EVALUATION OF TOTAL COLIFORM, FECAL COLIFORM, AND FECAL STREPTOCOCCI AS ADEQUATE INDICATORS IN MONITORING PUBLIC WATER SUPPLY QUALITY IN THE TROPICS

by

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DEDICATION

When I want to discover something, I begin by reading up everything that has been done along that line in the past - that's what all the books in the library are for. I see what has been accomplished at great labor and expense in the past. I gather the data of many experiments as a starting point and then I make many more. The three essentials to achieve anything worthwhile are, first, hard work; second, stick-to-it-iveness; third, common sense.

-Thomas A. Edison

To those who have thought they can't.

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ABSTRACT

Previous studies in tropical areas have demonstrated that high false positive and negative errors were commonplace when determining whether fecal contamination of source water has occurred. Other studies had shown that microbial pathogens can have higher survival rates relative to standard indicator bacteria. These violations to the criteria used to select an ideal fecal contamination indicator organism proposed doubts about the use of standard techniques in determining the quality of tropical public water supply systems.

To determine if Escherichia coli was an adequate indicator of tropical drinking water quality, influents and effluents from four water treatment plants in Puerto Rico were tested weekly for a three month period. Membrane filtration and multiple tube fermentation techniques were used to detect total coliforms, fecal coliforms, and fecal streptococci on appropiate selective media. All presumptive positive results were confirmed according to standard methodology. From each sample, an appropiate percentage of organisms giving confirmed fecal

coliform results were isolated and identified. Total bacterial densities were determined by acridine orange direct counts.

Results show that while plant effluents generally complied with current federal regulations, high densities of bacteria were present in drinking water. Among these bacteria, injured (viable but unculturable) coliforms were detected by m-T7 agar. High levels of false positive results were found for both the raw and drinking water samples. Turbidity levels apparently affected the disinfection process, the normal expression of indicator bacteria in standard media, and the methodologies used to assess water quality. Neither coliforms nor fecal streptococci were adequate indicators of tropical water quality.

INTRODUCTION

In recent years, the number of cases of waterborne diseases in Puerto Rico has increased every year (Puerto Rico Department of Health, 1986). The most probable cause for this, according to the United States Environmental Protection Agency (EPA), the Environmental Quality Board (EQB) and the Puerto Rico Acqueduct and Sewer Authority (PRASA), is the over capacity and antiquity of the island's water treatment and purification plants, as well as the increased quantities of raw sewage and other pollutants being discharged into the island's water supplies (Ruiz de la Mata, 1985). The mixture of pathogenic bacteria and industrial pollutants, along with the possible ineffectiveness of the water treatment process represent an increased risk of waterborne disease epidemics.

The standard techniques being used to assess the microbiological quality of treated water (coliform bacteria assays) were developed for use in temperate climates, where the target indicator organism, Escherichia coli, is thermotolerant, whereas the environmental bacterial flora is not. In the tropics, most assumptions about the indicator organism become invalid since some environmental flora can withstand the higher temperatures used to recover E. coli, thus resulting in high numbers of false positives (Santiago- Mercado and Hazen, 1987). Studies made in the Microbial Ecology Laboratory of The University of Puerto Rico have shown an increased pathogen survival rate relative to regrowth of the indicator organism in the environment (López-Torres, 1982; Hazen et al., 1982; Biamón and Hazen, 1983).

Accurate determination of the level of biological contamination is extremely important in tropical areas, since these areas possess larger numbers of waterborne diseases. This determination is more difficult in tropical source water than it is in temperate waters due to several factors. Among the physico-chemical variables that affect accurate determinations of biological pollution in the tropics, we find temperature, high organic content, high light intensity, and heavy rainfall (Hazen, 1988).

In temperate climates, water temperature ranges from freezing to 30°C, while tropical water temperature may be as high as 45° and never goes below 15°C (Hill and Rai, 1982). In the tropical forests of Puerto Rico, water temperature ranged between 18° and 24°C throughout the year (Carrillo et al., 1985; López et al., 1987). This thermal environment results in permanently stratified (oligomictic) water reservoirs as well as lower amounts of dissolved oxygen relative to temperate waters (Hutchinson, 1977). In Puerto Rico, large diurnal variations (1 to 8 mg/L) in the dissolved oxygen content of water have been measured (López-Torres et al., 1987; Carrillo et al., 1985; Pérez-Rosas and Hazen, 1988). This indicates that tropical source waters become anoxic faster than those of temperate climates, and thus, few organisms apart from mesophilic, facultatively anaerobic bacteria can thrive in the environment (Hazen, 1988; Carrillo et al., 1985).

Light intensity in the tropics can adversely affect our perception of the levels of indicator bacteria present in tropical source water. The constantly high light intensity, along with high temperatures, cause constant hypereutrophy of tropical surface waters. The higher densities of naturally occurring bacteria in tropical waters can limit the reliability

of assays that employ viable counts to determine the level of biological water contamination. Tropical source waters have a tendency to overgrow on standard media (López-Torres et al., 1987; Santiago-Mercado and Hazen, 1987). Also, non-indicator (background) bacteria can produce bacteriocins that inhibit the growth or the typical appearance of indicator bacteria on standard media (Means and Olson, 1981; Burlingame et al., 1986).

Tropical rainfall also affects dramatically the microbial content of water. Some watersheds in Puerto Rico may receive more than 10 cm of rain in 24 h (Carrillo et al., 1985). Heavy rainfall changes the nutrient and microbial content of water reservoirs by flushing the surrounding land and vegetation into the reservoir (Carrillo et al., 1985; Hill and Rai, 1982; Oluwande et al., 1983). The washing of soil into streams and lakes also increases the turbidity of reservoirs. Studies in Africa, Puerto Rico, and Hawaii have clearly demonstrated that increases in total bacterial densities coincide with increasing rainfall (Barrel and Roland, 1979; Oluwande et al., 1983; Carrillo et al., 1985; Fujioka and Shizumura, 1985).

When discussing the variables that make tropical water quality so different from that of temperate water, we must also take into consideration the fact that the quantity and quality of bacterial resources are different between these two environments. The hypereutrophic state of tropical waters, combined with the higher temperatures, create a high organic content environment dominated by mesophilic and thermotolerant microbes (Hill and Rai, 1982; Santiago-Mercado and Hazen, 1987). This microflora is similar to that of the gut of animals (Hazen, 1988). Tropical waters also have higher numbers of

both autochtonous and allochtonous microbes (Hill and Rai, 1982). The combination of the particular microbial population and environmental conditions of tropical source waters result in microbial survival and activity rates that are totally different from those of temperate waters (Hazen, 1988; McFeters and Stewart, 1972).

In the tropics there are a lot more pathogenic microorganisms than there are in temperate climate zones. In fact, many tropical diseases are totally unknown in temperate climates. Therefore, microorganisms to be used as indicators of tropical water contamination must cover a wider range and diversity of pathogens. However, tropical nations have adopted regulations developed for industrialized temperate nations without testing their validity, or the validity of techniques used to assess water quality under these regulations, in their particular circumstances. This often results in these nations not being able to meet their own water quality standards (Hazen, 1988). This problem arises in part from a number of social and economic factors. Although 65% of the world's population lives in the tropics, these people actually have less than 10% of the the world's wealth (Odum, 1971). Since resources are limited, funding for the costly research needed to find better indicators of water pollution are virtually non-existent.

False estimates of contamination can be avoided by studying the methods currently being used for water analysis in Puerto Rico as well as the environmental flora present in source water and in finished drinking water. Studies of this nature would reduce the possibilities of waterborne disease epidemics by allowing us to understand better what bacterial contamination indicators are telling us. Results of this study

could be used to modify existing regulations regarding the bacterial quality of finished drinking water in tropical climates.

STATEMENT OF PROBLEM:

Tropical waters have been identified as carriers of a large number of pathogenic microorganisms. In Puerto Rico, water reservoirs receive large quantities of industrial, agricultural, and domestic sewage effluents which, when combined with the potentially pathogenic microbes in the environment, represent an increased risk of waterborne disease outbreaks. The techniques commonly used to examine the microbiological quality of drinking water rely on assumptions made about the target indicator organism (Escherichia coli), especially thermotolerance (Bonde, 1977). These assumptions may be invalid in tropical climates. Regrowth of the indicator organism, as well as lower pathogen survival rates have been observed in tropical areas (Carrillo et al., 1985; López-Torres et al., 1987; Hagler et al., 1986; Fujioka et al., 1981; Lavoie, 1983; Hazen et al., 1982; Hazen et al., 1987; Santiago-Mercado and Hazen, 1987).

Water quality in Puerto Rico is a major problem. Various types of effluent, such as domestic and municipal sewage, industrial effluents and agricultural effluents and run-offs, are being discharged into most of the island's rivers and coastal areas. In 1978, the United States Water Resources Council reported that 96% of all samples collected from 24 sampling stations throughout the island violated the total coliform standards. The United States Geological Survey has since reported that 80.6% of all water sampling stations in Puerto Rico violated the recommended maximum contaminant levels for recreational waters (<1000 fecal coliforms / 100 ml) (Curtis et al., 1984). As contamination

of public water supplies increases, so has the number of cases of gastroenteritis (possibly waterborne) (Puerto Rico Department of Health, 1985). In 1976, improper chlorination of the drinking water supply in the town of Comerío resulted in 7,800 cases of gastroenteritis (U. S. Water Resources Council, 1978). During the summer of 1987, an outbreak of gastroenteritis and dysentery was documented in the town of Yauco (Puerto Rico Department of Health, 1987). The cause of the outbreak was linked to the drinking water supply from the water purification plant at Ranchera Ward, and, in fact, once the plant was cleaned and water flow returned to normal, the outbreak subsided (Puero Rico Department of Health, 1987; EPA, 1987). Deteriorating water quality may be the source of the marked increase in cases of gastroenteritis, dysentery, and other undiagnosed diseases being reported by The Puerto Rico Department of Health (from 29,455 cases in 1984 to approximately 73,722 in 1988).

