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Effect of Sunlight and Other Factors on the Population Dynamics of Coliform Bacteria

by

Cindy Ginés Sánchez
Terrestrial Ecology Division,
Center for Energy and Environment Research

Submitted to the School of Environmental Health,
University of Puerto Rico, Medical Sciences Campus
in partial fulfillment of the requirements for
the Master of Science Degree

December 10, 1984

CENTER FOR ENERGY AND ENVIRONMENT RESEARCH
UNIVERSITY OF PUERTO RICO — U.S. DEPARTMENT OF ENERGY

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A mi familia:
María, Bobby, Sandra,
y Kenny

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LIST OF ABBREVIATIONS

Abbreviation	Meaning
DO	Dissolved Oxygen
FC	Fecal Coliforms
LogFC	Logarithm of the Fecal Coliforms
LogTC	Logarithm of the Total Coliforms
TC	Total Coliforms
Temp	Water Temperature
STP	Sewage Treatment Plant

ABSTRACT

Effect of Sunlight and Other Factors on the Population Dynamics of Coliform Bacteria: Francisco Folch Castañer, Ph.D., Principal Advisor; William H. McDowell, Ph.D., Associated Advisor; Iraida Robledo, M.S., Associated Advisor.

The effect of sunlight on the population dynamics of coliform bacteria was studied in a stream receiving the effluent from Cagüas' Sewage Treatment Plant (STP). The effects of nutrients and chlorine were also studied. Five stations were established in El Caño (into which the STP discharges) and Río Cagüitas (which merges with El Caño approximately 0.5 kilometers downstream from the STP discharge). Samples were taken during the daytime and nightime, and dissolved oxygen, water temperature, light intensity, and chlorine were measured in the field. Samples were taken back to the laboratory for analysis of fecal and total coliforms, nitrates and nitrites, ammonia, and chloride.

The results of this study show that the water quality of these streams is extremely poor. Both the fecal coliforms and dissolved oxygen had a high rate of non-compliance with the water quality regulations. Moreover, Río Cagüitas, which does not receive a discharge from a STP, usually had a higher density of coliform bacteria than El Caño, whose waters are composed almost solely of the effluent from an STP. A multiple regression-correlation analysis demonstrated that there was no significant effect of either the nutrients or chlorine on the density of either coliform group.

An ANOVA analysis showed that the stream segment significantly affected the change in the density of coliforms between adjacent stations, but the hour of sampling did not. This result shows that segments of the streams had different rates of increase or decrease in the density of bacteria, probably due to the fact that Río Cagüitas had a higher density than El Caño. The fact that the hour of sampling did not have a significant effect shows that there was no difference between daytime and nightime samplings, therefore suggesting that sunlight did not cause substantial coliform die-off in these streams.

INTRODUCTION

The use of non-pathogenic bacteria as indicators of pollution with pathogenic bacteria has generated much interest in the population dynamics of coliforms once they are released into streams, lakes or the ocean. Public health officials need to be able to predict the population dynamics of these bacteria in the environment in order to determine what uses can be given to a body of water that receives fecal pollution. In Puerto Rico it is necessary to predict coliform population dynamics because the high human populational density creates a water pollution problem that is increased by industrial waste discharges. The small number of water bodies fit to serve as water supply sources and as recipients of wastes, makes water reuse a necessity that amplifies this problem.

Based on a review of the literature Chamberlin and Mitchell (1978) concluded that sunlight is the most important factor in the mortality of coliform bacteria released into the ocean. Exposure to sunlight can kill over 90% of a population of total coliforms in 8 hours (Grigsby and Calkins, 1980). Sunlight therefore appears to be an effective bactericidal agent that is not being utilized optimally to disinfect sewage effluents that are

released into the environment. However, other factors have also been found to affect coliform survival. The chlorine applied to sewage treatment plant (STP) effluent kills a large proportion of bacteria in the effluent (Camper and McFeters, 1979), and the density of these bacteria can also be affected by the concentration of nutrients in the water (Hazen and Aranda, 1980).

Therefore, it is obvious that the survival of bacteria released into the environment may be determined by more than one factor. This experiment was conducted to assess the effect of sunlight on the survival of fecal and total coliform bacteria (FC and TC, respectively) released by a STP into a small, tropical stream in Puerto Rico. Samplings were carried out at day and at night to determine the effect of sunlight on their mortality pattern. The effect of other factors, especifically chlorine and nutrients, was also examined.

OBJECTIVES

The purpose of this study is to assess the effect of sunlight on the population dynamics of total and fecal coliform bacteria, and to assess the effect of other factors, specifically chlorine and nutrients.

LITERATURE REVIEW

Coliform Population Dynamics

Measurement of Coliform Population Dynamics

The magnitude of the die-off of a population of bacteria once it is released into a body of water is measured using Chick's decay coefficient, k ($hour^{-1}$), which is a function of the initial density of coliforms (B_o), the final density (B), and the time elapsed (t, $hour^{-1}$) (Chamberlin and Mitchell, 1978):

$$k = - \frac{\log (B/B_o)}{t} \qquad (1)$$

The final density of bacteria must be corrected for dilution of the plume, and this procedure is explained in John (1978) and Hubbard, et al. (1982).

This equation is based on the assumption that bacteria released into an unfavorable environment will experience first-order die-off. This die-off pattern is characterized by the death of an equal proportion of the remaining population of bacteria in a given time period (See Fig. 1). However, bacteria populations do not always follow this pattern.

Population Dynamics in Temperate Regions

Studies carried out in temperate streams and lakes show a great variability in k values. In their review of the literature Chamberlin and Mitchell (1978) report that k values range from 0.010 to 1.0 and generally fall between 0.015 and 0.20 hour^{-1}. Another author (Velz, 1970) reports that during the warm weather period k values for temperate rivers ranged from 0.46 to 0.96. The discrepancy between these authors is not easily explained. One possible reason is that Chamberlin and Mitchell (1978) corrected the k coefficient for the dilution of the effluent, but Velz (1970) apparently did not. The most likely explanation is that the studies they cited lasted different time periods. The studies used by Velz (1970) lasted 1 to 2 days and were

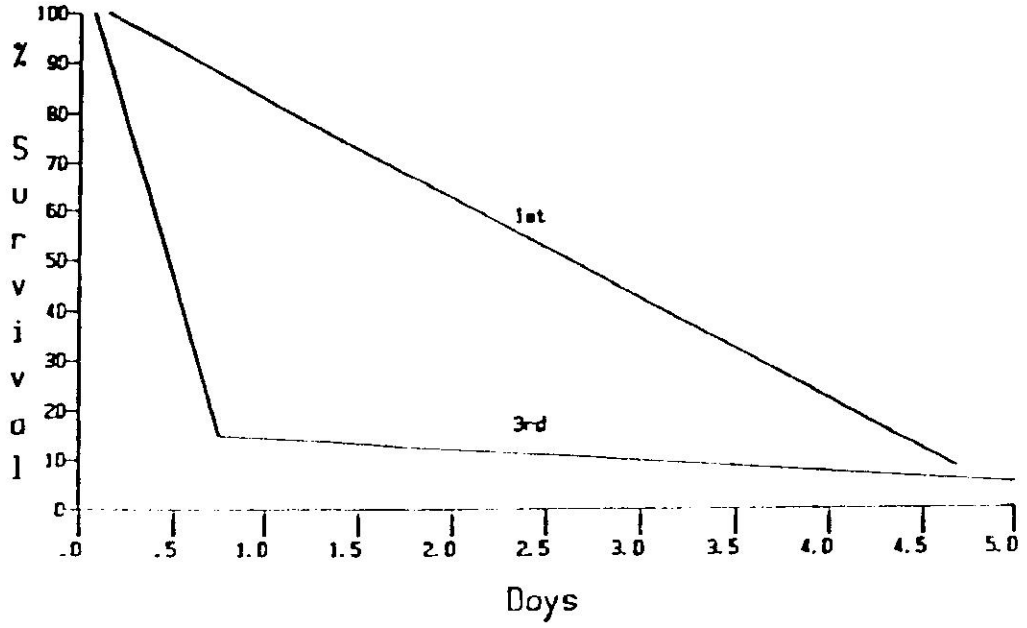


Fig. 1. Patterns of bacteria mortality. 1st indicates first-order and 3rd indicates third-order die-off. Adapted from Mancini (1978).

carried out during the summer, but Chamberlin and Mitchell (1978) do not state how long the studies they reviewed lasted or in what season they were done.

