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EVALUATION OF HEALTH EFFECTS ASSOCIATED  
WITH THE APPLICATION OF WASTEWATER TO LAND

SOUTHWEST RESEARCH INSTITUTE  
SAN ANTONIO, TEXAS

DECEMBER 1976

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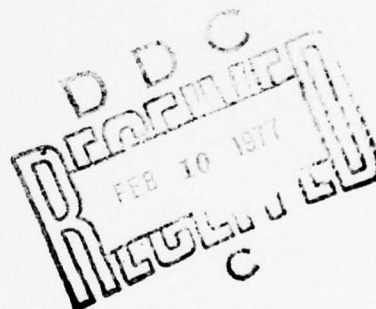
# EVALUATION OF HEALTH EFFECTS ASSOCIATED WITH THE APPLICATION OF WASTEWATER TO LAND

ANNUAL REPORT - PHASE I  
SwRI Project 01-4297-000

by

Donald E. Johnson  
James W. Register  
David E. Camann  
Conan H. Millstein  
Joe L. Gulinson

December 1976



Supported by:

**U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND**  
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**Contract No. DAMD17-75-C-5072**  
**Capt. John P. Glennon, Project Officer**

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spray site. Meteorological data are presented along with this information. Methods for collection, storage and analysis of effluents and aerosols for different parameters have been evaluated during the field survey. ←

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## SUMMARY

A preliminary environmental monitoring survey has been conducted at a spray irrigation site located in Pleasanton, California. This site was selected following a review of sites within the United States which utilize spray irrigation for disposal of secondarily treated municipal wastewater. This interim report contains the results of monitoring the effluent wastewater for inorganic and organic chemical parameters, bacteria and viruses. Data are also given for the quantities and types of bacteria and viruses present in ambient air around the spray site. Meteorological data are presented along with this information. Methods for collection, storage and analysis of effluents and aerosols for different parameters have been evaluated during the field survey.

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## I. INTRODUCTION

The use of irrigation, either ditch or spray, of secondary treated wastewater has become a very important alternative to tertiary wastewater treatment. The United States Environmental Protection Agency presently requires that land application be considered in the selection of mechanisms for disposal of treated municipal sewage. The use of irrigation for disposal of treated wastewater has some significant advantages in that the disposal of these wastes does not lead to pollution of rivers, streams and lakes and that the water is reused, which is especially important in arid areas of the United States.

There has been some concern expressed that the utilization of spray irrigation of wastewater near populated areas could have some adverse health effects. The possibility of health effects has been on two fronts: contamination of ground water with bacterial and viral pathogens as well as trace metals, and transport of these pollutants to populated areas via aerosol formation. This study is directed at examination of the transport of bacteriae, viruses and possibly trace metals via aerosols.

There have been some studies directed at examination of aerosols created by spray irrigation of wastewater, and one of the more recent is that of Sorber, et al<sup>(1)</sup> in which he developed a model to predict the pathogen concentration downwind from a spray source. This model considers the percentage of wastewater aerosolized, the number of pathogens in the wastewater, wastewater treatment effectiveness and prevailing meteorological conditions. Some preliminary testing of the model was done during a four-week field study conducted in the Southwestern part of the United States when a golf course was irrigated with treated wastewater. This study examined bacterial aerosols and it showed that there were relatively high concentrations of bacterial aerosols that can be transmitted for considerable distances and that these bacteria aerosols are in the respirable range.

This project was initiated to conduct a comprehensive study of the production and transport of bacterial and viral pathogens as well as possibly trace metals from spray irrigation of secondary treated municipal wastewater with the possibility that an epidemiological study would be conducted if there were sufficient quantities of these materials transported to an exposed population. One of the primary overall objectives of this

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study is to determine the quantities and types of pathogens aerosolized, the distances which they are transported downwind and whether or not these pathogens are in sufficient quantities to produce measureable health effects in exposed populations. The overall study will provide sufficient data such that if health problems are indicated, design parameters could be recommended for treatment of the wastewater to prevent possible health effects. This is extremely important, since it is apparent that the utilization of spray irrigation could have numerous advantages in different areas of the United States.

This project was designed to be conducted during a three-phase protocol. Phase I was to select a suitable site for the conduct of the study and to develop the optimum methods for sampling and analysis of aerosols and effluent samples for different types of bacteria, viruses and chemical constituents. Phase II was to involve an extensive environmental monitoring of the spray site to cover a period of some six months. This phase would accurately measure the quantities and types of pathogens and other constituents under a variety of meteorological conditions and hopefully with a range of source strengths of these materials in the wastewater. Following the completion of Phase II, the decision would be made as to whether or not there are sufficient numbers of these pathogens present to warrant conduct of Phase III, an epidemiology study of an exposed population nearby the spray fields versus a control population at some distance from the spray fields. This interim report includes the results of Phase I. The report will also include conclusions and recommendations for the design of Phase II. The information contained in this report will be used for the design of Phase II.

## II. EXPERIMENTAL

### A. Selection of Site

In our original proposal, site selection criteria were described on pages 23-27. These criteria were amended to include the recommendation that the wastewater for the spray irrigation site must be primarily domestic in origin (more than 75%); however, certain types of industrial effluent would not be acceptable even at 25% or less. Another criterion that was added was that the spray site would emphasize application of wastewater to agricultural rather than recreational land such as golf courses. Our original proposal listed a site near Walla Walla, Washington and possibly some additional sites in the California area which appeared to meet most of the criteria described. The proposal recommended that additional contacts be made via telephone to come up with the final recommendation as to potential sites, and that the final site be selected following on-site inspection by the project leader, a sanitary engineer and an epidemiologist. Following these inspections, one of the sites would be selected if it met all or most of the selection criteria.

Based on the evaluation of the information supplied via telephone contacts and examination of printed material, two sites were proposed for on-site inspection; one near Walla Walla, Washington, and the other near Pleasanton, California. A site visit was made to these two sites during the period of 13 to 17 July, 1975 by Major Charles A. Sorber from the Army, Mrs. E. Tompkins, from the Environmental Protection Agency at Research Triangle Park and Dr. D. E. Johnson, project leader, Southwest Research Institute.

The result of these site visits was that the Pleasanton, California, site was selected. A memorandum for the record was supplied by Major Charles Sorber on 23 July which compiles a considerable amount of information on these two sites. A summary of the information supplied by Major Sorber is that the Walla Walla site was found to be unsatisfactory for several reasons, but the most important is the fact that the wastewater is highly treated, resulting in an effluent containing less than 10 mg/liter BOD. Furthermore, the effluent is disinfected and mixed with creek water (varying ratio but averages about 50/50) such that the final effluent sprayed is of very high quality. Also, the irrigation water is delivered through a separate distribution system to some 425 farmers and other persons with rights to this water. Each applicator chooses whether to use the irrigation

water provided under this arrangement, so that it would be difficult to conduct an environmental study with a coordinated sampling program. The Walla Walla site did have the advantage that there were significant numbers of a middle class population living within the spray area, i.e. small farms located throughout the spray fields. This would have provided an exposed population present very close to the spray fields. Unfortunately, the effluent is highly treated and is further diluted by river water such that the final BOD perhaps averaged less than 5 mg/liter.

The selection of Pleasanton, California was approved by the Project Officer on the basis of the recommendation from the on-site survey. Spray irrigation at the Pleasanton site is conducted on a year-round basis and is under control of the City of Pleasanton, California. The wastewater treatment plant effluent, despite the fact that it has undergone secondary treatment, is relatively poor in quality, with BOD's in the range of 70-80 mg/liter at the plant effluent. Very small quantities of chlorine are added to the wastewater. This chlorine comes from a washdown operation within the plant. It is added to the secondary clarifier for odor control (10 mg/liter). These quantities of chlorine should have little disinfectant influence on the final effluent.

#### B. Description of Study Area

A schematic of the study area for Pleasanton is shown in Figure 1. A population of middle class socioeconomic characteristics is located within one mile to the east/southeast of the plant. This population is located in a recently completed (within three years) subdivision off Mission Drive. Mission Drive runs east-west, and the street begins on Sunol opposite the treatment plant. The prevailing winds in this area are from the southwest to northwest quadrant; thus, this populated area would be downwind of the spray fields. There is sufficient population in this subdivision to conduct an epidemiological study, and there is a more than adequate population of similar socioeconomic characteristics located away from the spray fields. The population in the subdivision near the treatment plant live within 700 to 1250 feet from the center of the spray fields. A part of the study plan of Phase I was to determine the micrometeorology in this area specifically to see what percentage of time this subdivision is downwind of the spray fields.

The Pleasanton water reclamation plant is under the control of the City government of Pleasanton, California. City personnel involved are as follows:



City Manager	William H. Edgar
Assistant City Manager	Alan B. Campbell
Public Works Director of Field Services	H. Arnold Eaton
Public Works Field Superintendent	Arthur N. Monaco

Arthur Monaco is directly responsible for the operation of the water reclamation plant, including the spray irrigation disposal system. The City has no immediate (within three years) plans to change the operation of this treatment plant with regards to the spray irrigation. The plant will be modified (estimated November 1975) by the addition of an activated biofilter process (ABF) following the trickling filter. It is anticipated that the addition of this process will result in an additional BOD reduction of 20 to 25 mg/l in the final effluent. It will also reduce the general organic loading to the aerated ponds, thereby reducing or completely eliminating odor problems. The treatment plant is operating at near capacity, and they are expected to add only a few new connections.

The operation of spray irrigation is under the direct control of the City of Pleasanton, and it appears to be in a favorable position to continue the current spray practices without significant change for at least the next three years. The spray operation is nearly a "break-even" cost operation for the City because of the income it derives from the leasing of the pastures to cattlemen. The beef cattle which graze on the grass (44% alta tall fescue, 33% Ariki perennial rye and 23% Potomac Grass Orchard) appear to grow well without supplemental food. The grazing of dairy cattle on the spray fields is prohibited.

Although the report by Major Sorber contains details of the treatment plant, the following is a brief description of the plant operations.

An average of 1.4 MGD of sewage is treated by trickling filtration and is stored in aeration ponds with a total retention volume of three million gallons. Approximately 600 gal/min is recycled during irrigation from Pond No. 2 outlet to Pond No. 1 inlet to promote further oxidation. Pumping into the irrigation system from Pond No. 2 begins daily between 8 and 9 AM and continues for a period of 16 to 18 hours depending on the early morning level of the pond, anticipated inflow and precipitation. For

optimum operation, the pond level is kept between 2.4 and 3.0 feet with the most desirable level being 2.6 to 2.7 feet. Their objective is to spray daily until about one-half of the wastewater present in the two ponds has been sprayed. The residence times in the two oxidation ponds have not been determined. A dye experiment is planned for Phase II to accurately measure this parameter. It appeared from visual observation (using a dye) that there was not adequate mixing in these ponds, and a considerable amount of "short circuiting" occurred within each pond.

A six-day test conducted in January of 1975 showed a 72% removal of BOD from a mean of 352 mg/liter in raw sewage to a mean of 98 mg/l in the secondary effluent, and total suspended solids removal of 79% from 537 mg/l to 112 mg/l. The results of our one-month study conducted August 14 through September 13, 1975, indicate that the mean BOD level and total suspended solids level of the pond effluent being used for irrigation are 35 mg/l and 27 mg/l, respectively. These values are somewhat lower than those reported for the plant (previous year's average). It is possible that the time period under study in Phase I (middle of August to middle of September) represents a nontypical time period for the plant.

There are four major industrial waste sources in the area. These are as follows:

1. The Cheese factory waste probably has the greatest effect on the overall BOD input to the plant. Data obtained by the Kennedy Engineers, Inc. for the City of Pleasanton indicate that the BOD level discharge waste from the cheese factory is approximately ten times that of normal domestic sewage. The cheese factory discharges approximately 0.016 MGD.
2. There is sizeable input (0.12 MGD) from the Kaiser Research Center, but available test data indicate it to be a waste of normal strength.
3. The Villa Armondo Winery contributes the majority of its discharge during the crushing season (fall and early winter) (0.01 MGD). Data on this discharge have not been evaluated as of this date.
4. The Alameda County Fair contributes a major portion of the industrial flow during its month-long operation each summer (0.25 MGD). Available data indicate the possibility of high strength wastes.

The City of Pleasanton contracted with Pacific Environmental Laboratory of San Francisco, California, to perform analyses on unused groundwater wells located around the spray irrigation fields. This work has been completed and the data are available.

C. Establishment of On-Site Facilities

Meteorology -- Two members of the SwRI meteorological team arrived in Pleasanton, California, August 5, 1975, to select a suitable site for the installation of two weather stations for collection of data at two- and ten-meter heights. Consideration was given to terrain, vegetation, construction, human activities and the location of the spray fields versus the location of the human population in the selection of the weather station site. The site selected represents the best compromise of all the above considerations. The weather stations were located on secured city property approximately half-way between the spray fields and the nearest populated area. The exact locations are shown on Figures 1 and 2. Data collection began August 11, 1975 and will continue through the duration of all three phases of this study.

Laboratory -- Two members of the SwRI environmental survey team arrived in Pleasanton, California, August 10, 1975, and immediately began to set up and equip the conference room set aside for project use by the City of Pleasanton Public Works Department as an on-site laboratory. A telephone, refrigerator, sterilizer, incubator and sink were installed. In addition, a fluorometer, pH meter and Hach kit were set up for on-site water analyses. A refrigerated timed-cycle sampler was set up next to the irrigation pumps located on the bank of effluent Pond No. 2 for collection of composite samples during the daily periods of irrigation.

D. Spray Irrigation Operation

The spray equipment is designed so that a volume of water equal to the amount of incoming raw sewage less evaporation losses is sprayed onto cattle pastures daily. This is accomplished by keeping the level of the final effluent Pond No. 2 (See Figure 2) receiving the secondary effluent as constant as possible by pumping more or less water to the irrigation fields. Two pumps, 100- and 75-HP, respectively, with a third standby pump, are used to move the water through 10-inch mains to the fields.

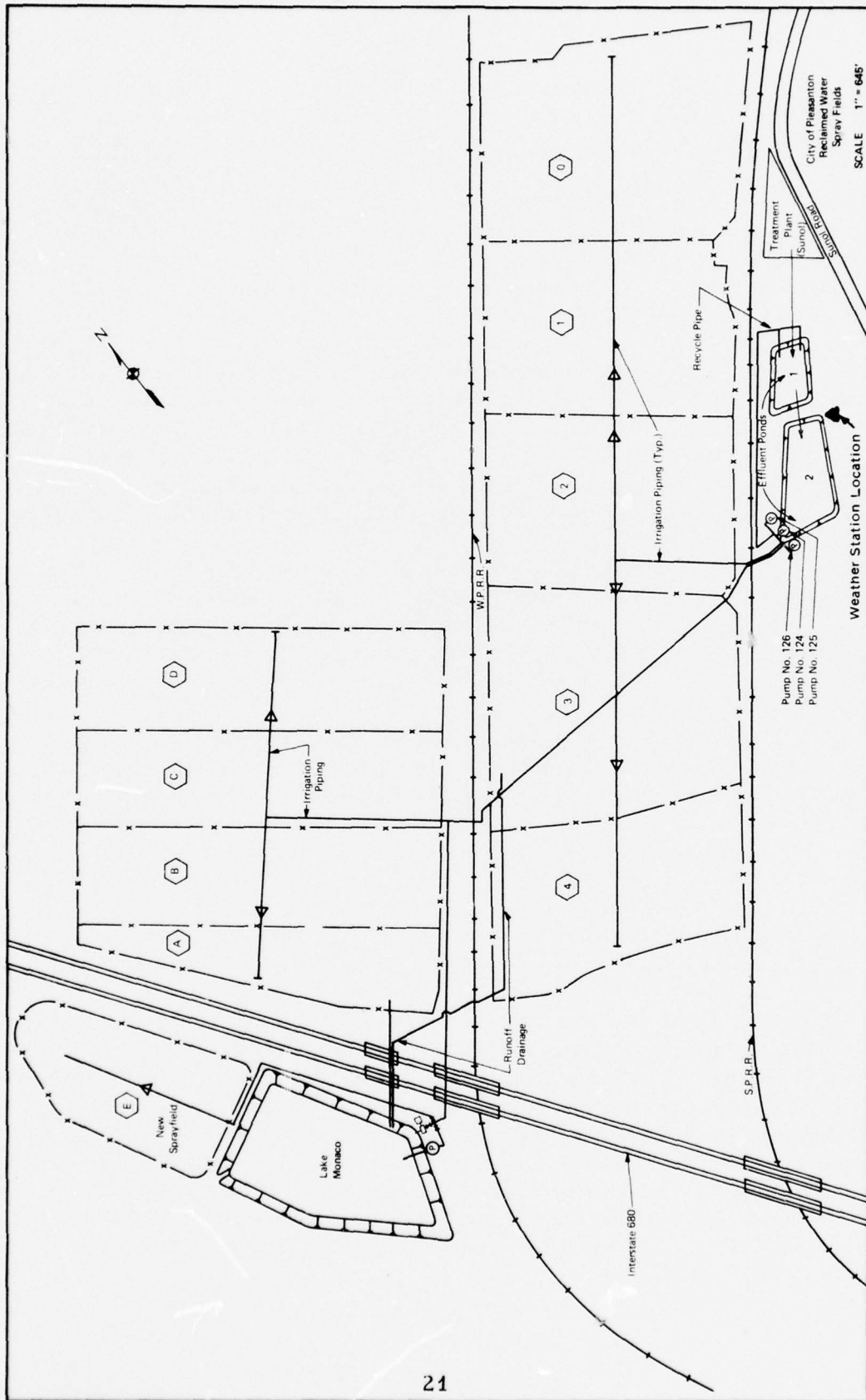


FIGURE 2. SPRAY FIELDS AND EFFLUENT PONDS

Table 1 lists the daily incoming raw sewage versus the volume sprayed for the months of August and September, 1975. The volume of wastewater sprayed daily is calculated from the levels in Ponds 1 and 2.

Two major parcels of land are subdivided into smaller fields for the purposes of cattle management. A third smaller field is also available when additional spray area is needed. The two larger areas are a 62-acre field labeled A, B, C, & D and a 100-acre field labeled 0, 1, 2, 3, 4. The third area is labeled E. (see Figure 2).

The water is applied to these fields through Rainbird No. 30 sprinkler heads which have been drilled to have  $7/32$ - and  $11/64$ -inch orifices. The spray heads are connected to 1-inch iron pipe 2 ft. high off 3-inch irrigation pipes. The sprays are located every 30 feet along the irrigation pipe. The spray wets an area approximately 18 meters in diameter and is directed upward approximately 5 meters at the highest point.

The transit time of the water in the pipes ranges from 4 minutes, 40 seconds to the center sprays in field 3; to 24 minutes, 40 seconds to the furthest sprays in field D. These transit times were measured by observing the appearance of dye at the sprays after its introduction at the pumps.

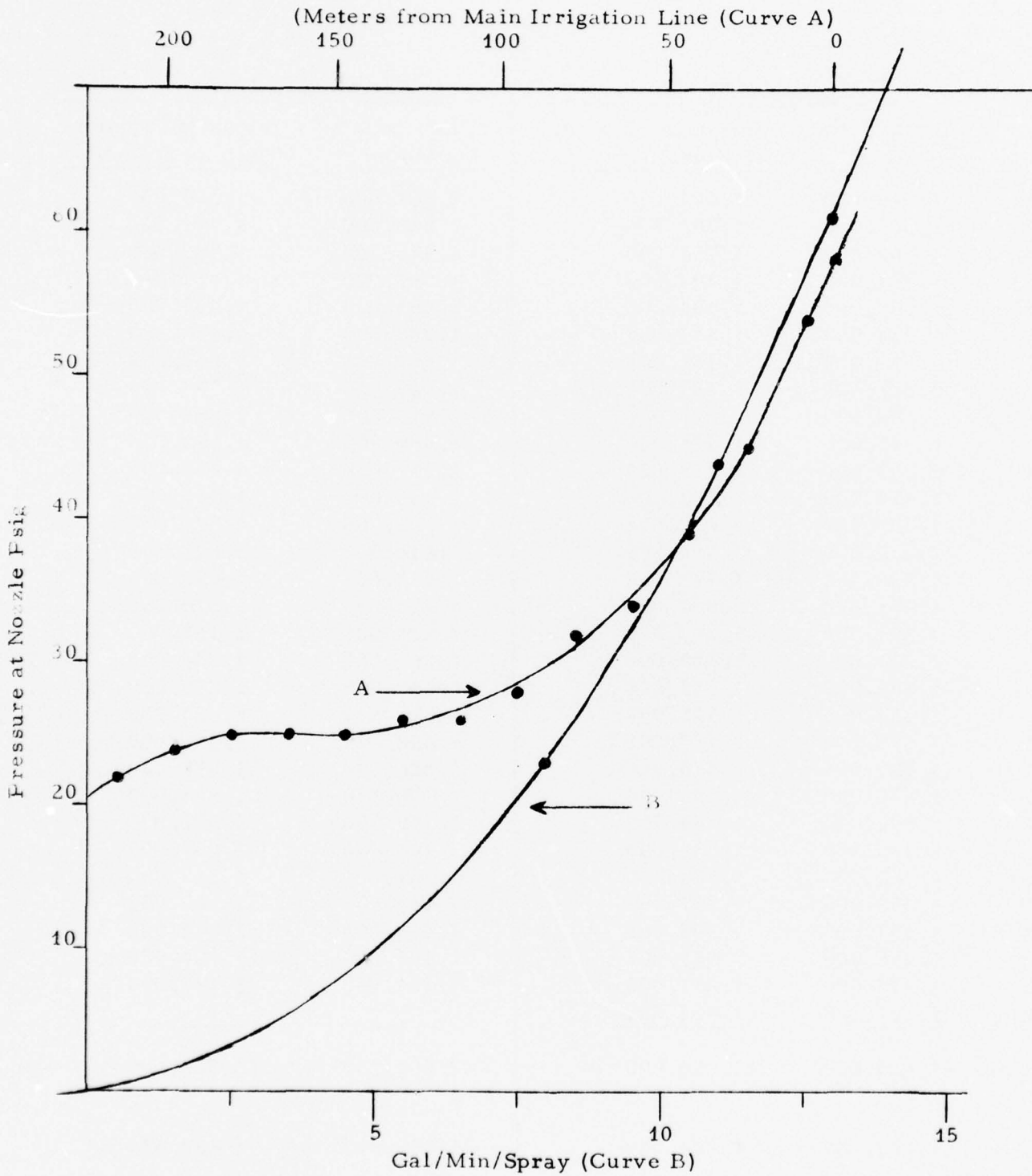
It should also be mentioned that the rate of application of water to the fields ranged from a high of 13 gal/min at the sprays next to the main water line to a low of 8 gal/min next to the spray furthest from the main line. Figure 3 shows a plot of spray nozzle pressure versus distance from the main irrigation line. These data points were measured values from the monitoring during August and September. The data show that the spray heads closest to the line were near 60 psi to just over 20 at the furthest distance from the line. The second plot on Figure 3 is a calculated curve for gallons per minute of spray with distance and/or pressure.

Fields A, B, C and D are irrigated with an average of 52 sprays, all in a straight line, moving the sprays 60 feet daily from left to right as viewed in Figure 2. Fields 0, 1, 2, 3 and 4 are irrigated with 40 to 89 sprays depending on the shape and contour of the field being sprayed. The sequence of spraying begins with Field 4 with two lines of sprays, one at the center of the field and one at the left end of the field. The spray lines are then moved 60 feet daily toward the right end of the field. After Field 4 is completed (approximately 4 days), the same sequence is followed for the remaining fields. The cattle are always kept one field ahead of the sprays in both spray fields.

TABLE I. PLANT FLOW DATA  
August and September, 1975

Date	August, 1975		September, 1975	
	Plant Inflow Gallons	Irrigation System Gallons Sprayed	Plant Inflow Gallons	Irrigation System Gallons Sprayed
1	1,525,000	1,607,000	1,164,000	1,363,000
2	1,507,000	1,702,000	1,363,000	1,371,000
3	1,502,000	1,754,000	1,471,000	1,487,000
4	1,454,000	1,109,000	1,587,000	1,681,000
5	1,609,000	1,534,000	1,481,000	1,435,000
6	1,434,000	1,157,000	1,435,000	1,452,000
7	1,357,000	1,388,000	1,352,000	1,249,000
8	1,388,000	1,194,000	1,349,000	1,749,000
9	1,494,000	1,441,000	1,549,000	1,089,000
10	1,341,000	1,461,000	1,389,000	1,388,000
11	1,363,000	1,478,000	1,388,000	1,433,000
12	1,478,000	1,508,000	1,433,000	1,409,000
13	1,408,000	1,272,000	1,509,000	1,518,000
14	1,372,000	1,499,000	1,418,000	1,522,000
15	1,399,000	1,773,000	1,422,000	1,552,000
16	1,431,000	1,394,000	1,452,000	1,826,000
17	1,394,000	1,347,000	1,526,000	1,167,000
18	1,247,000	1,359,000	1,367,000	1,304,000
19	1,459,000	1,334,000	1,304,000	1,075,000
20	1,334,000	1,443,000	1,375,000	1,378,000
21	1,384,000	1,130,000	1,378,000	1,416,000
22	1,430,000	1,370,000	1,416,000	1,578,000
23	1,473,000	1,493,000	1,478,000	1,355,000
24	1,393,000	1,488,000	1,555,000	1,501,000
25	1,388,000	1,385,000	1,401,000	1,657,000
26	1,485,000	1,398,000	1,457,000	1,545,000
27	1,498,000	1,257,000	1,545,000	1,349,000
28	1,357,000	1,395,000	1,449,000	1,144,000
29	1,395,000	978,000	1,444,000	1,704,000
30	1,478,000	1,251,000	1,504,000	1,189,000
31	1,251,000	1,464,000		
Total Gal.	44,028,000	43,368,000	42,961,000	42,886,000
Daily Avg.	1,420,258	1,398,968	1,432,033	1,429,533

FIGURE 3 . Nozzle Pressure & Flow versus Distance from Main Irrigation Line



Runoff from the spray fields drains through steel pipe to a holding pond (Lake Monaco) located southwest of Interstate 680 (Figure 2). This pond, as well as effluent pond No. 3, is used for additional holding capacity. This additional capacity is needed during rainy periods and as a reservoir for holding of effluent if problems occur with the spray operations. The plant may also irrigate from Lake Monaco when necessary.

E. Methods of Analysis

1. On-Site Analysis

Due to the proximity of local laboratories versus SwRI laboratories in San Antonio, Texas, it was decided that certain analyses would best be performed by SwRI personnel on-site and other analyses would be performed by a local laboratory equipped and staffed to conduct environmental air and water analyses.

After a site visit to Pacific Environmental Laboratories (PEL) located in San Francisco, it was decided that they would perform via subcontract the following analyses:

<u>Analysis</u>	<u>Sample</u>
Total and Fecal Coliform (MPN)	pond effluent
Total Plate Count	"
Biochemical Oxygen Demand (5-day)	"
Nitrite Nitrogen	"
Total Solids	"
Total Suspended Solids	"
Total and Fecal Coliform	air (Andersen 2000 sampler)
Total Plate Count	"

SwRI personnel performed on-site analyses for pH, hardness, total and free chlorine on the pond effluent. Methods for these above analyses are listed in Table II.