Many common bacteria of water, such as Klebsiella pneumoniae, Aeromonas hydrophila, Legionella pneumophila, Pseudomonas spp., Vibrio cholerae, Vibrio vulnificus, and Vibrio parahaemolyticus, as well as their indicator, Escherichia coli, are potential pathogens of man (Hazen et al., 1987). Many of these organisms can be isolated from polluted and unpolluted waters in Puerto Rico, although not all of them have been linked to waterborne disease occurrence (Hazen et al., 1987). Almost all pathogens are transmitted to water by fecal contamination. For this reason, bacteria found exclusively and universally in feces are assayed as indicators of possible pathogens in the water. Coliform bacteria assays are the most practical, since assaying for the presence of pathogens themselves is costly, difficult, and time consuming. The

pathogens may be in extremely low quantities in the water (which makes it difficult to isolate and identify them) and still be infective.

Coliform bacteria have been traditionally used as indicators of possible fecal contamination, since they were thought to be present only when feces had been deposited in the water (Hutchinson and Ridgway, 1977). Coliforms are subdivided into total and fecal coliforms. All are gram negative, non-spore forming, aerobic or facultatively anaerobic bacilli that ferment lactose at 35°C, producing gas (Bergey's Manual, 1974). More than 50 bacterial species have positive coliform reactions and can be found in the feces of warm blooded animals. Fecal coliforms also possess a characteristic higher thermal tolerance; that is, the ability to ferment lactose at 44.5°C. This characteristic allows the isolation of the group through the use of elevated temperature tests. E. coli, Citrobacter freundii, Enterobacter cloacae, and Klebsiella pneumoniae are members of this group (Bergey's Manual, 1974). Assays for fecal coliforms usually focus on Escherichia coli, first described in 1855 by Escherich. E. coli is found in high densities in the feces of warm blooded animals. This bacterium is the major component of both the total and fecal coliform group (Lavoie, 1983), and is nearly always found in fecal contaminated water. However, other coliforms may be found even when E. coli is absent. E. coli is also the coliform most affected by water treatment procedures (APHA, 1985). Among the most common coliforms found in both raw and treated water supplies, Enterobacter species are the most prevalent. Other genera of common occurrence, are Citrobacter and Klebsiella.

The underlying principles governing good indicator bacteria (as stated by Bonde in 1977) are:

1. Indicator bacteria must be present whenever pathogens are present.
2. Indicator bacteria must be present only when the pathogens are present.
3. Indicator bacteria must be significantly more abundant than pathogens.
4. Indicator bacteria must resist both the environment and desinfectants better than pathogens.
5. Indicator bacteria must grow easily on relatively simple media.
6. Indicator bacteria must yield characteristic reactions so they can be unambiguously identified.
7. Indicator bacteria must be randomly distributed in the water.
8. Indicator bacteria must grow independently of other organisms on artificial media.

The problem of using temperate water quality standards in the tropics has long been a source of discussion. In a study conducted by Feachem (1974) on the waters of New Guinea, fecal coliforms (FC) and fecal streptococci (FS) levels were greater than 100 CFU/100 ml, rendering all sites unacceptable as drinking water sources. He concluded these sites were grossly contaminated with feces. Yet FC and FS densities were lowest in areas with high human population. Evison and James (1975) reviewed literature from various tropical areas and concluded that E. coli densities at these areas did not correlate to sources of fecal contamination. Several other studies have demonstrated that supposedly universal indicators of fecal contamination were inappropriate for use as such in the tropics. These include studies showing no correlation between the indicator organisms

and the presence of Salmonella sp. in water (Wright, 1982a; Thomson, 1981); studies reporting high coliform and streptococci densities in non-contaminated streams (Fujioka and Shizumura, 1985); studies reporting the occurrence of alarmingly high coliform densities upstream from sewage contamination sites (Oluwande et al., 1983); and studies confirming the low accuracy of standard media for the isolation of E. coli from tropical source water (Lavoie, 1983).

In temperate waters, E. coli fits most of Bonde's principles. The assaying methods for this particular bacterium take advantage of its thermotolerance as opposed to the lack of thermal tolerance of the environmental bacterial flora to isolate it in growth media. In the tropics, this could be invalid, since components of the normal environmental flora can also be thermotolerant. Enteric organisms, being adapted to the high temperatures of the gut of warm blooded animals (usually around 37°C), can survive and grow in tropical waters (Bigger, 1937; Ragavachari and Iyer, 1939), especially since these waters are high in nutrient concentrations (Hazen et al., 1982; Hazen and Aranda, 1981). Studies have shown that E. coli can persist in tropical freshwaters (Carrillo et al., 1985; Biamón and Hazen, 1983; Pérez-Rosas and Hazen, 1988; Hazen et al., 1982). In fact, E. coli seems to be a part of the normal aquatic flora of the tropics (Hazen et al., 1987; Bermúdez and Hazen, 1988; Fujioka and Shizumura, 1985). Few studies have been done on the activity, survival, and density of coliforms in tropical environments (Hazen et al., 1982; Biamón and Hazen, 1983; López-Torres, 1982; Ortiz-Roque and Hazen, 1983; Fuentes et al., 1983; Carrillo et al., 1985; Peele et al., 1981). It has also been shown that many coliforms can subsist as free living saprophytes (Bergey's Manual,

1974), and, as such, can multiply and form slimes inside water distribution pipes. The isolation of high densities of the same group at one site may be indicative of such growth (APHA, 1985).

The findings by The U. S. Water Resources Council and The U. S. Geological Survey (where most sites sampled by both violated the maximum contaminant levels for coliforms) resulted in the condemnation of source water in Puerto Rico. Yet recent studies (Carrillo et al., 1985; Hazen and Aranda, 1981; López-Torres et al., 1987, Santiago-Mercado and Hazen, 1987, Rivera et al., 1988; Bermúdez and Hazen, 1988) indicate that even water from pristine sites in the Caribbean National Forest exceed the maximum contaminant levels for fecal coliforms, yet less than 40% of 300 fecal coliform isolates were actually E. coli (Santiago-Mercado and Hazen, 1987). Similar studies involving the same methods in temperate climate zones (Pagel et al., 1982) demonstrated that 90% of all fecal coliform isolates were E. coli. This reveals that the environmental flora in Puerto Rico has bacteria capable of producing false positive reactions when assaying for the presence of fecal coliforms. Comparisons of four fecal coliform assay methods in Canada and Puerto Rico yielded interesting results: The specificity (ability of the medium to restrict growth of organisms other than the target bacterium) of the media in Puerto Rico was significantly lower (at least by 20%) than that recorded by Canadian investigators (Pagel et al., 1982). It seems that, in Puerto Rico, all methods have significantly higher false negative errors, even though the accuracy of methods was the same on the island as it was in Canada. Santiago-Mercado and Hazen (1987) showed that high densities of thermophilic

and mesophilic background flora in tropical waters significantly reduced the levels of E. coli on standard coliform media.

In addition, it has been shown that, in Puerto Rico, many pathogens can be found in the complete absence of E. coli. These include Candida albicans (Valdés-Collazo et al., 1987), Klebsiella pneumoniae (López et al., 1987), Legionella pneumophila (Ortiz-Roque and Hazen, 1987), Vibrio cholerae (Pérez-Rosas and Hazen, 1988), Yersinia enterocolitica (Elías et al., 1988), and Aeromonas hydrophila (Hazen et al., 1981). Under these circumstances, results from recent studies on the culturability of certain pathogens become frightening. Studies by Xu et al (1982), Colwell et al (1985), and Roszak et al (1984) have shown that E. coli, as well as pathogens such as Salmonella enteriditis and Vibrio cholerae may survive and remain pathogenic in the environment, but are unculturable on standard media. Furthermore, the presence of enteric viruses in tropical source water has also been documented in the absence of E. coli (Berg and Metcalf, 1978; Toranzos and Gerba, 1988; Keswick et al., 1985; Rose et al., 1975; Cabelli, 1983; Hejkal et al., 1982), yet viral assays are rarely performed.

The survival characteristics of E. coli in the tropics also affect its performance as an indicator organism in these environments. In 1937, Bigger was the first to report the growth of coliforms in tropical water. Then, in 1939, Ragavachari and Iyer demonstrated the survival of coliforms for several months in tropical river waters. Recent studies in Puerto Rico (Carrillo et al., 1985; López-Torres et al., 1987; Valdés-Collazo et al., 1987; Hazen et al., 1987) have demonstrated the survival and proliferation of E. coli in tropical rain forest streams. Studies have also demonstrated that under the conditions prevailing in a tropical rain

forest, Candida albicans (Valdés-Collazo et al., 1987), Klebsiella pneumoniae (López-Torres et al., 1987), Legionella pneumophila (Ortiz-Roque and Hazen, 1987), Vibrio cholerae (Pérez-Rosas and Hazen, 1988), Yersinia enterocolitica (Elías et al., 1988), Aeromonas hydrophila (Hazen et al., 1981), and Salmonella typhimurium (Jiménez et al., In Press) have survival rates different from those of their indicator, E. coli. Differences in survival rates between E. coli and pathogens in tropical source waters hamper its ability to indicate the presence of pathogens in these waters. Escherichia coli always survives much longer *in situ* in tropical waters compared to temperate waters. The increased survival rates further stress the fact that temperate drinking water regulations are unapplicable to tropical waters (Hazen, 1988).

