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Presence of Enteric Microorganisms in the Air Environment at Wastewater Treatment Plants in Puerto Rico

Final Report

by

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ABSTRACT

In Puerto Rico there is a growing concern about the incidence of diarrhea and other enteric diseases and their autbreak at different times and in different regions around the island. As wastewater treatment plants in the island are established near population centers and as most areas around them are under the action of plant generated aerosols full of microorganisms and carried by the wind, the basic research was undertaken to study the presence of coliforms of possible potential health hazard in the vicinity of the plants, and their possible relation to the treatment given the wastewaters. The air environment around two trickling filter and two activated sludge wastewater treatment plants were studied.

The trickling filter plants in the investigation didn't show the production of any aerosolized coliform bacteria that could be detected by the use of impingers or by direct exposure of agar plates at a distance of 30.5 m (100 ft) downwind from the plants. In the activated sludge plants, on the contrary, coliform and fecal coliform bacteria were detected at the distance of 30.5m (100 ft) downwind from the plants, even thought their member was not great.

As all samples were taken during daylight hours when solar radiation was at its peak (10:00AM-2:00PM), the temperatures were high (33-36C), relative humidity around 69%, and wind velocity not high, these test conditions as a whole are possibly responsible for the results of low coliform counts obtained.

Both the trickling filter and the activated sludge types of wastewater treatment plants do not seem to be a public health risk as they are effective in reducing the number of coliforms of fecal origin from the aerosols produced by them, especially the trickling filter ones.

Based on the results of the investigation, recomendations are made as to the type of plant to be established (trickling filter), and the location (in arid coastal regions), away from population groups of great health risks as are children and elderly people.

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INTRODUCTION

In Puerto Rico there is a growing concern about the incidence of diarrhea and other enteric diseases and their outbreak at different times and in different regions around the island. As an overpopulated island, there is almost no uninhabited region and large amounts of wastewaters are generated all throughout the country. Wastewater treatment plants are mostly established near population centers to minimize the costs of the associated sewerage systems, all of them subjected to the winds characteristic of the tradewind area of the North Atlantic Ocean, where the island is geographically located.

It is a known fact that microorganisms and organic matter can be transferred from bodies of water to air by adsorption on gas bubbles that rise from the subsurface to the microlayer, and then burst at the water/air interface. The microorganisms are this way ejected into the environment where they can be dispersed and transferred to other sites. As a consequence, most areas around wastewater treatment plants are under the action of aerosols generated at the plants and transported by the wind.

Aerosols are defined as a system of colloidal particles dispersed in a gas, smoke or fog; and, as far as wastewater treatment is concerned, they can be created through various processes, especially in activated sludge, trickling filters and spray irrigations.

Much research has been done on this subject (1, 2) and a symposium, sponsored by the Health Effects Research Laboratory of the U.S. Environmental Protection Agency was held recently (3). No research on this subject had been done in Puerto Rico, though, and considering the situation previously presented, the basic study was undertaken, supported in part by funds provided by the United States Department of the Interior, Office of Water Research and Technology, as authorized by the Research

and Development Act of 1978 (PL 95-467), Project A-070-PR.

Bacterial aerosol particles vary in size. The smaller particles are the ones generally considered to constitute a health hazard because they penetrate the lower respiratory tract. The larger particles, though, should be considered of health significance, since, after having been caught in the upper respiratory tract, they may be subsequently swallowed. Where enteric bateria from sewage are concerned, these larger particles may thus be considered to be an even greater health risk (4).

Even though generally there are no health problems with the wastewater treatment plant workers related to their work, the distance from a treatment plant is important to consider when estimating exposure, as it is possible that persons living close to a treatment plant are more chronically exposed to aerosols than treatment plant workers, since their exposure could be 24 hours a day (5). It was with this in mind and having the problem previously presented that the study here presented was undertaken and developed.

MATERIALS AND METHODS

The methods finally used to sample the air around the wastewater treatment plants were the use of impingers with buffered water and direct exposure of agar plates. But, before the final decision to use impingers and direct exposure of agar plates was taken, some other methods were tried in the field.