Based on a review of the literature and on his own sampling, Velz (1970) concluded that the most common pattern of coliform mortality is third-order die-off. This pattern consists of rapid initial die-off followed by much slower mortality (See Fig. 1). He found that 99.51% of a coliform population had a half-life of 0.64 days, and the other 0.49% had a half-life of over 5 days. Therefore, he concluded that the mortality pattern was basically first-order since such a large fraction of a population of bacteria die during the first day in a river.

Population Dynamics in Puerto Rico

The dynamics of coliforms appear to be different in the tropics than in temperate regions. k values are much higher, and regrowth of bacteria has been reported. One study of coliform mortality carried out in Puerto Rico found k values that ranged from 0.8 to 2.9 day^{-1} (19.2 to 23.2 $hour^{-1}$) (Muñoz, et al., 1969). The k values reported are much higher than those reported for temperate regions and demonstrate that die-off in the tropics can be much quicker than in temperate regions. However, the same study also reports that in two Puerto Rican streams coliform density increased instead of decreased. Biamón and Hazen (1983) also report that colifoms in an effluent plume

dumped into the ocean showed an initial increase followed by a decrease in density. These findings may be explained in part by the fact that in both cases of regrowth the polluting material was the organic-rich waste material from sugar cane. This effluent may provide nutrients necessary for bacteria reproduction that are not provided by sewage effluent. Another possible explanation is that in the tropics phytoplankton is involved in the control of population levels of coliforms (Hazen and Aranda, 1980).

Population Dynamics of Pathogenic Bacteria

It is important to remember that the population dynamics of pathogenic bacteria do not necessarily have to be the same as the dynamics of coliforms. Several studies have demonstrated that pathogens have greater resistance to lethal agents than coliforms (Chamberlin and Mitchell, 1978; Jagger, 1981). In their study of bacteria in an industrial effluent, Biamón and Hazen (1983) found that the density of FC was not correlated with the density of Aereomonas hydrophila, a pathogenic bacteria. Therefore, care must be taken when making generalizations of bacteria mortality based on studies of coliforms.

Factors That Affect Coliform Population Dynamics

Sunlight

Based on an extensive review of the literature Chamberlin and Mitchell (1978) concluded that sunlight is the most important factor in the mortality of indicator bacteria released into the ocean. They found a significant linear relationship between the decay rate of coliforms and the depth-corrected intensity of sunlight. Evidence of sunlight's lethal action to bacteria in freshwater was provided by Grigsby and Calkins (1980) who placed samples of effluent from a sewage treatment plant in tanks exposed to the sun. They found that the density of TC decreased steadily during the day and increased slightly at night (See Fig. 2). Several other in vitro experiments have

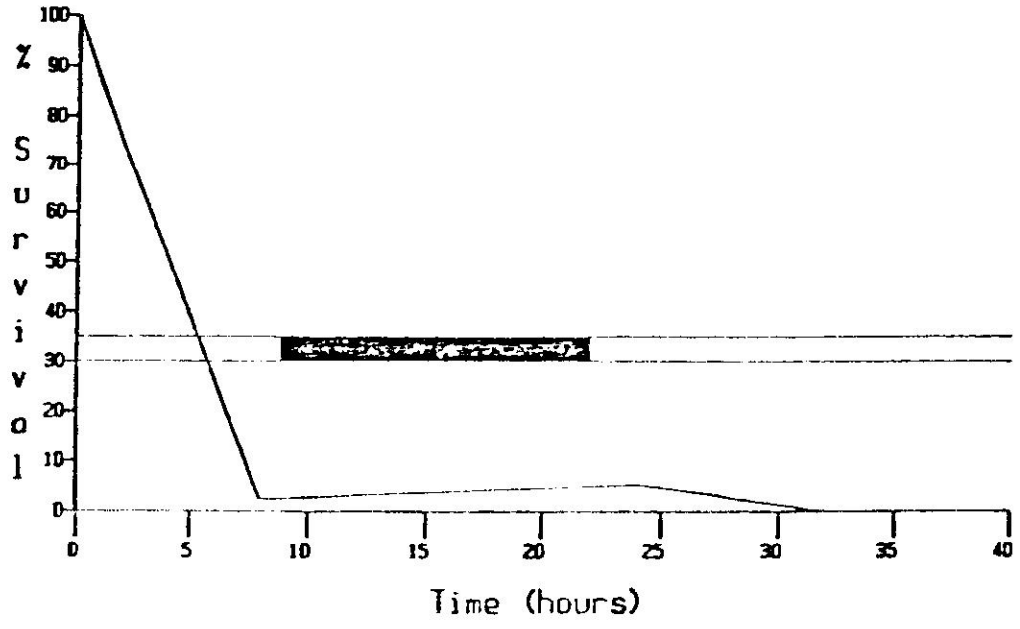


Fig. 2. Effect of exposure to sunlight on total coliforms. The bar represents daytime (empty) and nightime (filled) exposure. Adapted from Grigsby and Calkins (1980).

demonstrated that sunlight causes a decrease in the density of coliforms (Chojnowski, et al., 1979; Fujioka and Narikawa, 1982; Tuveson and March, 1980).

Therefore, there is widespread evidence that sunlight causes mortality in vitro, but there have been no experiments to our knowledge to test the effect of sunlight on coliform mortality in sewage plumes. Proving this effect may be difficult because the turbulence and turbidity of plumes may attenuate sunlight's lethal action. However, at least indirect evidence has been presented by Fattal, et al. (1983) who detected a negative correlation between the mean monthly FC density in a sewage plume and the mean monthly duration of sunlight.

The ultraviolet (UV) component of sunlight is at least partially responsible for its lethal effect. All three components of UV light [near (λ = 320-400 nm), mid (λ = 290-320 nm), and far (λ = 200-290 nm) UV] can kill bacteria (Peak, 1983). Their modes of action are different. The mid UV component causes mixed results, but the far and near UV cause the same end result: death of the bacteria due to DNA damage (Peak, 1983). The near UV can also cause sub-lethal damage to the permeability of the cell membrane and protein synthesis apparatus (Jagger, 1981).

Chlorination

Chlorine is the most commonly used disinfectant of waste water effluents. It rapidly reduces coliform

densities by as much as 90% (Camper and McFeters, 1979). However, recent experiments have shown that this decline is only temporary and, that after a lag period, the density of coliforms begins to increase. Following exposure to chlorine the density of indicator bacteria decreases for an hour (Camper and McFeters, 1979), but after this initial decline the density can increase for as long as five days (Kinney, et al., 1978).

The explanation of this "regrowth" lies in chlorine's mode of action. A large proportion of the bacteria exposed to chlorine are not killed; they are injured sub-lethally. Although chlorine damages the cell's genetic material and protein synthesis apparatus (Haas and Engelbrecht, 1980), the greatest damage occurs in the cell membrane. Pores are opened in the membrane that disrupt active transposrt and allow low molecular weight material to escape the cell (Haas and Engelbrecht, 1980). The net result is that the bacteria is not dead but is injured to such an extent that it is unable to grow on selective media such as m-FC and m-endo. Proof of this hypothesis was provided by an experiment in which the absorbance of a suspension of chlorine-injured coliforms was measured simultaneously with plate counts. The former remained unchanged while the latter decreased (Camper and McFeters, 1979). Given time, the cells repair the damage and are again able to grow on the plating media, creating the illusion of "regrowth".

All research involving indicator bacteria must take into consideration the fact that the most commonly used plating media do not detect these sub-lethally injured cells. m-endo can recover 95% of the uninjured and 66% of the chlorine-injured cells, and m-FC can recover 105% and 7%, respectively (McFeters, et al., 1982).