Escherichia coli has been found on epyphytic vegetation 15 m above the ground in a Puerto Rican rain forest (Rivera et al., 1988; Bermúdez and Hazen, 1988). This points to the possibility of naturally occurring E. coli strains in some tropical environments. These environmental isolates have biochemical and physiological characteristics, plasmid profiles, coliphage susceptibilities, and antibiotic sensitivities that are very similar to those of clinical E. coli isolates (Rivera et al., 1988). In fact, the environmental isolates were found to have more than 85% DNA homology and identical mol% G+C with E. coli B. Naturally occurring E. coli could account for the results of studies showing high coliform densities in the absence of a fecal contamination source. This finding is yet another reason why, in the tropics, E. coli does not fit the underlying assumptions of a good indicator organism.

The above mentioned studies, plus the fact that coliforms have been shown to be less resistant to chlorination than some human

pathogens (Berg and Metcalf, 1978), indicate that the criteria used for determining the bacterial quality of drinking water in Puerto Rico may be incorrect; that is, fecal contamination may be detected when in fact, there is none. With this in mind, it is not surprising that we find it difficult to meet the legislated standard for drinking water as stated in the Safe Drinking Water Act (Public Law 93-523, 1974; modified in 1986 to meet changes proposed by the U.S. E.P.A. in 1983). Two possibilities remain: either to change the indicator system and / or the maximum contaminant levels used, or to enumerate pathogens directly. Studies suggest that fecal streptococci (enterococci), enumerated with selective media, may be better indicators of fecal contamination than fecal coliforms (Hazen, unpublished data).

Enterococci are a subgroup of the fecal streptococci. The group is composed of Streptococcus faecalis, S. faecalis subsp. liquefaciens, S. faecalis subsp. zymogenes and S. faecium. These organisms are gram positive, non-spore formers that have a spherical shape and are arranged in pairs or chains (Bergey's Manual, 1974). They have been used as secondary indicators of water quality, but the problem with coliforms has renewed interest in them as primary indicators of water pollution (Dutka and Kwan, 1978; Cabelli, 1983). Fecal Streptococci have not been observed to regrow in natural waters (Evison and James, 1975). They have short survival times outside their natural habitat, so all detected pollution must be recent (APHA, 1985). Streptococcus faecalis is commonly found in human feces (Bergey's Manual, 1974). Other fecal streptococcus species, such as S. bovis and S. equinus are found in feces of warm blooded animals (Bergey's Manual, 1974). Enterococci have been proposed as indicators of human fecal

contamination (Berg and Metcalf, 1978; Cabelli, 1983; Cabelli et al., 1983). Fecal Streptococci densities correlate better than those of E. coli and total coliforms for the presence of enteric viruses (Berg and Metcalf, 1978; Cabelli, 1983).

Media used to enumerate enterococci, such as KF agar, contain sodium azide, which interferes with cytochrome oxidase in the electron transport chain of aerobic organisms, limiting unwanted growth (McFaddin, 1980). This is an important fact in the avoidance of over or underestimation of actual bacterial densities. It has been suggested that the densities of fecal coliforms and enterococci can be compared to determine the origin of contamination. Ratios of 4.0 or above indicate human fecal contamination, whereas ratios of 7.0 or less indicate animal fecal contamination (Geldreich and Kenner, 1969). The fecal coliform / enterococci ratio has been criticized because of differences observed in the survival rates of enterococci and fecal coliforms and because of differences found among individuals with differing diets. Hill et al. (1971) found that individuals with vegetarian diets exhibited greater densities of enterococci in their bacterial flora. Later, it was found that in environmental water above 20°C, E. coli survived longer than enterococci (Evison and James, 1975). These findings suggest that the fecal coliform / enterococci ratios can vary, affecting our perception of the actual source of contamination as well as the extent of pollution. The method has been used; however, to examine the quality of waters while trying to determine whether pollution was due to human fecal contamination or to storm waters (Feachem, 1976; Wheater et al., 1979).

This study will examine the presence and densities of total and fecal coliforms, as well as enterococci, as indicators of fecal

contamination of drinking water and different methods of enumeration of these bacteria in order to establish whether they are adequate indicators of bacterial pollution in tropical treated drinking water. Samples will be taken from raw water at various purification plants of the PRASA system (influent: water coming directly from the natural reservoirs) as well as finished drinking water processed in the plant (effluent). The study will determine if water treatment affects the levels of coliforms or enterococci present in finished drinking water and whether these organisms are good indicators of recent fecal contamination of tropical source waters. The objectives of this study are:

1. To determine which indicators best point to recent fecal contamination of tropical drinking water.
2. To determine which organisms are affected by treatment at potable water purification plants and whether treatment affects the levels of fecal coliforms.

MATERIALS AND METHODS

Study Sites. Four filtration plants operated by PRASA were selected for the study:

Rancheras Water System, located on road 371, Km 13.0 in the town of Yauco was selected for this study since it was the source of the etiological agent of a gastroenteritis outbreak that occurred during the summer of 1987. This treatment plant, with a processing capacity of 1,368,000 liters of water per day (GPD), serves a population of 1,440 individuals. The raw water, obtained from Duey River, does not receive aeration. The pH is adjusted through the addition of lime (CaO). Aluminum sulfate ($Al_2(SO_4)_3$) is added for flocculation purposes. Algae are controlled during periods of slow flow by the addition of copper sulfate. Records of the addition of these chemicals are not available. Raw water is prechlorinated at the water inlet using chlorine gas at a rate of 0.08 kg/24 h. The water is then deposited in a sedimentation tank for 1 hour and then filtered by gravity through a rapid sand filter. Post chlorination occurs at the pipe leading to the storage tank at a rate of 0.2 kg/24 h. The plant has storage facilities for 57,000 liters of finished water. This plant has never had problems with odor or color of finished water.

The Villalba Urban System, located on road 513, km 1.2, receives water from four sources: El Guineo Lake, which has sediments that affect the raw water quality (particularly the odor and taste); Aceituna Lake, which receives water from El Guineo Lake through a concrete channel; Jacaguas River, which is probably the main source of contamination, since there is a sewage plant discharge line about 4

miles upstream from the water intake line; and El Semil Dam, providing spring water coming through rock and discharged into a concrete channel that is not protected against grazing livestock (another possible contamination source). This plant was selected for the study because of its location relative to the sewage processing plant. Since a water intake is located downstream from a sewage discharge line, the raw water at the plant contains high levels of fecal contaminants. The water is sedimented for one hour, filtered through a rapid sand filter, and chlorinated by adding gas until an average concentration of 2.5 mg/L is achieved. Finished water is stored in 8 tanks having a total capacity of 2,698,000 liters.

Guaynabo Water Treatment Plant, located on road 873, Barrio Frailes Altos in Guaynabo, serves the town of Caguas and some sectors of Guaynabo. Raw water comes from tanks in Aguas Buenas, which in turn receive water from Cidra Lake. This plant was selected because of the contamination levels at Cidra Lake due to illegal sewage discharges as well as by the distance that the water has to travel before it reaches the plant (approximately 15 miles). Both of these factors might affect the raw water quality. Water processing is carried as previously described: no aereation is necessary, CaO and $Al_2(SO_4)_3$ are added, sedimentation occurs over one hour, and water is filtered through rapid sand filter beds. Chlorination is achieved by the addition of chlorine gas until an average final concentration of 1.4 mg/L is achieved in the water. Finished water is stored in 4 uncovered tanks with capacity for about 133,000 liters each.

La Plata System, located on road 872, km 5.2 in Toa Alta, serves various sectors of Toa Alta and Bayamón. This system receives water

directly from La Plata Dam, which in turn receives water from La Plata River. This river receives sewage discharges upstream from the dam and is also used for primary recreation (possible pollution sources). Water is treated with aluminum sulfate to form flocs, sedimented for one hour, pre-chlorinated (at 0.1 kg/24 h), filtered through rapid sand filters, and post-chlorinated using gas until the chlorine content of the waters reaches an average of 1 mg/L. Water is also aereated. In addition to lime and aluminum sulfate, polyphosphates (for the control of corrosion), and fluoride are added to the water. Finished water is stored in a subterranean tank with a capacity of 19 million liters.

Sampling. Water was grab sampled into sterile, one-liter Nalgene screw-cap bottles at the water intake and at the finished water primary distribution line. Samples from the finished water line were collected in the same type of container with an appropiate volume of a sterile 1% aqueous solution of sodium thiosulfate added to neutralize free chlorine. All samples were kept according to sample custody procedures described in Standard Methods for the Examination of Water and Wastewater (APHA,1985) and transported to the laboratory within 5 h of collection.