TABLE II. ANALYTICAL METHODS FOR WASTEWATER ANALYSES

Analysis	Analytical Laboratory (a)	Method		Remarks
		Ref. (b)	Page	
Chemical Oxygen Demand (COD - total)	SwRI - SA	1	495	Dichromate reflux
Total Organic Carbon (TOC)	SwRI - SA	2	237	Analysis performed with a modified Beckman Carbon Analyzer after acidification and N <sub>2</sub> stripping.
Biochemical Oxygen Demand (BOD)	PEL	1	489	5-day incubation at 20°C. Weston & Stack D. O. probe.
Total Suspended Solids (TSS)	PEL	2	268	Gooch crucible with glass fiber filter filtration and dryness at 103 to 105°C.
Total Solids	PEL	2	270	Evaporation at 103 to 105°C.
Hardness (CaCO <sub>3</sub> )	SwRI - PI	1	179	EDTA Titration.
Total Phosphorus (Total P)	SwRI - SA	1	523	Persulfate digestion, stannous chloride color development.
Nitrate Nitrogen (NO <sub>3</sub> - N)	SwRI - SA	1	461	Brucine method.
Nitrite Nitrogen (NO <sub>2</sub> - N)	PEL	2	215	Diazo dye method.
Ammonia Nitrogen (NH <sub>3</sub> - N)	SwRI - SA	1	222	Distillation, Nesslerization, optical density with spectrophotometer.
Organic Nitrogen (Organic N)	SwRI - SA	1	244	Determined on residue from ammonia distillation. Digestion, distillation and Nesslerization with spectrophotometer.
Total Chlorine	SwRI - PI	3	2-31	Orthotolidine method. Optical density determined by Hach DR-EL/2.
Free Chlorine	SwRI - PI	3	2-29	DPD method. Optical density determined by Hach DR-EL/2.
pH	SwRI - PI	1	276	Glass electrode and Orion specific ion meter model 407.
Mercury	SwRI - SA	2	118	Cold vapor technique utilizing LDC UV Monitor. Detection limit (5) 0.000032.
Arsenic	SwRI - SA	2	95	Atomic Absorption Spectrophotometry. Hydride generator method. Direct determination. Detection limit (5) 0.0013 mg/l.
Cadmium	SwRI - SA	4	151	Atomic Absorption Spectrophotometry. Graphite Furnace. Analysis by method of additions. Detection limit (5) 0.0002 mg/l.
Lead	SwRI - SA	2	112	Atomic Absorption Spectrophotometry. Graphite Furnace. Samples digested and concentrated before analysis. Detection limit (5) 0.0027 mg/l.

TABLE II. ANALYTICAL METHODS FOR WASTEWATER ANALYSES (Cont'd)

Analysis	Analytical Laboratory (a)	Method		Remarks
		Ref. (b)	Page	
Copper	SwRI - SA	2	108	Atomic Absorption Spectrophotometry. Air/acetylene flame analysis by method of additions. Detection limit <sup>(5)</sup> 0.015 mg/l.
Zinc	SwRI - SA	2	155	Atomic Absorption Spectrophotometry. Air/acetylene flame analysis by method of additions. Detection limit <sup>(5)</sup> 0.022 mg/l.
Total Coliform	PEL	1	662	Multiple-tube fermentation at 35 ± 0.5°C. Lactose broth (Difco No. 0004) presumptive and brilliant green bile (Difco No. 0007) confirmatory.
Fecal Coliform	PEL	1	669	Multiple-tube fermentation. Lactose broth (Difco No. 0004) presumptive at 35 ± 0.5°C and E. C. Medium (Difco No. 0314) confirmatory at 44.5 ± 0.2°C.
Standard Plate Count	PEL	1	660	Plate Count Agar (Difco No. 0479) and incubation at 35 ± 0.5°C.

References and Footnotes for ANALYTICAL METHODS FOR WASTEWATER ANALYSES - TABLE II.

(a) Analytical laboratories were:

- SwRI - SA - Southwest Research Institute, San Antonio Laboratories
- SwRI - PI - Southwest Research Institute, Pleasanton, California facility
- PEL - Pacific Environmental Laboratory, San Francisco, California

(b) References and footnotes for analytical tests were:

1. AWWA, APHA, WPCF, Standard Methods for the Examination of Water and Wastewater, Thirteenth edition, American Public Health Association, Washington, D. C., 1971.
2. Methods for Chemical Analysis of Water and Wastes, U. S. Environmental Protection Agency, Washington, D. C., 1974.
3. Hach Water and Wastewater Analysis Procedures Manual, Hach Chemical Company, Ames, Iowa, 1975.
4. Ediger, Richard D., "A Review of Water Analysis by Atomic Absorption," Atomic Absorption Newsletter, Vol. 12, No. 6 (1973).
5. The detection limit is defined as that concentration of the analyte which would yield an absorbance equal to twice the standard deviation of a series of measurements of a solution, the concentration of which is distinctly detectable above the baseline.

2. Analysis at SwRI Laboratories

Analyses of selected samples for the following parameters were performed at SwRI Laboratories located in San Antonio, Texas. The methods for these analyses are listed in Table II.

Heavy metals

cadmium  
copper  
zinc  
lead  
arsenic  
mercury

Total Organic Carbon  
Total Phosphorus  
Nitrate Nitrogen  
Ammonia Nitrogen  
Chemical Oxygen Demand  
Organic Nitrogen

3. Bacteria and Virus Analyses at SFRE Laboratories

All bacteria and virus analyses of pond effluents and aerosol samples were performed in San Antonio, Texas by Southwest Foundation for Research and Education. Bacteriological examinations were performed in accordance with standard procedures and techniques which have been established for identification of microorganisms by clinical diagnostic laboratories and by procedures detailed in Standard Methods for the Examination of Water and Wastewater published by the American Public Health Association. Identification was performed by cultural morphology, biochemical reactions, microscopic appearance and serologic testing, as required.

Several samples sent to SFRE for selective examination were also tested for coliforms. Effluent samples were plated out to determine total aerobic bacterial count. Total coliform counts were performed by standard methods, including use of selective media and biochemical tests. Identification of fecal coliform bacteria was performed as described in Standard Methods, including gas

production and growth at 44.5°C. MacConkey's Agar, Eosin Methylene Blue Agar, Shigella-Salmonella Agar, Phenylethanol Agar and Blood Agar were employed for isolation of bacteria from effluent samples collected on a selective basis. Isolates were identified by appropriate standard tests.

Although it had been anticipated that a simple spot plate technique would be adequate for isolation of coliphages, only about 10% of the samples were positive when tested by the following method. The sample to be tested was centrifuged at 5,000 rpm for 30 minutes to eliminate debris and most of the bacteria. The supernatant was filtered through a Millipore membrane HAWP02500 and 1.0 ml filtrate was pipetted onto each of four Nutrient Agar plates inoculated with a young (2-hour) broth culture of the four test organisms. These were a standard strain of Escherichia coli obtained from American Type Culture Collection, a human E. coli strain and a chimpanzee E. coli strain isolated in this laboratory and an E. coli strain used in T. phage (E. coli B) research. The plates were incubated at 37°C and examined at 24, 48 and 72 hours for presence of plaques. At this point, any plates not demonstrating plaques could have been considered negative. Plaques counted were reported as PFU/ml sample.

However, since establishment of the presence or absence of coliphages was considered significant in this program, the following additional procedure was performed (from C. Eklund, Laboratory Manual for Bacteriophages, University of Texas Press, Austin).

Four-tenths of a milliliter of the centrifuged and filtered sample (as above) was inoculated into those of a young broth culture of each E. coli strain to observe for lysis. If no lysis was observed within 6 hours, a soft agar (0.7%) overlay was prepared by inoculation of liquefied agar with the 6-hour broth culture-filtrate above, and this was poured over a solid agar (1.5%) base plate. After incubation at 37°C, the plate was examined at 24, 48 and 72 hours for evidence of plaques in the confluent bacterial growth.

At this point, all samples not demonstrating plaques were recorded as negative. All samples showing evidence of plaques were recorded as positive - less than 10 PFU per milliliter, since it is assumed that any sample which contained as many as 10 PFU/ml would have yielded plaques on the original spot plate tests.

Each set of tests included positive and negative controls.

Virus analyses were performed as shown below. Effluent samples and aerosol samples collected in Brain Heart Infusion Broth were

filtered through a talc- Celite (diatomaceous silica) mixture sandwiched between two AP2004200 filters in a millipore filter holder. A one-liter sample was used for effluents and 75 to 175 ml was used for aerosol samples. The adsorbed virus was then eluted in 4 ml of 2% casein in 0.5 M Tris Buffer at pH 8.3. The eluate was next filtered through an HAWP02500 filter to remove bacteria. The filtrate was inoculated into tubes of baboon kidney monolayers for determination of cytopathology. Baboon kidney monolayers in 1-ounce prescription bottles were also inoculated and an agar overlay employed for the detection of plaques. When it was suggested that there might be viruses present which would not grow on baboon kidney cells, a trial run was made utilizing a human cell line - W138 - in addition to the baboon cells. When several samples demonstrated cytopathology in W138 cells but not in baboon cell monolayer tubes or bottles, the decision was made to repeat all the effluent and aerosol examinations employing both cell lines in the repeated series.

(a) Comparison of Virus Isolation Techniques

Three methods of virus concentration have been tested in addition to the talc-Celite sandwich technique which was used for virus assay. They are:

- Wallis-Melnick Virus Concentrator  
(Carborundum prototype)
- Polyethylene glycol technique
- Bentonite-Colloid Association System

The Wallis-Melnick Concentrator is an assembly of clarifying filters, adsorbent filters and pump, mounted on a cart for easy portability. A small generator was utilized as a source of electricity and the filters were returned to the laboratory for concentration and elution of virus. The system is designed for use in the field and can process large volumes of relatively clear water or wastewater. However, particulate matter contained in raw sewage effluents quickly clogs the filters, decreasing the rate of flow and resulting in considerable loss of virus.

The polyethylene glycol technique is a procedure for removing virus from the fluid vehicle by dialysis. The fluid passes through the membrane into a polyethylene glycol solution and the virus is eluted from the inside of the dialysis tube. The bentonite system is dependent upon the adsorption of virus by bentonite clay. The solids are then collected, centrifuged and virus eluted from the pellet and assayed for infectivity.

The Wallis-Melnick Concentrator was employed as follows. A 100-liter (approximately 26.5 gallons) sample of non-chlorinated secondarily treated wastewater\* was pumped through a series of 3 clarifying filters, Honeycomb-type tubes identified as (1) a 5-micron 019R10S (Commercial Filters Corp., Lebanon, Indiana), (2) a 1-micron 039R10S and (3) a 1-micron W10A-72, into a plastic-lined 55-gallon drum. To the sample in the drum was added 0.5 M  $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$  at the rate of 1 ml/l sample. The pH was adjusted to 3.5 by addition of 25% HCl.

The adjusted sample was pumped through 2 additional tubular filters, (4) and (5) K27R10S and (6) a plate filter holder (10-1/2 inch) containing a 2.0 micron on top (upstream side) of a 0.45 micron filter. The filtrate was discarded. Elution of virus from filters 4, 5 and 6 was performed by passing through the filters 1 liter of 0.05M glycine solution at pH 11.5. The eluate was adjusted to pH 3.5 by addition of 1M HCl ( $\text{AlCl}_3$  having been added as above) and again filtered through 2.0-micron and 0.45 micron filters in a 5" filter holder. The filtrate was discarded, the virus eluted with 80 ml glycine solution and the eluate adjusted to pH 3.5. This eluate was filtered through 2.0 micron and 0.45-micron filters in a 2" filter holder and the filtrate discarded. The virus was eluted from the 2" filter by passing through it 10 ml glycine solution. This final eluate was adjusted to pH 6-7 and tonicity adjusted by addition of 3-M NaCl at the rate of 0.5 ml per 9.5 ml of concentrate.

This final concentrate was inoculated into 1-oz bottles containing baboon kidney cells as monolayers. Dilutions were prepared from  $10^{-1}$  to  $10^{-6}$  and 0.1-ml volumes inoculated into each of 10 bottles per dilution. Incubation was under 10%  $\text{CO}_2$ . Plaques were counted and calculated as PFU per ml of original sample. Bottles were held 3 weeks before being considered negative.

The polyethylene glycol concentration method was employed as follows. A 100-ml sample was placed in a single length of dialysis tubing (Union Carbide) which was then closed carefully to prevent leakage. The dialysis tube was immersed in sterile polyethylene glycol (PG) solution (100 g PG in 100 ml Phosphate-buffered saline (PBS) /100-ml sample)

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\* This sample was collected at a sewage treatment plant located in San Antonio. The sample was used as received; thus, the viruses present are those indigenous to the effluent.

and held overnight at 4°C. The dialysis tube was then removed from the PG and rinsed with sterile distilled water to remove PG from the outside of the tube. The virus was collected in PBS (containing 2% fetal bovine serum) by eluting with 2 ml eluent then repeating with a second elution of 2 ml for a total of 4 ml of eluate. Dilutions of eluate were prepared and 0.1-ml volumes inoculated into 1-oz bottles containing baboon kidney cell monolayers (10 bottles per dilution). Plaques were counted after suitable incubation as above and calculated as PFU/ml sample.

The bentonite concentration method<sup>(2)</sup> was used as follows. To 1 liter of sample was added 1.0 gram bentonite clay and 0.01-M CaCl<sub>2</sub> at pH 6. This was mixed magnetically for 30 minutes, transferred to a separatory funnel and allowed to settle overnight at 4°C. The settled solids were collected and pelleted by light centrifugation. The supernatant was discarded, the solids were resuspended in deionized water, eluted for 15 minutes and recentrifuged. The eluate was assayed for virus; however, instead of infectivity on HeLa cells, baboon kidney cells as described above were used and assayed for PFU to provide for a more uniform comparison study.

The talc-Celite (diatomaceous silica) procedure which was employed for this comparison was essentially the same as that used for assay of effluent and aerosol samples. A 1-liter sample was passed through a Millipore AP 2512450 clarifying filter in a plate filter holder to remove debris. The filtrate was then filtered through a talc-Celite mixture sandwiched between two AP 2004200 filters in a Millipore filter holder. The absorbed virus was then eluted from the sandwich in 4 ml of 2% casein in 0.05 M Tris Buffer at pH 8.3. The eluate was next filtered through an HAWP02500 filter to remove bacteria. This filtrate was diluted and the tenfold dilutions inoculated onto baboon kidney cell monolayers in 1-oz Rx bottles and an agar overlay employed for the detection of plaques. Incubation was at 37°C under 10% CO<sub>2</sub>. Results were calculated as PFU/ml sample. The results of this experiment are shown as follows:

	PFU/ml	
	Sample 1	Sample 2
Wallis-Melnick Concentrator	45	46
Polyethylene Glycol Technique	1.5 x 10 <sup>2</sup>	1.9 x 10 <sup>2</sup>
Bentonite-Colloid System	1.6 x 10 <sup>2</sup>	1.8 x 10 <sup>2</sup>
Talc-Celite Sandwich	1.6 x 10 <sup>2</sup>	1.7 x 10 <sup>2</sup>

It is apparent that less virus was recovered from the samples tested by means of the Wallis-Melnick concentrator than was recovered by any of the three other methods. Results obtained with the Polyethylene Glycol, Bentonite-Colloid and Talc-Celite methods were essentially equivalent.

The Wallis-Melnick Concentrator has the advantage of making it possible to test very large samples. Southwest Foundation for Research and Education has tested up to 50 gallons of wastewater on a single run. It can be used in the field and the concentrate returned to the laboratory for elution of virus from the filters. The machine is cumbersome, particularly if a generator must be operated as a source of electricity for the pump, and considerable attention is required.

The Polyethylene Glycol and Bentonite-Colloid techniques can be performed in the laboratory on relatively small samples (1-liter) brought in from the field. They are not difficult to set up, and they require much less time for processing and handling the samples. As with the talc-celite techniques, many samples can be processed simultaneously; for example, SFRE laboratories has processed 20 to 30 samples per day by the talc-celite procedure. This is particularly important in a survey situation.

## F. Methods of Sample Collection

### 1. Effluent Sampling

Three types of effluent samples were collected.

(a) A composite sample of the pond effluent was taken daily using a refrigerated timed-cycle sampler. Approximately 100 ml of effluent was taken every 15 minutes during the time the fields were being irrigated (10 to 18 hours per day). This sample was taken from a sample line tee off the main irrigation lines going to the spray fields.

(b) Hourly grab samples (diurnal study) were taken manually from the same sampling point over a three-day period during the hours of irrigation.

- (c) Grab samples were taken directly from the spray nozzles in spray fields before and after each aerosol sampling period.

All water samples were collected in pre-washed and/or sterilized polyethylene bottles, depending upon the parameter under study. Preservatives were immediately added to the samples requiring them and samples were placed in a refrigerator maintained at 1-4°C until analysis or shipment to SwRI or PEL.

## 2. Aerosol Sampling Equipment

Four different methods for sampling of aerosols were used.

- (a) Andersen 2000 sterile disposable samplers designed to accept two agar plates and to separate respirable and non-respirable particles were used for total viable organisms and total and fecal coliform measurements. Sampling times were varied from 30 seconds to 30 minutes at a flow of 1.0 cubic foot per minute.

- (b) High volume, all-glass impingers (AGI) were used for collection of aerosol samples for bacteriophage and virus determinations. These impingers (3/16-inch orifices approximately 1/2 inch above the bottom of the impingers) were designed for 28.4 liters per minute flow. Brain heart infusion (100 ml) was used as the collection medium and each sample was taken over a 30-minute period.

- (c) All-glass Porton impingers with 1.0-mm orifices approximately 30 mm above the bottom of the impingers designed for an air flow of 12.5 liters per minute flow and liquid volumes of 30 ml were used for collection of aerosol samples for both selected and routine bacterial analyses. Brain heart infusion (30 ml) was used as the collection medium. Each sample was collected over a 30-minute period.

- (d) A LEAP high volume sampler was also used for collection of aerosols for both virus and routine

and selected bacteria analyses. The collection medium was a brain heart infusion solution containing 0.1% Tween 80. Each sample was collected over a 30-minute period at 1000 lpm air flow and a high voltage setting of 12 to 14 Kv. The collection medium (100 ml) was circulated at 10 ml per minute. The 100-ml samples were subdivided into two 50-ml samples, one each for virus and bacterial analysis. After subdividing, the samples for virus analysis were sealed in a No. 3 steel can and placed immediately in an ice chest containing dry ice; the bacteria samples were placed in an ice chest and cooled with frozen Koolpacs.

The aerosol sampling equipment was operated out of two mobile units containing the necessary generators, vacuum pumps, sampling manifolds, flow meters, etc. to permit simultaneous collections from each type of sampling equipment.

#### G. Conduct of Monitoring

The monitoring program began with the collection of the first composite sample of Pond No. 2 effluent during the hours of spray irrigation on August 14, 1975 and ended with collection of the final effluent composite and aerosol samples on the morning of September 14, 1975. During this period, composite samples were taken from Pond No. 2 effluent during daily hours of irrigation. Hourly grab samples were taken over a three-day period to examine possible diurnal changes in effluent composition. These studies were initiated on Tuesday, August 19th, and continued through Thursday, August 21. Aerosol samples were collected during both daytime and nighttime hours.

There were some delays in the initiation of aerosol sampling due to generator and LEAP sampler failure. The cause of failure in the LEAP sampler was due to damage of the equipment during shipment to Pleasanton. SwRI personnel secured parts for the LEAP sampler and made the necessary repairs on site. The generator was repaired at a local machine shop.

During the course of the sampling, some difficulty was also encountered with the vacuum pumps used to draw the aerosol through the various impingers. These difficulties were due to poorly designed moisture traps which allowed moisture to enter the pumps, causing them to freeze up. Some changes were made in the design to correct the problem, but considerable time was lost in overhauling the pumps.

One of the most difficult problems encountered was the selection of upwind and downwind sampling sites. This was due to the large percentage of time during which the wind was calm or extremely variable. On numerous occasions, sampling runs had to be aborted due to an extreme change in wind direction or lack of wind.

Aerosol samples were taken on twelve different days from August 23 through September 13, 1975. During this time period, a total of 285 aerosol samples of various types were collected. The different types and number of samples of each type collected during the day or night are listed as follows:

<u>Type Sampler</u>		<u>No. of Samples Collected</u>		
		<u>Day</u>	<u>Nite</u>	<u>Total</u>
Andersen 2000	Total and fecal coliform	47	17	64
	Standard Plate Count	47	17	64
AGI Impinger	Virus	14	10	24
	Coliphage	34	10	44
Porton Impinger	Total and fecal coliform	6	4	10
	Standard Plate Count	6	4	10
	Selected Bacteria	16	10	26
LEAP	Virus	9	10	19
	Selected Bacteria	7	9	16
	Total and fecal coliform	3	1	4
	Standard Plate Count	3	1	4

Downwind stations were located by use of information from the meteorology station located at the site. In addition, smoke bombs were used in the field to study wind direction and to be certain that sampling locations were directly downwind from the spray fields. Whenever possible, upwind stations were selected in an area which had not been downwind from the spray fields at any time during the previous 4 to 6 hours.

A typical sampling schedule began at 6 A.M. with the collection of the composite sample from Pond No. 2. The sample was then subdivided and labeled for the various types of analyses and preservatives were added, where required. On-site analyses for pH and chlorine were completed while

various samples were being packed with Koolpacs for shipment to SwRI, SFRE and PEL. The samples for SwRI and SFRE were driven to San Francisco airport to make the 8 A.M. deadline for getting shipments on the only non-stop flight to San Antonio. After delivery of the shipment to the airport, the samples for Pacific Environmental Laboratories were delivered to their downtown San Francisco Laboratory. On the return trip to Pleasanton, dry ice was picked up for freezing virus samples collected during the remainder of that day. Upon return to Pleasanton, aerosol sampling equipment was loaded in the mobile units and sampling sites for the afternoon were selected. One person would man the LEAP sampler mobile unit and two persons would man the mobile unit set up for Andersen 2000, Porton and AGI samplers. Whenever possible, the two units would make simultaneous collections at the same sampling location. Grab samples were taken directly from the spray nozzles before the aerosol sampling began and again after aerosol sampling was completed.

### III. RESULTS

#### A. Meteorological Considerations

Inherent in any sampling program of airborne effluents to determine their content and concentration is the need to define the relationship between measurements made at specific points and what is occurring in the overall effluent plume. Plume travel and deposition rates are dependent on three factors: characteristics of the effluent, characteristics of the discharge and the discharge exit, and the existing meteorological conditions. All of these factors are interrelated, and the manner in which they interact is dependent on their characteristics.

Effluent characteristics include particle composition and size, weight and the chemical reaction with other atmospheric constituents and each other. Discharge exit characteristics include height of the exit above ground, exit velocity, temperature of exiting effluents and the physical dimensions of the discharge exit.

The third factor, meteorological conditions, includes wind velocity, wind direction and the characteristics (gustiness and variability) of both; the presence or absence of precipitation; relative humidity; and stability of the atmosphere. The last parameter, stability, is probably the most important of all of the characteristics since it, more than any other parameter, will most often affect the distribution and deposition rate of airborne pollutants. Wind velocity at the point of effluent discharge, together with the other characteristics of the discharge exit, will determine how rapidly the plume will be "bent".

Based on the above-mentioned considerations which are well documented by pollutant plume investigators, it is considered advisable to make measurements of meteorological conditions simultaneously with other observations and measurements. Based on overall program requirements of economy, unattended operation and reliability, meteorological data were gathered in the manner and with the instrumentation described below.

#### 1. Meteorological Measurements and Instrumentation

Simultaneous measurements were made at the two- and ten-meter levels. The following parameters were measured at the two-meter level: wind velocity and direction, temperature, relative humidity and

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precipitation (amount, rate and duration). The following parameters were measured at the 10-meter level: wind velocity and direction and temperature. Atmospheric stability can be inferred from both the wind direction and temperature differences at the two levels. Wind velocities assist in determining the probable downwind and crosswind axes of the plume. The relative humidity provides a means of inferring chemical interaction between the effluent and atmospheric moisture. Precipitation measurements also assist in inferring the interaction and possible "wash-out" of the effluent from the atmosphere.

Measurements were made and recorded on time-referenced strip charts by mechanical weather stations manufactured by Meteorology Research, Inc. Measurements were made at the 10-meter level by mounting the instrumentation on a guyed crank-up tower. This arrangement permitted easy access to the instrumentation for servicing and chart changing.

Starting threshold for both the anemometer and wind vane are less than three-fourths of a mile per hour. Wind direction measurement accuracy is  $\pm 1\%$  of full scale, while wind speed data are accurate to  $\pm 2\%$ . Humidity measurements are accurate to  $\pm 3\%$  over the entire range of humidities from 0 to 100%. Rainfall measurements are accurate within .01 inch per 2 inches of rainfall per hour. Temperature measurements are accurate to  $\pm 3\%$   $^{\circ}\text{F}$  absolute. However, the two temperature sensors were calibrated to each other against a standard thermometer traceable to NBS standards. Therefore, differences in temperatures of the thermometers -- the criterion of interest to establish the atmospheric stability -- should be accurate to within less than  $1^{\circ}\text{F}$ .

## 2. Meteorological Conditions During the Period of Test

The test site was located in a shallow valley bounded on the near west side by a low range of hills pierced by a valley oriented roughly east-west, located approximately five miles north of the test site. Another low range of hills lies to the southeast and is oriented northeast-southwest. These hills leave a valley to the south leading to San Jose. The terrain to the north slopes gradually upward.

The net result of the above described topography is such as to provide turbulent wind conditions except under northerly and southerly wind conditions. During warm-to-hot periods and low wind conditions a cyclonic circulation of the winds can be set up in the test site area without regard to the prevailing winds.