Nutrients

Little emphasis has been placed on the role of nutrients on the population dynamics of coliforms in the environment. In their review of factors influencing coliform mortality, Chamberlin and Mitchell (1978) concluded that nutrients could explain only a small fraction of coliform die-off. However, several experiments have demonstrated that a correlation can exist between nutrients and coliform density. Research conducted in a Swedish estuary showed that the density of FC was positively correlated with total nitrogen and nitrate (Hirn, et al., 1980). Hazen and Aranda (1980) found that the density of FC in several stations along a Puerto Rican stream was positively correlated with ortho-phosphate and nitrate concentrations. A study of the population dynamics of bacteria in a plume of organic-rich industrial effluent dumped into the sea showed that the density of the bacteria Aeromonas hydrophila was positively correlated with total phosphorous, total organic carbon, and orthophosphate; whereas the density of FC correlated only with conductivity

(Biamon and Hazen, 1983). Since a relationship between bacteria density and nutrients does not always exist, and since the nutrients involved vary, it is possible that the effect of nutrients is indirect or only one of many. One possibility is that phytoplankton may be modifying the effect of nutrients on coliforms (Hazen and Aranda, 1980).

Other

Various other factors have been proposed to cause coliform mortality, including predation. In one experiment, predation was found to remove 10^7 coliforms in 10 days (McCambridge and McMeekin, 1979), but in another experiment predation did not cause significant die-off (DeLeval and Remacle, 1979). This discongruity is probably due to the different methodologies used. The latter experiment removed the protozoans by filtering the water through a 0.8 μm membrane, but the former experiment demonstrated that it is this component of the fauna that is most active in predation.

Sedimentation could also affect coliform population dynamics. E. coli and Vibrio cholera can survive for considerable periods of time and even reproduce in sediments (Hood and Ness, 1982). Furthermore, activities such as dredging the bottom of a stream can resuspend coliforms bound to the sediment and increase their density in the water column (Grimes, 1980). Therefore, sediments may act as a sink or as a source of coliforms.

Still other factors have been proposed, including heavy metals, bacteriophages, algae, and bacterial toxins. However, their effect on the population dynamics of bacteria is deemed to be minimal (Chamberlin and Mitchell, 1978).

MATERIALS AND METHODS

Location

The source of fecal bacteria for this study was the city of Cagüas' STP. The trickling filter plant received mostly domestic wastes at an average flow of 6.9 Mgd (PRASA, 1984). The plant discharged the treated effluent into a channel locally called El Caño (See Fig. 3). During stable flow conditions the plant's effluent makes up the entire flow of El Caño, which runs through 0.5 km of pasture before merging with Río Cagüitas. Downstream from the point of merger with El Caño, Río Cagüitas flows by a sand and gravel quarry; however, this activity does not seem to affect the river's flow significantly.

Both Río Cagüitas upstream from El Caño, and the STP's effluent have highly variable water quality. At times both the river and the plant's effluent bear almost raw sewage and at other times the water they bear are moderately clean. For other studies of this river see Quiñones-Márquez (1980) and Font, et al. (1972).

Sampling Procedure

Five stations were established in a 1-km stretch of the Caguitas and El Caño (See Fig. 3). Station 1 was

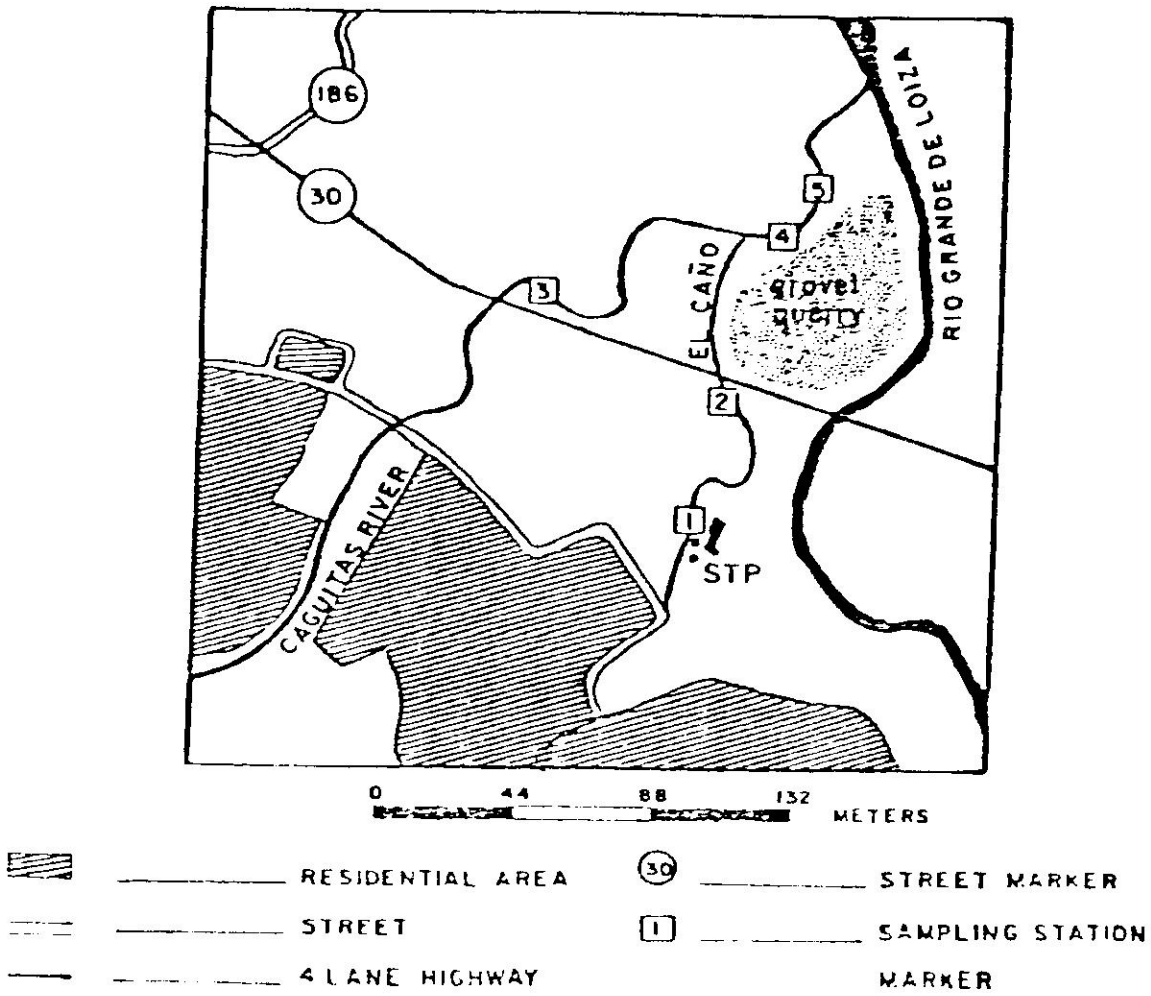


Fig. 3 Location of the sampling stations. Caguas' Sewage Treatment Plant (STP in the map) is located in the north eastern edge of the city. Adapted from USGS (1977).

located at the point of discharge of the STP into El Caño. Station 3 was located in Río Cagüitas upstream from its merger with El Caño and is equivalent to the Geological Survey's water quality station number 50055250 (Curtis, et al., 1984).

Samples were taken once a week beginning in March 1984. Five daytime samplings were followed by three nightime samplings. All samplings were carried out in stable flow conditions beginning at 9 a.m. and 9 p.m., respectively. The order in which the stations were sampled varied but was generally: 3, 1, 2, 4, 5.

Two samples were taken at each station. A sample for microbiological analysis was taken in a sterile bottle with 1 mL of 10% sodium thiosulfate to neutralize residual chlorine (APHA, 1980). A sample for chemical analysis was taken in a plastic bottle. The samples were taken simultaneously 10 cm from the water surface and were protected from sunlight immediately. All samples were placed on ice as soon as possible (but never more than five minutes after sampling) and were kept on ice until they were analyzed.

Several other parameters were measured in each station at a depth of 10 cm. Dissolved oxygen (DO) and water temperature (Temp) were measured using a Model 57 Meter (Yellow Springs Inst. Co.). Light intensity was measured using an underwater photometer (Protomatic). Total Chlorine was measured in Station 1 with a Model CN-66 meter (Hach Co.).