Water Quality. Water temperatures were measured *in situ* using a standard, centigrade scale thermometer. Total and free chlorine levels were also measured *in situ* with a Hach chlorine test kit employing the color comparator technique (Model CN-66, Hach Co., Loveland, Colorado). The pH was measured upon collection using a portable pH meter. Additional, small volume water samples were collected into sterile Whirl-Pak bags (Nasco, Ft. Wilkinson, Wisconsin) and transported to the

laboratory for turbidity analysis using a Model 16800 Turbidimeter (Hach).

Bacterial Analysis:

Total Direct Counts. Bacterial cell densities and the percentage of those active in protein synthesis were determined by acridine orange direct counts (AODC). For AODC, an appropiate volume of the sample was filtered through 47 mm diameter, 0.22 µm pore size, polycarbonate membranes pre-stained with 0.07% irgalan black (Nuclepore, Pleasantville, California). After filtration, 2 ml of acridine orange (0.1%, aqueous solution) was added and left on the filter for 2 minutes, filtered, and the excess washed off with sterile water. Stained filters were observed using the 100X objective of an epifluorescence microscope (Model 2071, American Optical Corp., Buffalo, New York). Total direct counts were determined by the average of the number of fluorescing bacteria in ten randomly selected fields on the membrane multiplied by 98177.77 (the number of fields on the filter). Acridine orange binds to nucleic acids and fluoresces red or green under the epifluorescent microscope depending on the amounts of RNA and DNA present in the cells (Hobbie et al., 1977).

Percent Activity. The percentage of cells active in protein synthesis were calculated indirectly from the total acridine orange direct counts. The ratio of red fluorescent cells (having greater amounts of RNA) to the total number of fluorescent cells as obtained in the acridine orange direct counts represents the proportion of cells actively transcribing their genetic code in order to produce proteins (Hobbie et al., 1977).

Membrane Filtration Studies. Levels of total and fecal coliforms, as well as fecal streptococci were measured by membrane filtration studies according to Standard Methods (APHA,1985). Volumes of 1, 10, 100, and 1000 ml were used in each case. All presumptive growth was confirmed by inoculating at least 10% of the colonies on each plate into appropiate Standard Methods (APHA,1985) media as follows:

Colonies presenting the typical green metallic sheen on m-Endo agar (Difco Laboratories, Detroit, MI) after 28h incubation at 37°C were confirmed according to their ability to grow and ferment lactose with the production of gas within 24 h on Brilliant Green Lactose Bile (2%) broth and Lauryl Tryptose broth at 37°C (APHA,1985). Colonies giving positive reaction on both media were subcultured on McConkey agar for 24 h at 37°C to verify their identity as lactose positive, gram-negative organisms (APHA,1985). All media were purchased from Difco Laboratories.

Fecal coliforms present a typical blue color on m-FC agar (Difco) after 24 h incubation at 45°C, while all other colonies are gray. m-FC agar is prepared according to the formulation published by Geldreich et al (1969) and supplemented with 10 ml of 1% rosolic acid in 0.2N NaOH solution per liter. Presumptive fecal coliforms were confirmed on EC broth (Difco), which contains 0.15% Bacto Bile Salts No. 3. These bile salts inhibit spore formers and fecal streptococci from growing while enhancing the growth of E. coli at 45°C (Hajna and Perry, 1943). Organisms positive for the production of gas on EC broth at 45°C after 24 h were streaked on McConkey Agar (Difco) and incubated at the same conditions to isolate lactose positive, gram negative colonies. Approximately 10% of these organisms per site per collection were

identified using the API-20E system for the identification of enterobacteriaceae and other gram negative bacteria (Analytab Products, Inc., Plainview, New York).

KF Streptococcus agar (Difco) was used to assay for the presence of fecal streptococci. This medium contains sodium azide, which interferes with the electron transport chain of aerobic organisms (Kenner et al., 1961). The addition of 1 ml triphenyltetrazolium chloride 1% per 100 ml of medium results in a red color of the colonies for easy identification. Color is produced as enterococci reduce the tetrazolium to an acid dye (Difco Laboratories, 1984). Presumptive enterococci after incubation of KF Agar at 37°C for 48 h were confirmed on Azide Dextrose broth (Difco) incubated under the same conditions. Azide Dextrose broth has the same selective agent as KF Agar. Turbidity of the medium after 48 h was considered as positive for the presence of streptococci. The broth was then streaked on m-Enterococcus agar (Difco), which does not recover such fecal streptococci as S. bovis and S. equinus (thus selecting streptococci of human fecal origin). This medium was found to be 100% selective when assaying heavily polluted waters (Difco Laboratories, 1984). Reddish colonies on this medium after 48 hours at 37°C were considered positive for the presence of fecal streptococci in the samples.

Injured fecal coliforms were assayed using m-T7 agar (Difco), a new selective medium that allows for the improved recuperation of these organisms from drinking water. In laboratory studies, this medium was able to recover 86 to 99% more injured coliforms than m-Endo agar (LeChevallier et al., 1983). m-T7 Agar recovered nearly three times more coliforms than did m-Endo agar from drinking water;

and less than 0.5% of all m-T7 Agar colonies gave false negative reactions (LeChevallier et al., 1983). Additional selectivity may be obtained through the aseptical addition of 0.1 µg of penicillin G per ml of medium after autoclaving (LeChevallier et al., 1983). The identity of the typical yellow m-T7 coliform colonies obtained after incubating for 24 h at 45°C was confirmed as described previously for fecal coliforms detected by m-FC agar.

Most Probable Number. The most probable numbers of total coliforms, fecal coliforms and fecal streptococci were determined using the five tube method for 0.1, 1.0, and 10.0 ml water samples and appropiate selective media (Lactose broth for total coliforms; EC broth for fecal coliforms; and Azide Dextrose broth for fecal streptococci), as described in Standard Methods for the Examination of Water and Wastewater (APHA,1985). All media was purchased from Difco Laboratories, Detroit, Michigan.

For total coliform bacteria, five tubes of sterile lactose broth were inoculated per dilution factor (i.e., 5 tubes with 10 ml water, 5 with 1 ml, 5 with 0.1 ml). The procedure was performed for both influent and effluent. The number of tubes from each dilution showing gas formation at 37°C after 48 h was recorded and the number of coliforms looked up on a MPN table in Standard Methods for the Examination of Water and Wastewater (APHA,1985). Positive tubes from the lowest dilution were confirmed in the same manner as colonies from m-Endo Agar (Membrane filtration studies). Fecal coliform bacteria MPN determinations were performed in the same manner, but the tubes were incubated at 45°C. Positive tubes from the lowest dilution were confirmed exactly like the colonies from m-FC and m-T7 agars.

The MPN technique to assay for fecal streptococci was also performed with five tubes per dilution factor and at 37 °C. Confirmation was carried out as with colonies isolated on KF agar.

Quality control. All media were prepared according to package directions. Unused m-Endo and m-FC agar plates were discarded after 2 days. Unused KF and m-T7 Agar plates were discarded after 30 days. Unused liquid media tubes were discarded after 90 days. All media were kept in a dark cold room at 4°C.

Sterility of all media was checked for each batch by incubating a sterile plate or tube at 37° or 45°C, depending on the media. Specificity of all media was checked for each batch of medium by inoculating one tube or plate with E. coli B (ATCC 23848) and another with Streptococcus faecalis (CBSC 15-5600A) and incubating under the desired experimental conditions.

Media Performance: The relative performances of the viable count methods were calculated for each site. Analyses of variance were performed on the average recovery efficiencies of each method. If a site revealed significant differences, a Student Newman-Keuls test was performed on the data to determine the nature of the differences. Results from these tests allowed the rating of methods according to their performance.

Specificity and Selectivity of the Media: Specificity was evaluated by confirming 10% of all positive (presumptive target) and negative (presumptive nontarget) colonies from plates containing 10 to 100 CFU. The false positive error (FPE) was defined as the number of false positive target colonies divided by the total number of presumptive target colonies. False negative error was defined as the number of false

negative target colonies divided by the number of verified negative target colonies plus false negative target colonies. All results were expressed as percentages. The procedure was repeated for both untreated influents and chlorinated effluents.

The selectivity index is both a measure of the inhibitory effect of background on target bacteria and a measure of the capability of the media to prevent background growth (Pagel et al., 1982; Clark, 1980; Ray and Specks, 1973; Franzblau et al., 1984). If low selectivity indexes are found, background bacteria are inhibiting the growth of indicator bacteria (Clark, 1980). This parameter was calculated by dividing the number of presumptive target colonies by the total number of isolates from each media. All results were expressed as percentages.

Data Analysis: Statistical analysis will be performed with programs developed for Apple Macintosh Plus and Macintosh SE computers. Multiple correlation and regression analyses to correlate bacteriological and physico-chemical data were performed using Statview 512+ software from Abacus Concepts. Analyses of variance and Tukey's tests to determine differences between sites, bacterial densities, and physico-chemical parameters were performed using TCH-Stats-ANOVA software developed by Dr. Terry C. Hazen. The log (X+1) transformation was used to make heteroscedastic data more homoscedastic. This transformation was selected due to the exponential variability of the data. Any statistical probability equal to or less than 0.05 was considered significant (Zar, 1984).

RESULTS

Water quality. Averages of water quality parameters at each site are presented on Table 1. These measurements were subjected to analysis of variance (ANOVA). If significant differences were detected by site, a Tukey's Test was performed on the data to determine which sites were different. Measurements of the total and free chlorine content of influents were not significantly different by site. Temperature, pH, and turbidity, however, differed by site (Table 1, Appendix I). Influent water temperature was equal only between Bayamón and Guaynabo (Tukey's Test: $Q=4.23$, $df=40$, $P>0.05$), while Villalba and Yauco had significantly lower values. Influent water pH was significantly lower at Guaynabo ($Q=3.71$, $df=40$, $P<0.05$), while equal at the other three sites. Influent turbidity was significantly higher at Yauco, but equal at Bayamón, Guaynabo and Villalba ($Q=8.54$, $df=40$, $P<0.01$).