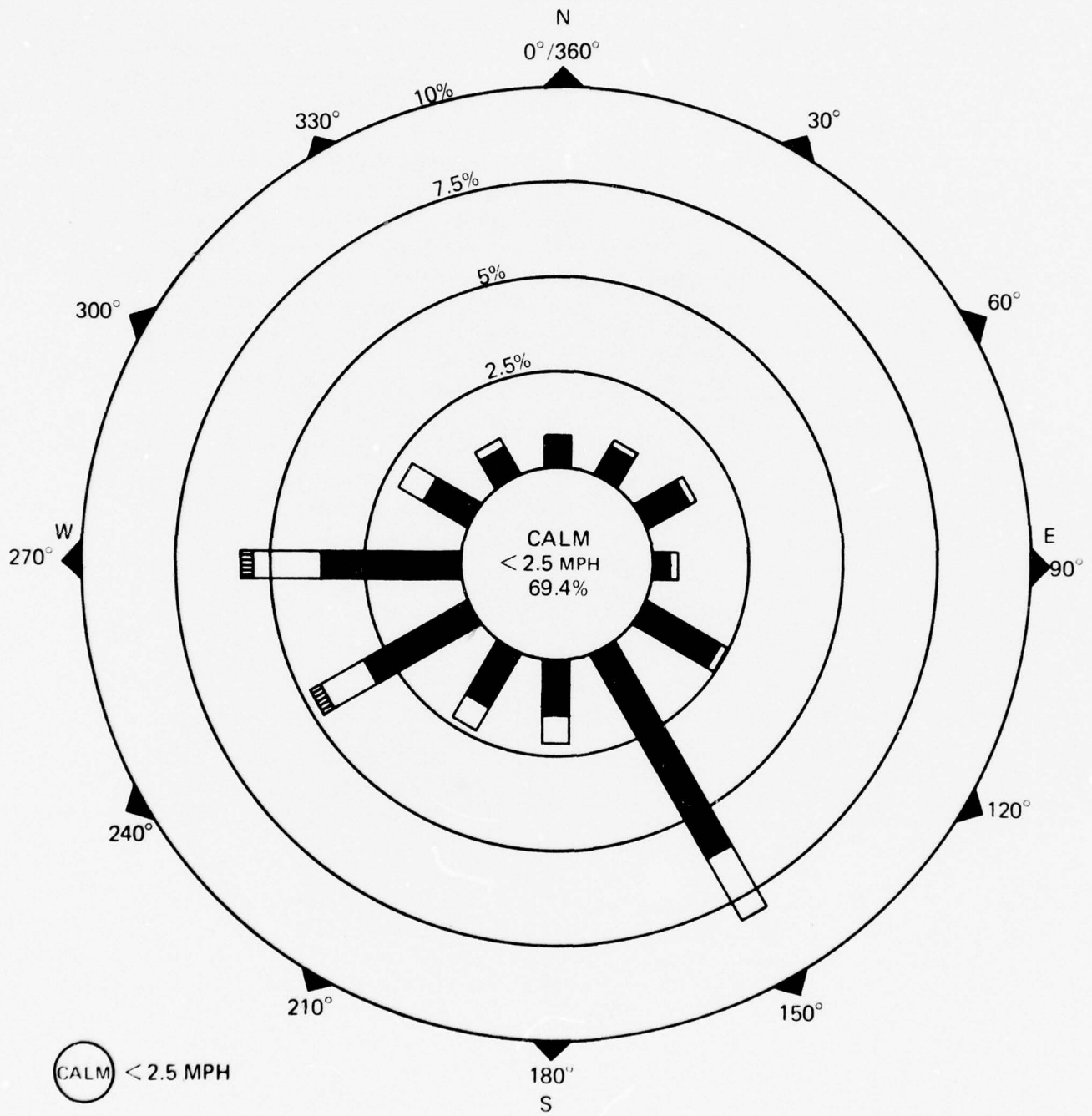
No reliable climatic data are available for the test site area. Based on National Weather Service northern hemisphere climatic charts for the month of August (Monthly Weather Review, Vol. 103, No. 11, November, 1975, pp. 1027-1032) the area was under the influence of a weak ridge of high pressure with a low pressure trough lying to the west just offshore of San Francisco. Temperatures were slightly below normal during the test period (approx. 2° F). The test site appeared to have been traversed by four mildly cool outbreaks during the test period. These occurred on August 18, 26, 30 and September 8, and were evidenced by stronger winds from the west and north than were the case during the rest of the period and by cooler temperatures. Winds during most of the period were very light--some 3 to 5 miles per hour lower than would normally be expected for this period--and erratic in direction. Figure 4 shows the windrose pattern at the site for the sampling period in August and September.





Based on the temperature differences between the 2- and 10-meter levels, the atmosphere was neutrally stable for most of the test period. On August 13, the atmosphere was slightly unstable during the afternoon hours. On August 27, after a cool outbreak as mentioned above, the atmosphere gradually returned to a neutral stability by the 28th. On September 4 and 5, after an early morning during which the atmosphere was slightly unstable, the air became quite stable. This is an unusual condition and could have been caused by evaporative cooling of the lowest levels due to the spray and stronger than normal winds during most of this period. This phenomenon is also evidenced by the sometime 1° to 2° F cooler temperatures at the low level compared to the 10-meter level.

Again on the afternoon of September 9, after another cool outbreak, the afternoon atmosphere was slightly unstable followed by an early morning period of slightly stable air on September 10. Finally, the atmosphere was slightly unstable on the afternoon of September 13.

Normally, one can expect that almost all pollution from an effluent emission under slightly unstable to unstable conditions will be deposited very close to the source. Under neutral stability conditions, "coning" occurs and the effluent plume travels farther than in the unstable cases. The condition is called "coning" since the effluent plume resembles a conical cylinder. Under very stable conditions, the plume travels a great distance in an essentially undiluted state before it reaches the ground. In summary, the greater the instability, the closer to the source the

FIGURE 4.  
 PERCENTAGE DISTRIBUTION OF WIND DIRECTIONS AND WIND SPEEDS



-  CALM < 2.5 MPH
-  2.5-5 MPH
-  5-7.5 MPH
-  7.5-10 MPH

LOCATION: Pleasanton, Calif.  
 Wastewater Treatment Plant  
 PERIOD: Aug 11-Sept 14, 1975  
 NO. of OBSERVATIONS: 1626  
 FREQUENCY: 1/2 hour

effluent deposition will be. The more stable the atmosphere is, the greater the distance the plume will travel before it reaches the ground.

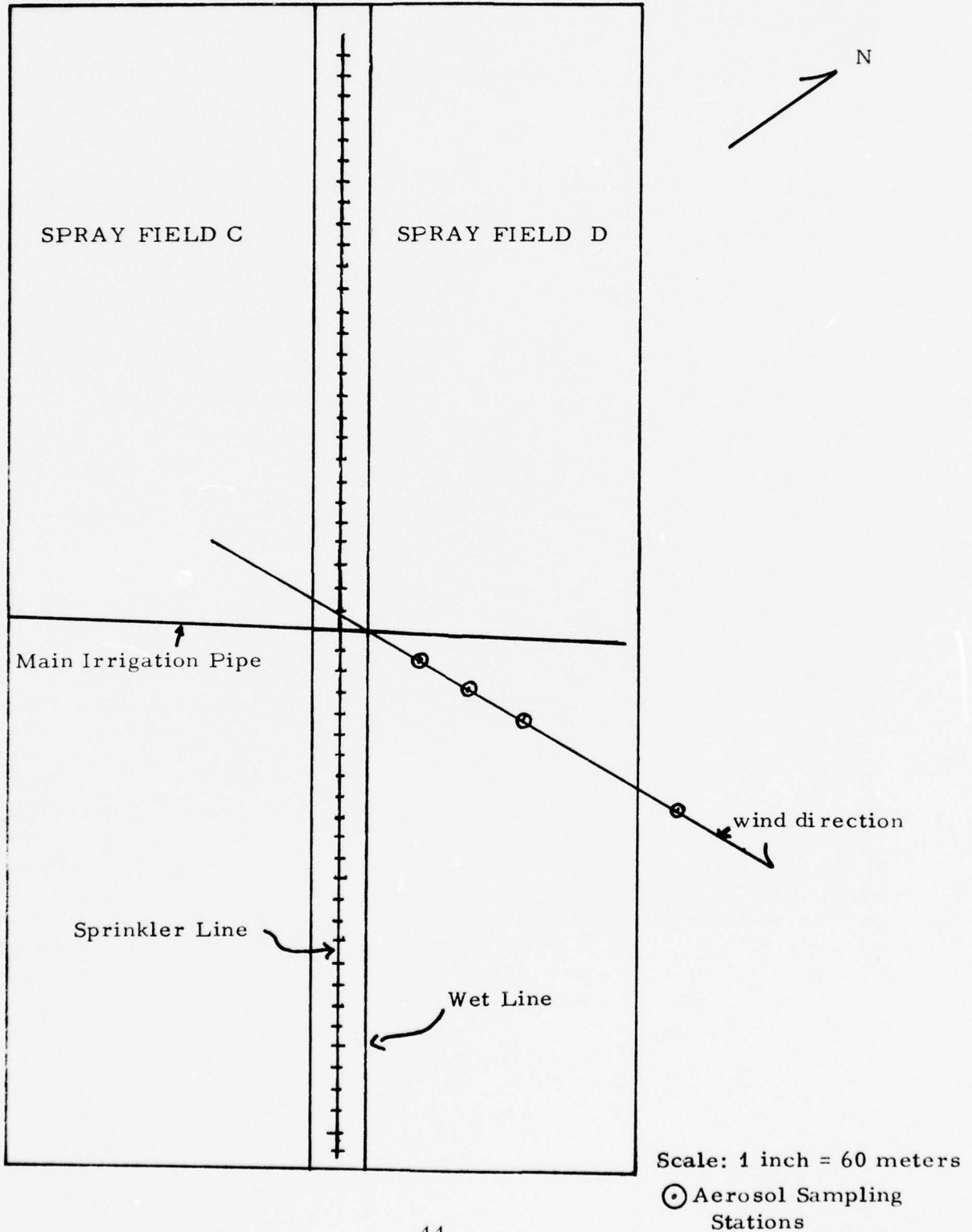
## B. Percent Aerosolization of Wastewater

### 1. Experimental

The percent aerosolization of wastewater sprayed in the fields was estimated from the results of a dye experiment utilizing a suitable modification of Turner's continuously emitting infinite line source atmospheric dispersion model.<sup>(3)</sup> Three dye experiment runs were performed in a spray field in which 52 Rainbird #30 sprinklers were located on a line along a 470 m. length (see Figure 5). Each sprinkler covered a circular area of radius 9.14 m. The distance between adjacent sprinklers was 9.2 m. The wastewater was pumped from the main pipeline into the middle of the sprinkler line. The pressure in both orifices at every other sprinkler along the line was measured in a nearby identically configured field. The pressure decreased from approximately 60 psig at the middle of the sprinkler line to 22 psig at the end sprinklers, with a corresponding flow rate decrease from 13 gal/min at the middle to 8 gal/min at the ends (see Figure 3).

Each run consisted of injecting a known quantity of dye into the main pipeline supplying the field's sprinklers over a ten-minute period and sampling the total amount of dye contained in the aerosol at four downwind locations. The aerosol dye concentration was computed by dividing the mass of dye collected by the volume of air passing through the air sampler during the effective ten minute sampling period. In two of the dye experiment runs, the aerosol samplers were placed basically downwind from the center of the sprinkler line at distances of 25 m , 50 m , 75 m and 150 m from the edge of the spray wet line, at an angle of  $60^\circ$  with the sprinkler line. On these two runs, the wind was aligned at angles of  $50^\circ (\pm 30^\circ)$  and  $65^\circ (\pm 30^\circ)$ , respectively, with the line of sprinklers. These two runs were used in making the percent aerosolization estimates. The third dye run was performed with each of the four samplers at a distance of 50 m from the edge of the wet line; the samplers were spaced along one end of the line of sprinklers. This run was discarded because the direction and variability of the wind relative to the sprinkler line ( $5^\circ \pm 90^\circ$ ) prevented adequate dispersion modeling.

FIGURE 5 . AEROSOL SAMPLING STATIONS FOR PERCENT AEROSOLIZATION STUDY



The atmospheric dispersion model utilized applies to a continuously emitting infinite line source. The model is:

$$\chi(x, y, z; H) = \frac{q}{\sin \theta \sqrt{2 \pi} \sigma_z u} \left\{ \exp \left[ -\frac{1}{2} \left( \frac{z-H}{\sigma_z} \right)^2 \right] + \exp \left[ -\frac{1}{2} \left( \frac{z+H}{\sigma_z} \right)^2 \right] \right\} \quad (1)$$

where

- q = aerosol dye source strength,  $\mu\text{g}/\text{m sec}$
- x = distance downwind from the sprinkler line, m
- y = distance perpendicular to wind direction, m
- z = vertical distance above the ground, m
- H = height of aerosol centerline at the source, m
- $\chi$  = aerosol dye concentration at (x, y, z),  $\mu\text{g}/\text{m}^3$
- u = mean wind velocity, m/sec.
- $\theta$  = angle of the wind with the sprinkler line
- $\sigma_z$  = standard deviation of the vertical dye concentration distribution, m

$\sigma_z$  depends on the Pasquill stability class and the distance downwind x.

Most of the model's assumptions (an infinite line source, constant and continuous emissions, constant and uniform wind velocity, aerosol diameter of less than 20 microns, normally distributed dispersion in the vertical and horizontal directions, negligible dispersion in the downwind direction, level terrain,  $\theta > 45^\circ$ ) are reasonably well satisfied by the dye experiment conditions. Considering the small distances of the air samplers from the sprinkler line relative to the length of the sprinkler line, the infinite line source assumption is realistic. The high rotation rate of the Rainbird sprinklers (approximately 25 seconds) relative to the ten-minute dye injection period makes the continuous emission assumption reasonable. However, the model's assumption of no deposition on the ground is probably invalid.

The model described by equation (1) was used to estimate q from the sampler dye concentration  $\chi$  and the other variables at each air

sampler location at both runs. The "no deposition" assumption may lead to under-estimates of  $q$  at the farther downwind air sampler distances.

The wastewater dye source strength values  $Q$  were calculated rather than experimentally determined. The calculations were based on a known amount of dye injected and the measured amount of water mixed with the dye during the ten minute sampling period. The actual volume of water was measured using a Rockwell in-line three-inch (7.62 cm) water meter in series with the sprinkler line. Source strength  $Q$  was calculated for each sampler on both runs for the section of the sprinkler line that was directly upwind from the air sampler location. The formula used to estimate  $Q$  was:

$$Q = \frac{M}{LT} \left( \frac{F_s}{F} \right) \quad (2)$$

where

$M$  = mass of dye sprayed through the line of sprinklers,  $\mu\text{g}$

$L$  = length of the sprinkler line, m.

$T$  = dye spraying period, sec.

$F_s$  = wastewater flow rate per sprinkler of the three or four sprinklers directly upwind from the air sampler,  $\text{m}^3/\text{sec}$

$F$  = wastewater flow rate per sprinkler of the entire sprinkler line,  $\text{m}^3/\text{sec}$

The correction factor ( $F_s/F$ ) was introduced into equation (2) to correct for the increased dye level in the middle of the sprinkler line because of the higher flow rate there.

The percent of the sprayed wastewater converted to aerosol form was estimated as:

$$A = \frac{100 \text{ g}}{Q} \quad (3)$$

A percent aerosolization estimate was obtained for each of the four air sampler dye concentrations on both runs.

## 2. Results

The percent aerosolization of the sprayed wastewater has been estimated through a dye experiment using an infinite line source atmospheric dispersion model according to the previously described procedure. Because the model applies only to the aerosol's dispersion from its source, it does not consider such aerosol formation factors as sprinkler type, spray pressure, temperature, and relative humidity. Thus, the percent aerosolization estimates herein obtained must be considered in light of the aerosol formation conditions during the dye experiment runs.

The dye experiment conditions during the two runs are shown in Table III. Both runs were taken within a single hour in the afternoon at 50% relative humidity. The temperature was 31°C on run 1 and 29°C on run 2.

The atmospheric dispersion model parameters pertaining to all four sampler distances on each run are also displayed in Table III. The remaining model parameters that vary with sampler distance are presented in Table IV. The aerosol dye source strength estimates  $q$  calculated from the model equation (1) are also shown in Table IV. The estimated aerosol dye source strength decreased with sampler distance on run 1: from 11.0  $\mu\text{g}/\text{m sec}$  at 40.2 m. to 6.5  $\mu\text{g}/\text{m sec}$  at 181.5 m. On run 2, the estimates appear to vary randomly with downwind distance, between 6.7 and 7.9  $\mu\text{g}/\text{m sec}$ .

Table V displays the data from which the dye source strength  $Q$  in the sprayed wastewater was calculated. There was a slight geometric discrepancy between the 60° angle of the air sampler line with the wet line edge and the angles of 50° on run 1 and 65° on run 2 of the mean wind direction with the sprinkler line. Consequently, the identity of the sprinklers providing most of the dye to an air sampler varied with the run and the sampler distance. The most probable upwind sprinklers for each aerosol sample are given in Table V. There were 52 sprinklers in the line; hence sprinklers 26 and 27 were located in the center of the sprinkler line nearest the main pipeline. The wastewater flow rate per sprinkler,  $F_s$ , for the most probable upwind sprinklers was derived from Figure 5 for each aerosol sample. Based on the parameters presented in Table V, the wastewater dye source strength  $Q$  was calculated using equation (2).

The percent aerosolization was estimated by inserting the aerosol dye source strength  $q$  and the wastewater dye source strength  $Q$  for each sampler and run in equation (3). The percent aerosolization

TABLE III

## DYE EXPERIMENT CONDITIONS

	Run 1	Run 2
Date	9/9/75	9/9/75
Time	1540-1600	1613-1633
Temperature	30.6°C	29.4°C
Relative Humidity	50%	50%
ATMOSPHERIC DISPERSION MODEL PARAMETERS:		
Z, Effective Sampler Height	1.5m	1.5m
H, Effective Aerosol Source Height	2.5m	2.5m
U, Mean Wind Velocity	3.6m/sec	2.8m/sec
Ø, Angle of Wind with Sprinkler Line	50°	65°
Pasquill Wind Stability Class	B/C	B/C

TABLE IV

ESTIMATION OF AEROSOL DYE SOURCE STRENGTH FROM  
THE ATMOSPHERIC DISPERSION MODEL

Run	Air Sampler Distance		$\sigma_z$ Vertical Dispersion Standard Deviation m.	$\chi$ Aerosol Dye Con- centration Sampled $\mu\text{g}/\text{m}^3$	$Q$ Estimated Aerosol Dye Source Strength $\mu\text{g}/\text{m sec.}$
	From Center of Wet Line Edge m.	$x$ Downwind from Sprinkler Line m.			
1	25	40.2	3.62	0.664	11.0
	50	68.5	6.17	0.438	10.3
	75	96.7	8.70	0.279	8.8
	150	181.5	16.0	0.117	6.5
2	25	34.0	3.06	0.530	7.5
	50	57.9	5.21	0.410	7.9
	75	81.8	7.36	0.265	6.7
	150	153.4	13.8	0.173	7.7

TABLE V.

## ESTIMATION OF WASTEWATER DYE SOURCE STRENGTH

Run	Air Sampler Distance Downwind From Sprinkler Line, m.	Most Probable Upwind Sprinklers, Numbers	M Dye Mass Sprayed Through Sprinkler Line, g	L Effective Length of Sprinkler Line, m	T Effective Dye Spraying Period, Sec	Wastewater Flow		Q Wastewater Dye Source Strength $\mu\text{g}/\text{m} \text{ sec}$
						$F_s$ Probable Upwind Sprinklers $\text{m}^3/\text{sec}$	$F$ Entire Sprinkler Line $\text{m}^3/\text{sec}$	
1	40.2	24-26	321	479	600	$6.81 \times 10^{-4}$	$6.81 \times 10^{-4}$	1461
	68.5	23-26						1430
	96.7	23-25						1396
	181.5	21-23						1299
2	34.0	25-28	333	479	600	$6.81 \times 10^{-4}$	$6.81 \times 10^{-4}$	1552
	57.9	25-28						1552
	81.8	25-28						1552
	153.4	26-29						1535

estimates obtained are presented in Table VI. On run 1, the percent aerosolization values decreased with their air sampler distance: from 0.75% at 40.2 m to 0.72% at 68.5 m, to 0.63% at 96.7 m and to 0.50% at 181.5 m. On run 2, all of the estimates of the percentage of the wastewater aerosolized were near 0.5%: 0.48%, 0.51%, 0.43% and 0.50%.

### 3. Discussion

The percentage of the wastewater aerosolized in the Pleasanton spray irrigation fields on hot afternoons at 50% relative humidity appears to be in the range of 0.5% to 0.7%. The relative consistency of the percent aerosolization estimates at different air sampler distances and wind directions suggests that the modified infinite line source atmospheric dispersion model herein employed is a reasonable approximation to aerosol dispersion near the Pleasanton spray fields for the conditions occurring during the dye experiment.

It is recommended that a variety of dye experiments be conducted in the Phase II environmental monitoring program. It would appear useful to determine the percent aerosolization under a representative range of temperature and humidity conditions in Pleasanton. It is also pertinent to estimate percent aerosolization in some of the other spray fields, especially the 0-1-2-3-4 fields in which different geometric and spray pressure conditions may prevail. Conducting dye experiments under a variety of conditions would also test the generality and suitability of the modified infinite line source model for estimating aerosol dispersion (and also pathogen levels, when appropriate die-off rates are determined) near the spray fields. The dye experiments should only be conducted when a steady wind direction prevails.

#### C. Effluent Data

There were several objectives in the sampling and analysis of effluent. The overall objective was to provide a detailed characterization of the wastewater which was being sprayed. This information would be used as the data base for assessing the relationships between wastewater quality, aerosol concentrations and eventually health effects. Another objective was to determine the daily variations in composition. This information is necessary to design an in-depth environmental monitoring protocol for Phase II.

TABLE VI.

## ESTIMATED PERCENT AEROSOLIZATION OF SPRAYED WASTEWATER

Run	Air Sampler Distance Downwind from Sprinkler Line, m.	q Aerosol Dye Source Strength $\mu\text{g}/\text{m sec}$	Q Wastewater Dye Source Strength $\mu\text{g}/\text{m sec}$	A Percent Aerosoli- zation
1	40.2	11.0	1461	0.75%
	68.5	10.3	1430	0.72%
	96.7	8.8	1396	0.63%
	181.5	6.5	1299	0.50%
2	34.0	7.5	1552	0.48%
	57.9	7.9	1552	0.51%
	81.8	6.7	1552	0.43%
	153.4	7.7	1535	0.50%

To facilitate the design of Phase II, a diurnal study was conducted. This provided information as to possible cyclic changes in composition that occur daily (during spray operations). Since these samples were collected at the exit of the second oxidation pond, it was not anticipated that measureable differences would be seen. The ponds were expected to provide somewhat of a uniform composition of effluent at the exit. Different results would be expected if samples were taken before the ponds. The oxidation ponds also provide an additional step in improving water quality.

Grab samples of effluent were collected at the spray heads before and after each aerosol collection run. These effluent samples describe the composition (chemical and biological) of effluent being aerosolized. Tables VII, VIII and IX show a summary of the effluent data for routine and selective parameters.

1. Daily Composite Samples

Table X presents the results of the routine analyses of the daily composites, including the average and standard deviation of the samples. Table XI shows the results of selected analyses of the daily composites.

The total and free chlorine data are suspect as the analyses were performed on composite samples, and the results of one-half of the analyses have free chlorine values greater than total chlorine. Chlorine is added to the secondary clarifier primarily as an odor-controlling agent at very low levels (<10 mg/l). The secondary effluent is then discharged to the aeration ponds. The likelihood of finding chlorine under these conditions is very remote; thus, the values reported are probably incorrect. Improved procedures for collection and analysis of chlorine will be utilized for Phase II.

The organic parameters analyzed during these tests exhibited fluctuations which are not expected from a plant which utilizes aeration ponds. These variations may be due to sampling and/or analysis techniques or short circuiting within the ponds. Overall, these parameters indicate relatively poor quality of wastewater.

Figures 6 and 7 show daily plots of TOC, BOD and TSS. The data do not suggest any daily trends. The data show TOC values greater than COD values for 9/8/75 to 9/10/75 which is not possible. The remainder of the samples had TOC values consistent with expected variations. The ratio of TOC/COD should be relatively constant; however,

TABLE VII. DATA SUMMARY FOR ROUTINE EFFLUENT PARAMETERS

Routine Analysis									
Oxygen Demand and Physical Parameters - 24-Hr Composites (8/14 - 9/13)									
	TOC, mg/l	TSS, mg/l	Total Solids mg/l						
Mean X	48	28	713						
Std Dev X	23	10	37						
CV % X	47	37	5						
Inorganic and Bacteriological Parameters - 24-Hr. Composites (8/14 - 9/13)									
	Total Chlorine, mg/l	Free Chlorine, mg/l	pH	Total Coliform, MPN/100 ml	Fecal Coliform, MPN/100 ml	Standard Plate Count, organisms/100 ml			
Mean X	0.06	0.07	8.47	$6.8 \times 10^6$	$1.3 \times 10^6$	$1.9 \times 10^8$			
Std Dev X	0.02	0.04	0.17	$2.6 \times 10^7$	$4.4 \times 10^6$	$1.9 \times 10^8$			
CV % X	41	79	2	390	340	100			
Oxygen Demand and Physical Parameters		Inorganic and Bacteriological Parameters - Diurnal Study							
	TOC, mg/l	TSS, mg/l	Total Solids, mg/l	Total Chlorine, mg/l	Free Chlorine, mg/l	pH	Total Coliform, MPN/100 ml	Fecal Coliform, MPN/100 ml	Standard Plate Count, No./100 ml
8-19-75									
Mean	37	27	725	<0.05	<0.02	8.31	$3.4 \times 10^5$	$6.4 \times 10^4$	$4.2 \times 10^7$
Std Dev	3	10	59	-	-	0.08	$1.9 \times 10^5$	$3.9 \times 10^4$	$2.0 \times 10^7$
CV %	9	38	8	-	-	1	56	61	48
8-20-75									
Mean	35	18	752	<0.05	<0.02	8.36	$2.0 \times 10^5$	$4.5 \times 10^4$	$6.1 \times 10^7$
Std Dev	6	4	49	-	-	0.11	$1.1 \times 10^5$	$2.6 \times 10^4$	$4.5 \times 10^7$
CV %	17	24	7	-	-	1	53	58	75
8-21-75									
Mean	37	27	689	<0.05	<0.02	8.54	$3.1 \times 10^5$	$1.3 \times 10^5$	$1.0 \times 10^8$
Std Dev	9	12	30	-	-	0.07	$2.4 \times 10^5$	$1.4 \times 10^5$	$1.2 \times 10^8$
CV %	23	43	4	-	-	1	76	111	110
Oxygen Demand and Physical Parameters - Grab Samples (8/25 - 9/13) (Aerosol Study)									
	TOC, mg/l	TSS, mg/l	Total Solids mg/l						
Mean X	52	33	729						
Std Dev X	26	18	24						
CV %	49	56	3						
Inorganic and Bacteriological Parameters Grab Samples (8/25 - 9/13) (Aerosol Study)									
	Chlorine, mg/l	Free Chlorine, mg/l	pH	Total Coliform, MPN/100ml	Fecal Coliform, MPN/100ml	Standard Plate Count, No.			
Mean X	0.28	0.08	8.42	$5.4 \times 10^6$	$4.4 \times 10^5$	$3.0 \times 10^8$			
Std Dev X	0.16	0.05	0.16	$9.7 \times 10^6$	$7.3 \times 10^5$	$6.3 \times 10^8$			
CV % X	58	69	2	180	170	210			

TABLE VIII. DATA SUMMARY FOR SELECTED COMPOSITE AND GRAB EFFLUENT PARAMETERS

Selected Analysis							
<i>Oxygen Demand and Physical Parameters - 24 Hr Composites (8/17-9/10)</i>							
	COD, mg/l	TOC, mg/l	BOD, mg/l	TSS, mg/l	Total Solids, mg/l		
Mean X	95	54	35	27	722		
Std Dev X	29	24	15	13	29		
CV % X	31	45	43	48	4		
<i>Inorganic and Nutrient Parameters - 24 Hr Composites (8/17-9/10)</i>							
	Hardness, mg/l Ca CO <sub>3</sub>	Total Phosphorus, mg/l	Nitrate Nitrogen, mg/l	Nitrite Nitrogen, mg/l	Ammonia Nitrogen, mg/l	Organic Nitrogen, mg/l	Total Chlorine, mg/l
Mean X	281	6.45	0.21	0.06	35.33	24.08	0.05
Std Dev X	17	8.59	0.19	0.02	29.43	18.80	0.03
CV % X	6	133	93	41	83	78	60
<i>Heavy Metals, mg/l - 24 Hr Composites (8/17-9/10)</i>							
	Mercury	Arsenic	Cadmium	Lead	Copper	Zinc	
Mean X	$1.4 \times 10^{-4}$	0.0022	0.0004	0.0055	0.0272	0.2050	
Std Dev X	$3.1 \times 10^{-5}$	0.0010	0.0003	0.0037	0.0065	0.3308	
CV % X	23	45	75	66	24	161	
Selected Analysis							
<i>Oxygen Demand and Physical Parameters - Grab Samples (8/25-9/13) (Aerosol Study)</i>							
	COD, mg/l	TOC, mg/l	BOD, mg/l	TSS, mg/l	Total Solids mg/l		
Mean	130	49	27	30	718		
Std Dev	84	28	14	20	13		
CV %	65	59	52	68	2		
<i>Inorganic and Nutrient Parameters - Grab Samples (8/25-9/13) (Aerosol Study)</i>							
	Hardness, mg/l	Total Phosphorus, mg/l	Nitrate Nitrogen, mg/l	Nitrite Nitrogen, mg/l	Ammonia Nitrogen, mg/l	Organic Nitrogen, mg/l	
Mean	280	2.7	0.16	0.27	52.3	29.4	
Std Dev	5	0.7	0.11	0.14	25.0	19.6	
CV %	2	27	67	50	48	67	
<i>Heavy Metals, mg/l - Grab Samples (8/25-9/15) (Aerosol Study)</i>							
	Mercury	Arsenic	Cadmium	Lead	Copper	Zinc	
Mean	$1.29 \times 10^{-4}$	0.0017	0.0006	0.004	0.024	0.18	
Std Dev	$2.94 \times 10^{-5}$	0.0002	0.0006	0.002	0.007	0.16	
CV %	23	9	106	39	31	90	