Coliform Enumeration

Coliform densities in the samples taken for microbiological analysis were determined using the membrane filtration method as described in Standard Methods (APHA, 1980). M-endo and M-Fc broths (Difco) were used to determine total coliform and fecal coliform densities, respectively. These media were filter sterilized through 0.20 μm pore diameter membranes (Type SM, Sartorius Co.) and dispensed into sterile petri dishes with absorbent pads (Millipore Co.).

A sample was chosen randomly for analysis. Using sterile phosphate buffer (APHA, 1980), two dilution series (one for each medium) were made. The final filtration volume was always 10 mL, and these dilutions were filtered in less than 30 minutes. A sterile filtration unit and 0.45 μm pore diameter membranes (Type HA, Millipore Co.) were used. The four dilutions for one medium were filtered beginning with the most dilute. The filter was then sanitized by rinsing it with ethanol, was allowed to air dry, and was rinsed with sterile buffer before beginning the remaining filtrations for that station. The same procedure was followed for each station, and the maximum time elapsed between collection and examination of the samples was 8 hours.

TC were incubated at 35^{o}C for 24 hours, and red colonies with green metallic sheen were counted. FC were

incubated at 44.5°C for 24 hours, and blue colonies were counted.

Chemical Analysis

Samples were analyzed for nitrite and nitrate, ammonia, and chloride, using automated analysis (AutoAnalyzer II, Technicon Instrument Co.) as described in Standard Methods (APHA, 1980). The samples were prepared by filtering them through Whatman filter paper (W & R Balston Co.) and then through 0.45 μm pore diameter membranes (Type HA, Millipore Co.). The filtered samples were diluted 1 in 10 using distilled-deionized water (Nanopure, Barnstead Co.) before analysis.

A portion of each diluted sample was stored at 4°C for determination of nitrogen as nitrate and nitrite ($NO_2^=/NO_3^-$) and, chloride ion (Cl^-) (holding time $\leq$ 5 days, and 3 weeks, repectively). A second portion was acidified by adding concentrated sulfuric acid and stored at 4°C for analysis of nitrogen as ammonia (NH_4^+) (holding time ($\leq$ 6 days).

Data Analysis

Data analyses were done using the SPSS statistical package (Nie, et al., 1975).

RESULTS[1]

Water Quality

A summary of the values obtained for several indicators of the water quality of Río Cagüitas and El Caño appears in Table 1. As expected, the high densities of FC and TC, low concentrations of DO, and extremely high concentrations of NH_4^+, corroborated that both streams receive sewage effluent.

Two of these parameters (FC and DO) had a high rate of non-compliance with the applicable water quality regulations (EQB, 1983). Whereas the standard is that no more than 20% of all samples taken in a given location shall have more than 40 CFU/mL of fecal coliforms, 80% of the samples in this study exceeded that value. Over 90% of all samples analyzed for DO had a concentration lower than that stipulated by the standard, 4.0 ppm. On the other hand, both Cl^- and $NO_2^=/NO_3^-$ had 100% compliance.

1. All values obtained in this study appear in Table 4 in the Appendix.

Table 1. Water quality summary for Rio Cagüitas and El Caño. Results based on all samples taken during this study.

Parameter	Minima-Maxima	Average ± Std. Dev.
FC (CFU/mL)	1.9 - 34000	2353 ± 5906
TC (CFU/mL)	11 - 91000	10393 ± 20245
Temp (^{o}C)	25.5 - 31.0	28.3 ± 1.2
DO (ppm)	1.0 - 5.6	2.8 ± 1.1
% DO Sat.	13.0 - 68.0	35.7 ± 13.7
$NO_2^{=}/NO_3^{-}$ (ppm)	0.08 - 1.06	0.41 ± 0.27
NH_4^{+} (ppm)	9.9 - 14800	1350 ± 2971
Cl^{-} (ppm)	39 - 94	65 ± 13
Chlorine (ppm)[1]	0.2 - 4.0	1.5 ± 1.3
STP Discharge (m^3/hr)[1]	792 - 1000	902 ± 82

1. Measured only in the STP outfall (Station 1).

Effect of Physico-Chemical Factors

A multiple regression-correlation analysis was carried out to detect any correlations between the logarithm of either coliform group and the physico-chemical factors studied. The correlation coefficients appear in Table 2, but there was no significant multiple correlation with the logarithm of the bacteria ($p=0.05$). In addition, the logarithm of neither coliform group in the STP was significantly correlated with the concentration of chlorine in the STP (See Fig. 4).

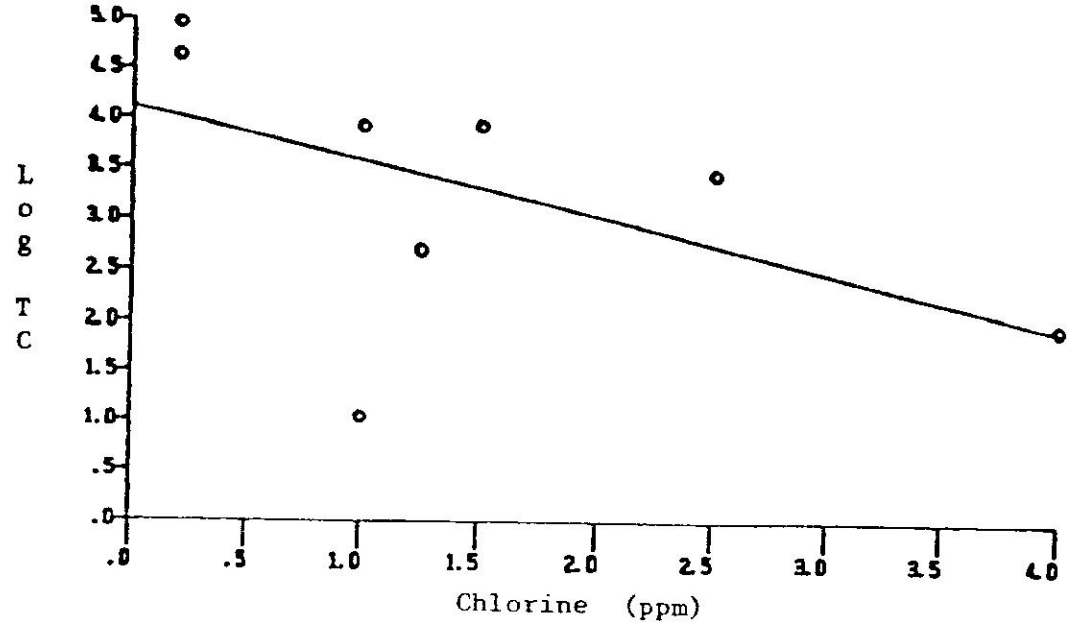
Coliform Population Dynamics

Although the number of bacteria fluctuated greatly with each sampling, a prevalent populational trend was evident (See Fig. 5 and 6). In general, there was a decrease in density between the STP and Station 2, which was followed by an increase between Stations 2 and 3 and a moderate decrease between Stations 4 and 5. However, instead of the expected decrease in density between the first (Station 1) and last stations (Station 5), there was a slight increase in density. This increase may be attributed to the fact that Río Cagüitas (Station 3) usually had a much higher density of coliforms than El Caño and therefore greatly increased their density in Stations 4 and 5 (See Fig. 5 and 6).

Several other trends could be detected. A foreseeable result is that the total coliforms had a higher density of

Table 2. Coefficients for the correlation of the physico-chemical parameters with the logarithm of fecal or total coliforms. A stepwise multiple regression-correlation analysis with forward inclusion proved that none of the parameters were significantly correlated to either coliform group (p=0.05).