Effluent pH did not differ significantly by site (Table 1, Appendix II). Effluent temperature, turbidity, and chlorine content was significantly different by site (Table 1, Appendix II).

ANOVA between influents and effluents by site revealed a significant difference in temperatures at Yauco ($F=10.56$, $df=1$ and 20, $P<0.01$). Significant differences were also found in the total and free chlorine content ($F=55.5$, 182.2, 188.4, 231.3; $df=1$ and 20; $P<<0.001$; and $F=40.9$, 162.4, 229.3, 202.2; $df=1$ and 20; $P<<0.001$, respectively) between influents and effluents at all sites.

Bacteriological methods. Average bacterial densities at each site are presented on Table 2. ANOVA of the viable count methods used

revealed no significant differences between m-T7 agar and m-KF agar influent results by site (Table 2, Appendix III). In Bayamón, significantly lower densities of total coliforms, fecal coliforms, and fecal streptococci were detected with MPN methods than in any of the three other sites ($Q=7.4, 6.8, 5.5$; $df=40$; $P<0.001$; see Figs. 2 and 6). None of the methods used was significantly different by site when assessing influents (Appendix IV).

ANOVA between methods used to detect the same group of organisms in influent waters revealed that total coliform densities were significantly different between membrane filtration and MPN ($F=4.28$, $df=1.86$, $P<0.05$; see Figs. 2-5). Fecal coliform detection methods were also different (Figs. 6-9). While no significant difference was found between m-FC agar and fecal coliform MPN, membrane filtration with m-T7 agar produced significantly lower results ($Q=6.05$ and 5.31, $df=129$, $P<0.01$). None of the other comparable viable count procedures for total coliforms and fecal streptococci showed significant differences when assaying effluents.

ANOVA of methods pooling sites (Appendix V) revealed, in the case of influent water, the variability of results from the same method when used to assess water from different sources. Only m-T7 agar and m-KF agar gave consistent results between sources of water. In the case of effluent water, similar tests revealed that for most methods, water quality from the four plants was consistent (Appendix V). Notable exceptions were MPN for both total and fecal coliforms; some plants performed better than others in coliform removal.

AODC and percent cells active in protein synthesis. Average AODC and percent activity are presented on Table 3. ANOVA of influent AODC

did not exhibit significant differences by site ($F=2.41$, $df=3$ and 41, $P>0.05$; Figure 11). Percent activity of the influents was similar only between Bayamón and Villalba, while those of Yauco and Guaynabo were significantly lower, as revealed by Tukey's test ($Q=4.86$ and 7.54; $df=40$; $P<0.05$ and 0.01, respectively). Effluent AODC was significantly lower at Guaynabo ($Q=4.62$, $df=40$, $P<0.05$), while similar at the other three sites. Effluent bacterial activities were significantly different between all sites ($F=10.53$, $df=3$ and 40, $P<<0.001$). Further analysis (Tukey's test) revealed significant similarity between the effluent activities of Bayamón and Villalba ($Q=1.34$, $df=40$, $P>0.05$), while those of Guaynabo and Yauco ($Q=1.21$, $df=40$, $P>0.05$) were also similar between themselves, but lower than the other two.

AODCs were also used as measurements of bacterial background relative to the indicator organisms assayed. The higher the ratios between the indicators and AODC, the lower the amount of background bacteria present in the sample.

Among influents (Table 3), the highest average ratio was obtained when assaying total coliforms in Guaynabo. In fact, this site's influents had the lowest overall level of background bacteria relative to the other sites. On the other hand, Influents from Bayamón contained the highest levels of background bacteria. In the effluents, the highest indicator / AODC ratios were those of MPN methods at Guaynabo. MPN methodology generally had higher ratios than those of membrane filtration. The lowest ratios were those of Bayamón's effluents.

Correlation of bacterial parameters and water quality of influents and effluents. Two correlation matrices were performed to determine the relationship between viable count methods, AODC, the percentage of

cells active in protein synthesis, and water quality parameters of combined influents and combined effluents. All correlations mentioned were significant at the $P<0.05$ level.

In the influents, all viable count methods were positively correlated with each other (Table 4). In the effluents (Table 5), total coliforms, as detected by membrane filtration, were positively correlated with fecal coliforms detected by membrane filtration with m-FC agar, and total coliform MPN correlated positively with fecal streptococci MPN.

Correlation of bacterial parameters and water quality at each site. Two correlation matrixes; one for influent, one for effluent, were performed by site to determine the relationships between water quality viable count methods, AODC and percent activity. All correlations mentioned were significant at the $P<0.05$ level.

At Bayamón, (Table 6) the influent total coliform levels (as detected by membrane filtration) correlated positively to all the other viable count techniques assessed, AODC, and pH. Fecal coliforms detected by membrane filtration using m-FC agar were positively correlated to results of fecal coliforms from m-T7 agar, total coliform, fecal coliform, and fecal streptococci MPN, and pH. Fecal coliforms detected by m-T7 agar correlated positively with total coliform and fecal streptococci MPN. There are positive correlations between total coliform MPN and fecal streptococci MPN, fecal coliform MPN and fecal streptococci MPN, and AODC and percent activity. Temperature correlated positively with total chlorine content, and negatively with total and fecal coliforms detected by membrane filtration, total coliform, fecal coliform, and fecal streptococci MPN, and AODC.

In the Bayamón effluents (Table 7), fecal streptococci levels determined by membrane filtration were positively correlated with fecal streptococci MPN, total and free chlorine levels, and fecal coliforms detected by m-T7 agar. Fecal streptococci MPN were positively correlated to total chlorine levels. Turbidity was positively correlated to m-T7 agar fecal coliforms, fecal streptococci (detected by both membrane filtration and MPN), and free chlorine content. A positive correlation also existed between total and free chlorine levels.

The Guaynabo influents (Table 7) displayed positive correlations between membrane filtration total coliforms and membrane filtration fecal coliforms (using m-FC agar); membrane filtration fecal coliforms (using m-FC agar) and m-T7 agar, membrane filtration fecal streptococci, total coliform MPN, and fecal coliform MPN; m-T7 agar and both fecal coliform and fecal streptococci MPN; total coliform MPN and fecal streptococci MPN; and fecal coliform MPN and both fecal streptococci MPN and percent activity. Influent temperature correlated negatively with pH, m-T7, and all MPN methods. Turbidity was positively correlated to membrane filtration total coliforms, membrane filtration fecal streptococci, and AODC. Percent activity correlated negatively with pH.

In the effluent from the Guaynabo plant (Table 9), membrane filtration total coliforms were positively correlated to AODC. Also, total and free chlorine levels were positively correlated.

The correlation matrix for Villalba influent (Table 10) reveals positive correlations between total coliforms detected by membrane filtration and fecal coliforms detected by m-FC agar and m-T7 agar, fecal streptococci detected by membrane filtration, fecal coliform MPN

and fecal streptococci MPN. Fecal coliforms detected by membrane filtration using both m-FC agar and m-T7 agar were also positively correlated with fecal coliform and fecal streptococci MPN. Fecal coliform MPN were positively correlated with fecal streptococci MPN, as were AODC and percent activity. Temperature of influent water was positively correlated to total coliforms detected by membrane filtration, fecal coliforms detected by membrane filtration using m-T7 agar, and fecal streptococci MPN. Influent pH correlated negatively with total coliforms detected by membrane filtration. In the Villalba effluents (Table 11), total coliforms detected by membrane filtration correlated positively with fecal coliforms detected by membrane filtration using m-FC agar, as did fecal coliform MPN and turbidity. Effluent total and free chlorine content were also positively correlated. Effluent water temperature was negatively correlated to pH.

In the influent at Yauco, all the viable count methods for all of the bacterial groups assessed were positively correlated to one another (Table 12). Temperature also correlated positively to all methods except membrane filtration for the detection of fecal coliforms using m-T7 agar. AODC correlated negatively with the percentage of cells active in protein synthesis. Other negative correlations existed between influent pH and membrane filtration fecal coliforms using m-FC agar, as well as with all MPN methods (total coliforms, fecal coliforms, and fecal streptococci).

The correlation matrix for the effluent at Yauco (Table 13) showed positive correlation between total coliform MPN and total coliform membrane filtration, fecal coliform membrane filtration on both m-FC agar and m-T7 agar. Fecal streptococci MPN densities were also

positively correlated to all these parameters. Fecal streptococci membrane filtration densities were positively correlated to total coliform and fecal coliform (m-FC) membrane filtration. Membrane filtration for the detection of fecal coliforms using m-T7 agar were also positively correlated with these two parameters. AODC correlated positively to both total coliform and fecal streptococci MPN, while correlating negatively with percent activity. Turbidity correlated negatively with pH. The total and free chlorine content of the effluent were positively correlated.