TABLE IX. DATA SUMMARY FOR SELECTED DIURNAL EFFLUENT PARAMETERS

Selected Analysis						
<i>Oxygen Demand and Physical Parameters - Diurnal Study</i>						
	COD, mg/l	TOC, mg/l	BOD, mg/l	TSS, mg/l	Total Solids mg/l	
8-19-75						
Mean	79	37	58	30	715	
Std Dev	9	3	5	7	60	
CV %	12	9	8	23	8	
8-20-75						
Mean	110	37	23	21	758	
Std Dev	44	4	4	2	41	
CV %	40	12	17	8	5	
8-21-75						
Mean	103	29	36	19	700	
Std Dev	3	2	6	4	42	
CV %	3	7	16	19	6	
Selected Analysis						
<i>Inorganic and Nutrient Parameters - Diurnal Study</i>						
	Hardness, mg/l	Total Phosphorus, mg/l	Nitrate Nitrogen, mg/l	Nitrite Nitrogen, mg/l	Ammonia Nitrogen, mg/l	Organic Nitrogen, mg/l
8-19-75						
Mean	258	12.8	0.08	0.02	23.3	13.8
Std Dev	10	0.2	0.02	0.01	9.3	10.1
CV %	4	1	23	41	40	73
8-20-75						
Mean	274	6.0	0.08	0.03	21.0	7.2
Std Dev	5	9	0.04	0.02	2.0	6.7
CV %	2	15	46	72	10	93
8-21-75						
Mean	268	5.1	0.06	0.03	26.0	9.5
Std Dev	4	0.4	0.01	0.01	3.8	5.9
CV %	1	8	24	28	15	63
Selected Analysis						
<i>Heavy Metals - Diurnal Study</i>						
	Mercury, mg/l	Arsenic, mg/l	Cadmium, mg/l	Lead, mg/l	Copper, mg/l	Zinc, mg/l
8-19-75						
Mean	$1.08 \times 10^{-4}$	0.0029	0.0004	0.005	0.030	0.59
Std Dev	$7.83 \times 10^{-6}$	0.0003	0.0002	0.001	0.002	0.74
CV %	7	10	54	22	7	126
8-20-75						
Mean	$1.22 \times 10^{-4}$	0.0027	0.0006	0.005	0.030	0.26
Std Dev	$2.59 \times 10^{-5}$	0.0012	0.0003	0.002	0.007	0.25
CV %	21	43	43	38	23	97
8-21-75						
Mean	$1.00 \times 10^{-4}$		0.0015	0.004	0.021	1.43
Std Dev	0		0.0017	0	0.005	1.13
CV %	0		113	0	24	79

TABLE X. ROUTINE DAILY COMPOSITE SAMPLES COLLECTED DURING HOURS OF IRRIGATION

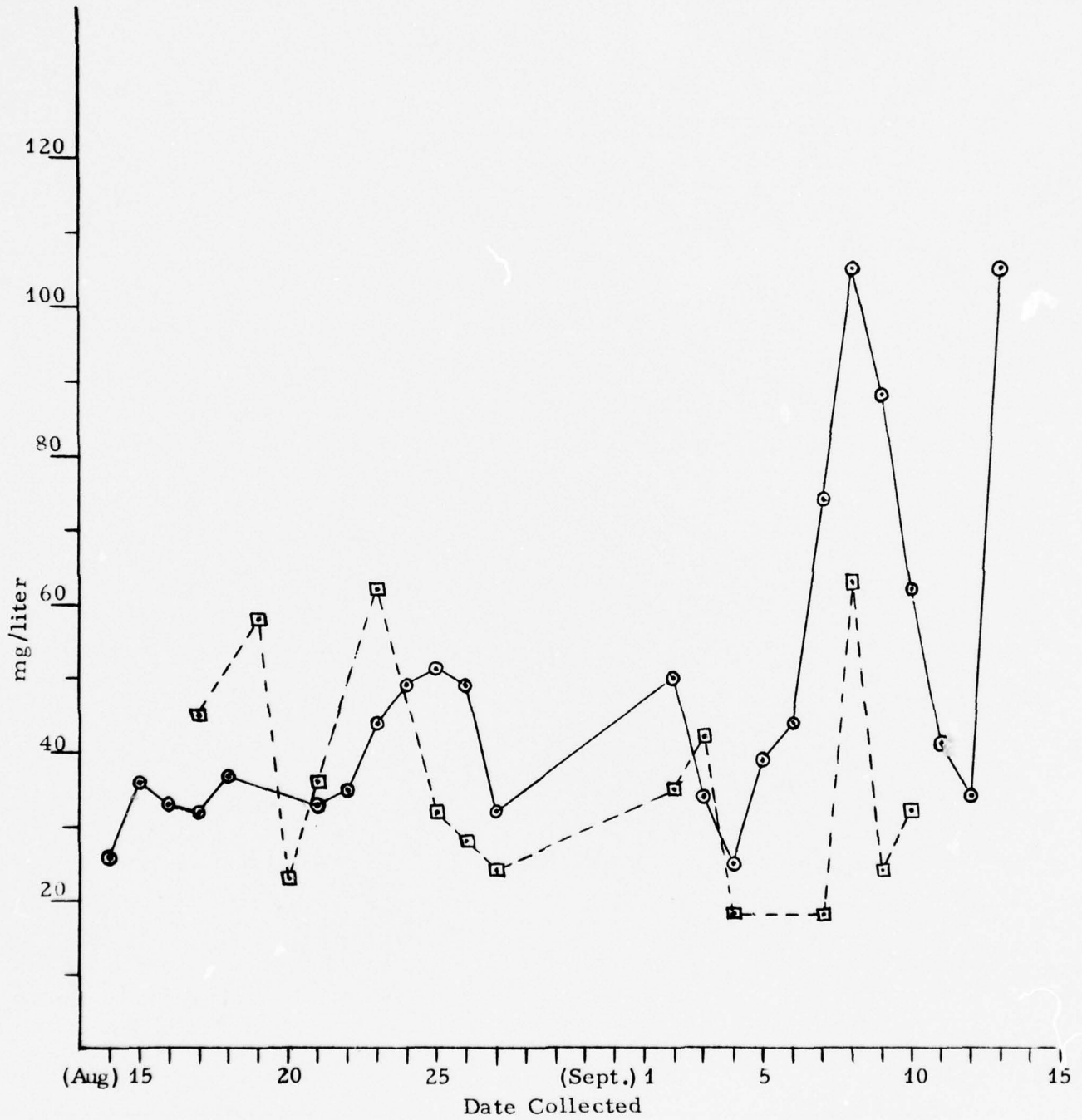
Sample Date	Oxygen Demand and Physical Parameters			Inorganic Parameters			Bacteriological Parameters		
	TOC, mg/ℓ	TSS, mg/ℓ	Total Solids, mg/ℓ	Total* Chlorine, mg/ℓ	Free † Chlorine, mg/ℓ	pH	Total Coliform, MPN/100 mℓ	Fecal Coliform, MPN/100 mℓ	Standard Plate Count, No./100 mℓ
8-14-75	26	37	650	N.D.	N.D.	8.44	$5.6 \times 10^4$ ‡	$7.9 \times 10^4$ ‡	$8.7 \times 10^7$
8-15-75	36	35	640	N.D.	N.D.	8.40	$3.3 \times 10^5$	$4.9 \times 10^4$	$1.7 \times 10^8$
8-16-75	33	26	660	N.D.	N.D.	8.33	$4.6 \times 10^5$	$3.1 \times 10^5$	$1.7 \times 10^8$
8-17-75	32	32	650	N.D.	N.D.	8.41	$4.9 \times 10^5$	$1.4 \times 10^5$	$2.7 \times 10^8$
8-18-75	37	24	680	N.D.	N.D.	8.16	$1.3 \times 10^6$	$1.3 \times 10^6$	$2.0 \times 10^8$
8-21-75	33	19	700	N.D.	N.D.	8.34	$7.9 \times 10^5$	$3.3 \times 10^5$	$6.5 \times 10^7$
8-22-75	35	19	700	N.D.	N.D.	8.62	$2.3 \times 10^6$	$7.9 \times 10^5$	$8.5 \times 10^7$
8-23-75	44	44	740	N.D.	N.D.	8.55	$7.0 \times 10^5$	$1.7 \times 10^5$	$1.4 \times 10^8$
8-24-75	49	33	700	N.D.	N.D.	8.57	$7.0 \times 10^5$	$7.0 \times 10^5$	$1.7 \times 10^7$
8-25-75	51	39	720	N.D.	N.D.	8.57	$1.7 \times 10^6$	$3.3 \times 10^5$	$6.3 \times 10^7$
8-26-75	49	24	740	0.08	N.D.	8.52	$1.1 \times 10^7$	$1.7 \times 10^6$	$7.0 \times 10^8$
8-27-75	32	22	730	0.04	0.13	8.43	$2.8 \times 10^6$	$4.9 \times 10^5$	$1.4 \times 10^8$
9-02-75	50	28	700	0.08	0.05	8.60	$1.1 \times 10^6$	$4.6 \times 10^5$	$1.1 \times 10^8$
9-03-75	34	38	690	0.04	0.05	8.44	$4.9 \times 10^5$	$3.3 \times 10^5$	$7.6 \times 10^7$
9-04-75	25	45	730	0.1	N.D.	8.62	$7.9 \times 10^5$	$2.2 \times 10^5$	$2.5 \times 10^8$
9-05-75	39	27	700	0.02	N.D.	8.48	$1.3 \times 10^8$	$2.2 \times 10^7$	$8.3 \times 10^8$
9-06-75	44	34	740	0.10	0.09	8.64	$7.9 \times 10^5$	$4.9 \times 10^5$	$2.3 \times 10^8$ ‡
9-07-75	74	13	740	0.04	0.07	8.72	$7.0 \times 10^5$	$4.9 \times 10^4$	$1.4 \times 10^8$
9-08-75	105	15	760	0.03	0.07	8.16	$7.9 \times 10^5$	$1.3 \times 10^5$	$1.1 \times 10^7$
9-09-75	88	11	730	0.04	0.07	8.28	$7.9 \times 10^5$	$4.9 \times 10^5$	$1.6 \times 10^8$
9-10-75	62	11	730	0.1	0.11	8.38	$4.9 \times 10^5$	$4.9 \times 10^4$	$1.1 \times 10^8$
9-11-75	41	28	770	0.08	0.13	8.81	$4.9 \times 10^5$	$1.1 \times 10^5$	$5.0 \times 10^7$
9-12-75	34	18	760	0.08	0.09	8.60	$2.3 \times 10^6$	$2.2 \times 10^5$	$2.0 \times 10^8$
9-13-75	105	39	740	0.1	0.13	8.24	$1.3 \times 10^6$	$2.2 \times 10^5$	$1.9 \times 10^8$
Mean X	48	28	713	0.06	0.05	8.47	$6.8 \times 10^6$	$1.3 \times 10^6$	$1.9 \times 10^8$
Std Dev X	23	10	37	0.02	0.04	0.17	$2.6 \times 10^7$	$4.4 \times 10^6$	$1.9 \times 10^8$
CV% X	47	37	5	41	79	2	390	340	100

\*N.D. = <0.05 mg/ℓ †N.D. = <0.02 mg/ℓ ‡Analytical Quality Control Analysis

TABLE XI. SELECTED COMPOSITE SAMPLES OF POND NO. 2 EFFLUENT COLLECTED DURING HOURS OF IRRIGATION

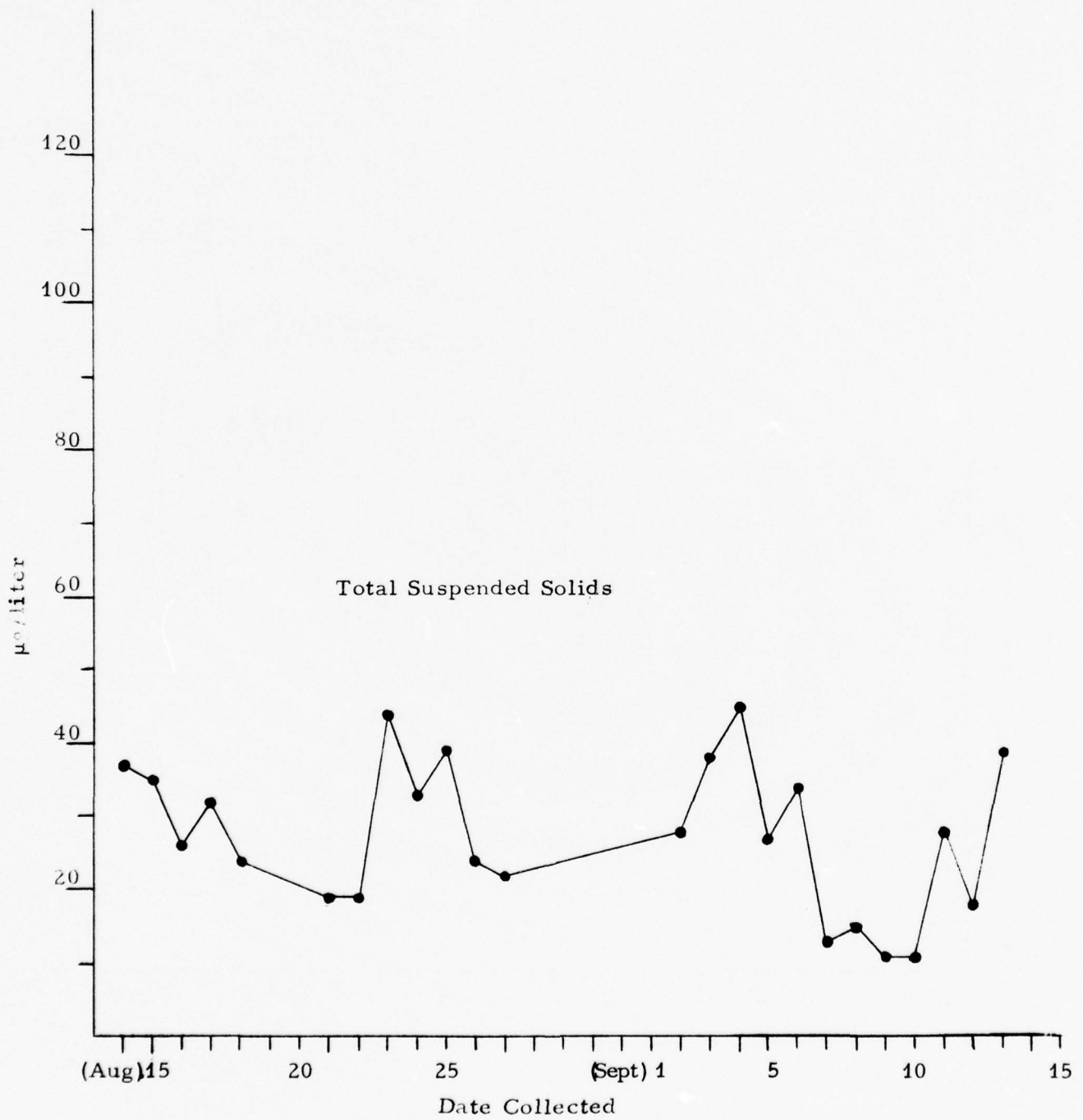
Sample Date	Oxygen Demand and Physical Parameters					
	COD, mg/l	TOC, mg/l	BOD, mg/l	TSS, mg/l	Total Solids, mg/l	
8-17-75	139	32	45	32	650	
8-23-75	94	44	62	44	740	
8-25-75	72	51	32	37	720	
8-26-75	120	49	28	24	740	
8-27-75	113	32	24	22	730	
9-2-75	72	50	35	28	700	
9-3-75	144	34	42	38	690	
9-4-75	70	25	18*	45	730	
9-7-75	103	74	18*	13	740	
9-8-75	84	105	63	15	760	
9-9-75	79	88	24*	11	730	
9-10-75	51	62	32	11	730	
Mean X	95	54	35	27	722	
Std Dev X	29	24	15	13	29	
CV % X	31	45	43	48	4	
*Analytical Quality Control Analysis						
Sample Date	Inorganic and Nutrient Parameters					
	Hardness mg/l CaCO <sub>2</sub>	Total Phosphorus, mg/l P	Nitrate Nitrogen, mg/l	Nitrite Nitrogen, mg/l	Ammonia Nitrogen, mg/l	Organic Nitrogen, mg/l
8-17-75	230	29.6	0.10	—	18.7	7.7
8-23-75	280	4.3	0.14	0.03	22.6	19.8
8-25-75	290	2.8	0.10	0.04	23.3	18.6
8-26-75	280	0.8	0.03	0.04	6.8	0.3
8-27-75	280	4.2	0.10	0.04	14.6	33.0
9-2-75	280	3.4	0.14	0.07	27.0	5.6
9-3-75	285	3.3	0.20	0.06	22.4	65.3
9-4-75	290	2.4	0.70	0.06	19.5	43.6
9-7-75	290	18.4	0.14	0.11	31.0	10.5
9-8-75	290	2.8	0.17	0.05	96.0	37.0
9-9-75	290	3.4	0.51	0.08	93.0	15.0
9-10-75	290	2.0	0.18	0.09	49.0	32.5
Mean X	281	6.45	0.21	0.06	35.33	24.08
Std Dev X	17	8.59	0.19	0.02	29.43	18.80
CV % X	6	133	93	41	83	78
Sample Date	Heavy Metals					
	Mercury, mg/l	Arsenic, mg/l	Cadmium, mg/l	Lead*, ng/l	Copper, mg/l	Zinc, mg/l
8-17-75	1.21 × 10 <sup>-4</sup>	0.0019	<0.0002	0.004	0.039	0.09
8-23-75	1.33 × 10 <sup>-4</sup>	<0.0013	<0.0002	0.005	0.021	0.15
8-25-75	1.81 × 10 <sup>-4</sup>	0.0014	<0.0002	0.016	0.030	0.05
8-26-75	1.20 × 10 <sup>-4</sup>	0.0033	0.0002	0.004	0.029	0.04
8-27-75	1.54 × 10 <sup>-4</sup>	0.0017	0.0009	0.004	0.026	0.07
9-2-75	2.02 × 10 <sup>-4</sup>	0.0019	<0.0002	0.003	0.035	0.05
9-3-75	1.09 × 10 <sup>-4</sup>	0.0023	0.0005	0.003	0.030	0.77
9-4-75	1.30 × 10 <sup>-4</sup>	0.0019	0.0003	0.004	0.032	0.04
9-7-75	1.47 × 10 <sup>-4</sup>	<0.0013	0.0002	0.005	0.017	1.03
9-8-75	1.11 × 10 <sup>-4</sup>	<0.0013	0.0010	0.009	0.020	0.03
9-9-75	1.29 × 10 <sup>-4</sup>	0.0044	0.0009	0.005	0.022	0.07
9-10-75	<1.00 × 10 <sup>-4</sup>	0.0031	0.0003	0.004	0.025	0.07
Mean X	1.4 × 10 <sup>-4</sup>	0.0022	0.0004	0.0055	0.0272	0.2050
Std Dev X	3.1 × 10 <sup>-5</sup>	0.0010	0.0003	0.0037	0.0065	0.3308
CV % X	23	45	75	66	24	161
Limit of Detection	1.00 × 10 <sup>-4</sup>	0.0013	0.0002	0.003	0.015	0.02
*Lead Values Reported on Digested/Concentrated Samples						

FIGURE 6 . Daily Composite Samples Collected During Hours of Irrigation (10-18 hours)



○—○ Total Organic Carbon  
□- - -□ Biochemical Oxygen Demand

FIGURE 7 . Daily Composite Samples Collected During Hours of Irrigation (10-18 hours)



this ratio varied from 0.18 to 1.74. These variations indicate a possibility of problems in these measurements. Similar comments could be made regarding the ratio of BOD to TOC. The BOD values are higher than TOC for five of the composite samples, while for the other 10, the BOD's are lower.

Total solids and hardness data should indicate fluctuations of influent except that the lime added for pH control could influence these. The total phosphorus values are consistent with expected values from a municipal sewage plant (with the exception of days 8/17/75, 8/26/75 and 9/7/75). The ammonia and organic nitrogen values indicate a high nitrogen source either as influent or poor nitrogen removal through the plant. The low nitrate and nitrite values suggest poor nitrogen removal as would be expected with a trickling filter process. This situation may be changed somewhat with the new activated biofilter process. High algal activity in the aeration ponds could also influence these values.

The effluent bacteriological parameters gave consistent results with the values being rather high. The means for total coliform  $6.8 \times 10^6$ /100 ml, fecal coliform  $1.3 \times 10^6$ /100 ml and standard plate count  $1.9 \times 10^8$ /100 ml are indicative of a treated sewage discharge without disinfection. These results indicate that total coliform, fecal coliform and standard plate count could serve as effluent constituents which would provide meaningful and convenient indicators of effluent quality. The levels of trace metals are low in all of the effluent samples.

Table XII reports the coliphage analyses for all of the effluent samples. The quantities of coliphage found are low--less than 10--except for an occasional sample (high value 420 pfu/ml). All effluent samples were positive for coliphage. Table XIII reports the results of virus and selected bacteria on effluent samples. Three of the effluents yielded Proteus mirabilis, an organism which might be considered a pathogen under certain conditions. No other pathogenic organisms were found. This finding is difficult to understand in view of the relatively large quantities of bacteria measured in the effluents. Other studies have found pathogenic organisms consistently in secondary treated effluent, especially when disinfection was not practiced.

About 40 percent of all the effluent samples tested yielded positive responses in one or more of the three cell lines used for viruses. Only one of 10 diurnal effluent samples was positive, which is not consistent with the other data. The composites for 8/17/75 and 8/23/75 were positive while the diurnal study was conducted on 8/19/75, 8/20/75 and

TABLE XII. COLIPHAGE ISOLATION FROM EFFLUENT SAMPLES

Date	Daily Composite PFU/mℓ	Diurnal Study						Grab Samples During Aerosol Collections			
		Time	PFU/mℓ	Time	PFU/mℓ	Time	PFU/mℓ	Aerosol Run No.	Date	Time	PFU/mℓ
		8-19-75		8-20-75		8-21-75					
8-14-75	<10	0930	<10	0800	<10	1000	<10	2	8-25-75	1330	<10
8-15-75	<10	1030	<10	0900	<10	1100	<10		1830	<10	
8-16-75	170	1130	<10	1000	<10	1200	<10	3	8-26-75	1300	<10
8-17-75	<10	1230	<10	1100	<10	1300	<10		1900	<10	
8-18-75	150	1330	<10	1200	<10	1400	<10	4	8-27-75	1315	<10
8-21-75	<10	1430	<10	1300	<10	1500	<10		1900	<10	
8-22-75	<10	1530	<10	1400	<10	1600	160	5	9-2-75	1930	<10
8-23-75	200	1630	<10	1500	<10	1700	<10		2300	<10	
8-24-75	<10	1730	130	1600	<10	1800	<10	6	9-3-75	2200	170
8-25-75	<10	1830	<10	1700	<10	1900	<10		9-4-75	0200	<10
8-26-75	<10	1930	<10	1800	<10	2000	<10	7	9-5-75	2100	<10
8-27-75	<10	2030	<10	1900	..*	2100	<10				
9-2-75	<10	2130	<10	2000	<10	2200	<10	8	9-10-75	1600	<10
9-3-75	<10	2230	<10						2230	420	
9-4-75	<10							9	9-11-75	2100	<10
9-5-75	<10								2300	<10	
9-6-75	<10							10	9-12-75	1800	370
9-7-75	<10								2130	<10	
9-8-75	<10							11	9-13-75	1345	180
9-9-75	<10								1345	150	
9-10-75	<10										
9-11-75	<10										
9-12-75	400										
9-13-75	350										

\*Sample Lost

TABLE XIII. VIRUS, BACTERIA AND COLIPHAGE ISOLATES FROM EFFLUENT SAMPLES

Sample Date	Time	Virus Isolates* BKC-Plaques PFU/ml	CPE-BKC Positive	CPE-WI-38 Positive	Identified Bacteria*										Coliphage Isolates	
					Proteus	Strep-gamma	Enterobacter	Escherichia	Bacillus	Micrococcus	Citrobacter	Alkaligenes	Diphtheroid	Providencia	Mima	PFU/ml
<i>Daily Composites</i>																
8-17-75		5	+	+		+	+	+	+	+			+		<10	
8-23-75			+	+	+	+	+	+	+	+					200	
8-25-75		1	+				+	+	+	+	+		+		<10	
8-26-75							+	+	+	+	+				<10	
8-27-75								+	+	+	+		+		<10	
9-2-75								+	+	+	+		+		<10	
9-3-75		1		+			+	+	+	+			+		<10	
9-4-75					+		+	+	+	+	+				<10	
9-7-75			+	+			+	+	+	+			+		<10	
9-8-75		1						+	+	+	+		+		<10	
9-9-75		1						+	+	+	+		+		<10	
9-10-75				+				+	+	+	+				<10	
<i>Diurnal Study</i>																
8-19-75	1130						+	+	+	+		+			<10	
	1530						+	+	+	+	+				<10	
	1830						+	+	+		+		+		<10	
	2230						+	+	+	+	+		+		<10	
8-20-75	0900						+	+	+	+	+		+	+	<10	
	1300	1					+	+	+	+	+		+		<10	
	1700						+	+	+		+				<10	
	2000							+	+	+		+			<10	
8-21-75	1000						+	+	+		+				<10	
	1400						+	+	+	+		+			<10	
<i>Grab Samples During Aerosol Collections</i>																
9-3-75	2200	3	+	+				+	+	+			+		170	
9-4-75	0200	2	+	+				+	+	+			+		<10	
9-10-75	2230			+											420	
9-11-75	2100			+				+	+	+			+		<10	
9-12-75	2130			+				+	+	+	+		+		<10	
*Blanks indicate negative findings except for lost grab sample for bacteria sample date 9-10-75.																

8/21/75. One possible explanation is that the diurnal samples might not have been cooled fast enough. A large number of one-liter samples was collected over a short time during the diurnal study and placed into a refrigerator. The other effluent samples were rapidly cooled (refrigerated composite sampler was used for 24-hour composites and grab samples during aerosol collections were placed immediately on wet ice).