The use of membrane filters have been experimented for many years and filters were finally perfected to permit their use in filtering air and specifically in filtering microbiological aerosols (6). Two general problems have guided the experiments, though: 1- the degree of retention of air-borne organisms, and 2- the preservation of viability of retained organisms.

Air filtering through dry membrane filters

The first method used was filtering the air through Nalgene disposable filter units with membrane filters with pore size of .45 micron, dry and wet (Filter unit PS 245-0045 Sybron/Nalge, Nalge Company, Rochester, New York, U.S.A. 14602). At the same time and site, 2 plates with "Plate Count Agar" were exposed as control.

A dry filter unit was connected to a flowmeter (Flowmeter SHO-RATE, Type 1355-00A1AAM, Brooks Instrument Division, Emerson Electric Co., Hatfield Pennsylvania, U.S.A. with Matheson flowtube No. 604, Matheson Instruments, 430 Caredean Drive, Horsham, PA 19044) and this to a vacuum pump. Ambient air was filtered through the unit for 25 minutes, the reading on the flowmeter being 90, for a total volume of 0.215 cubic meters (215 liters) of air filtered. After exposure, the membrane filter in the filter unit was washed with 6 succesive 10 cc (ml) portions of sterile saline solution water (9g NaCl/1,000 ml H_2O) into a sterile jar. The filter was removed (cut out with a sterile scalpel) and put into a sterile "M-FC Agar" plate.

Both the plate and the saline solution of the filter's washings were kept in an ice chest until they were finally assayed in the laboratory. There, the plates were incubated at 44.5C in a water bath (inside plastic bags) for 24 hours, after which the typical fecal coliform colonies were counted. The "Plate Count Agar" control plates (also put in an ice chest until taken to the laboratory) were incubated at 35C for 48 hours and the colonies counted. One 10 ml lactose broth fermentation tube (single strengh) was inoculated with each colony and incubated at 35C for 24-48 hours to check if the colonies were of coliforms.

The saline solution of the filter's washing was used to prepare a standard plate count and a total coliform test.

The number of sites to be incorporated into an ambient air monitoring network depends largely on the amount of data required. At a small source where one wind direction usually predominates (as is the case of wastewater treatment plants in Puerto Rico), monitors are usually operated at two sites: one to monitor the effects of the source, and the other to provide upwind background concentrations (7). Based on this fact, only two sites were originally monitored.

The first tests were done on a sunny day in the activated sludge Moca Wastewater Treatment Plant. Using an imaginary line (transect) through the plant and in the direction of the wind, a sample was taken 100 ft before the plant (upwind), and the other 120 ft downwind after the plant. The results are presented in Table 1.

As no colonies were isolated in the filters using "M-FC Agar" plates incubated at 44.5C for 24 hours, the same procedure was followed using "Endo Agar" to incubate the filter in an air incubator at 35C for 48 hours, the downwind test being done again in the Moca Wastewater Treatment Plant, but only 100 ft away of the tanks. The medium was changed so as to try to

isolate total coliforms or other types of microorganisms to later check if they were of fecal origin. After the change was done, a report by Grabow et al. (8) appeared in the August 1981 issue of the Applied and Environmental Microbiology where they presented the advantages of using M-FC agar without rosolic acid, but it had already been decided to change the use of filters to the use of impingers, later presented in this work. The results of this second test are also presented in Table 1.

At this point of the investigation it was clear that the second general problem stated before (the preservation of viability of retained organisms) was present. As it is a known fact that bacteria are usually killed by desication during collection in filters (9), a similar round of tests was also done in the Moca Wastewater Treatment Plant using Nalgene disposable filter units with .45 micron membrane filters, this time wet.

Air filtering through wet membrane filters

A device was prepared using three slant-cut rubber tubes (protruding from a three-holed plastic cup) conected, with glass tubing, to a single piece of rubber hose with a 12 inch piece of glass tubing at its end. Each slant-cut rubber tube had its own flow control, as did the rubber hose, too. In this way, a very slow dripping was attained when the system was used. The whole device was autoclave-sterilized before use. When in use, the rubber hose was filled with sterile saline water and the glass tubing at its end put into a jar with more sterile saline water. The saline water dripped, by gravity, to the filter unit directly below the three slant-cut rubber tubes, maintaining the filter, in this way, slightly wet all the time.