Media Performance. Media performance when screening for target bacteria in the influents was not significantly different at Guaynabo and Yauco (Table 14). At Bayamón, membrane filtration on m-Endo agar was able to recover higher bacterial densities than any of the other methods. This would be expected, since this group should be the most abundant of the bacteria being assessed. At Villalba, not only was m-Endo agar able to recover high levels of coliforms, but MPN analyses performed better than fecal coliform and fecal streptococci membrane filtration analyses. Evaluation of media performance relative to the levels of nontarget bacteria reveal that, at Bayamón, m-Endo agar performed better than any other method (Table 15). At Yauco, m-Endo, m-KF, and total coliform MPN performed higher than the rest of the methods as far as nontarget organism recovery. Guaynabo and Villalba showed no differences among media.

Selectivity and specificity of the methods when assaying influents are summarized in Table 16. If we accept 15% as the upper level permissible for false positives, like Pagel et al (1982) did when evaluating the performance of fecal coliform membrane filtration

methods in a temperate climate zone, none of the methods is acceptable, no matter how well the media performed. Media selectivity for influents ranged from 29 to 66% (Table 16). Selectivity of the media in tropical waters was therefore much lower than those reported by Pagel et al (1982) in temperate waters.

Performances in the assessment of both target and nontarget bacteria in chlorinated effluents did not vary among media (Tables 14 and 15). However, false positive and negative errors in effluents were higher than those of influents, and thus, also unacceptable. Selectivities in chlorinated effluents ranged from 47 to 79% (Table 17). While still unacceptable, these selectivities are higher than those of the same media when assessing influents.

Among the species isolated and identified from influents, E. coli was the most frequent (42.7%; Table 18). The media isolating this bacterium most often was m-T7 agar. MPN methodology recovered the lowest percentage of E. coli. Other frequently isolated species were Enterobacter sp. (27%) and Citrobacter freundii (21%). Enterobacter sp. was detected most frequently by m-FC agar, while C. freundii was observed more frequently on m-T7 agar.

The species most commonly isolated in chlorinated effluents was also E. coli (61.1%; Table 19). m-T7 agar isolated this bacterium far better (50%) than m-FC agar (27.3%) or FC MPN (22.7%). The next most frequently isolated species was Pseudomonas sp. (16.7%). m-FC agar, apart from detecting practically all Pseudomonas sp. encountered (94.4%), also detected C. freundii and E. cloacae.

DISCUSSION

Bacterial densities and media performance are affected in various ways by a great number of physical and chemical parameters that can act by themselves, synergistically, or in antagonistic ways (Badge et al., 1982). Our results confirm this fact, as can be observed in the multiple correlations between the collected data. The bacterial counts we see are the effects of multivariate events. In some cases, these cannot be explained by the available data; however, those that can are not only interesting, but of great relevance to the issue of drinking water quality in the tropics.

Influent temperature seems to be a very important factor affecting the levels of bacteria. Specific patterns are evident, particularly in results for the most probable numbers (MPN) of the bacteria under study. Almost all of the viable count procedures performed on the influents of La Plata and Los Filtros filtration plants (Bayamón and Guaynabo, respectively) correlate negatively with temperature (Tables 6, 8, 10, and 12). There were no significant differences between the influent temperatures at these two sites, while those of Villalba and Yauco were significantly lower. Influent bacterial levels at Bayamón, as measured by MPN, were significantly lower than those of the three other plants. All of this leads us to beleive that high influent temperature depresses the amount of organisms present in raw water, particularly at Bayamón. This agrees with results obtained by Badge et al (1982) from raw waters in New Delhi. Badge and his colleagues demonstrated that above a specific maximum temperature level, rapid microbial growth could not occur due to unrepairable

damage to the cytoplasmic contents. This finding is particularly important since it has been demonstrated that E. coli can die very rapidly with slight temperature increases, while other bacteria, such as Pseudomonas aeruginosa and Citrobacter freundii, which are opportunistic pathogens, (both were found in influent samples from the four study sites; See Table 18) survive and can produce false positives in culture media (Thomson, 1981).

Temperature is also positively correlated with almost all of the viable count methods performed on the Villalba and Yauco influents, whose temperatures were well below those encountered at Bayamón and Guaynabo. We suggest that this phenomenon is caused by an increased survival rate of the microorganisms during periods of higher water temperature at these sites. Our suggestion is backed by the work of Wright (1982a), who observed and described a similar phenomenon while working on the survival of fecal microorganisms in the waters of Sierra Leone, as well as by studies done at our laboratory (Carrillo et al., 1985; Biamón and Hazen, 1983; Pérez-Rosas and Hazen, 1988; Hazen et al., 1982; Hazen et al., 1987; Bermúdez and Hazen, 1988; López-Torres, 1982; Ortiz-Roque and Hazen, 1983; Fuentes et al., 1983; Carrillo et al., 1985; Santiago-Mercado and Hazen, 1987; Hazen, 1988).

Another factor significantly affecting the microbial content of water is turbidity. Among the studied influents and effluents, increases in turbidity bring about increasing total coliform, fecal coliform, fecal streptococci, and AODC levels, particularly at Guaynabo. Observations made during samplings point towards rainfall as the cause of increased influent turbidity. The clay particles and organic matter that make up turbidity can adsorb ions, molecules, and biological agents, thus

affecting transport and release of toxic materials, bacteria and viruses (National Academy of Sciences, 1977; Hoff, 1978).

Influents from Guaynabo and Yauco were the most succeptible to elevated bacterial densities due to increased turbidity caused by rainfall. The sources of these influents are not protected against roaming cattle and other domestic animals, or from human activities such as laundering. We beleive these increased bacterial contents are caused by the leaching of fecal and other waste materials from river and lake banks into source water by rainfall. The FC/FS ratios at these sites under these conditions, point to mixed human-animal fecal contamination during rainfall events in a pattern is similar to that reported by Barrel and Rowland (1977) in Western Africa. However, routine use of FC/FS ratios might be unacceptable under normal conditions due the the prolonged survival and normal presence of fecal coliforms in tropical source water. Sediment disturbances caused by increased water flow is another factor contributing to the elevated bacterial counts. Riverbed and lake sediments usually have higher bacterial levels than water due to the adsorption of cells to clay and other organic and inorganic particles (National Academy of Sciences, 1977). Benthic bacteria washed away by heavy precipitation may also contribute to the elevated counts, since, in the tropics, these might include bacteria capable of false positive reactions on standard media (Hazen, 1988).

A direct correspondence between increasing turbidity of influents and effluents was observed. Increases in effluent turbidity (above 1.5 NTU) were also related to increasing bacterial counts at all sites, although these were statistically non-significant. The statistical

discrepancy must be related to the lack of data points, since the above mentioned situation occurred a limited number of times at each site. However, increases in effluent turbidity bring about increased detection rates of fecal streptococci relative to the detection of coliforms. Detection of bacteria under these circumstances is possible due to the protection against chlorination that turbidity can provide. As stated previously, clay, organic, and inorganic particles can adsorb bacterial cells. These particles also have a tendency to bind together, forming clumps. Bacteria inside these clumps cannot be effectively affected by chlorine; instead, they remain sublethally and reversibly injured (National Academy of Sciences, 1977; McFeters et al., 1986; World Health Organization, 1987). These injured bacteria remain viable but unculturable by standard methodology. It has been demonstrated that the majority of viable bacteria in chlorinated drinking water are attached to particulate matter (LeChevallier et al., 1988; World Health Organization, 1987). Furthermore, the fraction of turbidity composed by organic material can form compounds with the free chlorine content of the water, thus inactivating it and permitting the survival of bacterial cells (National Academy of Sciences, 1977; Goytía, 1978; Hoff, 1978). This can result in the eventual recovery of bacteria in the water distribution systems due to increased nutrient availability and favorable temperatures (McFeters et al., 1986).

In the case of fecal streptococci, we suggest not only the possibility of bacteria protected against chlorine through turbidity, but survival rates higher than those of coliforms in the presence of chlorine, as has been reported with Klebsiella sp. (Ptak et al., 1973) and some enteropathogens (LeChevallier and McFeters, 1985), since streptococci

were found in greater numbers than coliforms whenever these occurred. Defense mechanisms against chlorination have recently been described for several bacteria (LeChevallier et al., 1988). Further research is needed to fully understand the behavior of fecal streptococci in chlorinated effluents. Correlations between the detected densities of fecal streptococci, chlorine content, and turbidity (Table 9) confirm these possibilities. Turbidity values were a highly significant factor affecting both the chlorine content and fecal streptococci levels at Bayamón. At the same time, chlorine had a highly significant effect on fecal streptococci levels. All of these correlations point towards turbidity as the factor determining the occurrence of fecal streptococci in drinking water through interference with chlorination. Fecal coliforms were also detected in Bayamón effluents by m-T7 agar, in a pattern similar to that of fecal streptococci in terms of the relationship between bacterial density, turbidity, and chlorine content. The fact that fecal coliforms detected at this site existed in an injured state (they were detected only by m-T7 agar) is further evidence of the protection against chlorination that turbidity particles can confer to bacteria.

Wright (1986) stated that the flushing produced by heavy rainfall resulted in periods of increased health hazards due to the introduction of pollutants into bodies of water used as drinking water supplies. Our observations suggest that heavy rains bring about periods of highly uncertain water quality. If E. coli is naturally occurring in the tropics, it would be difficult to determine whether increases in its density during heavy rainfall are due to the flushing of strains from fecal material or to the flushing of strains from the environment into the reservoirs. Heavy rains also flush soil surrounding the reservoirs and disturb sediments.