## 2. Diurnal Samples

The results of effluent analysis for the diurnal study are shown in Tables XIV and XV. Figures 8 through 14 show plots of some of the data. Because of the two oxidation ponds which have a theoretical two days retention time, diurnal changes from the plant should be considerably dampened. There are, however, several operations which could produce cyclic daily changes in effluent (from the ponds) quality. If there is insufficient mixing in the ponds during times that spraying is taking place, there will be short circuiting such that a significant portion of water from the plant will proceed directly to the pumps and to the spray fields.

Another operation which could produce a daily cycle is the spraying of effluent for part of the day and the refilling of the ponds the rest of the day. During refilling operations, the effluent gets better mixing, and it has slightly longer residence times in the oxidation ponds. Another operation which could produce daily cycles is algal activity in the ponds with the production of carbon dioxide. The quantities of carbon dioxide produced at night should be higher than during the daytime.

There are wide fluctuations in some of the measurements, and overall, only pH is clearly cyclic during the day. The pH of the water apparently increases during the day. The pH of the water coming into the ponds is reported to be kept near 8.6. The TOC values appear to increase slightly from morning to night. It's possible that during the analysis of samples the CO<sub>2</sub> is not completely flushed out; thus, changes in CO<sub>2</sub> content in the effluent (algal activity) may be reflected in these data.

TOC and TSS appear to increase from the start of spraying to the end. This may be the result of some of the factors mentioned above. Total solids also tend to decrease within the same time period.

The bacterial data are rather uniform for the diurnal study. It is probable that much more consistent data for coliforms would have been achieved (improved sensitivity and accuracy) if the millipore technique

TABLE XIV. ROUTINE HOURLY GRAB SAMPLES FROM POND NO. 2 EFFLUENT  
DURING HOURS OF IRRIGATION - DIURNAL

Time	Oxygen Demand and Physical Parameters			Inorganic Parameters			Bacteriological Parameters		
	TOC, mg/ℓ	TSS, mg/ℓ	Total Solids, mg/ℓ	Total* Chlorine, mg/ℓ	Free † Chlorine, mg/ℓ	pH	Total Coliform, MPN/100 mℓ	Fecal Coliform, MPN/100 mℓ	Standard Plate Count, No./100 mℓ
8-19-75									
0930	38	12	780	N.D.	N.D.	8.18	$4.9 \times 10^5$	$2.8 \times 10^4$	$2.0 \times 10^7$
1030	35	14	770	N.D.	N.D.	8.27	$3.3 \times 10^5$	$1.1 \times 10^5$	$3.1 \times 10^7$
1130	35	24	750	N.D.	N.D.	8.31	$3.3 \times 10^5$	$7.9 \times 10^4$	$3.1 \times 10^7$
1230	34	18	770 ‡	N.D.	N.D.	8.28	$2.3 \times 10^5$	$7.9 \times 10^4$	$4.4 \times 10^7$
1330	32	15	790	N.D.	N.D.	8.29	$1.8 \times 10^5 ‡$	$4.3 \times 10^4 ‡$	$3.2 \times 10^7$
1430	36	18	780	N.D.	N.D.	8.34	$3.3 \times 10^5$	$1.7 \times 10^5$	$6.0 \times 10^7$
1530	40	24	780	N.D.	N.D.	8.38	$4.6 \times 10^5$	$7.0 \times 10^4$	$3.6 \times 10^7$
1630	36	27	760	N.D.	N.D.	8.36	$7.9 \times 10^4$	$2.3 \times 10^4$	$4.5 \times 10^7$
1730	37	32	630	N.D.	N.D.	8.37	$1.7 \times 10^5$	$7.0 \times 10^4$	$3.5 \times 10^7$
1830	33	34	650	N.D.	N.D.	8.42	$2.2 \times 10^5$	$3.5 \times 10^4$	$2.5 \times 10^7$
1930	38	40	680	N.D.	N.D.	8.40	$7.9 \times 10^5$	$4.3 \times 10^4$	$2.8 \times 10^7$
2030	46	41	670	N.D.	N.D.	8.35	$4.9 \times 10^5$	$4.9 \times 10^4$	$3.1 \times 10^7$
2130	36	35	660	N.D.	N.D.	8.21	$4.9 \times 10^5$	$3.3 \times 10^4$	$7.0 \times 10^7$
2230	39	37	680	N.D.	N.D.	8.21	$1.7 \times 10^5$	$7.0 \times 10^4$	$9.3 \times 10^7$
8-20-75									
0800	32	27	690	N.D.	N.D.	8.06	$1.7 \times 10^5$	$7.0 \times 10^4$	$5.5 \times 10^7$
0900	32	19	770	N.D.	N.D.	8.27	$1.3 \times 10^5$	$4.9 \times 10^4$	$2.9 \times 10^7$
1000	27	19	760	N.D.	N.D.	8.27	$7.9 \times 10^4$	$1.3 \times 10^4$	$1.5 \times 10^7$
1100	39	13	840	N.D.	N.D.	8.28	$1.5 \times 10^5 ‡$	$2.0 \times 10^4 ‡$	$2.0 \times 10^7$
1200	37	18	800	N.D.	N.D.	8.36	$3.3 \times 10^5$	$4.9 \times 10^4$	$2.0 \times 10^7$
1300	42	19	810	N.D.	N.D.	8.41	$1.1 \times 10^5$	$2.3 \times 10^4$	$4.1 \times 10^7$
1400	35	13	790	N.D.	N.D.	8.44	$7.9 \times 10^4$	$1.1 \times 10^4$	$4.0 \times 10^7$
1500	36	17	690	N.D.	N.D.	8.39	$2.2 \times 10^5$	$7.0 \times 10^4$	$6.0 \times 10^7$
1600	30	17	770	N.D.	N.D.	8.42	$7.9 \times 10^4$	$2.3 \times 10^4$	$1.5 \times 10^8$
1700	39	22	720	N.D.	N.D.	8.43	$3.3 \times 10^5$	$7.9 \times 10^4$	$1.4 \times 10^8$
1800	23	18	700	N.D.	N.D.	8.44	$3.3 \times 10^5$	$7.9 \times 10^4$	$9.0 \times 10^7$
1900	44	10	710	N.D.	N.D.	8.45	$3.3 \times 10^5$	$7.0 \times 10^4$	$1.0 \times 10^8$
2000	35	22	730	N.D.	N.D.	8.43	$2.8 \times 10^5 ‡$	$3.1 \times 10^4 ‡$	$3.1 \times 10^7$
8-21-75									
1000	27	16	730	N.D.	N.D.	8.34	$3.3 \times 10^5$	$7.0 \times 10^4$	$2.5 \times 10^7$
1100	32	16	710	N.D.	N.D.	8.47	$2.3 \times 10^5$	$2.3 \times 10^4$	$2.9 \times 10^7$
1200	37	17	700	N.D.	N.D.	8.49	$3.3 \times 10^4$	$1.3 \times 10^4$	$4.2 \times 10^7$
1300	34	25	650	N.D.	N.D.	8.57	$4.6 \times 10^4$	$3.8 \times 10^4$	$6.2 \times 10^7$
1400	30	21	670	N.D.	N.D.	8.54	$1.7 \times 10^5$	$1.7 \times 10^4$	$5.7 \times 10^7$
1500	31	23	670	N.D.	N.D.	8.61	$1.7 \times 10^5$	$7.0 \times 10^4$	$5.5 \times 10^7$
1600	61	23	660	N.D.	N.D.	8.58	$1.3 \times 10^5$	$4.9 \times 10^4$	$4.0 \times 10^7$
1700	34	23	670	N.D.	N.D.	8.56	$7.0 \times 10^5$	$2.2 \times 10^5$	$1.4 \times 10^8$
1800	35	23	660	N.D.	N.D.	8.59	$2.8 \times 10^5$	$1.3 \times 10^5$	$2.2 \times 10^8$
1900	40	30	670	N.D.	N.D.	8.58	$7.9 \times 10^5$	$4.9 \times 10^5$	$1.2 \times 10^8$
2000	43	53	740	N.D.	N.D.	8.61	$4.9 \times 10^5$	$7.9 \times 10^4$	$3.5 \times 10^7$
2100	37	30	720 ‡	N.D.	N.D.	8.56	$2.3 \times 10^5$	$1.3 \times 10^5$	$9.0 \times 10^7$
2200	41	49	710	N.D.	N.D.	8.54	$4.9 \times 10^5 ‡$	$3.3 \times 10^5 ‡$	$4.5 \times 10^8$
*N. D. = <0.05 mg/ℓ      †N. D. = <0.02 mg/ℓ      ‡Analytical Quality Control Analysis									

TABLE XV. SELECTED HOURLY GRAB SAMPLES FROM POND NO. 2 EFFLUENT DURING HOURS OF IRRIGATION - DIURNAL STUDY

Oxygen Demand and Physical Parameters						
Sample Date	Time	COD, mg/ℓ	TOC, mg/ℓ	BOD, mg/ℓ	TSS, mg/ℓ	Total Solids, mg/ℓ
8-19-75	1130	87	35	59	24	750
	1530	68	40	51	24	780
	1830	87	33	62	34	650
	2230	75	39	58	37	680
8-20-75	0900	68	32	21	19	770
	1300	137	42	27	19	810
	1700	76	39	18	22	720
8-21-75	2000	157	35	24	22	730
	1000	101	27	40	16	730
	1400	105	30	32	21	670

Inorganic and Nutrient Parameters							
Sample Date	Time	Hardness, mg/ℓ CaCO <sub>3</sub>	Total Phosphorus, mg/ℓ P	Nitrate Nitrogen, mg/ℓ	Nitrite Nitrogen, mg/ℓ	Ammonia Nitrogen, mg/ℓ	Organic Nitrogen, mg/ℓ
8-19-75	1130	250	12.8	0.09	0.01	16.6	13.3
	1530	250	13.0	0.06	0.02	14.0	27.6
	1830	270	12.8	0.07	0.03	32.3	10.6
	2230	260	12.6	0.10	0.02	30.3	3.6
8-20-75	0900	270	7.0	0.04	0.02	18.3	1.3
	1300	280	6.2	0.06	0.02	22.0	1.6
	1700	270	4.9	0.10	0.03	23.0	12.0
8-21-75	2000	275	5.9	0.12	0.06	20.6	14.0
	1000	265	5.4	0.07	0.02	23.3	13.7
	1400	270	4.8	0.05	0.03	28.7	5.3

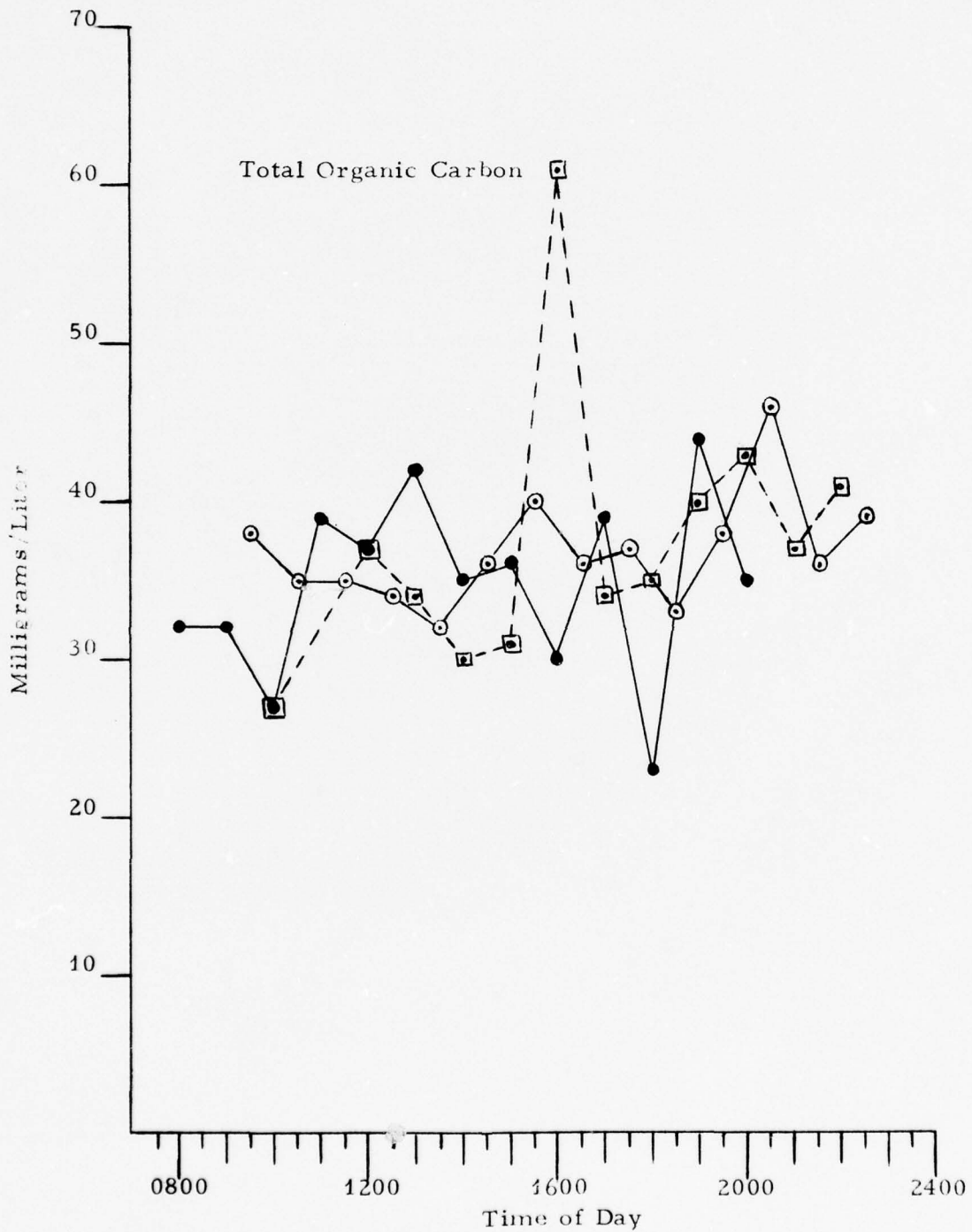
  

Heavy Metals							
Sample Date	Time	Mercury, mg/ℓ	Arsenic, mg/ℓ	Cadmium, mg/ℓ	Lead, mg/ℓ	Copper, mg/ℓ	Zinc, mg/ℓ
8-19-75	1130	$1.04 \times 10^{-4}$	0.0033	0.0002	0.003	0.031	0.05
	1530	$1.18 \times 10^{-4}$	0.0027	0.0007	0.005	0.031	1.67
	1830	$1.10 \times 10^{-4}$	0.0030	0.0003	0.005	0.031	0.42
	2230	$1.19 \times 10^{-4}$	0.0027	0.0004	0.005	0.027	0.20
8-20-75	0900	$<1.00 \times 10^{-4}$	$<0.0013$	0.0003	0.007	0.024	0.33
	1300	$<1.00 \times 10^{-4}$	0.0033	0.0005	0.003	0.029	0.06
	1700	$1.38 \times 10^{-4}$	0.0022	0.0006	0.004	0.039	0.06
8-21-75	2000	$1.50 \times 10^{-4}$	0.0039	0.0009	0.004	0.026	0.58
	1000	$<1.00 \times 10^{-4}$	—	0.0027	0.004	0.024	0.63
	1400	$<1.00 \times 10^{-4}$	0.0041	0.0003	0.004	0.017	2.23

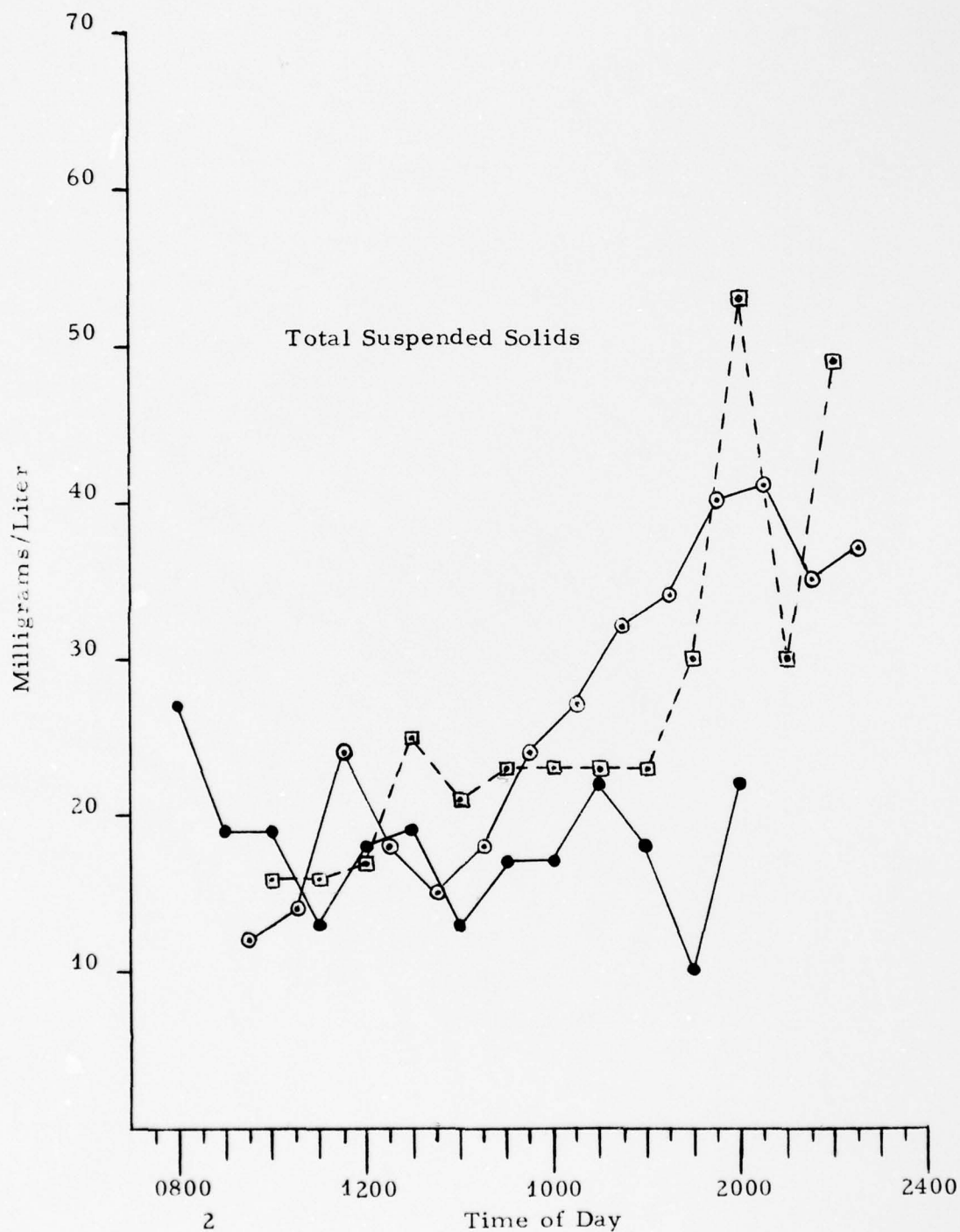
*Limit of Detection		$1.00 \times 10^{-4}$	0.0013	0.0002	0.003	0.015	0.02
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FIGURE 8 . Hourly Grab Samples of Pond No. 2 Effluent During Hours of Irrigation



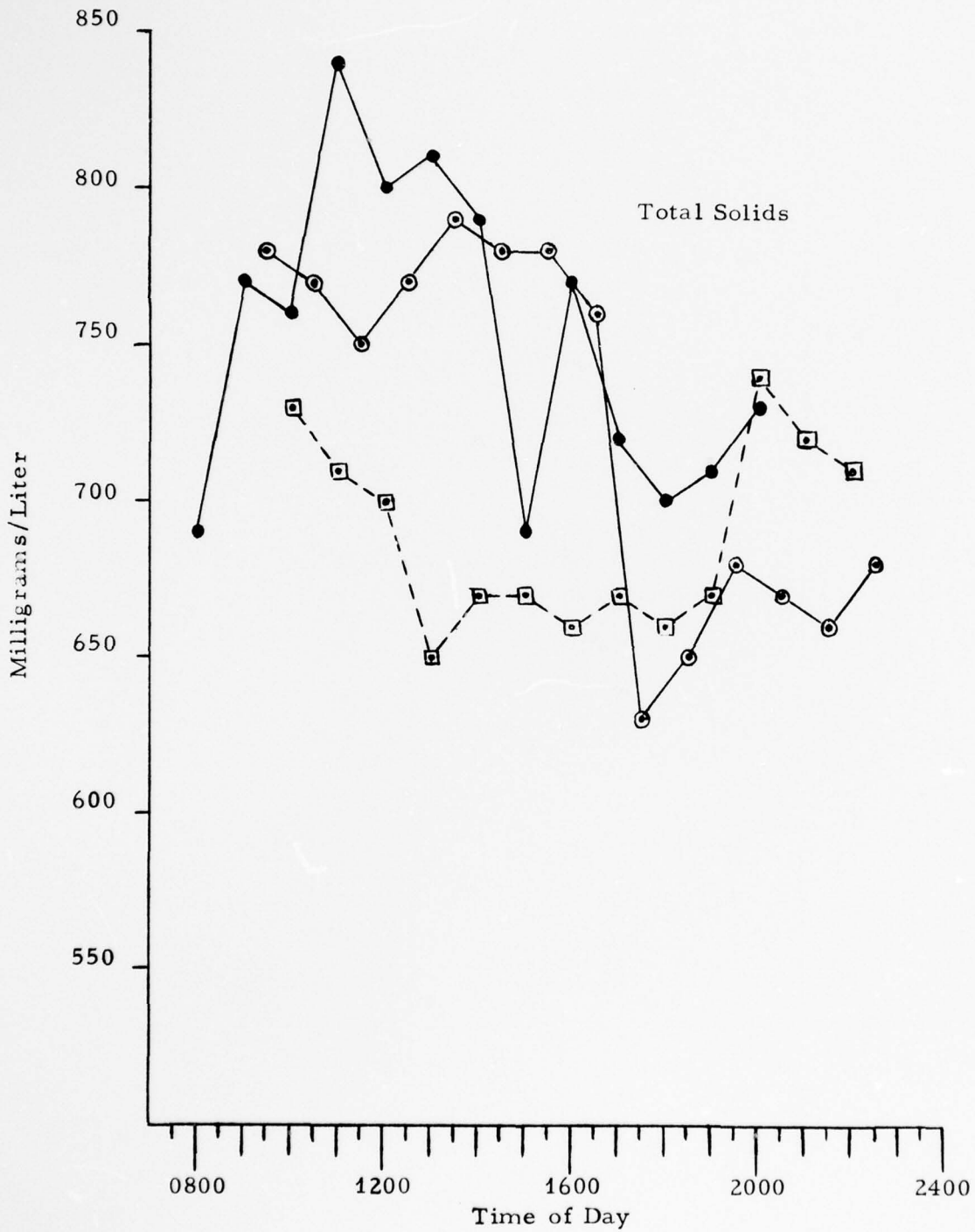
Sample Date:   
 ○—○ 8-19-75 67   
 ●—● 8-20-75   
 □- - -□ 8-21-75

FIGURE 9. Hourly Grab Samples of Pond No. 2 Effluent During Hours of Irrigation



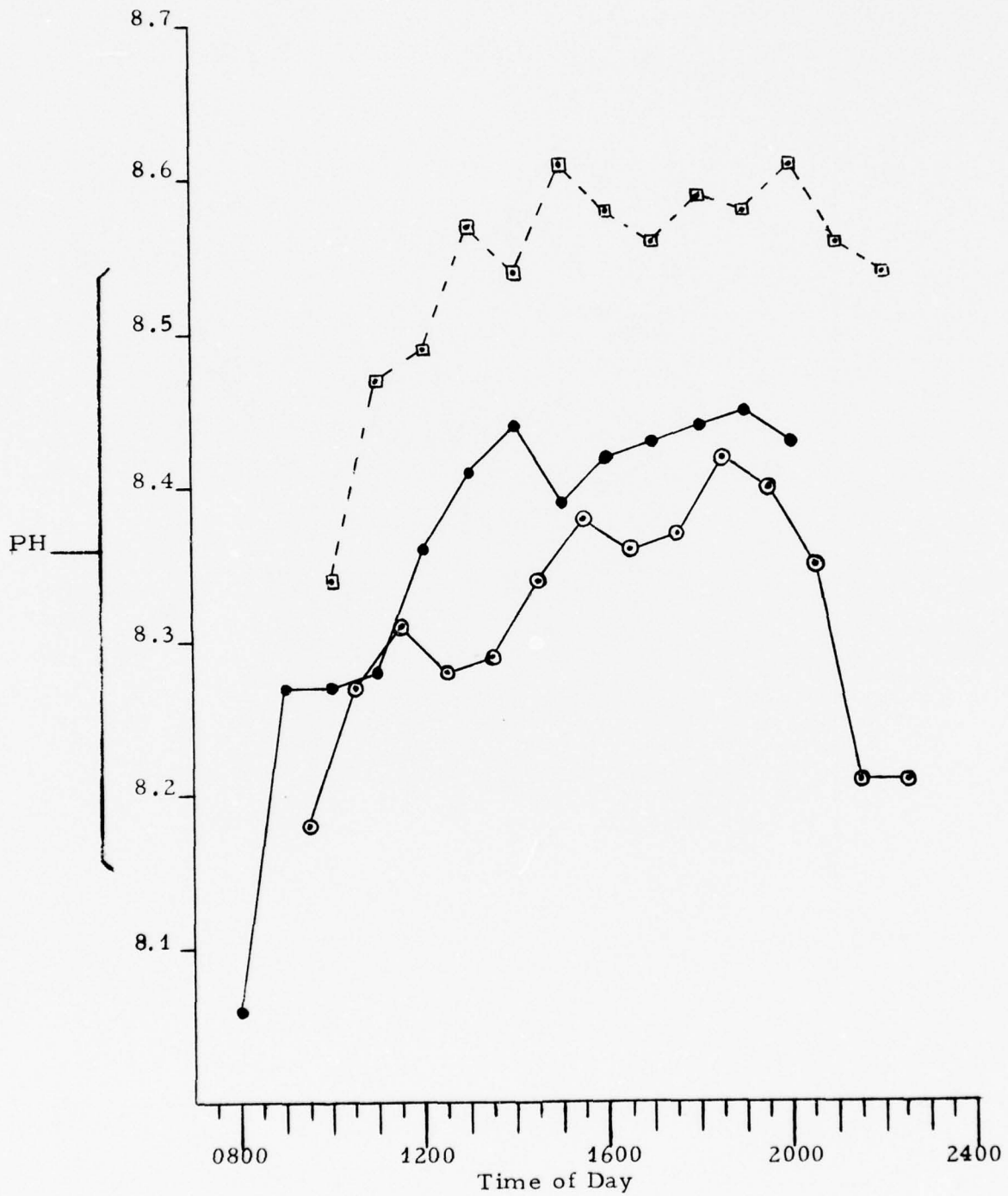
Sample Date:   
 ○ — ○ 8-19-75 68   
 ● — ● 8-20-75   
 □ - - □ 8-21-75

FIGURE 10 . Hourly Grab Samples of Pond No. 2 Effluent During Hours of Irrigation



Sample — 8-19-75 69  
 Date: 8-20-75  
 8-21-75

FIGURE 11 . Hourly Grab Samples of Pond No. 2 Effluent During Hours of Irrigation