The first thing noticed with the wet filter was that the volume of air filtered was less than with the dry one. In 25 minutes of filtering, only .082 cubic meters (82 liters), the highest possible reading attained in the flowmeter was 30, were filtered.

The tests done were similar to the ones performed with the dry filter, this time again using "Endo Agar" plates to incubate the filters. The results are presented in Table 2.

Direct exposure of agar plates

As stated before, every time a round of tests was done, open petri dishes with sterile "Plate Count Agar" were exposed at the same site the filters were used. This was done in order to be sure there were viable microorganisms in the air and to try to isolate the coliforms present. The plates were exposed for 60 minutes and the results are presented in each test as "Plate Count-Control". With every single isolated colony appearing in the plates, one 10 ml "Lactose Broth" fermentation tube was inoculated to determine if the isolated bacteria were of the coliform type. Plans were to use the positive tubes to try to identify the bacteria as fecal coliforms, inoculating "EC Medium" fermentation tubes and incubating them at 44.5C in a water bath for 24 hours. The results are presented in each test as "Coliform colonies from the plate count (%) and, when fecal coliforms were detected, as "Fecal coliform colonies from the plate count (%).

Even though up to this point no coliforms were detected using the plates with later inoculation of "Lactose Broth" fermentation tubes, the results demonstrate once more the known fact that the wind carries viable microorganisms from the plants to the surroundings. On the other side, at this moment and with the results obtained using wet and dry filters, it was decided to abandon their use because no visible quantitative or qualitative results of any type were obtained.

Sampling with impingers

As the two air sampling devices recommended by contributors at the First International Symposium of Aerobiology were the all-glass impinger (AGI-30) and the Andersen-type six-stage microbial impactor (10, 11, 12), the next available mean of air sampling was the use of all-glass impingers. According to Fannin, they are simple, inexpensive and dependable; and, even

though their air sampling capacity is low, they can be easily sterilized (13). Tyler et al., on the other hand, believe that, although quite efficient, the all-glass impingers cause significantly great killing of vegetative bacteria, variable with species, relative to other samplers (14). But in his studies with Shipe, the logical conclusion was that the all-glass impinger (AGI) was superior to other liquid samplers tested (15).

The most important reason for selecting the impingers, though, is the fact that liquid impinger samplers are used to determine the number of viable microorganisms in aerosols, rather than the number of viable aerosol particles (13).

After some preliminary tests with Midget impingers and plastic impingers (available in the laboratory), the plastic (polypropylene) ones were selected as the ones to be used. They were prepared to have a jet-to-base distance of 30mm, as the AGI-30 recommended as standard by a committee of aerobiologists (13). On sampling downwind at on approximate distance of 100 feet from the source, it was noticed that aerosols produced by the plants charged the surrounding environment with viable microorganisms. No sampling was done at higher distances from the source, though, since no significant number of fecal coliform microorganisms were detected at this distance. The earlier decision of sampling in only two sites, one upwind and one downwind, was finally taken as enough for the study.

Tests were made on the wastewater treatment plants of Añasco and San Sebastián, of the trickling filter type, and on the Moca and San Germán ones, of the activated sludge type.

An impinger containing 25 cc (ml) of buffered water prepared under the specifications of the Standard Methods for the Examination of Water and Wastewater, page 892 (16) was connected to a flowmeter and this to

a vacuum pump. Ambient air was passed through the impinger for 60 minutes, the reading on the flowmeter being 95, for a total volume of 0.540 cubic meters (540 liters) of air passed. The impinger was then put in an ice chest until finally assayed in the laboratory. The liquid in the impinger was completed to a final total volume of 40 cc (ml) and then used to prepare a total and fecal coliform test and a Standard Plate Count. The results are presented in Table 3 as total coliform MPN Index/100 ml of the liquid in the impinger, Fecal Coliform MPN Index/100 ml of the liquid in the impinger, and Standard Plate Count of the liquid in the impinger.