The resulting high turbidity affects the efficiency of chlorination and supports high levels of background bacteria (which interfere with coliform recovery on standard media). High turbidity also clogs filter membranes and makes it difficult to properly observe microbial growth in fermentation tubes. Special care should be taken when analyzing potable water samples coming from high turbidity sources, rainy season or not; particularly if their turbidity is even slightly higher than normal. We also note that while bacterial counts are increased in both influents and effluents after the onset of rains, so are the numbers of false positives and negatives found. We agree with Wright (1986) that membrane filtration is the method of choice under these circumstances, since it provides relatively pure colonies that aid in the process of confirmatory subculturing in appropiate media. However, since high turbidity can clog filters, special care should be taken in the preparation of suitable dilutions to overcome this problem. We go further, to suggest an actual identification of a suitable amount of nontarget organisms from each fecal coliform negative sample to be absolutely sure of the absence of fecal contamination of potable water. It is important to remember that while high turbidity will not necessarily be an indicator of potential health hazards, low turbidity is not an inequivocal indicator of the bacterial quality and safety of potable water.

All of the methods used to monitor the presence of indicator organisms in tropical source waters seem to be unacceptable due to their high rates of false positive and negative errors (Tables 16 and 17). Tropical waters have autochtonous bacteria capable of producing positive coliform reactions when standard methodology is used

(Santiago-Mercado and Hazen, 1987; Hazen, 1988). These bacteria cause high numbers of false positives on standard media, which results in overestimation of pollution. The acceptable limit for false negatives used by Pagel et al (1982) in temperate waters was 5%. In tropical waters, we found false negatives ranging from 12 to 50% (Table 16). Thus, according to specificity indexes, all the methods tested are grossly unacceptable in tropical source waters. In addition, the high levels of background bacteria present in tropical waters are capable of producing metabolic interferences with the normal reactions of indicator organisms on standard media (Means and Olson, 1981; Burlingame et al., 1984; Santiago-Mercado and Hazen, 1987; Hazen, 1988). This interference can result in underestimation of the levels of pollution in tropical waters. The levels of background bacteria relative to each of the indicators measured can be determined indirectly from the ratio between the indicator and the total direct count (determined by AODC). These ratios range from 0 to 1.08×10^{-4}. The indicator bacteria being assessed by standard media are only 0 to 0.01% of the total microbial flora present in the analyzed waters (Table 3). The low selectivity indexes recorded (29 to 66% for influents, 47 to 75% for effluents; see Tables 16 and17) relative to the performance of fecal coliform assessment methods in temperate zones (Pagel et al., 1982) are further evidence of the inhibitory nature of background flora (Clark, 1980).

Final identification of colonies from fecal coliform methods are yet another measure of the high density and variability of background bacteria. Among influents, E. coli was successfully isolated 42.7% of the time out of 170 isolates (Table 18). Other common nontarget bacteria in influents were Enterobacter sp. (15.8%) and Citrobacter freundii (12.3%).

The Enterobacter and Citrobacter genera have positive fecal coliform reactions, but are not fecal specific (Bonde, 1977; Hoadley et al., 1975; Santiago-Mercado, 1987). Identification of organisms recovered from chlorinated effluents by fecal coliform methods resulted in the isolation of E. coli 61% of the times out of a total of 43 isolates (Table 19). Other bacteria frequently isolated from effluents were Pseudomonas sp. (16.7%) and Enterobacter cloacae (13.9%). The occurrence of Pseudomonas sp. in chlorinated effluents is important, since this bacterium, if ingested by humans, might cause various sorts of infections.

Confirmation rates of both target and nontarget bacteria (Tables 18 and 19) reveal that a maximum of 61% of all colonies were actually E. coli by any fecal coliform method. This is lower than results recorded by Santiago-Mercado and Hazen (1987), who found that a maximum of 70% of tested colonies were E. coli in various types of tropical freshwater. By comparison, Pagel et al (1982) recorded confirmation rates that were at least 27% higher in contaminated temperate freshwater. Pagel et al (1982) found high numbers of E. coli among the nontarget colonies assayed. She attributed her results to the thermal stress imposed on the indicator by the colder waters of temperate climates.

Comparisons of the densities of target fecal coliforms recovered from influents reveal that m-FC agar had higher recovery rates at Bayamón and Guaynabo (Table 14). At Villalba and Yauco, however, fecal coliform MPN had better recovery rates, as would be expected from highly polluted waters (Santiago-Mercado and Hazen, 1987). The high bacterial densities in heavily polluted waters mask fecal coliforms

by inhibiting the expression of their normal characteristics. Densities of total coliforms were higher at all sites when m-Endo agar was used (Table 14). The highest overall total coliform recovery was recorded on the Villalba influent, which is grossly polluted by a sewage outfall.

Influent fecal streptococci were recovered most efficiently by m-KF at Bayamón and Guaynabo (Table 14). At Villalba; however, the fecal streptococci MPN method performed better. This could also be explained by the better overall performance of MPN methodology in grossly contaminated waters (Santiago-Mercado and Hazen, 1987). It is interesting that at Yauco, the membrane filtration and MPN methods had similar performances.

Recovered bacterial densities in chlorinated drinking waters by the methods tested reveal that MPN methods generally had better performances (Tables 14 and 15). This fact had been noted previously by Grabow et al (1981) in temperate waters. Notable exceptions were m-KF recovered fecal streptococci at Bayamón and Yauco. At these two sites, membrane filtration was able to detect fecal streptococci when MPN failed to do so. This could represent the survival of fecal streptococci to standard treatment.

Influent nontarget bacteria recovered on fecal coliform media were lowest when m-T7 agar was used (Table 15). The only site where this was not true was at Guaynabo. The water source at Guaynabo is Cidra Lake, which has been said to be grossly polluted due to illegal domestic sewage discharges. Since the lake is very distant from the treatment facility, water has to travel inside pipes for approximately 15 miles before reaching the plant. It is possible that the high pollution level of the lake, combined with a possibly thick biofilm inside the

pipes, provide optimum conditions for the growth of bacteria not normally associated with water sources. These unusual bacteria might be the cause of the higher nontarget levels detected by m-T7 agar at Guaynabo. This fact is supported by the observation that most of the unidentifiable bacteria reported in Table 18 came from Guaynabo influents.

Apart from the low levels of influent nontarget bacteria reported on m-T7 agar, this medium also recorded the highest selectivity index among the fecal coliform methods tested (Table 16). The specificity of m-T7 agar was also the closest to the limits adopted by Pagel et al (1982) for temperate waters. Perhaps the low performance of this medium when assessing nontarget colonies (Tables 15 and 16) is due to the ability of m-T7 agar to suppress some of the background growth associated with tropical source waters. m-T7 agar also recorded low levels of confirmed nontarget colonies (Table 16). This might be an indication that m-T7 agar, while still unacceptable for fecal pollution assessment in tropical source water, might be useful for fecal coliform isolation in this environment with a better chance of inequivocal recovery in one step.

Studies have shown the influence of the type of water analyzed, the media used, and techniques employed on the bacterial levels detected at particular sites (Evans et al., 1981b). We found that although the levels of total coliforms detected on the influents by membrane filtration (MF) and MPN techniques were not different at Villalba (Figure 5), they did differ at the other study sites (Figures 3-5). A similar pattern was evident with methods for the detection of fecal coliforms. Bayamón displayed no differences between methods for fecal

coliform assessment (Figure 6), while at the other three sites, the methods disagreed on the results (Figures 7-9). Fecal streptococci assays revealed differences between the MF and MPN methods only in the Villalba effluent (Figure 10), the other three sites having similar levels detected by each of the two methods used in both influents and effluents. All influents had high total bacterial densities, as determined by AODC (Figure 14).

In the sites exhibiting differences in the influent total coliform levels detected by MF or MPN, MPN values were slightly lower than those obtained by MF. This was caused by non-coliform interference on the culture media used. The MPN technique has been criticized for its failure to detect total coliforms under particular circumstances (Evans et al., 1981a; Evans et al 1981b; Jacobs et al., 1986). Evans demonstrated that if the total bacterial densities exceed 500 cells per ml, the MPN technique results in decreased coliform densities being reported due to competition of non-coliforms for nutrients, as well as by the production by these of coliform inhibitory substances. The influents studied had high total bacterial densities, plus high percentages of false positives and negatives resulted from their assays, the lower densities of coliforms in source waters as detected by MPN must be a result of inhibition and masking by non-coliform organisms. The same explanation applies to differences between the MF and MPN methods used in the detection of fecal coliforms in tropical source water.

At Villalba and Yauco, membrane filtration using m-T7 agar resulted in significantly lower densities than MPN or MF with m-FC agar. As already discussed, high numbers of false positives and negatives occur when assaying for the presence of fecal coliforms in

tropical source waters (Santiago-Mercado and Hazen, 1987). Examining the confirmation rates we obtained for our fecal coliform assay methods, we agree with these results. We also found that while the fecal coliform densities detected by MF with m-T7 agar were lower than those detected by m-FC agar, this medium has a higher reliability of actually detecting the target fecal coliform (Escherichia coli) than other methods. MF with m-T7 agar produced the highest confirmation percentage of any of the three methods used. This is in agreement with results obtained by LeChevallier at al (1983), who stated that m-T7 agar not only recovered fecal coliforms from chlorinated water, but could also be used to detect coliforms in untreated surface waters with a high reliability. MPN, on the other hand, produced the highest percentage of unidentifiable microorganisms plus low confirmation rates, confirming its unreliability due to common components of tropical bacterial flora being able to grow in the media.