	Dependent Variable	
Independent Variable	Logarithm Fecal Colif.	Logarithm Total Colif.
Dissolved Oxygen	-0.60	-0.48
Temperature	0.53	0.37
Light	-0.03	0.12
$NO_2^{=}/NO_3^{-}$	0.44	0.64
NH_4^{+}	-0.50	-0.68
Discharge	-0.34	-0.55
Chlorine	-0.62	-0.50



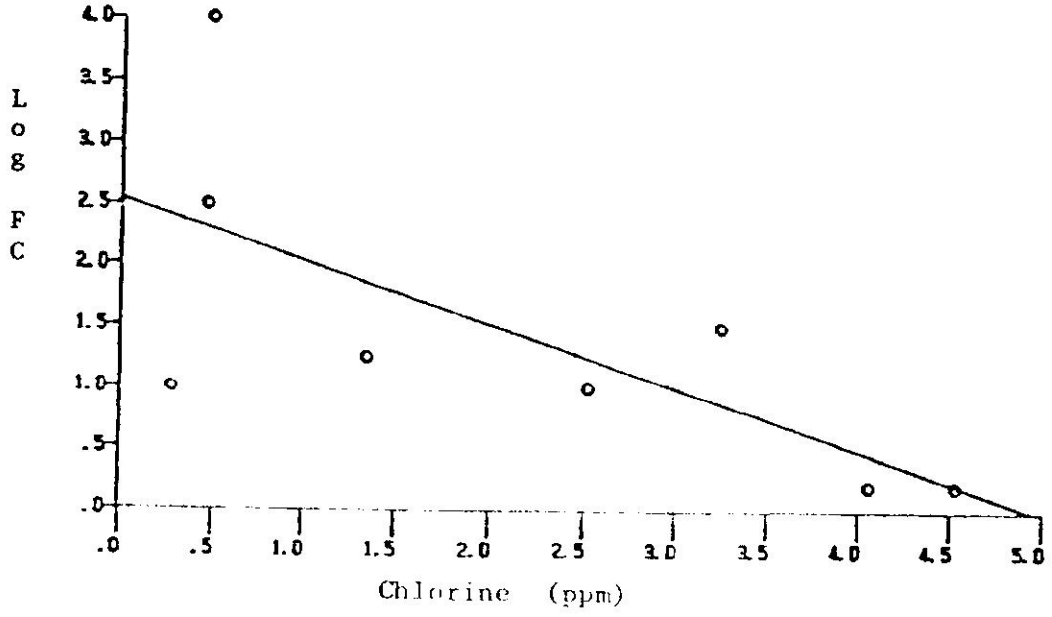


Fig. 4. Correlation of chlorine with the logarithm of coliforms in the Sewage Treatment Plant outfall. Neither correlation was significant (p=0.05).

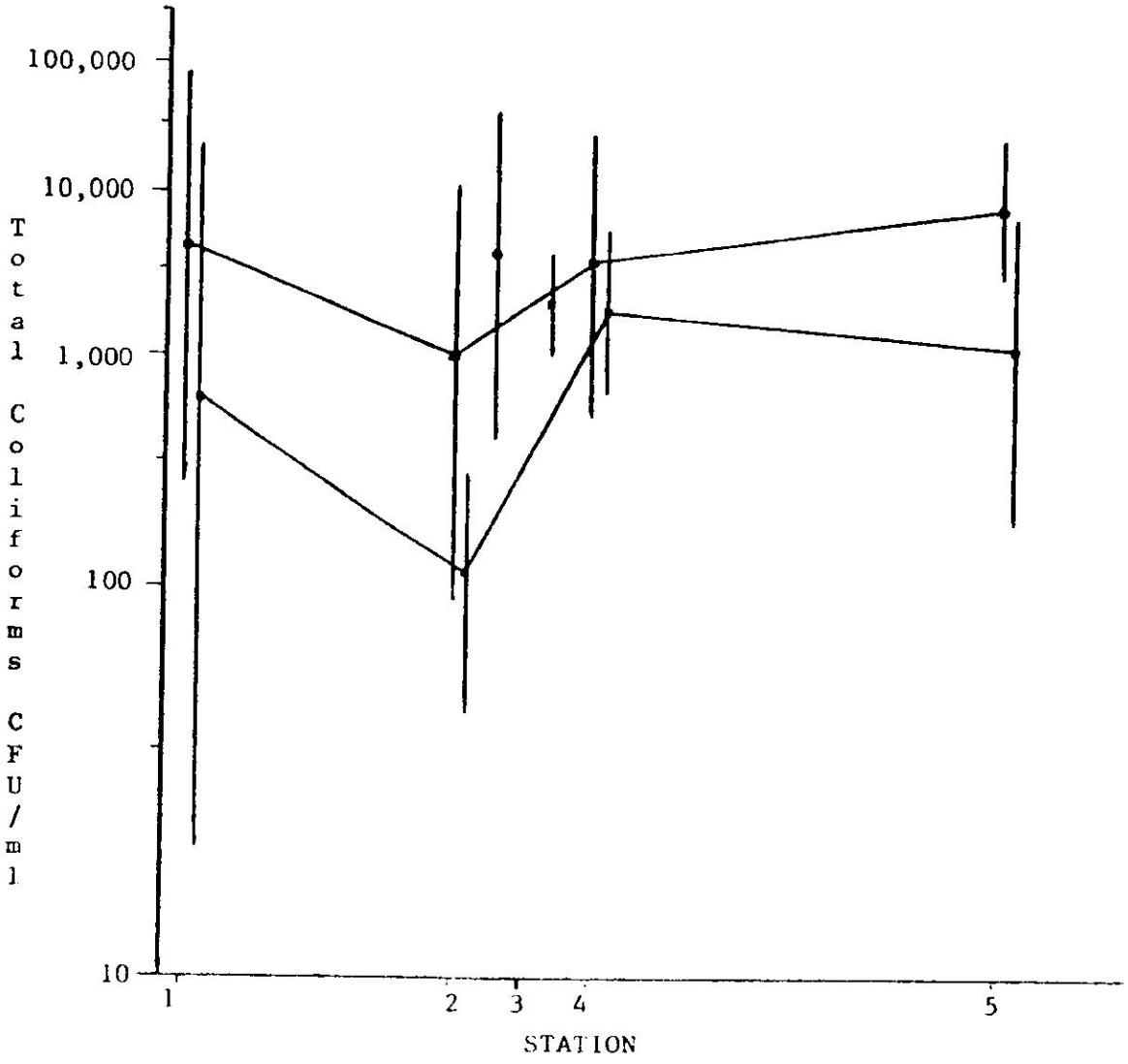


Figure 5. Total coliform density in the Cagüitas River and El Caño. Values represent the average for each sampling station with one standard deviation for daytime (•) and nightime (■) samplings.