Once again, every time a round of tests was done, open petri dishes with sterile "Plate Count Agar" were exposed at the same site the impingers were used. The plates were put in an ice chest until taken to the laboratory, where they were incubated at 35C for 48 hours and the colonies counted. One 10 ml "Lactose Broth" fermentation tube (single strengh) was inoculated with each colony and incubated at 35C for 24-48 hours to check if the colonies were of coliforms. The percentage of coliform colonies from the total number of colonies in the plate is presented in Table 3 as Total coliform colonies from the plate count (%). Each fermented tube was used to inoculate an "EC Medium" fermentation tube incubated at 44.5C for 24 hours to check if the coliform present was of fecal origin. The percentage of fecal coliform colonies from the total number of colonies in the plate is presented in Table 3 as Fecal coliform colonies from the plate count (%).

The results of the sampling done with the impingers and the agar plates as control appear in Table 3. The type of wastewater treatment plant, distance from source of bacterial aerosols, test conditions and holding time for the samples is presented, as well as the Most Probable Number (MPN) of total and fecal coliform per cubic meter of air, total

viable microorganisms per cubic meter of air, plate count and percent of coliform and fecal coliform colonies from the plate count.

Most probable number (MPN) of total or fecal coliforms per cubic meter of air

To find the MPN of total or fecal coliforms per cubic meter of air, the following procedure was followed:

1. The reading on the flowmeter, 95, was checked against the flowmeter calibration chart and the flow rate, 540 liters/minute, was found. This number was multiplied by the time in minutes, 60, air was passed through the flowmeter and impinger system. The number thus obtained is the total number of liters of air passed through the impinger. This is then changed to cubic meters multiplying by .001.
2. A proportion is made between the results of the total (or fecal) coliform test-as taken from Table 908: II in pages 924-925 of the Standard Methods for the Examination of Water and Wastewater (16), given as the Most Probable Number (MPN) Index per 100 ml of sample, in this case the liquid in the impinger- and the final volume in the impinger, 40 ml, to find the MPN of total (or fecal) coliforms in the total volume of liquid in the impinger.
3. The result in number 2 above is then divided by number 1 above and the result is expressed as the Most Probable Number (MPN) of coliforms (or fecal coliforms) per cubic meter of air.

Example from Table 3-Añasco trickling filter wastewater treatment plant:

1. Reading on the flowmeter: 95

   Flow rate (from the flowmeter calibration chart): 9 liters/min.

   Time in minutes: 60 minutes

   Total volume of air passed (in liters):

9 liters/min x 60 min.= 540 liters

Total volume of air passed (in cubic meters):

$$540 \text{ liters} \times \frac{.001 \text{ cubic meter}}{\text{liter}} = 0.540 \text{ cubic meters}$$

2. Total (or fecal) coliform MPN Index/100 ml: <3

Final total volume of liquid in the impinger: 40 ml

$$\frac{<3 \text{ coliforms}}{100 \text{ ml}} = \frac{x \text{ colonies}}{40 \text{ ml}}$$

$x = <1.2$ coliforms

3. <1.2 coliforms ÷ 0.540 cubic mt = <2.22

$$<2.22 \quad < \frac{2 \text{ coliforms}}{\text{cubic meter}}$$

Total viable microorganisms per cubic meter of air

To find the Total viable microorganisms per cubic meter of air, the following procedure was followed:

1- The total number of colonies in the agar plate (Standard Plate Count of the liquid in the impinger) represents the number of viable microorganisms in 1 ml (the inoculum) of the liquid in the impinger. This number is multiplied by the final total volume of liquid in the impinger to find the total number of viable microorganisms in the sample.

2- This total number of viable microorganisms is then divided by the quantity of air passed through the impinger (found earlier in number 1 of the procedure to find the MPN of total or fecal coliforms per cubic meter of air). The result is expressed as Total viable microorganisms per cubic meter (Table 3).

Example from Table 3- Añasco trickling filter wastewater treatment plant:

Colony count: 6 colonies/ml

Final volume of the liquid in the impinger: 40 ml

Total number of viable microorganisms (colonies) in the sample:

6 colonies/ml x 40 ml = 240 colonies

Volume of air passed through the impinger: 0.540 cubic mt

240 colonies $\div$ 0.540 cubic meters = 444 colonies/cubic mt.

RESULTS

For technical reasons and equipment limitations it was not possible to take the samples simultaneously, but test conditions were meticulously checked in each sampling site.