Sample Date:   
 ○ — ○ 8-19-75 70   
 ● — ● 8-20-75   
 □ - - □ 8-21-75

FIGURE 12 . Hourly Grab Samples of Pond No. 2 Effluent During Hours of Irrigation

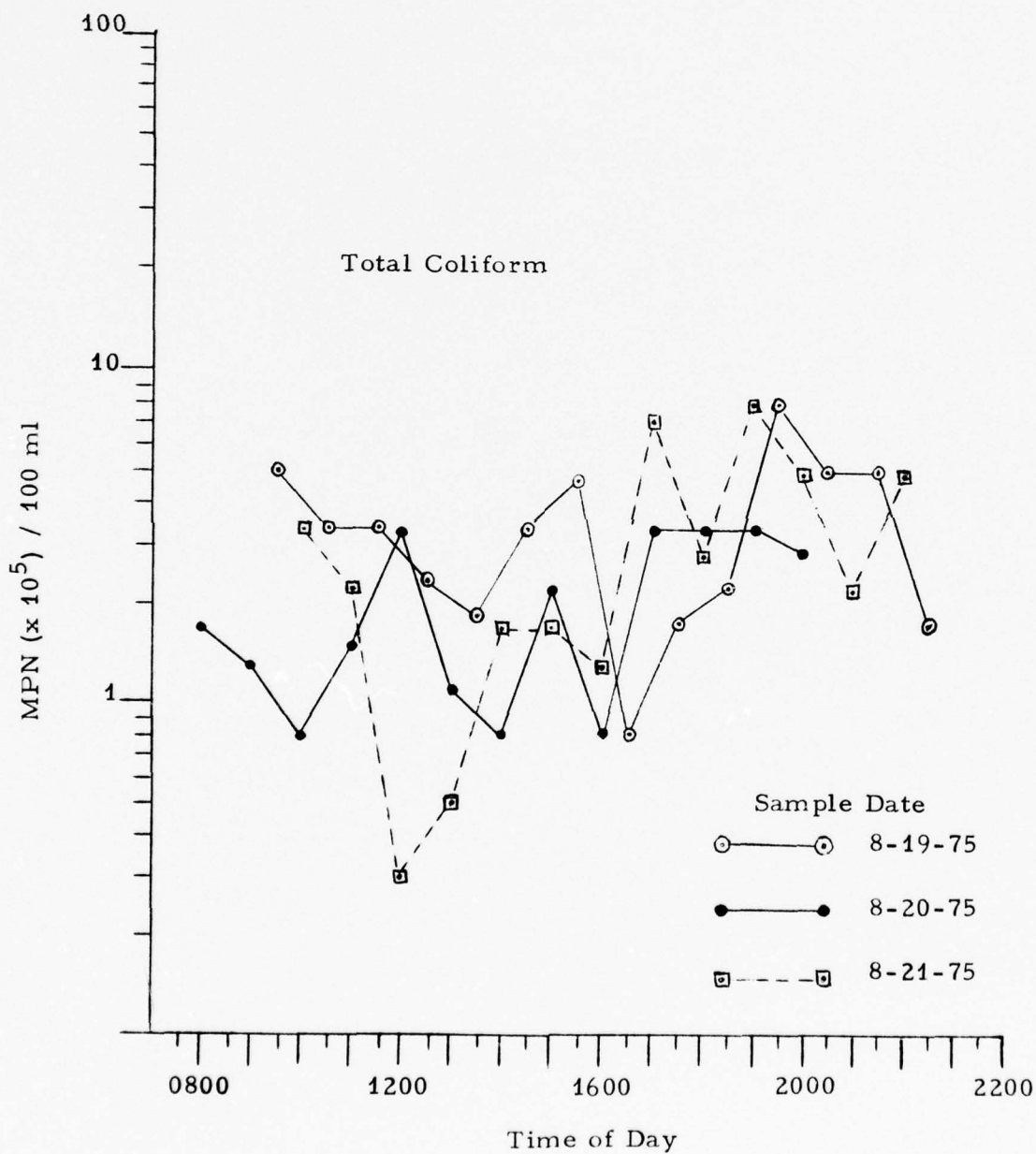


FIGURE 13. Hourly Grab Samples of Pond No. 2 Effluent During Hours of Irrigation

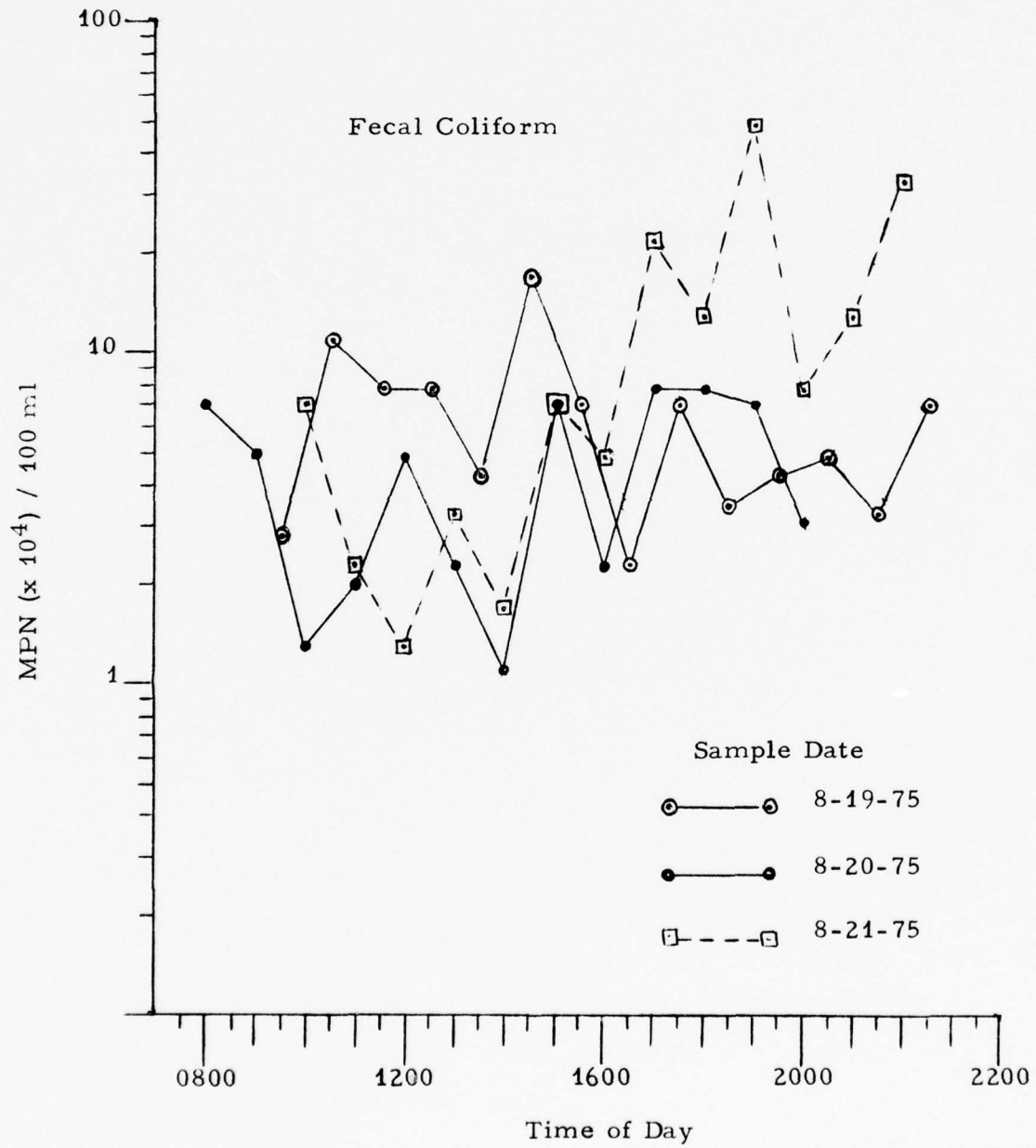
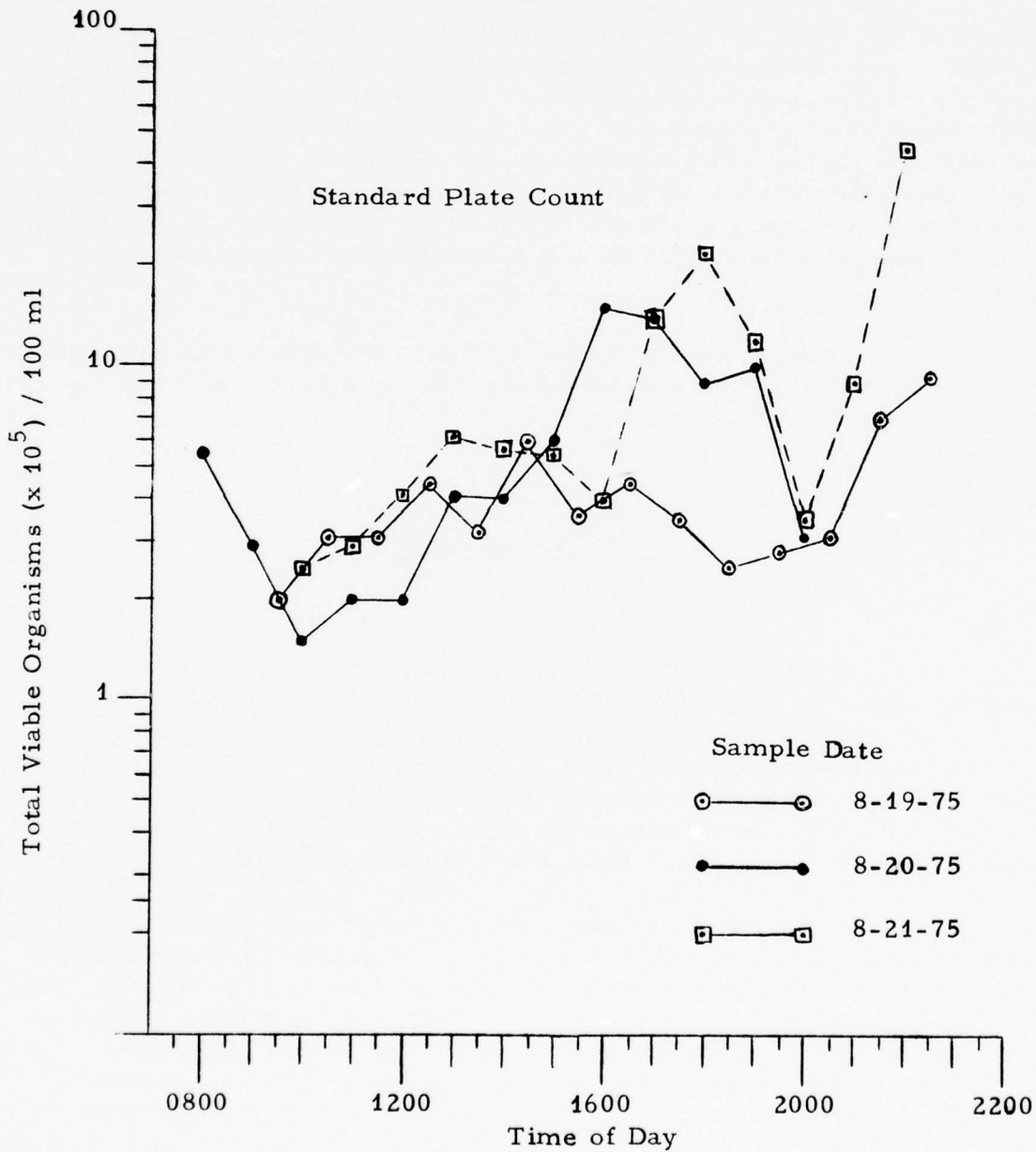


FIGURE 14 . Hourly Grab Samples of Pond No. 2 Effluent During Hours of Irrigation



had been used instead of the MPN method. Virus and coliphage results would also have been more consistent if a larger sample size had been used, thus providing greater sensitivity.

### 3. Grab Samples

The results for routine analysis of grab sample are shown in Table XVI. Selected data are shown in Table XVII. Some of the results of bacterial and virus analyses are reported with the aerosol results in order to describe source strength data. In general, the data seen for the grab samples are very similar to the data for the composite daily samples. This indicates that significant differences in effluent quality do not exist at the spray heads versus the exit of the aeration ponds (daily composites). Little or no daily trends in effluent quality were observed so that perhaps the daily composite data would be sufficient to describe effluents being aerosolized.

The enumeration of types of viruses present in effluent samples and aerosols indicates that approximately 80% were entero with the remainder Reo and Adeno types.

### D. Aerosol Data

#### 1. Comparison of Aerosol Sampling Methods

##### a. Experimental

Various sampling methods have been employed in collecting the aerosol samples. An objective of the initial study has been to select the appropriate aerosol sampling methods for use in the full environmental monitoring study during Phase II. The methodology utilized was to obtain paired aerosol samples from the compared samplers at the same time and downwind distances on certain run samples. Both paired samples were subjected to the same microbiological analysis.

This methodology was used to compare the bacterial concentrations (viable particles, viable coliforms and fecal coliforms) obtained from the Porton and the Andersen samplers at 11 locations. The bacterial concentrations obtained from the LEAP and Andersen samplers at 4 locations were also compared. The bacteria groups positively identified as being present in the aerosol samples were Strep-gamma, Enterobacter, Escherichia, Bacillus, Micrococcus, Citrobacter and Diphtheroid. The identification frequencies of each of these bacteria groups

TABLE XVI. ROUTINE GRAB SAMPLES FROM SPRAY NOZZLES  
BEFORE AND AFTER AEROSOL SAMPLING

Sample Date	Time	Air Sample Run No.	Oxygen Demand and Physical Parameters			Inorganic Parameters			Bacteriological Parameters		
			TOC, mg/ℓ	TSS, mg/ℓ	Total Solids, mg/ℓ	Total Chlorine, mg/ℓ	Free Chlorine, mg/ℓ	pH	Total Coliform, MPN/100 mℓ	Fecal Coliform, MPN/100 mℓ	Standard Plate Count, No./100 mℓ
8-25-75	1330	2	57	27	740	0.19	<0.02	8.50	$4.9 \times 10^5$	$3.3 \times 10^5$	$9.3 \times 10^7$
	1830		67	65	730	<0.05	<0.02	8.50	$4.9 \times 10^6$	$1.3 \times 10^5$	$9.2 \times 10^7$
8-26-75	1300	3	61	33	750*	0.15	<0.02	8.42	$7.9 \times 10^6$	$1.3 \times 10^6$	$7.2 \times 10^8$
	1900		48	38	740	0.25	<0.02	8.56	$4.9 \times 10^6$	$4.6 \times 10^5$	$4.1 \times 10^8$
8-27-75	1315	4	34	7	720	0.29	<0.02	8.23	$3.3 \times 10^5$	$1.1 \times 10^5$	$3.4 \times 10^7$
	1900		42	32	730	0.38	<0.02	8.37	$3.5 \times 10^6$	$2.2 \times 10^5$	$1.5 \times 10^8$
9-02-75	1930	5	48	16	690	0.54	0.11	8.18	$3.3 \times 10^5$	$1.3 \times 10^5$	$7.5 \times 10^7$
	2300		38	20	690	0.16	0.13	8.27	$1.3 \times 10^6$	$1.7 \times 10^5$	$1.2 \times 10^8$
9-03-75	2200	6	37	55	710	0.18	0.06	8.41	$2.8 \times 10^7$	$4.9 \times 10^5$	$6.5 \times 10^7$
9-04-75	0200		42	49	740	0.23	0.05	8.22	$9.0 \times 10^6$ *	$4.8 \times 10^5$ *	$2.5 \times 10^8$
9-05-75	2100	7	34	28	730*	0.30	0.09	8.38	$3.5 \times 10^7$	$1.1 \times 10^6$	$4.5 \times 10^8$
9-10-75	1600	8	34	50	740	0.35	0.16	8.56	$3.1 \times 10^5$	$1.3 \times 10^5$	$9.5 \times 10^7$
			99	12	710	0.18	0.11	8.54	$3.3 \times 10^5$	$1.7 \times 10^4$	$4.0 \times 10^7$
9-11-75	2100	9	31	16	710*	0.18	0.07	8.77	$2.2 \times 10^5$	$7.9 \times 10^4$	$3.5 \times 10^7$
	2300		29	12	700	0.10	0.07	8.34	$4.9 \times 10^5$	$3.3 \times 10^4$	$2.9 \times 10^7$
9-12-75	1800	10	99	29	750	0.36	0.05	8.22	$4.6 \times 10^5$	$1.7 \times 10^5$	$1.9 \times 10^8$
	2130		34	18	720	0.27	0.09	8.63	$3.3 \times 10^6$	$3.3 \times 10^6$	$2.8 \times 10^9$
9-13-75	1345	11	45	55	770	0.54	0.18	8.44	$4.9 \times 10^5$	$1.3 \times 10^5$	$1.0 \times 10^6$
	1345		118	63	780	0.69	0.14	8.48	$4.9 \times 10^5$	$1.1 \times 10^5$	$9.6 \times 10^5$
		Mean X	52	33	729	0.28	0.08	8.42	$5.4 \times 10^6$	$4.4 \times 10^5$	$3.0 \times 10^8$
		Std Dev X	26	18	24	0.16	0.05	0.16	$9.7 \times 10^6$	$7.3 \times 10^5$	$6.3 \times 10^8$
		CV % X	49	56	3	58	69	2	180	170	2.0

\*Analytical Quality Control Analysis

TABLE XVII. SELECTED GRAB SAMPLES FROM SPRAY NOZZLES BEFORE AND AFTER AEROSOL SAMPLING

Oxygen Demand and Physical Parameters							
Sample Date	Time	COD, mg/ℓ	TOC, mg/ℓ	BOD, mg/ℓ	TSS, mg/ℓ	Total Solids, mg/ℓ	
9-3-75	2200	205	37	46	55	710	
9-4-75	0200	231	42	38	49	740	
9-10-75	1600	57	99	22	12	710	
9-11-75	2100	48	31	15	16	710*	
9-12-75	2130	108	34	15	18	720	
*Analytical Quality Control Analysis							
Inorganic and Nutrient Parameters							
Sample Date	Time	Hardness, mg/ℓ CaCO <sub>3</sub>	Total Phosphorus, mg/ℓ P	Nitrate Nitrogen, mg/ℓ	Nitrite Nitrogen, mg/ℓ	Ammonia Nitrogen, mg/ℓ	Organic Nitrogen, mg/ℓ
9-3-75	2200	275	2.3	0.08	0.38	29.3	5.3
9-4-75	0200	280	3.5	0.04	0.11	22.8	21.6
9-10-75	1600	285	1.7	0.14	0.14	64.0	37.0
9-11-75	2100	275	2.8	0.28	0.36	80.5	58.0
9-12-75	2130	285	3.2	0.26	0.38	65.0	25.0
Heavy Metals							
Sample Date	Time	Mercury, mg/ℓ	Arsenic, mg/ℓ	Cadmium, mg/ℓ	Lead, mg/ℓ	Copper, mg/ℓ	Zinc, mg/ℓ
9-3-75	2200	$1.32 \times 10^{-4}$	—	0.0016	0.004	0.025	0.12
9-4-75	0200	$1.75 \times 10^{-4}$	—	<0.0002	0.007	0.036	0.44
9-10-75	1600	$<1.00 \times 10^{-4}$	0.0019	<0.0002	0.003	0.021	0.05
9-11-75	2100	$1.31 \times 10^{-4}$	0.0016	0.0005	<0.003	0.019	0.24
9-12-75	2130	$1.07 \times 10^{-4}$	0.0017	0.0003	0.004	0.018	0.06
Limit of Detection		$1.00 \times 10^{-4}$	0.0013	0.0002	0.003	0.015	0.02

among the LEAP and the Porton samples were compared at 13 locations. The virus isolation frequencies for the LEAP sampler and the all glass impinger (AGI) sampler were compared at 18 locations. Virus isolation was determined by plaque formation in baboon kidney cells, cytopathogenic effect in baboon kidney cells, and cytopathogenic effect in human embryonic lung cells.

The procedure in each of the preceding sampling method comparisons was to tabulate the analysis results on all samples taken by the compared methods at the same times and locations. No summarization of the sampler method comparison was attempted for the bacterial concentrations outside the detectable range of the samplers for the sampling period utilized. However, for the bacteria and virus identification based comparisons, the percentage of positively identified samples was calculated for both sampling methods. In calculating the identification percentages, each comparison location was considered to comprise a single sample. For example, when two LEAP samples from the same location/sampling time were compared with the one Porton sample taken at that location/sampling time, each LEAP sample would be weighted half the Porton sample.

The differences in positive identification frequencies on the paired samples from the samplers being compared were tested for significance by the Brownlee fixed sample size frequency comparison test,<sup>4</sup> assuming underlying binomial frequency distributions. The null hypothesis of equal identification rates for the compared samplers was tested against the two-sided alternative of unequal rates using a standardized test statistic that asymptotically has a normal distribution. The Brownlee test was used to compare the LEAP and Porton sampler identification rates for each bacteria group, and to compare the AGI and LEAP sampler isolation rates for each virus isolation technique.

The shipment method of the aerosol samples from the Pleasanton site to the San Antonio laboratory for virus analysis was also investigated. Depending upon the time of sampling, from 16 to 46 hours elapsed from the collection to the initial laboratory processing of these aerosol virus samples. The virus samples from the first five aerosol runs were shipped on wet ice; those from the last six aerosol runs were, with a single exception, shipped on dry ice. The shipment evaluation procedure was to compare the virus isolation frequencies of the wet ice samples versus the dry ice samples separately for the AGI samples and for the LEAP samples. The significance of differences between the wet and dry ice virus isolation frequencies was also determined by the two-sided Brownlee frequency comparison test for fixed sample sizes.

b. Results

The comparison of the Porton and Andersen sampler bacterial concentrations on the paired samples is presented in Table XVIII. When no viable factors were detected, the lower detection limit of the method has been reported as a < number. The Porton sampler yielded much higher total viable particle concentration in the sampled air by a standard plate count than the Andersen sampler did. However, the Andersen sampler gave higher viable coliform air concentrations from a plate count than the Porton sampler did by the most probable number procedure. One of the Porton samples yielded fecal coliform (3MPN/m<sup>3</sup>); none of the Andersen samples were positive for fecal coliform.

Comparison of the LEAP and Andersen sampler bacterial concentrations on the paired samples is shown in Table XIX. The LEAP sampler yielded much higher viable particle and viable coliform concentrations than the Porton sampler did. Positive fecal coliform were obtained by the MPN method on two of the LEAP samples ( $\leq 8\text{MPN/m}^3$  and  $\leq 1\text{MPN/m}^3$ ); none of the Andersen plates yielded fecal coliform.

The comparison of the LEAP and Porton samplers with respect to bacteria identification on paired samples is presented in Table XX. Only for the *Escherichia* and *Bacillus* groups is there reasonably consistent identification agreement between the LEAP and Porton samples, but the LEAP sampler appears more sensitive to detecting both of these bacteria groups. In fact, the percentage of positive (identified) samples with the LEAP sampler was at least as high as with the Porton sampler for all seven bacteria groups. The significance of the percentage difference was tested for each bacteria group; the results are summarized in Table XXI. Using a two-sided test, the greater identification sensitivity of the LEAP sampler was significant for *Escherichia* ( $P = 0.049$ ) and approaching significance for *Citrobacter* ( $P = 0.077$ ) and *Bacillus* ( $P = 0.099$ ) at the 0.05 significance level.

A tabulated comparison of the all glass impinger (AGI) and LEAP samplers relative to virus isolation on paired samples is displayed in Table XXII. Visual inspection fails to disclose any reasonable pattern of agreement between paired AGI and LEAP samples analyzed by the same virus isolation technique. The AGI sampler gave a slightly higher positive plaque formation frequency than did the LEAP sampler. The AGI sampler also yielded a much higher mean PFU/m<sup>3</sup> level than did the LEAP sampler. Table XXIII shows that none of the slight differences in isolation frequency between the AGI and LEAP samplers are significant.

