidity, wind speed, and light intensity. Therefore, it does not appear feasible that a model may be constructed which would be useful for predicting emission rates as a function of climatic variables. This must be considered in the design of field tests used to determine control measures for plants at particular locations.
5. The absence of epidemiological evidence of a health hazard by contact with bacterial aerosols from sewage treatment plants does not confer legitimacy on policies which ignore this potential hazard. This study shows the bacteria from the sewage treatment plant are deposited in the respiratory tract of mammals, and that the most common respiratory pathogens in such aerosols, Klebsiella pneumoniae, do not lose their pathogenicity by aerosolization.
6. Decorative fountains may emit hazardous aerosols if they splash into pools contaminated by pathogenic bacteria. Since human contact is the most important source of such contamination, there is little justification for increasing the airborne burden of pathogens

when regulations prohibiting wading and similar forms of contact would eliminate this danger.

7. The use of CFU from plates incubated directly after exposure in an air sampler is not a reliable method for enumerating the viable bacteria in aerosol droplets. At present, there is no apparent reliable constant for converting the CFU to viable cell counts; therefore, less reliance should be made on the numbers of colonies as an index of the health hazard, since this hazard is a function of the numbers of cells contacted.

B. Recommendations.

1. If one combines the hazards of poor effluent quality from the package plants in Jefferson County, Kentucky[20] with the dangers of bacterial aerosols, there is little doubt that this community would be better served by alternate methods of sewage treatment which would produce better quality effluent and which would reduce the extent of bacterial emissions in aerosols. The only solution other than centralization of treatment facilities is to increase the quality of the effluents from these small plants and to construct barriers around each plant to prevent the dispersal of aerosols. The cost of both in terms of construction and man-hours

required for enforcing regulations makes this solution practically impossible. Therefore, this study should encourage those attempting to enforce the expansion of a centralized treatment facility for Jefferson County. Perhaps it could also help inform the public, which has been opposed to such expansion of the centralized system, of the reasons why this newer system is needed.

2. There is no reasonable way to invoke immediate solutions to the problems of aerosols from the numerous treatment plants in Jefferson County. However, residents near these plants should be warned that edible garden products should not be eaten raw.

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