The results of the earlier samplings and assays are presented in Table 1. "M-FC agar" plates were used in the first sampling to incubate the filters, but it was later changed to "Endo Agar" so as to isolate coliforms and other types of microorganisms, but not necessarily fecal coliforms.

TABLE 1

Coliform counts in the air environment surrounding the Moca wastewater treatment plant[1]

Test	Station Upwind	Downwind	Downwind
Standard Plat Count of the saline washing.	0 colonies/ml	0 colonies/ml	0 colonies/ml
Total coliform MPN Index per 100 ml of the saline washing.	<2	<2	<2
Colony Count in the filter.	0[a]	0[a]	2[b*]
Plate Count-Control	46	122	115
Coliform colonies from the plate count (%)	0	0	0

[1] Obtained by filtering air through dry membrane filters and by direct exposure of agar plates.

[a] Filter incubated in "M-FC agar" plates at 44.5C for 24 hours.

[b] Filter incubated in "Endo Agar" plates at 35C for 24-48 hours.

[*] One 10 ml "Lactose Broth" fermentation tube (single strength) was inoculated with each colony to check if they were coliforms, but none fermented.

Table 2 shows the same type of sampling and assay, but this time using wet membrane filters and "Endo Agar", once again, to incubate them.

TABLE 2

Coliform counts in the air environment surrounding the Moca wastewater treatment plant[1]

Test	Station	
	Upwind	Downwind
Standard Plate Count of the saline washing.	0 colonies/ml	Spreader*
Total coliform MPN Index per 100 ml of the saline washing.	<2	<2
Colony count in the filter	0	Spreader*
Plate Count-Control	46	115
Coliform colonies from the plate count (%)	0	0

[1] Obtained by filtering air through wet membrane filters and by direct exposure of agar plates.

* A fast growing mold covered the whole plate. No other visible growth was noticed. In this downwind station, the dripping device was not sterile any more after handling it from one station to the other.

The results of the later samplings, using impingers and direct exposure of agar plates are presented in Table 3. As can be seen from the table, only one set of samples was taken in the San Sebastián plant, because in the other visits the conditions were not normal or suitable for sampling.

TABLE 3. Coliform Counts in the Air Environment Surrounding Wastewater Treatment Plants Obtained by the Use of Impingers and Direct Exposure of Agar Plates

Plant	Type	Station	Distance from Source	Sunny or Cloudy	Wind Speed (Km/hr)	Relative Humidity (%)	Temperature (°C)	Time of day
Añasco	Trickling filter	Upwind-Control	74	Sunny	5	78	34	11:05 AM
			170	Cloudy	3	70	33	10:20 AM
		Downwind	15	Sunny	5	78	34	10:00 AM
			100	Cloudy	4	66	35	12:10 PM
San Sebastián	*Trickling filter	Upwind-Control	100	Cloudy	0	70	33	2:00 PM
		Downwind	50	Cloudy	3	70	33	12:30 PM
Moca	Activited Sludge	Upwind-Control	78	Sunny	3	67	36	10:30 AM
			100	Sunny	2	69	34	10:30 AM
		Downwind	39	Cloudy	1	64	38	11:35 AM
			75	Sunny	0	66	35	1:00 PM
San Germán	Activated Sludge	Upwind-Control	100	Cloudy	6	69	33	11:15 AM
			100	Sunny	10	67	30	10:35 AM
		Downwind	100	Sunny	7	68	34	12:30 PM
			100	Sunny	8	69	31	11:47 AM

*Only one set of samples was taken in this plant because in the other visits the conditions were not normal or suitable for sampling.

TABLE 3. (CONT.)