TABLE XVIII. COMPARISON OF PORTON AND ANDERSEN AEROSOL SAMPLER BACTERIAL CONCENTRATIONS

Date (Run Number)	Distance of Samplers Downwind from Source, m.	Sampling Time Period, Hours		Total Viable Particles, particles/m <sup>3</sup>		Total Viable Coliforms		Viable Fecal Coliforms	
		Porton	Andersen	Porton	Andersen	Porton MPN/m <sup>3</sup>	Andersen Particles/m <sup>3</sup>	Porton MPN/m <sup>3</sup>	Andersen Particles/m <sup>3</sup>
9/10/75 (8)	Upwind	1505-1535	1505-1535	180,000	> 470	< 3	> 310	< 3	< 1
	20	1623-1653	1624-1625 1630-1635	Insuffi- cient Sample	390 > 1400	12	740 760	< 3	< 35 < 7
	75	1720-1750	1723-1724 1730-1735 1740-1755	120,000	2800 1200 > 750	8	570 84 530	< 3	< 35 < 7 < 2
	390	2130-2200	2135-2136 2140-2145 2150-2205	Insuffi- cient Sample	600 1100 > 940	< 3	71 35 140	< 3	< 35 < 7 < 2
9/11/75 (9)	Upwind	2137-2207	2137-2142 2145-2200	52,000	> 2300 > 940	< 3	350 190	< 3	< 7 < 2
	825	2223-2253	2223-2228 2230-2245 2250-2320	140,000	1100 > 940 > 470	3	49 35 54	< 3	< 7 < 15 < 1
	500	2040-2110	2040-2041 2045-2050 2055-2110	100,000	420 1900 > 760	< 3	35 300 > 59	< 3	< 35 < 7 < 2
	1500	1919-1949	1919-1924 1930-1945	34,000	900 > 470	< 3	500 > 49	< 3	< 7 < 2
9/12/75 (10)	1600	1817-1847	1817-1818 1820-1825 1830-1845	< 18,000	420 460 230	< 3	< 35 56 26	< 3	< 35 < 7 < 2
	1	1116-1146	1116-1117 1120-1121 1125-1130 1135-1150	34,000	140 1000 540 70	8	< 140 280 92 16	3	< 35 < 35 < 7 < 2
	1600	0925-0955	0925-0930 0925-0955 0950-0955 0950-1020	210,000	660 940	< 3	< 3 7	< 3	< 1 < 1
	1600	0925-0955	0925-0930 0925-0955 0950-0955 0950-1020	210,000	660 940	< 3	< 3 7	< 3	< 1 < 1

TABLE XIX. COMPARISON OF LEAP AND ANDERSEN AEROSOL SAMPLER BACTERIAL CONCENTRATIONS

Date (Run Number)	Distance of Samplers Downwind from Source, m.	Sampling Time Period, Hours		Total Viable Particles particles/m <sup>3</sup>		Total Viable Coliforms		Viable Fecal Coliforms	
		LEAP	ANDERSEN	LEAP	ANDERSEN	LEAP MPN/m <sup>3</sup>	ANDERSEN Particles/m <sup>3</sup>	LEAP MPN/m <sup>3</sup>	ANDERSEN Particles/m <sup>3</sup>
8/23/75 (1)	Upwind	1230-1300	1250-1305	400,000	21	3,700	<1	58	<1
			1250-1325						
8/26/75 (3)	200	1552-1622	1520-1550	1,700,000	210	1,600,000	<1	51	<1
8/27/75 (4)	100	1530-1600	1440-1510	1,100,000	47	1,100,000	<1	<1	<1
9/3,4/75 (6)	80	0003-0048	2353-0008	260,000	>940	110,000	>470	<1	<1
			2353-0023						

TABLE XX. COMPARISON OF PAIRED LEAP AND PORTON AEROSOL SAMPLER IDENTIFIED BACTERIA

Date (Run Number)	Distance of Samplers Downwind From Source, m.	Sampling Time Period, Hours		Identified Bacteria															
				LEAP Sampler					Porton Sampler										
				Strep-gamma	Enterobacter	Escherichia	Bacillus	Micrococcus	Citrobacter	Diphtheroid	Strep-gamma	Enterobacter	Escherichia	Bacillus	Micrococcus	Citrobacter	Diphtheroid		
8/23/75 (1)	Upwind	1230-1300	1335-1405	o	o	+	+	o	+	o	o	o	o	o	o	+	o	o	o
8/26/75 (3)	200	1552-1622	1520-1550	o	o	+	o	o	+	o	o	+	+	o	o	o	o	o	o
8/27/75 (4)	100	1530-1600	1440-1510	o	o	+	+	o	o	o	o	o	+	o	o	o	o	o	o
9/3,4/75 (6)	80	2242-2312 0003-0048	2353-0023	+	+	+	+	o	+	+	o	o	+	o	o	o	+	o	+
9/4/75 (6)	Upwind	0325-0355	0315-0345	o	+	+	+	+	+	o	o	o	o	+	o	o	+	o	+
9/4/75 (6)	200	0142-0217	0138-0208	o	o	+	+	o	+	+	o	o	o	+	o	o	o	o	o
9/5/75 (7)	100	2120-2150	2115-2145	o	o	+	o	+	o	o	o	+	+	o	o	+	o	o	o
9/10/75 (8)	75	1640-1710	1720-1750	o	+	+	+	o	o	o	o	+	o	o	o	o	o	o	o
9/10/75 (8)	390	2134-2204	2130-2200	o	+	+	+	o	+	o	o	o	o	o	o	o	o	o	o
9/11/75 (9)	Upwind	2052-2122	2137-2207	o	+	+	+	o	o	o	o	o	o	+	o	o	o	o	o
9/11/75 (9)	825	2226-2256	2223-2253	o	o	o	+	o	o	o	o	o	o	+	o	o	o	o	o
9/12/75 (10)	1600	1824-1856	1817-1847	o	o	o	+	o	o	o	o	o	o	o	o	o	o	o	o
9/12/75 (10)	500	1926-1956	2040-2110	o	o	o	+	o	o	o	o	o	o	+	o	o	o	o	o
Number of Samples				13	13	13	13	13	13	13	13	13	13	13	13	13	13	13	13
Number of Positive Samples				1	4	5	10	11	2	5	6	2	0	3	4	6	0	1	2
Percentage of Positive Samples				8	35	77	85	19	46	15	0	23	31	46	0	8	15		

TABLE XXI

SIGNIFICANCE OF BACTERIA IDENTIFICATION FREQUENCY  
DIFFERENCES BETWEEN PAIRED LEAP AND PORTON SAMPLES

Bacteria Group	LEAP Sampler Identification Frequency	Porton Sampler Identification Frequency	Significance of Frequency Difference P
Strep-gamma	1/13 = 8%	0/13 = 0%	> 0.5
Enterobacter	4.5/13 = 35%	3/13 = 23%	> 0.5
Escherichia	10/13 = 77%	4/13 = 31%	0.049
Bacillus	11/13 = 85%	6/13 = 46%	0.099
Micrococcus	2.5/13 = 19%	0/13 = 0%	0.3
Citrobacter	6/13 = 46%	1/13 = 8%	0.077
Diptheroid	2/13 = 15%	2/13 = 15%	> 0.5

TABLE XXII. COMPARISON OF PAIRED ALL GLASS IMPINGER (AGI) AND LEAP SAMPLERS IN ISOLATING VIRUSES

Date (Run Number)	Distance of Samplers Downwind from Source m.	Sampling Time Period, Hours		Virus Isolates					
				AGI Sampler			LEAP Sampler		
				AGI Sampler	LEAP Sampler	BKC Plaques* PFU/m <sup>3</sup>	CPE- BKC**	CPE- WI- 38***	BKC Plaques PFU/m <sup>3</sup>
8/23/75 (1)	Upwind	1335-1405	1230-1300	0	+	+	0	0	0
8/26/75 (3)	200	1520-1550	1552-1622	0	+	+	0	0	+
8/27/75 (4)	100	1440-1510	1530-1600	0	0	0	0	0	0
9/2/75 (5)	Upwind	1828-1858	1828-1858	0	0	0	0	0	0
9/2/75 (5)	310	2238-2308	2300-2330	1030	0	0	0	0	+
9/3&4/75(6)	80  (2nd LEAP)	2353-0023	2242-2312	0	0	0	13	0	0
			0003-0048	0	0	0	0	0	
				0	0	0	0	0	
9/4/75 (6)	Upwind	0315-0345	0325-0355	1030	+	0	0	0	+
9/4/75 (6)	200	0138-0208	0142-0217	0	0	0	0	0	0
9/5/75 (7)	100 (2nd LEAP)	2115-2145	2120-2150 2120-2150	0	0	0	0	0	0
9/10/75 (8)	Upwind	1505-1535	1457-1527	0	0	0	0	0	0
9/10/75 (8)	75	1720-1750	1640-1710	0	0	0	0	+	0
9/10/75 (8)	390	2130-2200	2134-2204	820	0	+	83	+	+
9/11/75 (9)	Upwind	2137-2207	2052-2122	710	0	+	0	0	0
9/11/75 (9)	825	2223-2253	2226-2256	0	0	0	0	+	+
9/12/75 (10)	1600	1817-1847	1824-1856	120	0	0	0	0	0
9/12/75 (10)	1500	1919-1949	1926-1956	2000	+	+	7	+	+
9/12/75 (10)	500	2040-2110	1926-1956	590	0	+	20	+	+
9/13/75 (11)	1600	0925-0950	0950-1020	0	0	+	0	0	+
				67	0		0	0	0
Number of Samples				18	18	18	18	18	18
Number of Positive Samples				6	3.5	7	3.75	5	6.5
Percentage of Positive Samples				33	19	39	21	28	36
Mean PFU/m <sup>3</sup>				293			8		

\* Baboon Kidney Cell Plaques, Plaque Forming Units per Cubic Meter

\*\* Cytopathogenic Effect - Baboon Kidney Cells

\*\*\* Cytopathogenic Effect - Human Embryonic Lung Cells

TABLE XXIII

SIGNIFICANCE OF VIRUS ISOLATION FREQUENCY DIFFERENCES  
BETWEEN PAIRED ALL GLASS IMPINGER (AGI) AND LEAP SAMPLES

Virus Isolation Technique	AGI Sampler Isolation Frequency	LEAP Sampler Isolation Frequency	Significance of Frequency Difference P
Baboon Kidney Cell Plaque Formation	6/18 = 33%	3.75/18 = 21%	> 0.5
Baboon Kidney Cell Cytopathology	3.5/18 = 19%	5/18 = 28%	> 0.5
Human Embryonic Lung Cell Cytopathology	7/18 = 39%	6.5/18 = 36%	> 0.5

The virus isolating frequencies obtained for samples shipped on wet ice and on dry ice are presented in Table XXIV. While there appears to be some advantage to shipping the LEAP samples on dry ice, there is no definite pattern. As Table XXV shows, no significant differences between wet ice and dry ice shipment are evident. Because most of the wet ice samples were collected in the day versus dry ice samples collected at night, it was not possible to break out this variable.

## 2. Factors Affecting Aerosol Levels

The aerosol sampling runs were conducted under meteorological conditions that yielded erratic microbiological aerosol levels. The wind velocity was generally under 2 m/sec. The wind direction varied greatly, frequently by as much as  $\pm 45^\circ$  and occasionally by more than  $\pm 90^\circ$  during the 15- and 30-minute sampling periods. Because of the spray fields' location downwind beside a large hill, the wind tended to swirl rather than blow steadily. Given the unstable wind conditions, many of the upwind aerosol samples were taken too close to the sprinkler line to constitute valid background level samples. For these reasons, the following analysis of factors affecting aerosol levels pertains only to the meteorological conditions present at Pleasanton during the Phase I sampling. The analysis was conducted primarily to develop a methodology for identifying factors and quantifying their effect, and to suggest those factors which will be examined in depth in Phase II.

### a. Experimental

The effect of distance from the sprinkler line on the positive identification frequency of seven bacteria groups, three virus types, and coliphage plaques was examined. The samples were grouped into three distance classes: upwind of the sprinkler line; 5 to 200 meters downwind from the sprinkler line; and greater than 200 meters downwind. The result classifications were positive (identified) and negative. A  $2 \times 3$  chi-square test of the null hypothesis of no interaction of distance on obtained result was performed for each group at the 0.05 significance level.

The effects of all the measured meteorological, sampling, and effluent factors on the bacterial concentrations in the Andersen aerosol samples were also investigated. The continuously emitting infinite line source atmospheric dispersion model given by equation (1) was utilized as the basis of the hypothesized Andersen sampling model. The dispersion model was extended by expressing the source strength and downwind concentration in viable counts, and by developing plausible functional

TABLE XXIV

COMPARISON OF WET ICE AND DRY ICE SHIPMENT OF AEROSOL  
 SAMPLES FOR VIRUS ANALYSIS

Method of Shipment	Virus Isolates					
	AGI Sampler			LEAP Sampler		
	BKC Plaques PFU/m <sup>3</sup>	CPE- BKC	CPE- WI- 38	BKC Plaques PFU/m <sup>3</sup>	CPE- BKC	CPE- WI- 38
<u>Transported on Wet Ice</u>						
Number of Samples	11	11	11	8	8	8
Number of Positive Samples	1	4	5	1	0	2
Percentage of Positive Samples	9	36	45	13	0	25
Mean PFU/m <sup>3</sup>	94			8		
<u>Transported on Dry Ice</u>						
Number of Samples	19	19	19	20	20	20
Number of Positive Samples	7	2	6	4	5	7
Percentage of Positive Samples	37	11	32	20	25	35
Mean PFU/m <sup>3</sup>	324			6		

TABLE XXV

SIGNIFICANCE OF VIRUS ISOLATION FREQUENCY DIFFERENCES  
BETWEEN WET ICE AND DRY ICE SHIPPED AEROSOL SAMPLES

Aerosol Sampler	Virus Isolation Technique	Virus Isolation Frequency		Significance of Frequency Difference P
		Samples Shipped on Wet Ice	Samples Shipped on Dry Ice	
LEAP	BKC-Plaques	1/8 = 13%	4/20 = 20%	>0.5
	BKC-CPE	0/8 = 0%	5/20 = 25%	0.3
	WI-38-CPE	2/8 = 25%	7/20 = 35%	>0.5
All Glass Impinger (AGI)	BKC-Plaques	1/11 = 9%	7/19 = 37%	0.2
	BKC-CPE	4/11 = 36%	2/19 = 11%	0.2
	WI-38-CPE	5/11 = 45%	6/19 = 32%	>0.5

relationships for such unincorporated aerosolizational and bacterial survival factors as temperature, relative humidity, solar radiation and sampling period. The resulting hypothesized model had the form:

$$VP_A = \frac{f_1(T, RH) f_2(T, RH, HF, TP) VP_E}{\sqrt{2\pi} \sin \phi f_3(S) D U} \left[ 1 + \exp\left(-\frac{C}{f_3^2(S) D^2}\right) \right] \quad (4)$$

where

- $VP_A$  = adjusted Andersen sampler aerosol viable particle concentration (actual count less background count), particles/m<sup>3</sup>.
- $VP_E$  = average effluent grab sample viable particle concentration, particles/ml.
- $f_1(T, RH)$  = aerosolization factor dependent on temperature T(°F) and relative humidity RH (%).
- $f_2(T, RH, HF, TP)$  = bacterial survival factor dependent on temperature, relative humidity, heat flux number HF (surrogate solar radiation factor) and Andersen sampling period TP (min).
- $\phi$  = angle of the wind with the sprinkler line.
- $D$  = sampler distance downwind from wet line edge, m.
- $f_3(S)$  = vertical dispersion factor dependent on the Pasquill atmospheric stability class, S.
- $U$  = wind velocity, m/sec.

Taking the logarithm of equation (4) converts it from a multiplicative to an additive form:

$$\begin{aligned} \ln(VP_A) = & \ln f_1(T, RH) + \ln f_2(T, RH, HF, TP) + \ln VP_E \\ & - \ln \sqrt{2\pi} - \ln \sin \phi - \ln f_3(S) - \ln D \\ & - \ln U + \ln \left[ 1 + \exp\left(-\frac{C}{f_3^2(S) D^2}\right) \right]. \quad (5) \end{aligned}$$

Stepwise multiple linear regression was utilized to express the aerosol concentration dependent variable  $\ln VP_A$  in terms of those variables on the right hand side of equation (5) with which it was related in the Andersen aerosol sampling data. The potential regressor variables derived from the right side of equation (5) are shown in Table XXVI. The aerosolization factor was assumed to consist of temperature and relative humidity power factors  $f_1 = T^a RH^{-\beta}$ , so that  $\ln f_1 = a \ln T - \beta \ln RH$ . The bacteria survival factor was allowed to consist of a temperature factor, linear and quadratic temperature factors,<sup>5</sup> solar radiation factors (heat flux and stability class) and a sampling time factor. Thus the potential bacteria survival regressor variables were  $\ln f_2 = - \ln T + \ln RH + \ln [1 + (RH - 50\%)^2] - \ln (HF + 3) + \ln S - \ln TP$ . The vertical dispersion factor  $f_3(S)$  was found to have an approximately negative exponential relationship to the stability over the sampling distance range of the aerosol runs:  $f_3(S) = .222 \exp(-0.4S)$ . Thus  $\ln f_3(S) = \ln (.222) - 0.4S$ . Consequently all of the potential regressor variables listed in Table XXVI have at least a semi-theoretical basis.

The 11 aerosol sampling runs yielded 42 usable observations of the Andersen viable particle concentration and its concomitant variables. Because the range of viable particle concentrations obtainable from an Andersen sampler was limited by the necessity to distinguish colonies, the upper detection limit was frequently exceeded. In these cases, the formula "twice the upper limit minus the background level" was used to estimate the adjusted sampled bacterial concentration; such observations were given half the weight of the enumerated bacterial concentrations during the regression analysis. The aerosol sampling was at low wind speeds with frequent wide variations in wind directions. Those observations over which the wind direction variation exceeded  $\pm 90^\circ$  were also weighted at half the other observations. The resulting observation weights were standardized so that the total observations weights equaled the total number of observations. The weight, dependent variable value and independent variable values for each observation have been tabulated.

The LNVA dependent variable was regressed against the Table XXVI regressor variables on the 42 aerosol sampling observations by stepwise multiple linear regression. The object was to identify the model factors actually related to the aerosol viable particle levels sampled in Pleasanton. The variable admission and deletion F levels were monitored to insure that only those regressor variables with a substantial contribution to the explained dependent variable variation were entered and retained in the regression equation. The F-level statistic has the F distribution with

TABLE XXVI. POTENTIAL REGRESSOR VARIABLES IN THE BACTERIAL CONCENTRATION REGRESSIONS

Regression Variable Name	Represented Physical Factor	Calculation Formula
<u>Dependent:</u>		
LN <sub>VPA</sub>	Sampled Aerosol Viable Particle Concentration	$\ln VP_A$
(LN <sub>VCA</sub> )	(Sampled Aerosol Viable Coliform Concentration)	( $\ln VC_A$ )
<u>Potential Regressor</u>		
LN <sub>VPE</sub>	Effluent Concentration Effect	$\ln VP_E$
(LN <sub>VCE</sub> )	(Viable Coliform Effluent Concentration Effect)	( $\ln VC_E$ )
LN <sub>DIST</sub>	Downwind Distance Effect	$\ln (D + 9.14/\sin \phi)$
LN <sub>U</sub>	Wind Velocity Effect	$\ln U$
LN <sub>SIN</sub>	Wind Angle Effect	$\ln (\sin \phi)$
LN <sub>NEXP</sub>	Vertical Source/Sampler Alignment Dispersion Effect	$\ln \left[ 1 + \exp \left( -\frac{162.3}{D^2 e^{-0.35}} \right) \right]$
S	Vertical Wind Stability Dispersion Effect	S
LN <sub>S</sub>	Solar Radiation Effect	$\ln S$
LN <sub>HF3</sub>	Solar Radiation Effect	$\ln (HF + 3)$
LN <sub>T</sub>	Temperature Effect	$\ln T$
LN <sub>RH</sub>	Linear Relative Humidity Effect	$\ln RH$
LN <sub>RHSQ</sub>	Quadratic Relative Humidity Effect	$\ln [1 + (RH - 50\%)^2]$
LN <sub>TP</sub>	Andersen Sampling Time Period Effect	$\ln T_p$
(LN <sub>TC</sub> )	(Andersen Sampling Time Period Effect)	( $\ln T_c$ )

1 and  $n-m-1$  degrees of freedom, where  $n$  = number of observations and  $m$  = number of regression variables in the equation. Admission and deletion F-levels above 2 or 3 are generally considered indicative of a substantial variable. The best regression equations generated in a stepwise run were identified by the significance of the regression equation F-ratio statistic which has the F distribution with  $m$  and  $n-m-1$  degrees of freedom.

The factors affecting the Andersen sampler viable coliform concentration levels were also examined by stepwise multiple linear regression. The viable coliform concentration dependent variable was  $LNVC_A = \ln(VC_A)$ , analogous to  $LNVP_A$  for the viable particle regression. The effluent grab sample viable coliform concentration  $VC_E$  was used in place of  $VP_E$ , and the coliform sampling time  $T_C$  in place of  $T_P$ . Otherwise the  $LNVC_A$  regression analysis was conducted identically to the  $LNVP_A$  regression, with Table XXVI providing the list of regressor variables. The  $LNVC_A$  regression was conducted on 47 observations from the 11 aerosol runs.

b. Results

Tables XXVII and XXVIII provide a tabulation of the aerosol data for all parameters.

The effect of distance from the wet line on the percent of the aerosol samples in which viable microorganisms were identified is shown in Table XXIX. In general, the upwind identification rates are surprisingly high in comparison with the near downwind rates. Only with the formation of plaques in baboon kidney cells (BKC) is the null hypothesis of no interaction between distance and percent of positive identifications strongly rejected ( $P = 0.005$ ), indicating a definite distance effect. However, although one would expect the highest percentage identification in the 5-200 meter distance classification, the effect of distance on BKC plaque formation appears only at distances greater than 200 m downwind. There is a nearly significant effect ( $P = 0.07$ ) of distance on viral identification by cytopathogenic effect in human embryonic lung cells (WI-38). However, again the outstanding identification percentage is at distances beyond 200 m in contradiction of our expectations. The fact that most virus sampling beyond 200 m downwind was conducted at night, while the majority of upwind and close downwind virus sampling was performed during the day, may explain this anomaly. With identification of the genus *Escherichia*, there is a nearly significant distance effect ( $P = 0.06$ ); in this case the relationship of percentage identification to distance is more reasonable. Table XXIX shows that very little effect of distance from the sprinkler line









TABLE XXVIII. BACTERIOLOGICAL DATA FOR EFFLUENTS AND AEROSOLS

Starting Time	Wind Velocity, m/sec		Temp. °F	RH, %	Sampler Distance Downwind from Source, m	Sampler Type	Effluent Source Avg., particles/m <sup>3</sup>	Total Viable Particles		Viable Coliforms		Fecal Viable Coliforms	
	Mean	Range						Air Samples Net, particles/m <sup>3</sup>	Gross, particles/m <sup>3</sup>	Air Samples Net, particles/m <sup>3</sup>	Gross, particles/m <sup>3</sup>	Air Samples Gross, particles/m <sup>3</sup>	Air Samples Gross, particles/m <sup>3</sup>
<i>Run No. 1 (8-23-75)</i>													
1250	2.23	1.12-2.23	90	37	(upwind)	Andersen		2.1 × 10 <sup>1</sup>					
1230	1.70		90	39	(upwind)	LEAP			2.0 × 10 <sup>7</sup>				
1458	2.23		89	32	5	Andersen		1.4 × 10 <sup>3</sup>					
1535	2.23		89	32	25	Andersen		3.3 × 10 <sup>3</sup>					
1612	2.23		89	32	50	Andersen		6.6 × 10 <sup>2</sup>					
1700	2.23		87	34	100	Andersen		3.8 × 10 <sup>1</sup>					
1720	1.12		86	39	150	Andersen			7 × 10 <sup>0</sup>				
<i>Run No. 2 (8-25-75)</i>													
1220	1.12	1.12-2.23	81	59	(upwind)	Andersen	(1) 9.3 × 10 <sup>5</sup>	6.0 × 10 <sup>1</sup>					
1400	1.70		84	51	10	Andersen	(2) 4.9 × 10 <sup>4</sup>	3.9 × 10 <sup>1</sup>					
1510	1.70		87	46	50	Andersen		3.0 × 10 <sup>2</sup>					
1610	1.70		85	47	100	Andersen			2.9 × 10 <sup>1</sup>				
1700	1.12		84	47	200	Andersen							
1840	1.12		83	45	500	Andersen		1.2 × 10 <sup>2</sup>					
1750	1.12		85	47	700	Andersen		7.5 × 10 <sup>1</sup>					
<i>Run No. 3 (8-26-75)</i>													
1220	0.84	0.56-1.12	67	67	(upwind)	Andersen	(1) 5.7 × 10 <sup>6</sup>	3.4 × 10 <sup>1</sup>					
1330	1.70	1.12-2.23	68	62	20	Andersen	(2) 6.4 × 10 <sup>4</sup>	3.1 × 10 <sup>2</sup>					
1420	2.23		68	61	100	Andersen		3.2 × 10 <sup>2</sup>					
1520	2.23		67	62	200	Andersen		1.8 × 10 <sup>2</sup>					
1552	2.79	2.23-3.35	67	63	200	LEAP			8.5 × 10 <sup>7</sup>				
1645	2.23		64	64	600	Andersen			2.6 × 10 <sup>1</sup>				
1735	2.79		63	66	1000	Andersen			>4.0 × 10 <sup>2</sup>				
1820	1.70	1.12-2.23	60	69	1600	Andersen		4.8 × 10 <sup>1</sup>					
<i>Run No. 4 (8-27-75)</i>													
1240	1.70	1.12-2.23	66	68	(upwind)	Andersen	(1) 9.2 × 10 <sup>5</sup>	4.7 × 10 <sup>1</sup>					
1345	1.70	1.12-2.23	66	68	40	Andersen	(2) 1.9 × 10 <sup>4</sup>						
* (1) Total viable particles. (2) Viable coliforms.													

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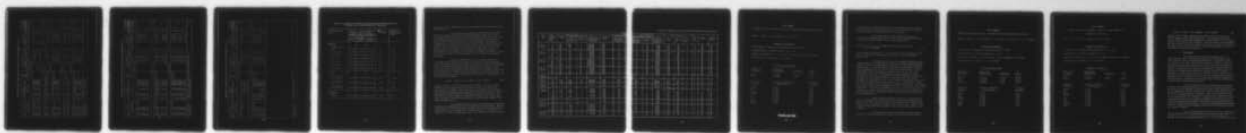
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TABLE XXVIII. BACTERIOLOGICAL DATA FOR EFFLUENTS AND AEROSOLS (Cont'd)

Starting Time	Wind Velocity, m/sec		Temp. °F	RH. %	Sampler Distance Downwind from Source, m	Sampler Type	Effluent Source Avg #, particles/ml	Total Viable Particles			Viable Coliforms			Fecal Viable Coliforms		
	Mean	Range						Net, particles/m <sup>3</sup>	Gross, particles/m <sup>3</sup>	Sampling Time, min	Net, particles/m <sup>3</sup>	Air Samples Gross, particles/m <sup>3</sup>	Sampling Time, min	Air Samples Gross, particles/m <sup>3</sup>		
<i>Run No. 4 (8-27-75) Continued</i>																
1440	1.70	1.12-2.23	67	67	100	Andersen LEAP			4.7 × 10 <sup>1</sup>							
1530	2.79	2.23-3.35	71	62	100	Andersen			5.5 × 10 <sup>7</sup>							
1645	2.23		68	61	450	Andersen		1.5 × 10 <sup>1</sup>			4					
1735	1.70	1.12-2.23	67	61	1000	Andersen		2.4 × 10 <sup>1</sup>			5					
1825	1.70		65	62	1450	Andersen			>3.5 × 10 <sup>2</sup>		36					<1
<i>Run No. 5 (9-2-75)</i>																
1828	1.70	1.12-2.23	82	43	(upwind)	Andersen	(1) 9.8 × 10 <sup>5</sup>									
2238	1.70		66	49	310	Andersen	(2) 8.1 × 10 <sup>3</sup>				1.3 × 10 <sup>1</sup>					
<i>Run No. 6 (9-3-4-75)</i>																
0315	<0.56		52	80	(upwind)	Andersen	(1) 1.6 × 10 <sup>6</sup>									
2232	<0.56	0.56-1.12	61	73	50	Andersen	(2) 1.9 × 10 <sup>5</sup>									
2242	<0.56				80	LEAP										
2353	<0.56		59	79	80	Andersen			1.6 × 10 <sup>7</sup>							
0138	<0.56		57	79	200	Andersen			>9.4 × 10 <sup>2</sup>							
<i>Run No. 7 (9-5-75)</i>																
2115	0.84	0.56-1.12	67	59	100	Andersen	(1) 4.5 × 10 <sup>6</sup>									
2120	0.84	0.56-1.12	67	59	100	Andersen	(2) 3.5 × 10 <sup>5</sup>									
<i>Run No. 8 (9-10-75)</i>																
1505	2.23	0.56-2.23	74	63	(upwind)	Andersen	(1) 6.8 × 10 <sup>5</sup>									
1505	2.23		74	63	(upwind)	Porton										
1623	1.12		72	62	20	Porton										
1624	1.12	0.56-1.12	72	62	20	Andersen	(2) 3.2 × 10 <sup>3</sup>									
1630	1.12		72	62	20	Andersen										
1720	0.84		71	62	75	Porton										

\* (1) Total viable particles.

(2) Viable coliforms.

† I.S. = Insufficient sample for analysis.