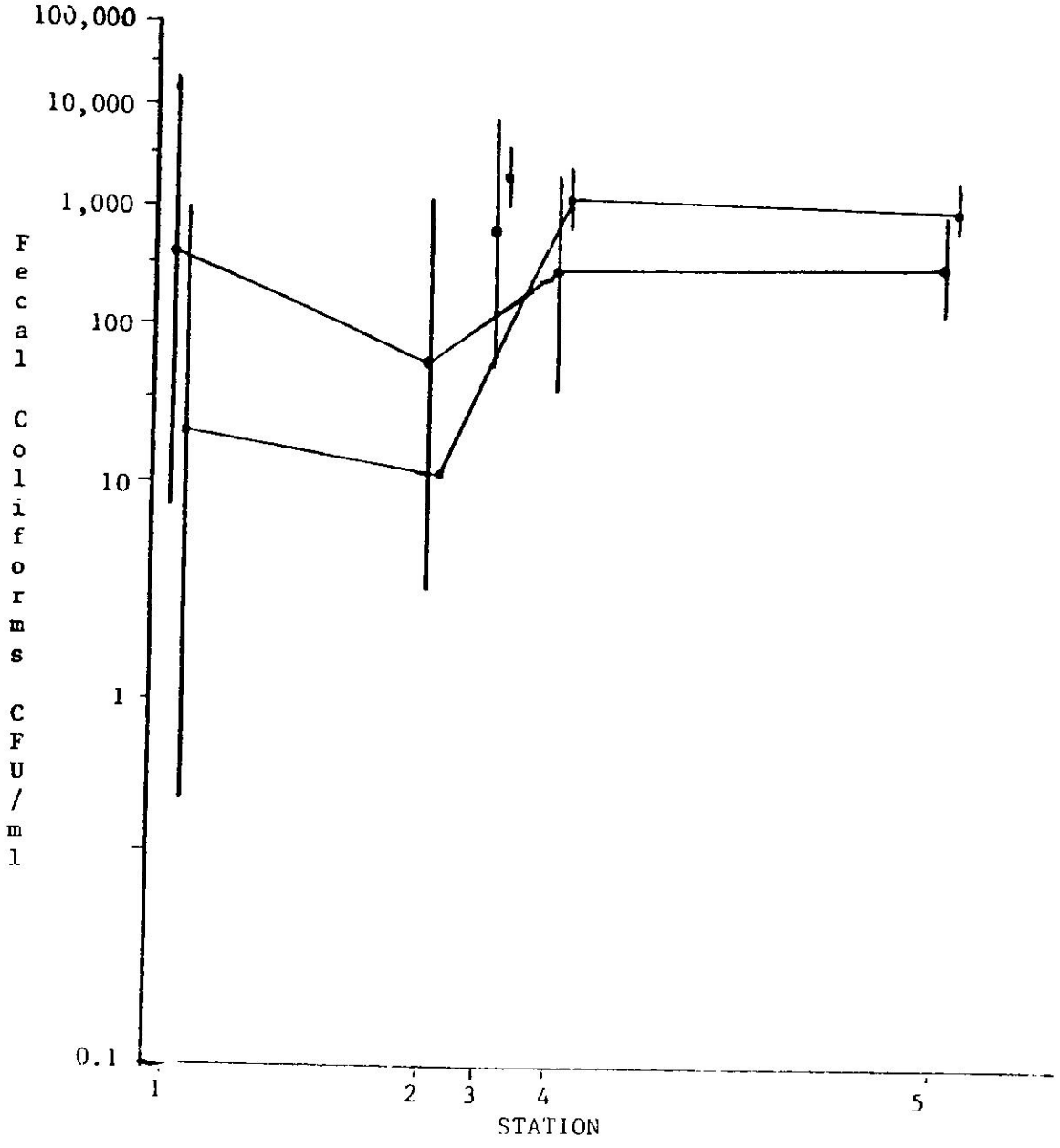


Figure 6. Fecal coliform density in the Cagüitas River and El Caño. Values represent the average for each sampling station with one standard deviation for daytime (●) and nighttime (■) samplings.

bacteria than the fecal coliforms. Contrary to what was expected, the average density of both groups of bacteria was greater during the daytime samplings than during the nightime samplings.

An ANOVA analysis was carried out to determine if the stream segment, the group of bacteria, or the hour of sampling affected the change in the logarithm of coliform bacteria between adjacent stations (See Table 3). Of the patterns that were apparent in the graphs only the difference between stations was statistically significant ($p<0.05$), thereby proving that the increases or decreases in the density of coliforms were not the same for all reaches of the river. Since there was no effect of the hour of sampling, the apparent differences between daytime and nightime samplings that could be observed in Fig. 5 and 6 were not statistically significant. Likewise, the apparent differences in the change in the number of bacteria measured by the two coliform groups were not substantial.

As expected, the logarithms of the two groups of bacteria (FC and TC) were highly correlated (See Fig. 7). The logarithm of TC accounted for 64.5% of the variation of the logarithm of FC.

Table 3. ANOVA used to examine the effect of the stream segment, the hour of sampling, and the group of bacteria on the change in the logarithm of coliform bacteria between adjacent stations. The stream segments were classified into the areas between Station 1 and 2, 2 and 3, 3 and 4, and 4 and 5. The hour of sampling was classified into daytime and nightime, and the group of bacteria was classified into total and fecal coliforms. The dependent variable was the difference in the logarithm of coliforms between these stations.

Source of Variation	Sum of Squares	DF	Mean Square	F	Signif. of F
Hour	0.69	1	0.69	0.63	0.432
Segment	29.00	3	9.67	8.78	0.000
Group	0.12	1	0.12	0.11	0.746
Two-Way Interactions:					
Segment and Hour	3.19	3	1.06	0.97	0.416
Segment and Group	1.74	3	0.58	0.53	0.665
Hour and Group	0.78	1	0.78	0.71	0.403
Three-Way Interactions:					
Segment, Hour and Group or Error	0.10	3	0.03	0.03	0.992
Residual	52.83	48	1.10		
Total	88.45	63	1.40		

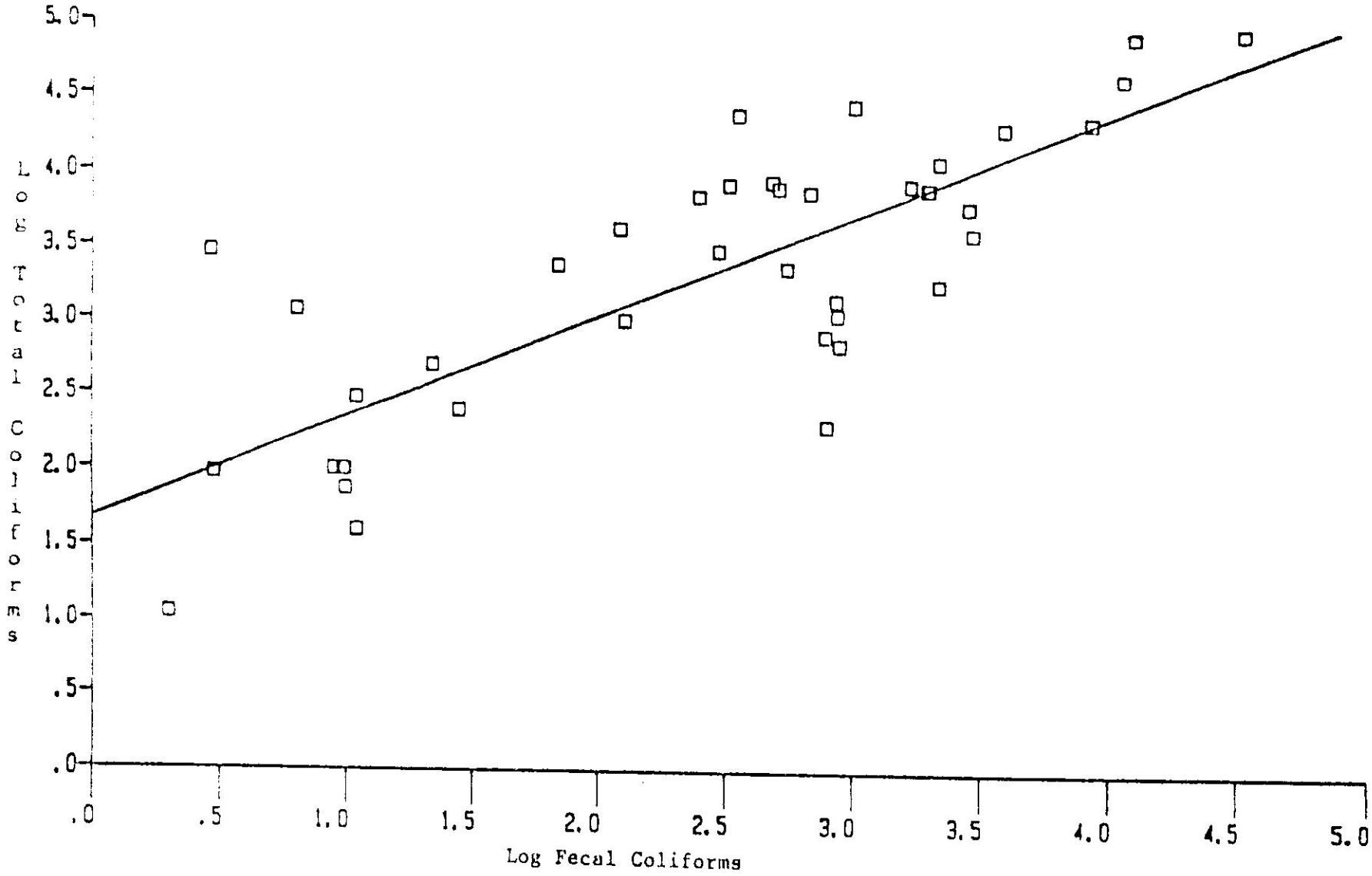


Figure 7. Correlation of the logarithm of the total coliforms with the logarithm of the fecal coliforms. The relationship was highly significant (r^2 = 0.645, p 0.01).