Plant	Holding Time (hr)	Total Coliform MPN Index/100 ml of the Liquid in the Impinger	MPN of Coliforms per Cubic Meter of Air	Fecal Coliform MPN Index/100 Ml of the Liquid in the Impinger	MPN of Fecal Coliforms per Cubic Meter of Air	Standard Plate Count of the Liquid in the Impinger (Colonies/ml)
Añasco	2.5	<3	<2	<3	<2	6
	3.0	<3	<2	<3	<2	2
	3.5	<3	<2	<3	<2	14
	1.5	<3	<2	<3	<2	Spreader
San Sebastián	1.5	<3	<2	<3	<2	2
	3.0	<3	<2	<3	<2	22
Moca	3.5	<3	<2	<3	<2	Spreader
	4.8	<3	<2	<3	<2	0
	2.5	4	<2	<3	<2	6
	3.3	<3	<2	<3	<2	11
San Germán	2.5	<3	<2	<3	<2	3
	3.0	<3	<2	<3	<2	5
	1.5	9	7	4	3	9
	2.0	9	7	4	3	18

MPN = Most Probable Number

TABLE 3. (Cont.)

Plant	Total viable microorganisms per cubit meter of air	Plate count control	Coliform colonies from the plate count (%)	Fecal coliform colonies from the plate count (%)
Añasco	444	26	0	0
	148	22	8	0
	1,037	34	0	0
	Spreader	Spreader	—	—
San Sebastián	148	118	0	0
	1,629	208	0	0
Moca	Spreader	36	0	0
	0	17	7	0
	444	99	0	0
	814	73	4	2
San Germán	222	72	0	0
	370	52	0	0
	666	76	0	0
	1,333	67	2	2

DISCUSSION

As can be seen from Table 3, the comparison between the upwind (control) station and the downwind one in all the plants demonstrates once more that wind carries viable microorganisms from the wastewater treatment plants and load the environment with aerosols containing them. The plants seem to be effective in removing most of the coliform bacteria present though, as almost no coliform or fecal coliform bacteria could be detected in the downwind sampling sites.

Even though the presence of coliform bacteria have been sampled to a distance of 1,287 m (4,224 ft) downwind from a trickling-filter sewage treatment plant (17) and to a distance of 350 m (1,150 ft) downwind from a wastewater spray irrigation line (4), the trickling filter plants studied in this investigation didn't show the production of any aerosalized coliform bacteria that could be detected by the use of impingers or by direct exposure of agar plates at a distance of 30.5 m (100 ft) downwind from the plants. In the activated sludge plants, on the contrary, coliform and fecal coliform bacteria were detected at the distance of 30.5 m (100 ft) downwind from the plants, even though their number was not great.

The results of the agar plates directly exposed to the environment tend to confirm these observations or findings as no coliform or fecal coliform bacteria were detected in the downwind stations of the trickling filter plants sampled, and only very low percents of coliform and fecal coliform bacteria were detected in the downwind stations sampled in the activated sludge plants.

There's an instance where data seem not to be congruent and it's in the second round of samples taken in the Moca wastewater treatment plant. In the upwind-control site the percent of coliform colonies from

the plate count is 7, whereas in the downwind site, after the plant, the percent is 4. One possible explanation is the fact that the plant is surrounded by pasture ground at a higher level than the plant, and where cattle grazing is common. A small stream is also located nearby, upwind from the upwind-control station. But then we have the fact that the percent of fecal coliform colonies is 0. Proper identification and characterization of the colonies, not done in this study, could clear up this point. Another instance, similar to this one, where data seem not to be congruent is in the second round of samples taken in the Añasco wastewater treatment plant. In the upwind-control site, 8 percent of the total number of colonies appearing in the agar plates were coliforms, even though none were of fecal origin. There's no comparative value of the downwind station though, since a spreader covered the whole plate in less than 48 hours, leaving no visible isolated countable colonies to be transferred.

On a somewhat similar study by Adams and Spendlove (17), they detected higher counts of viable particles and higher counts of coliform particles per cubic meter of air using an Andersen Sampler, but test conditions were different to the ones in our study (lower temperatures and relative humidity, higher wind speed and, most important, less solar radiation). Generally speaking, high wind velocities, high relative humidity, darkness, and low temperatures would be expected to give the greatest recoveries of microorganisms, both close to the plants and at greater downwind distances (17, 18, 3, 4).

Solar radiation is considered by some workers to be the most important factor in bacterial decline, including sewage bacteria and coliforms. The effect may be due to short-wave radiation, or to light-induced damage directly through the absorption of light by chromophores, or by reaction

with oxidesto form superoxides which in turn may cause damage to the cells (18).