TABLE XXVIII. BACTERIOLOGICAL DATA FOR EFFLUENTS AND AEROSOLS (Cont'd)

Starting Time	Wind Velocity, m/sec		Temp, °F	RH, %	Sampler Distance Downwind from Source, m	Sampler Type	Effluent Source Avg*, particles/m <sup>3</sup>	Total Viable Particles		Viable Coliforms		Fecal Viable Coliforms	
	Mean	Range						Net, particles/m <sup>3</sup>	Air Samples Gross, particles/m <sup>3</sup>	Net, particles/m <sup>3</sup>	Air Samples Gross, particles/m <sup>3</sup>	Net, particles/m <sup>3</sup>	Air Samples Gross, particles/m <sup>3</sup>
<i>Run No. 8 (9-10-75) Continued</i>													
1723	0.84	0.56-1.12	71	62	75	Andersen							
1730	0.84		71	62	75	Andersen		2.8 × 10 <sup>3</sup>				5.7 × 10 <sup>2</sup>	1
1740	0.84		71	62	75	Andersen		1.2 × 10 <sup>3</sup>				8.4 × 10 <sup>1</sup>	5
2135	0.56		58	80	390	Andersen		>7.5 × 10 <sup>2</sup>				5.3 × 10 <sup>2</sup>	15
2140	0.56		58	80	390	Andersen		6.0 × 10 <sup>2</sup>				7.1 × 10 <sup>1</sup>	1
2150	0.56		58	80	390	Andersen		1.1 × 10 <sup>3</sup>				3.5 × 10 <sup>1</sup>	5
2135†	0.56		58	85	390	Andersen		>9.4 × 10 <sup>2</sup>				1.4 × 10 <sup>2</sup>	15
2130	0.56		58	85	390	Porton		3.7 × 10 <sup>3</sup>				<2	30
								I.S.‡				<5	30
<i>Run No. 9 (9-11-75)</i>													
2137	0.56	0.56-1.12	53	88	(upwind)	Andersen	(1) 3.2 × 10 <sup>5</sup>						5
2145	0.56		53	88	(upwind)	Andersen	(2) 3.5 × 10 <sup>3</sup>						15
2137	0.56		53	88	(upwind)	Porton		>2.3 × 10 <sup>3</sup>				3.5 × 10 <sup>2</sup>	30
								>9.4 × 10 <sup>2</sup>				1.9 × 10 <sup>2</sup>	5
								5.2 × 10 <sup>4</sup>				<5	30
2223	1.12		53	91	825	Porton		1.4 × 10 <sup>5</sup>				<5	30
2223	1.12		53	91	825	Andersen		1.1 × 10 <sup>3</sup>				4.9 × 10 <sup>1</sup>	5
2230	1.12		53	91	825	Andersen		>9.4 × 10 <sup>2</sup>				3.5 × 10 <sup>1</sup>	15
2250	1.12		53	91	825	Andersen		>4.7 × 10 <sup>2</sup>				5.4 × 10 <sup>1</sup>	30
<i>Run No. 10 (9-12-75)</i>													
2040	1.70	1.12-2.23	55	82	500	Andersen	(1) 1.5 × 10 <sup>7</sup>						1
2045	1.70		55	82	500	Andersen	(2) 1.9 × 10 <sup>4</sup>						5
2055	1.70		55	82	500	Andersen		4.2 × 10 <sup>2</sup>				3.5 × 10 <sup>1</sup>	15
2040	1.70		55	82	500	Porton		1.9 × 10 <sup>3</sup>				3.0 × 10 <sup>2</sup>	5
1919	2.23		60	59	1500	Porton		>7.6 × 10 <sup>2</sup>				>5.9 × 10 <sup>1</sup>	15
1919	2.23		60	59	1500	Andersen		1.0 × 10 <sup>5</sup>				<5	30
1930	2.23		60	59	1500	Andersen		3.4 × 10 <sup>4</sup>				<5	30
1817	2.23		66	60	1600	Andersen		9.0 × 10 <sup>2</sup>				5.0 × 10 <sup>2</sup>	5
1817	2.23		66	60	1600	Porton		>4.7 × 10 <sup>2</sup>				>4.9 × 10 <sup>1</sup>	15
1820	2.23		66	60	1600	Andersen		1.8 × 10 <sup>4</sup>				<5	30
								4.2 × 10 <sup>2</sup>				<1	1
								4.6 × 10 <sup>2</sup>				5.6 × 10 <sup>1</sup>	5

\* (1) Total viable particles.  
 (2) Viable coliforms.  
 † Field Control—No air drawn through Sampler, check on sample handling technique.  
 ‡ I.S. = Insufficient sample for analysis.

TABLE XXVIII. BACTERIOLOGICAL DATA FOR EFFLUENTS AND AEROSOLS (Cont'd)

Starting Time	Wind Velocity, m/sec		Temp, °F	RH, %	Sampler Distance Downwind from Source, m	Sampler Type	Effluent Source Avg <sup>†</sup> , particles/m <sup>3</sup>	Total Viable Particles			Viable Coliforms			Fecal Viable Coliforms		
	Mean	Range						Net, particles/m <sup>3</sup>	Gross, particles/m <sup>3</sup>	Sampling Time, min	Net, particles/m <sup>3</sup>	Air Samples Gross, particles/m <sup>3</sup>	Sampling Time, min	Air Samples Gross, particles/m <sup>3</sup>		
<i>Run No. 10 (9-12-75) Continued</i>																
1830	2.23	1.12-2.23	66	60	1600 Lab Control †	Andersen										
					Lab Control †	Andersen										
					Lab Control †	Andersen										
<i>Run No. 11 (9-13-75)</i>																
1116	1.12	0.56-1.12	65	82	1	Andersen	(1) 9.8 × 10 <sup>5</sup>									
1116	1.12		65	82	1	Porton										
1120	1.12		65	82	1	Andersen	(2) 4.9 × 10 <sup>5</sup>									
1125	1.12		65	82	1	Andersen										
1135	1.12		65	82	1	Andersen										
0925	1.12		57	92	1600	Porton										
0925	0.56		57	92	1600	Andersen										
0950	0.56		60	90	1600	Andersen										

\* (1) Total viable particles.  
 (2) Viable coliforms.  
 † Lab Control—On site laboratory air.

TABLE XXIX. SIGNIFICANCE OF THE EFFECT OF DISTANCE FROM THE SPRINKLER LINE ON THE PRESENCE OF VIABLE MICROBIOLOGICAL ORGANISMS

Microbiological Group	Percent of Aerosol Samples in which Viable Microorganisms Were Identified			$\chi^2$ Test Statistic	Significance of Distance Effect
	Sampler Distance from Wet Line Edge				
	Upwind	Downwind 5-200m	Downwind > 200m		
<u>Bacteria:</u>					
Streptococcus-gamma	0/9=0%	2/19=11%	1/14=7%	1.02	> 0.5
Enterobacter	2/9=22%	5/19=26%	2/14=14%	0.70	> 0.5
Escherichia	4/9=44%	12/19=63%	3/14=21%	5.67	0.06
Bacillus	8/9=89%	10/19=53%	9/14=64%	3.50	0.17
Micrococcus	1/9=11%	3/19=16%	1/14=7%	0.58	> 0.5
Citrobacter	2/9=22%	5/19=26%	2/14=14%	0.70	> 0.5
Diphtheroid	2/9=22%	4/19=21%	1/14=7%	1.38	0.5
<u>Viruses:</u>					
Saboon Kidney Cell-Plaques	2/12=17%	2/25=8%	9/18=50%	10.64	0.005
Cytopathogenic Effect:BKC	2/12=17%	4/25=16%	5/18=28%	1.01	> 0.5
Cytopathogenic Effect:WI-38	3/12=25%	6/25=24%	10/18=56%	5.23	0.07
<u>Coliphage -</u>					
Plaques	2/8=25%	3/21=14%	3/15=20%	0.50	> 0.5

was evident in the identification of microorganisms at the Pleasanton spray irrigation site.

The aerosol regression data is presented in Table XXX. The meteorological data for the sampling distances within 200 m of the wet line were obtained from the 2-m station located in the spray field. For the sampling distances beyond 200 m, the meteorological data was taken from the 10-m station located next to the holding pond. The Pasquill stability class, ranging from 1 (very unstable) to 6 (very stable) with 4 the neutral class, was determined from the surface wind velocity, the solar altitude and the extent of cloud cover, by the standard procedure. The heat flux number, a measure of solar radiation, was based on field observation. It ranged from -1 for nighttime with  $\leq 3/8$  cloudiness, to 0 for overcast conditions or low solar altitudes, to 2 for daytime with strong insolation.

Stepwise multiple linear regression was utilized to investigate which factors were related to the total and coliform bacteria concentrations obtained from the Andersen samplers in the aerosol sampling runs. The viable particle regression attempted to explain the variation in LNVPA as a linear combination of the viable particle, sampling and meteorological factors listed as potential regressor variables in Table XXVI. The best predictive LNVPA regression equation generated from the 42 viable particle observations is presented and described in Table XXXI. The best regression equation,

$$\text{LNVPA} = 9.934 - 2.59 \times \text{LNHF3} - 0.61 \times \text{LNTP}, \quad (6)$$

explains only  $R^2 = 37.6\%$  of the variation in LNVPA. However, with an F-ratio of 11.73 with 2 and 39 degrees of freedom, it is a significant regression ( $P = 0.0001$ ). Both the heat flux term, LNHF3, with an F-level of 9.54 and the sampling period term, LNTP with an F-level of 7.07 make a substantial contribution toward explaining the variation in LNVPA. The heat flux term with its negative coefficient appears to reflect the proportional decrease in the upper detection limit of the Andersen sampler with longer sampling times.

An examination of the terms that do not make a substantial contribution to explaining the LNVPA variation is also noteworthy. Most of the atmospheric dispersion factors had little effect on the sampled aerosol viable particle concentration; these include distance ( $F = 1.12$ ), wind velocity ( $F = 0.79$ ) and wind angle ( $F = 0.16$ ). There also was no evidence

Run (Date) Time Period	D Sampler Distance Downwind from Source m.	Viable Particle Run				Viable		
		Observation Weight	Andersen Aerosol Sampling		VPE Effluent Grab Sample Particle Concentration number/ml	Observation Weight	Andersen Aerosol	
			VPA Net Particle Concentration number/m <sup>3</sup>	Tp Sampling Time min.			VCA Net Particle Concentration number/m <sup>3</sup>	S
1 (8/23/75) 1250-1750	5	0.66	1380	1	1,400,000	0.63	1	
	25	0.66	3280	1	1,400,000	0.63	1	
	50	0.66	640	3	1,400,000	0.63	5	
	100	0.66	19	3	1,400,000	0.63	2	
	150	0.66	1	5	1,400,000	0.63	1	
2 (8/25/75) 1220-1910	10	1.31	59	15	925,000	1.26	4	
	50	1.31	320	15	925,000	1.26	14	
	100	1.31	1	30	925,000	1.26	3	
	200	1.31	140	30	925,000	1.26	14	
	500	1.31	95	30	925,000	1.26	3	
	700	1.31	21	30	925,000	1.26	3	
3 (8/26/75) 1220-1850	20	0.66	304	15	5,650,000	0.63	1	
	100	1.31	314	30	5,650,000	1.26	21	
	200	1.31	174	30	5,650,000	1.26	1	
	600	1.31	1	30	5,650,000	1.26	5	
	1000	0.66	760	30	5,650,000	1.26	47	
	1600	1.31	42	30	5,650,000	1.26	1	
4 (8/27/75) 1240-1855	40	0.66	1340	15	920,000	1.26	33	
	100	1.31	7	30	920,000	1.26	1	
	450	1.31	22	30	920,000	1.26	4	
	1000	1.31	31	30	920,000	1.26	5	
	1400	1.31	660	30	920,000	1.26	36	
5 (9/2/75) 1828-2308	310	0.66	900	30	975,000	0.63	680	
6 (9/3/75) 2232-0345	50	0.66	1840	15	1,575,000	0.63	900	
	80	0.33	1840	15	1,575,000	0.32	900	
	200	0.66	900	30	1,575,000	0.63	600	
7 (9/5/75) 2115-2150	100	0.33	4960	5	4,500,000	0.63	530	
	100					0.63	310	
8 (9/10/75) 1505-2205	20	0.66	350	1	675,000	0.63	740	
	20	0.33	2760	5	675,000	0.63	760	
	75	0.66	2760	1	675,000	0.63	570	
	75	0.66	1160	5	675,000	0.63	84	
	75					0.63	530	
	390	1.31	560	1	675,000	1.26	71	
390	1.31	1060	5	675,000	1.26	35		
390					1.26	140		
9 (9/11/75) 2137-2320	825	1.31	1060	5	320,000	1.26	49	
	825					1.26	35	
	825					1.26	54	
10 (9/12/75) 1817-2055	500	1.31	380	1	14,950,000	1.26	35	
	500	1.31	1860	5	14,950,000	1.26	300	
	1500	1.31	860	5	14,950,000	1.26	500	
	1600	1.31	380	1	14,950,000	1.26	1	
	1600	1.31	420	5	14,950,000	1.26	56	
	1600	1.31	190	15	14,950,000	1.26	26	
11 (9/13/75) 0925-1150	1600	1.31	620	5	980,000	0.63	2	
	1600	.33	1840	15	980,000	0.63	7	

TABLE XXX. ANDERSEN BACTERIAL CONCENTRATION SAMPLING REGRESSION DATA

Viable Coliform Run			Wind Conditions			HF Heat Flux Number	T Temperature °F	RH Relative Humidity %
Andersen Aerosol Sampling		VC <sub>E</sub>	U	β	S			
VC <sub>A</sub> Net Particle Concentration number/m <sup>3</sup>	TC Sampling Time min.	Effluent Grab Sample Particle Concentration number/ml	Velocity m/sec	Angle with Sprinkler Line	Pasquill Stability Class			
1	5	7,000	2.23	65°	1	2	89	32
1	10	7,000	2.23	65°	2	2	89	32
5	15	7,000	2.23	65°	2	2	89	32
2	20	7,000	2.23	65°	3	2	87	34
1	30	7,000	1.12	65°	2	2	86	39
4	30	27,000	1.68	25°	1	2	84	51
14	30	27,000	2.23	65°	2	2	87	46
3	30	27,000	1.68	65°	2	2	85	47
14	30	27,000	1.12	65°	2	2	84	47
3	30	27,000	1.12	125°	2	2	83	45
3	30	27,000	1.12	155°	3	2	85	47
1	30	64,000	1.68	65°	1	2	68	62
21	30	64,000	2.23	65°	1	2	68	61
1	30	64,000	2.23	35°	2	2	67	62
5	30	64,000	3.35	35°	3	2	64	64
47	30	64,000	3.35	35°	3	1	63	66
1	30	64,000	1.68	65°	3	1	60	69
33	30	19,150	1.68	35°	1	1	66	68
1	30	19,150	1.68	35°	2	1	67	67
4	30	19,150	2.23	5°	3	1	68	61
5	30	19,150	1.68	35°	3	0	67	61
36	30	19,150	2.23	35°	4	0	65	62
680	30	8,150	1.68	155°	6	-1	66	49
900	30	185,000	0.28	95°	6	-1	61	73
900	30	185,000	0.28	125°	5	-1	58	79
600	30	185,000	0.28	175°	6	-1	57	79
530	5	350,000	0.84	65°	5	-1	67	59
310	15	350,000	0.84	125°	5	-1	67	59
740	1	3,200	0.84	95°	3	0	72	62
760	5	3,200	0.84	95°	3	0	72	62
570	1	3,200	0.56	155°	3	1	71	62
84	5	3,200	0.56	155°	3	1	71	62
530	15	3,200	0.56	155°	3	1	71	62
71	1	3,200	0.56	85°	5	0	58	80
35	5	3,200	0.56	85°	5	0	58	80
140	15	3,200	0.56	85°	5	0	58	80
49	5	3,550	1.12	155°	6	-1	53	91
35	15	3,550	1.12	155°	6	-1	53	91
54	30	3,550	1.12	155°	6	-1	53	91
35	1	18,800	1.68	155°	6	-1	55	82
300	5	18,800	1.68	155°	6	-1	55	82
500	5	18,800	2.23	155°	6	-1	60	59
1	1	18,800	2.23	155°	4	1	66	60
56	5	18,800	2.23	155°	4	1	66	60
26	15	18,800	2.23	155°	4	1	66	60
2	25	4,900	0.56	5°	4	0	57	92
7	30	4,900	0.56	35°	4	0	60	90

TABLE XXXI

SUMMARY ON THE BEST VIABLE PARTICLE PREDICTIVE REGRESSION EQUATION

$$\text{LNVPA} = 9.934 - 2.59 \times \text{LNHF3} - 0.61 \times \text{LNTF}$$

Equation Statistics

Coefficient of Multiple Determination,  $R^2 = 0.376$

Standard Error of the Estimate,  $SE = 1.75$

Regression F-Ratio,  $F = 11.73$  with 2 and 39 degrees of freedom

Significance of F-Ratio,  $P = 0.00010$

Variable Statistics

<u>Regression Variable</u>	<u>Regression Coefficient</u>	<u>T Statistic</u>	<u>F Level</u>
LNHF3	-2.5859	-3.09	9.54
LNTF	-0.6099	-2.66	7.07

<u>Regressor Variable</u>	<u>Partial Correlation Coefficient</u>	<u>Potential F Level</u>
LNVPE	-0.017	0.01
LNDIST	-0.169	1.12
LNU	-0.143	0.79
LNSIN	0.065	0.16
LNNEXP	-0.212	1.80
S	-0.194	1.49
LNS	-0.250	2.54
LNT	0.013	0.01
LNRH	-0.010	0.00
LNRHSQ	0.027	0.03

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of any effect of the effluent viable particle concentration on the sampled aerosol viable particle concentration ( $F = 0.01$ ). Similarly, the temperature and relative humidity seemed to have no effect.

The best predictive viable coliform regression equation generated for LNVCA from the 47 viable coliform observations is presented in Table XXXII. The regression equation is

$$\text{LNVCA} = 43.761 - 1.12 \times \text{LNDIST} + 2.94 \times \text{LNS} - 8.29 \times \text{LNT} - 0.55 \times \text{LNRHSQ}. \quad (7)$$

This equation accounts for  $R^2 = 59.3\%$  of the variation in LNVCA. Its F-ratio, 15.27 with 4 and 42 degrees of freedom, is very significant ( $P < 0.001$ ).

The best LNVCA regression equation indicates that the viable coliform levels obtained from Andersen samples in Pleasanton were related to distance, solar radiation, temperature and relative humidity. The distance term LNDIST makes a very substantial contribution to the LNVCA regression ( $F = 27.64$ ). Its regression coefficient of  $-1.12$  is remarkably close to the theoretical model value of  $-1.0$ . The solar radiation/stability term, LNS, also makes a large contribution to the regression ( $F = 26.34$ ). Its positive regression coefficient indicates that the higher aerosol coliform levels occurred during the more stable atmospheric conditions (at night). The temperature term LNT, with a 10.4 F-level, has a large negative coefficient,  $-8.29$ . This suggests a rapid decrease in viable aerosol coliforms with higher temperatures. The quadratic relative humidity term, LNRHSQ, with an F-level of 8.11, has a negative coefficient,  $-0.55$ . Since LNRHSQ has its lowest value at 50% relative humidity, its negative coefficient suggests that the aerosol coliform levels were highest at about 50% relative humidity and decreased at higher and lower humidity levels.

Again an examination of the regressor variables which make no additional contribution to the LNVCA regression equation (7) is informative. The F-levels in Table XXXII indicate that the effluent viable coliform level, the mean wind velocity, the wind direction, and the length of the sampling period have no effect on the aerosol viable coliform level sampled.

The second best LNVCA regression equation generated from the 47 viable coliform observations is presented in Table XXXIII:

TABLE XXXII

SUMMARY ON THE BEST VIABLE COLIFORM PREDICTIVE REGRESSION EQUATION

$$\text{LNVCA} = 43.761 - 1.12 \times \text{LNDIST} + 2.94 \times \text{LNS} - 8.29 \times \text{LNT} - 0.55 \times \text{LNRHSQ}$$

Equation Statistics

Coefficient of Multiple Determination,  $R^2 = 0.593$

Standard Error of the Estimate,  $SE = 1.43$

Regression F-Ratio,  $F = 15.27$  with 4 and 42 degrees of freedom

Significance of F-Ratio,  $P = 0.00000009$

Variable Statistics

<u>Regression Variable</u>	<u>Regression Coefficient</u>	<u>T Statistic</u>	<u>F Level</u>
LNDIST	-1.1230	-5.26	27.64
LNS	2.9359	5.13	26.34
LNT	-8.2923	-3.22	10.40
LNRHSQ	-0.5494	-2.85	8.11

<u>Regressor Variable</u>	<u>Partial Correlation Coefficient</u>	<u>Potential F Level</u>
LNVCE	-0.046	0.09
LNU	-0.128	0.68
LNSIN	0.070	0.20
LNNEXP	-0.006	0.00
S	0.044	0.03
LNHF3	-0.188	1.49
LNRH	-0.020	0.02
LNTC	-0.046	0.09

TABLE XXXIII

SUMMARY ON THE SECOND BEST VIABLE COLIFORM PREDICTIVE  
REGRESSION EQUATION

$$\text{LNVCA} = 10.987 - 0.41 \times \text{LNDIST} - 4.56 \times \text{LNHF3}$$

Equation Statistics

Coefficient of Multiple Determination,  $R^2 = 0.516$

Standard Error of the Estimate,  $SE = 1.52$

Regression F-Ratio,  $F = 23.47$  with 2 and 44 degrees of freedom

Significance of F-Ratio,  $P = 0.00000012$

Variable Statistics

<u>Regression Variable</u>	<u>Regression Coefficient</u>	<u>T Statistic</u>	<u>F Level</u>
LNDIST	-0.4104	-2.29	5.25
LNHF3	-4.5601	-6.85	46.94

<u>Regressor Variable</u>	<u>Partial Correlation Coefficient</u>	<u>Potential F Level</u>
LNVCE	-0.016	0.01
LNU	-0.187	1.55
LNSIN	0.086	0.32
LNNEXP	-0.019	0.02
S	0.233	2.46
LNS	0.271	3.40
LNT	0.042	0.08
LNRH	0.001	0.00
LNRHSQ	-0.226	2.32
LNTC	-0.140	0.85

$$\text{LNVCA} = 10.987 - 0.41 \times \text{LNDIST} - 4.56 \times \text{LNHF3} \quad (8).$$

Only the heat flux/solar radiation term LNHF3 and the distance term LNDIST appear in this regression equation. The negative coefficient, -4.56, of LNHF3 suggests decreasing viable coliform levels in the air during the day with increasing solar radiation. Thus the LNHF3 term in equation (8) plays the same role as the LNS and LNT terms in equation (7). However, the LNHF3 term masks the quadratic relative humidity effect, while the combination of LNS and LNT does not.

c. Discussion

The major factors affecting the bacterial and other microorganism levels around the Pleasanton spray fields appear to be solar radiation, distance downwind, temperature and relative humidity. The evidence for a solar radiation effect was quite consistent, while that for distance, temperature, and relative humidity was less consistent. It appears that the aerosol formation and bacterial survival variables (solar radiation, temperature, and relative humidity) had a stronger effect on bacteria levels than did the atmospheric dispersion model variables. In particular, the effluent bacterial level, the wind velocity and the wind direction relative to the sprinkler line had no effect on the sampled aerosol bacterial levels. The lack of evidence regarding effluent level and wind velocity may have resulted from the limited ranges of these variables that existed during the aerosol sampling runs.

A large proportion of the aerosol bacterial concentration variation could not be accounted for by the measured aerosol formation, bacterial survival, atmospheric dispersion, effluent and sampling factors. The unexplained variation is 62.4% for the viable particle regression and 40.7% for the viable coliform regression. There is the possibility that some major factor(s) affecting aerosol bacterial levels at Pleasanton were not measured in Phase I. However, it is plausible to ascribe much of the unexplained variation to the sampling variability of the Andersen sampler under the prevailing meteorological conditions and to the analytical variability of the plate count procedure. There were insufficient data collected on which to apportion the unexplained variation between sampling variation and analysis variation.

It is recommended that the Phase II aerosol sampling runs only be conducted when a steady wind at approximately a right angle to the sprinkler line occurs. Furthermore, in order to fairly evaluate the possible effect of every potential model factor, the runs should be performed

over as wide a range of each measured variable as possible. Sampling should be conducted during the day and at night, with high and low wind speeds and with high and low wastewater bacterial levels. It would also be desirable to sample over a wide range of temperature and relative humidity conditions.

It is also recommended that specially designed experiments be conducted for every important aerosol and effluent sampling and analysis method selected for use in Phase II. The objective of these experiments would be to quantify the sampling variability associated with each sampling method, and the analysis repeatability and reproducibility variation associated with each analysis method.

#### IV. CONCLUSIONS AND RECOMMENDATIONS

1. The wastewater at the Pleasanton site is of poor quality which is consistent with data collected previously by the City of Pleasanton. The high quantities of bacteria present in the effluent reflect domestic wastewater quality without disinfection.

2. Although statistically there were no significant diurnal changes seen in chemical or biological parameters, some trends were noticed. These trends warrant a closer look at the residence time of the effluent in the ponds for possible short circuiting by injecting a dye at the inlet to Pond No. 1 and measuring the concentration of dye at various points in Pond No. 1 and Pond No. 2 with time. The utilization of the two aeration ponds at the end of the treatment plant appear to dampen cyclic changes which may occur through the plant. This will make the study easier, since a rather uniform quality wastewater is being aerosolized.

3. The micrometeorology at the site complicates the air sampling and the interpretation of the aerosol data. The climatic conditions may, however, be near ideal for study of health effects in an exposed population. Thus, the high percentage of time inversion and calm conditions prevail should increase the levels of aerosols near the site (out to 1.6 km) at ground level. Conversely, these conditions complicate the study of transport properties of aerosols from a point source relative to wind speed and direction.

4. Aerosols generated from the treatment plant should not complicate the study of aerosols from the spray fields because the head works, aeration chamber and trickling filter are covered to control odors. There is a possibility that the aeration ponds generate aerosols. More data are needed to evaluate this point source.

5. Although relatively high levels of bacteria were found in effluents and in the air, no pathogenic organisms were isolated. This finding suggests that there are problems in the methods of sampling and/or analysis for these organisms, since other studies of domestic wastewater have consistently found pathogenic organisms.

6. The effluent data indicate that daily composite samples should be the primary samples used to describe effluent quality. Grab samples should be collected at the spray nozzles before and after aerosol collections for routine bacteriological parameters only. Additional extensive sample analysis of effluent samples for daily changes are not needed. It is, however, recommended that some grab samples of pond effluent be collected before and after the spraying periods to determine whether or not significant differences in water quality parameters are seen.

7. One of the objectives of Phase I which has not been completed is the enumeration of virus and pathogenic bacteria present in effluent samples. It is recommended that large samples (gallon size) of effluent be collected and analyzed for viruses and pathogenic bacteria. This effort would provide additional data as to the predominant types of organisms present in the effluent.

8. It appears that many of the upwind samples collected during Phase I were not indicative of background levels of microorganisms. The apparent cause was the shifting wind directions. It is recommended that future upwind air samples be collected much further upwind (approximately 2 km) to ensure that background levels are being measured.

9. It is recommended that the LEAP and AGI samplers be used for all the air sampling except for detection of certain bacterial strains using the Andersen sampler. Data from Phase I indicate that the LEAP sampler provides the highest sensitivity (attributed to efficiency of collection and quantities of air sampled) for bacteria, coliphage and viruses. The AGI sampler provides almost as effective sampling, and it has the advantage in low cost of samplers and ease of operation. The AGI samplers should be used to supplement the LEAP samplers.

10. The data from analysis of effluents and aerosols for coliphage and virus indicate that additional sensitivity is needed. This can be achieved by collection and analysis of a larger sample size and perhaps by using a larger quantity of concentrate in the assay.

11. Additional experiments utilizing dye in the effluent are needed to better define percentage aerosolization at the Pleasanton site. Specifically, data are needed for different humidities, wind speeds, day and night and distance from the spray fields. The calculated values reported here are higher than have been seen in other studies. This may be due to the methods used in this study to calculate source strength of dye in the wastewater.

12. Examination of factors which may influence the quantities of aerosols (bacteria, coliphage, virus) suggests that certain of these factors play a much larger role than do others. These findings should be considered as very preliminary. As mentioned, many of the upwind samples collected were obviously not indicative of background levels of microorganisms. The primary objective of this phase was to perfect methodologies for collection, assay and evaluation of the data using statistical procedures. Useful procedures were developed for statistical examination of the aerosol data as were methods for collection and assay of effluents and aerosols.

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