DISCUSSION

Water Quality

The results of this study agree with the findings of another study carried out in Río Cagüitas (Quiñones-Márquez, 1980). The values of dissolved oxygen, chloride, and total and fecal coliforms detected in both studies were in the same range. But the values obtained for $NO_2^{=}/NO_3^{-}$ and especially for NH_4^{+} were much higher than the values reported by Quiñones-Márquez. The averages obtained in this study were 0.41 and 1350 ppm, respectively, whereas the averages obtained by Quiñones-Márquez were approximately 2.2 and 4 ppm, respectively. This discrepancy could be due to the fact that they only sampled the Cagüitas upstream from its merger with El Caño (equivalent to Station 3 in this study). This station generally had a lower concentration of NH_4^{+} than the other stations. Another possible explanation might be found in the large fluctuation in these parameters with each sampling (See Table 4). Whereas in one sampling the NH_4^{+} concentration was 87 ppm, the next sampling it was 2290 ppm. With such large fluctuations, the discrepancy could

be explained. Of course, the best explanation may be that the quality of the river has worsened since Quiñones-Márquez studied it.

The high concentrations of nutrients, low concentrations of dissolved oxygen, and the high densities of coliforms found in Río Cagüitas and El Caño corroborate Quiñones-Márquez's (1980, p.51) conclusion that "... the quality of its water ranks among the worst in Puerto Rico." The similarity in the values obtained in both studies proves that the quality of the river has not improved in four years despite a doubling of the STP's capacity in 1983. In fact, it appears to have worsened as far as NH_4^+ and $NO_2^=/NO_3^-$ are concerned. A disturbing conclusion that can be drawn from these data is the fact that Río Cagüitas, which does not receive STP effluent, is equally as polluted as El Caño, whose waters are composed almost solely by the effluent from a sewage treatment plant.

Coliform Population Dynamics

The two groups of coliforms, the fecal and total coliforms, behaved as expected in detecting fecal pollution (APHA, 1980). A lower number of bacteria grew on the fecal coliform medium than in the total coliform because it is a more selective medium. On the other hand, the number of bacteria detected by the two plating media were always proportionate because they both measure fecal pollution.

Although there was a significant effect of the stream segment on the density of coliforms detected, it was impossible to detect any clear-cut die-off or regrowth of the bacteria. The decrease in the density of coliforms between Stations 1 and 2 suggests that the bacteria released by the STP experienced die-off. However, the overall trend inthese streams was an increase in the density of bacteria, as indicated by the increase between the STP and Station 5. An ANOVA showed that the stream segment significantly affected the change in coliform bacteria (See Table 3). This result is probably due to the fact that Río Cagüitas had such a higher density of bacteria than El Caño that it resulted in a change in density between Stations 2 and 3 that was significantly different from the other segments. Therefore, it is impossible to tell whether the increase between Stations 1 and 5 was due to the addition of bacteria by the Caguitas or the regrowth of chlorine-injured cells.

To determine if the bacteria released by the STP experienced die-off or regrowth, a mathematical calculation must be carried out to correct for the number of bacteria added by Río Cagüitas (Velz, 1970). This study attempted to carry out this correction using the chloride that is naturally found in these waters as a conservative tracer, but the calculation was not successfull apparently due to the addition of sub-surface flow that diluted the chloride.

Effect of Nutrients and Chlorine

Neither NH_4^+, nor $NO_2^=/NO_3^-$, nor chlorine affected the density of the coliform bacteria. This result does not agree with a study conducted in Puerto Rico that found a correlation between $NO_2^=/NO_3^-$ and the fecal coliforms (Hazen and Aranda, 1980). However, the authors hypothesized that this relationship is indirect and is due to a direct symbionism between algae and the coliforms. Their hypothesis is corroborated by other studies (Cole, 1982).

Hazen and Aranda studied a clear mountain stream where there should be an abundant population of algae. Río Cagüitas and El Caño, on the other hand, which carry a heavy load of suspended and dissolved solids (Curtis, et al., 1984) which filters out sunlight and may inhibit algae from growing. Therefore, the correlation of nutrients with coliform density should not be expected in these streams.

A surprising result is the lack of an effect of chlorine on the density of the coliforms. It was not correlated with the density of either group of bacteria in the STP outfall. The lack of correlation shows that the plant's operators applied excessive quantities of chlorine to the effluent. The concentration of total chlorine in the outfall surpassed 3.0 ppm more than once. This excess did not cause a proportionate decrease in the density of coliforms but instead may have caused damage to the stream's biota (Esvelt, et al., 1973).

Effect of Sunlight

Sunlight did not affect the dynamics of the coliforms in this stream. The non-significant effect of the hour of sampling on the change in the density of the bacteria between stations indicates that there was no difference between day and night samplings (See Table 3). In addition, the average density of both groups was higher during the daytime than during the nightime (See Fig. 5 and 6). If sunlight had acted as a bactericidal agent as suggested by Grigsby and Calkins (1980), then the coliform density should be lower during the daytime. Therefore, sunlight was not an effective bactericidal agent in this stream.

Sunlight's bactericidal action has only been proven in highly controlled experiments so it is not too surprising that no effect was found in this experiment. The turbidity and turbulence of the stream's water may attenuate the effect of sunlight by filtering out the UV rays and moving the bacteria to the bottom of the stream where the UV radiation does not penetrate. Tuveson and March (1980) found that clear water filtered out UV rays and attenuated its lethal effect. The reduction of UV lethality should be even greater in these streams that carry a heavy load of dissolved and suspended solids (Curtis, et al., 1984).

This finding demonstrates that the hypothesis put forth by Chamberlin and Mitchell (1978) that sunlight is the most important factor in the mortality of coliform

bacteria released into the ocean cannot be extrapolated to all streams. El Caño and Río Cagüitas do not behave as expected probably due to the high degree of turbity of their waters.

CONCLUSIONS AND RECOMMENDATIONS

The results of this study show that there was no significant effect of sunlight on the population dynamics of coliform bacteria released into El Caño. There were also no effect of nutrients or of chlorine. In addition, the water quality of these two streams was extremely poor.

It would be useless to suggest ways to enhance the bactericidal effect of sunlight when the water quality of both streams is so poor. Sunlight-induced mortality should be used as a second line of defense against fecal pollution; the first line of defense must be the efficient functioning of the sanitary system. Not only is the sewage treatment plant not functioning correctly, but the Cagüitas receives pollution from sources other than the STP that give it a higher degree of fecal pollution than El Caño.

The first step in correcting the gross pollution of this area is to identify and eliminate the source or sources of fecal pollution in Río Cagüitas. The next step must be to increase the efficiency of Cagüas' STP, which now barely provides primary treatment. Only when this plant removes the high load of solids in its effluent can sunlight begin to be a contributing factor in the control of indicator and pathogenic bacteria.

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APPENDIX

Table 4a. Sampling results. NA represents missing values.

Date 3/21/84-Diurnal
STP Discharge (m^3/hr) 900
Chlorine Applied (kg/hr) 7.56

Parameter	Station				
	1	2	3	4	5
Time	0930	1050	0905	1035	1010
Fecal Coli. (CFU/ml)	3	6.4	4000	490	1030
Log Fecal Coli.	0.48	0.81	3.60	2.69	3.01
Total Coli. (CFU/mL)	94	1160	20000	8800	28000
Log Total Coli.	1.97	3.06	4.30	3.94	4.45
Dissolved Oxygen (ppm)	5.60	4.00	2.80	2.60	2.80
Temperature (^oC)	27.0	28.0	25.5	26.0	26.0
Oxygen Saturation (%)	68	51	34	32	34
Light Inten. (Candle/ft^2)	500	2500	1600	3400	1200
$NO_2^=/NO_3^-$ (ppm)	0.53	0.80	0.74	0.36	0.26
NH_4^+ (ppm)	52.0	71.4	9.9	18.8	38.2
Cl^- (ppm)	59	58	39	55	48
Chlorine (ppm)	4.00	NA	NA	NA	NA

Table 4b. Sampling results. NA represents missing values.