On experiments with spray irrigations with wastewater, up to 10 times more aerosolized bacteria were detected during night irrigation than with day irrigation and the correlation between the aerosol densities and solar irradiation is significant at the 1% level (4).

The work here presented was done during the summer (June-August 1981) and all samples were taken during daylight hours, when solar radiation was at its peak (10:00 A.M.-2:00 P.M.). Temperatures were high, between 33-36C (92-96F) most of the time; relative humidity was around 69%; and wind velocity was not high. These test conditions as a whole are possibly responsible for the results (low coliform counts) obtained.

Both trickling filter and activated sludge wastewater treatment plants seem to be effective in removing the gross of the fecal coliform bacteria or at least in arresting the growth and development of them, as conditions within seem to favor the activities and development of other microorganisms including molds and yeasts. Natural environmental conditions around the wastewater treatment plants in Puerto Rico also tend to arrest the viability of fecal coliform cells, as the number of viable cells can be greatly reduced in natural aerosols, especially the non-spore forming microorganisms (as are all coliform cells), contrary to microorganisms that do form spores (some bacteria, yeasts and molds).

Conditions within the water in the plants and in the environment around them seem to be such that non-coliform bacteria and other microorganisms can multiply better than others. Aerosols produced by these plants do not contain significantly greater amounts of coliform bacteria as the air upwind from the plants.

In respect to coliforms of fecal origin and the incidence of diarrhea and other gastrointestinal diseases, it can be said with almost absolute assurance that wastewater treatment plants in Puerto Rico, both trickling filter and activated sludge types, are not a public health risk and that, on the contrary, they are effective in reducing the number of coliforms of fecal origin from the aerosols produced by the plants during daylight hours.

As Cecil Lue-Hing, Director of Research and Development with the Metropolitan Sanitary District of Greater Chicago once judged: "A wastewater treatment plant is one of the best neighbors you can have-it doesn't pollute, make noise, mug you, or argue with you. What better kind of neighbor could you ask for?" (5).

Though this study has not demonstrated wastewater aerosols produced by wastewater treatment plants to be a possible cause of the diarrhea and other gastrointestinal diseases autbreaks, this does not necessarily indicate that a health hazard or risk does not exist. It merely means that under the limitations of the study, there is inconclusive evidence (5).

As a tropical country, Puerto Rico has an average of 12 hours of sun each day, and solar irradiation is important in the microorganisms' dispersion and die-off. As wastewater treatment plants in the island generally work 24 hours a day, and as the present field study, done during daylight hours, has not revealed any health hazards or risks, a similar study as the one here presented can and should be done to assess the real health risks of wastewater treatment plants working during the night, after which, depending on the results, health authorities can do a comprehensive epidemiological study.

We expect this work will provide a comprehensive environmental framework to be used in future investigations related to the incidende of

diarrhea and other enteric diseases, and their possible relation with the microbial aerosols produced by the wastewater treatment plants. We also hope it will promote the establishment of higher mechanical barriers, especially in activated sludge wastewater treatment plants, to avoid the wide dispersion of aerosolized microorganisms by wind.

Henry Longest, EPA Deputy Assistant Administrator for Water Program Operations once said scientists studying wastewater aerosols had important responsibilities and cautioned them to consider three factors when conducting scientific investigations: risks, cost impacts and explicit recommendations (19). Risk assessment relates to the degree of precaution that one should take to protect people from aerosols and the concomitant costs (5).

With this in mind, and as long as monetary costs permit, plants should be established in arid coastal regions (high temperatures, low relative humidity, high solar irradiation) in places somehow protected from strong wind currents.

Neither this nor any other similar study has shown wastewater treatment plants to be a health risk, but the opposite-that wastewater treatment plants do not constitute a health risk - has not been shown either, since sickness cases have been reported (4, 5), especially among children and elderly people. A final recommendation, as a preventive measure and as long as costs permit, would be to establish new wastewater treatment plants as far as possible from population groups of great health risks as are children and elderly people; for example: as far away as possible from schools and from centers for the elderly, and that trickling filter wastewater treatment plants be the chosen type, as they seem to disperse less coliforms to the surrounding environment, as compared to the activated sludge type.

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