Date 3/28/84-Diurnal
STP Discharge (m^3/hr) 792
Chlorine Applied (kg/hr) 9.45

Parameter	Station				
	1	2	3	4	5
Time	1010	1130	0945	1115	1045
Fecal Coli. (CFU/mL)	330	10	28	<10	70
Log Fecal Coli.	2.52	1.00	1.45	<1	1.85
Total Coli. (CFU/mL)	8400	75	250	<100	2400
Log Total Coli.	3.92	1.88	2.40	<2	3.38
Dissolved Oxygen (ppm)	3.50	3.40	3.40	1.80	1.80
Temperature (^oC)	28.3	29.2	28.0	28.9	28.1
Oxygen Saturation (%)	44	44	43	23	23
Light Inten. (Candle/ft^2)	800	78	570	2900	2700
$NO_2^=/NO_3^-$ (ppm)	0.83	0.51	0.31	0.27	NA
NH_4^+ (ppm)	83.6	34.2	14.8	26.1	NA
Cl^- (ppm)	71	73	47	69	NA
Chlorine (ppm)	1.00	NA	NA	NA	NA

Table 4c. Sampling results. NA represents missing values.

Date 4/8/84-Diurnal
STP Discharge (m^3/hr) 890
Chlorine Applied (kg/hr) 4.54

Parameter	Station				
	1	2	3	4	5
Time	0930	1000	0850	1030	1100
Fecal Coli. (CFU/mL)	34000	122	128	250	360
Log Fecal Coli.	4.53	2.09	2.11	2.40	2.56
Total Coli. (CFU/mL)	91000	4200	1000	7000	24000
Log Total Coli.	4.96	3.62	3.00	3.85	4.38
Dissolved Oxygen (ppm)	3.05	2.80	2.95	1.15	1.80
Temperature (^oC)	29.4	28.0	27.0	29.0	31.0
Oxygen Saturation (%)	40	35	36	15	24
Light Inten. (Candle/ft^2)	340	250	400	2700	2800
$NO_2^=/NO_3^-$ (ppm)	0.62	0.48	0.30	0.15	0.10
NH_4^+ (ppm)	83.0	73.0	110.0	150.0	68.0
Cl^- (ppm)	73	74	48	73	65
Chlorine (ppm)	0.20	NA	NA	NA	NA

Table 4d. Sampling results. NA represents missing values.

Date 5/2/84-Diurnal
STP Discharge (m^3/hr) 792
Chlorine Applied (kg/hr) 5.67

Parameter	Station				
	1	2	3	4	5
Time	0940	1010	0910	1030	1100
Fecal Coli. (CFU/mL)	22	9.0	12700	690	520
Log Fecal Coli.	1.34	0.95	4.10	2.84	2.72
Total Coli. (CFU/mL)	500	100	85000	7500	8000
Log Total Coli.	2.70	2.00	4.93	3.88	3.90
Dissolved Oxygen (ppm)	3.75	4.20	4.35	1.75	1.60
Temperature (^oC)	29.0	30.0	28.0	29.0	29.0
Oxygen Saturation (%)	48	55	55	22	21
Light Inten. (Candle/ft^2)	2000	500	1000	2500	1500
$NO_2^=/NO_3^-$ (ppm)	0.87	1.06	0.34	0.28	0.17
NH_4^+ (ppm)	140.0	150.0	180.0	NA	NA
Cl^- (ppm)	64	64	60	62	68
Chlorine (ppm)	1.25	NA	NA	NA	NA

Table 4e. Sampling results. NA represents missing values.

Date 5/9/84-Diurnal
STP Discharge (m^3/hr) 946
Chlorine Applied (kg/hr) 7.18

Parameter	Station				
	1	2	3	4	5
Time	0930	1005	0910	1030	1045
Fecal Coli. (CFU/mL)	11500	8700	560	2200	300
Log Fecal Coli.	4.06	3.94	2.75	3.34	2.48
Total Coli. (CFU/mL)	43000	22000	2300	12000	3000
Log Total Coli.	4.63	4.34	3.36	4.08	3.48
Dissolved Oxygen (ppm)	NA	NA	NA	NA	NA
Temperature (^{o}C)	NA	NA	NA	NA	NA
Oxygen Saturation (%)	NA	NA	NA	NA	NA
Light Inten. (Candle/ft^2)	300	2000	3000	3500	1000
$NO_2^{=}/NO_3^{-}$ (ppm)	0.64	0.61	0.12	0.23	0.08
NH_4^{+} (ppm)	48.0	150.0	38.0	100.0	68.0
Cl^{-} (ppm)	65	66	50	73	69
Chlorine (ppm)	0.20	NA	NA	NA	NA

Table 4f. Sampling results. NA represents missing values.

Date 6/20/84-Nocturnal
STP Discharge (m^3/hr) 890
Chlorine Applied (kg/hr) 8.98

Parameter	Station				
	1	2	3	4	5
Time	2052	2250	2310	2132	2200
Fecal Coli. (CFU/mL)	1710	11	2200	900	800
Log Fecal Coli.	3.23	1.04	3.34	2.95	2.90
Total Coli. (CFU/mL)	8500	300	1800	700	200
Log Total Coli.	3.93	2.48	3.26	2.85	2.30
Dissolved Oxygen (ppm)	NA	NA	NA	NA	NA
Temperature (^oC)	NA	NA	NA	NA	NA
Oxygen Saturation (%)	NA	NA	NA	NA	NA
$NO_2^=/NO_3^-$ (ppm)	0.11	0.15	0.83	0.56	0.46
NH_4^+ (ppm)	185.0	162.0	87.0	116.0	128.0
Cl^- (ppm)	75	79	46	64	63
Chlorine (ppm)	1.50	NA	NA	NA	NA

Table 4g. Sampling results. NA represents missing values.

Date 6/28/84-Nocturnal
STP Discharge (m^3/hr) 1003
Chlorine Applied (kg/hr) 3.92

Parameter	Station				
	1	2	3	4	5
Time	2110	2245	2315	2154	2215
Fecal Coli. (CFU/mL)	1.9	11.2	3000	2900	2000
Log Fecal Coli.	0.28	1.05	3.48	3.46	3.30
Total Coli. (CFU/mL)	11	40	4000	6000	8000
Log Total Coli.	1.04	1.60	3.60	3.78	3.90
Dissolved Oxygen (ppm)	3.50	3.80	2.20	1.00	2.00
Temperature (^oC)	29.0	28.0	27.0	28.0	28.0
Oxygen Saturation (%)	45	48	27	13	25
$NO_2^=/NO_3^-$ (ppm)	0.10	0.24	0.29	0.26	0.22
NH_4^+ (ppm)	3940	2960	2290	14800	5260
Cl^- (ppm)	91	85	94	53	64
Chlorine (ppm)	1.00	NA	NA	NA	NA

Table 4h. Sampling results. NA represents missing values.

Date 7/2/84-Nocturnal
STP Discharge (m^3/hr) 1003
Chlorine Applied (kg/hr) 3.92

Parameter	Station				
	1	2	3	4	5
Time	2100	2255	2315	2125	2150
Fecal Coli. (CFU/mL)	2.9	9.8	880	870	790
Log Fecal Coli.	0.46	0.99	2.94	2.94	2.90
Total Coli. (CFU/mL)	2900	100	1100	1400	800
Log Total Coli.	3.46	2.00	3.04	3.15	2.90
Dissolved Oxygen (ppm)	3.50	4.00	1.70	2.00	1.70
Temperature (^oC)	29.0	29.0	28.0	30.0	29.0
Oxygen Saturation (%)	45	51	22	26	22
$NO_2^=/NO_3^-$ (ppm)	NA	NA	NA	NA	NA
NH_4^+ (ppm)	1300	2330	NA	5650	7680
Cl^- (ppm)	NA	NA	NA	NA	NA
Chlorine (ppm)	2.50	NA	NA	NA	NA