Examination of the species isolated from source waters by fecal coliform assays (Table 18) reveals that m-T7 agar was able to detect E. coli 42.7% of the time in the influents, as well as other organisms of clinical importance, such as Shigella dysenteriae and Klebsiella pneumoniae. All of this suggests that m-T7 agar may be more accurate in the detection of the target fecal coliforms in tropical waters. This medium performed equally well on chlorinated drinking water.

The occurrence of fecal coliforms in finished drinking water was rare and most frequently related to increases in effluent turbidity. However, there were a few cases when m-T7 agar was able to detect fecal coliforms in the chlorinated effluents when m-FC agar and MPN could not. In cases when E. coli was detected in the effluents by MF

using m-FC agar, m-T7 agar was able to detect 45% more E. coli than m-FC and 55% more than MPN. This confirms the performance of m-T7 agar in tropical source waters in levels similar to those stated by LeChevallier (1983) for temperate climates. However, since E. coli might be a normal inhabitant of tropical source water, the "accuracy" of m-T7 agar may be useless in the tropics. Since pathogenic bacteria such as Klebsiella pneumoniae, Shigella dysenteriae, and Pseudomonas sp. isolated from m-T7 agar were isolated in higher proportions than in the other fecal coliform methods (Tables 18 and 19), colonies from m-T7 could be screened for the presence of certain pathogens in tropical source water. This approach towards the direct assessment of pathogens could save time and effort, so its possibilities should be further evaluated.

Results from the fecal streptococci assays on effluents also suggest them as adequate indicators of fecal contamination of waters. We have already discussed the occurrence of fecal streptococci in source waters and the relationship between increases in their levels with rainfall. At first, the occurrence of fecal streptococci in chlorinated drinking water seemed to be strictly correlated to the occurrence of coliforms, but in later samplings, fecal streptococci were detected in the effluents from Guaynabo and Villalba even in the absence of coliforms. These occurrences could not be correlated to any of the parameters under study. Three possibilities exist: either the fecal streptococci detected were able to withstand chlorination and recover from injury on the complex media used, fecal coliforms were so injured that they failed to recover even on m-T7 agar; or, as is the case with coliforms, components of the normal microbiological flora at the study sites can

produce false positives on these media. Since all media used to isolate and confirm the presence of fecal streptococci contain sodium azide, any organism capable of producing false positives on these media must not rely on an electron transport chain with cytochrome oxidase for energy, or they must be resistant to this chemical. The high selectivity indexes of FS methods relative to other assays studied confirm this fact. However, studies in Puerto Rico have demonstrated the prolonged survival rate of Streptococcus faecalis, the target organism, in a tropical rain forest stream (Muñiz et al., In Press). High densities of S. faecalis were also found to survive in tropical marine waters contaminated with petroleum (Muñiz et al., In Press). All of these findings discard the possibility of fecal streptococci as adequate indicators of tropical drinking water quality.

The issue of injured bacteria in tropical drinking water quality is very important. Injured bacteria have been known to recover in distribution systems due to favorable temperatures and increased nutrient availability, posing health hazards to the community (McFeters et al., 1986; Wright, 1982b). In the four filtration plants studied, the total bacterial densities, determined by AODC were equal among influents and effluents (Figures 12-15). This suggests that chlorination might only be injuring coliforms, thus disabling them to grow on culture media, but it is not effective in the disinfection of water. Several mechanisms for the survival of bacteria in chlorinated water have been proposed. Attachment or association of bacteria with various surfaces can promote increased resistance to chlorine disinfection (Levy et al., 1984; Tracy et al., 1966; Silverman et al., 1983; Camper et al., 1986; Hejkal et al 1979; Hoff, 1978; LeChevallier et al., 1981). The production

of extracellular capsules can also protect some bacteria against chlorine (Reilly and Kippen, 1983; Clark, 1984). Members of the natural microbiological flora of tropical waters may be capable of surviving the standard disinfection procedures for the production of potable water. Effluent bacterial activity was lower than that of influents at all sites; however, at Guaynabo, Villalba, and Yauco activities were not significally different between influents and effluents (Figures 16-18), providing further evidence of the little impact of the disinfection procedures on the aquatic microbial communities at these sites. This is startling, since high levels of total and free chlorine residuals (ranging from 1.68 to 2.45 mg/L; See Table 1) were recorded in the effluents at all sites. The concentration at Yauco was high enough to eliminate Giardia sp. cysts (Rodríguez et al., unpublished data). Since these cysts are resistant to regular chlorination, the possibility of overchlorination cannot be ruled out. Neither Giardia sp. nor Cryptosporidium sp. cysts were found at any of the other sites. However, problems along the distribution line, such as broken pipes and collapses of bacterial biofilm inside pipes may be hampering the performance of residual chlorine, thus allowing high densities of bacteria to repopulate the finished water. Overchlorination can result in yet another problem: the formation of trihalomethanes. The occurrence of trihalomethanes is due to the oxidation reactions in water treatment (chlorine disinfection) with organic substances present in the water (World Health Organization, 1987). The most common trihalomethane, chloroform ($CHCl_3$), produces cancer in laboratory animals (World Health Organization, 1987). The carcinogenic potential of other common trihalomethanes, such as $CHBrCl_2$, $CHBr_2Cl$, and $CHBr_3$ (formed in the

casual presence of the bromide ion) is still being tested (World Health Organization, 1987). The formation rate of trihalomethanes is accelerated by high temperatures and pH as long as free residual chlorine is still present in the water. Total trihalomethane concentrations in drinking water can go up to 1000 µg/L (World Health Organization, 1987).

Effluent activity at Bayamón was slightly lower than that of the influents (Figure 19). This could be due to a slightly better performance of the treatment process at this site. However, this plant also adds fluoride to effluents as part of the treatment process, and the impact of this chemical on the microorganisms studied in this research was not measured. This is an area needing further investigation. We also note that the percentage of bacteria active in protein synthesis in any sample was highly variable at each site, probably due to the complex effects of a high number of physico-chemical and bacterial parameters and the interactions between them. Another possibility may be the seeding of coliform bacteria in finished water by the sand filter beds used in the treatment process. McFeters et al (1986) have shown that in temperate waters, up to 6,300 coliforms can be associated with each grain of sand in a filter bed. These coliforms can actually be inoculated into finished drinking water, as water pressure dislodges clumps from the filter bed that can survive chlorination due to their close association with each other (McFeters et al.,1986). The higher densities of both coliforms and false positive coliforms in tropical waters might accumulate in much higher numbers in the filter beds, especially if these are not backwashed every day. Thus, in the tropics, coliforms seeded into

drinking water may be a common occurrence. This adds further uncertainty to the problem of drinking water quality in the tropics.

These findings point towards the need for closer and careful monitoring of the methodology currently being used to determine drinking water quality in the tropics. We also see the need for the identification and proper management of water resources of relatively variable and reliable high quality to be used as potable water sources; this conclusion has been supported by other investigations of both tropical and temperate drinking water quality (National Academy of Sciences, 1977). Further research on the effects of physical and bacterial parameters and their interactions on water quality would also be needed at each of these water sources to develop and manage them in the most effective way. A better indicator of water quality is needed to solve the problems of water pollution control and drinking water quality in the tropics. Direct enumeration of pathogens, while costly, could be a solution. However, other indicators have been proposed (Dutka, 1978).

Bifidobacterium sp. have been proposed as tropical water quality indicators (Evison and James, 1975). Since Bifidobacterium sp. are obligate anaerobes, they are not able to survive in most extraenteral environments. In addition, Bifidobacteria are commonly found in the gut of humans, but not in animals (Hazen, 1988). Carrillo et al (1985) showed that the densities of Bifidobacterium adolescentis decreased one order of magnitude per day in a tropical rain forest stream in Puerto Rico. These investigators also found that the densities of bifidobacteria-like organisms were greater than those of E. coli at all sites, except those at a sewage outfall. The media developed for the isolation of

bifidobacteria (YN-6) had low specificity if background anaerobic bacterial densities were high (Carrillo et al., 1985). In fact, less than 80% of isolates from a Puerto Rican rain forest stream were actually bifidobacteria. Bifidobacteria shows promise as an indicator of human fecal contamination in terms of their specificity and lack of survival *in situ*. The development of media with better specificity in tropical waters would make bifidobacteria excellent indicators.

While Clostridium perfringens, another anaerobe, has been proposed as yet another indicator alternative (Fujioka and Shizumura, 1985; Bisson and Cabelli, 1980), its densities in human feces from the tropics are much lower than those of the currently used indicators (Wright, 1982a). In addition, clostridial spores can resist disinfection if the chlorine concentrations and pH are inadequate. These facts propose serious doubts as to the ability to detect C. perfringens easily when an eminent danger of pathogens exists (Wright, 1982a; Hazen, 1988). Wright(1982a) could not correlate the isolation of C. perfringens with the occurrence of Salmonella sp. in tropical source water. Furthermore, Carrillo et al (1985) found that the densities of total anaerobes were high in sites that had received torrential rainfall. This, along with the increased densities of C. perfringens reported in uncontaminated sites after rainfall (Fujioka and Shizumura, 1985), suggests that C. perfringens might have an extraenteral source in the tropics (Hazen, 1988). While bifidobacteria and Clostridium perfringens might be more suitable indicators for the tropics, we must also take into account that techniques involving the assessment of these anaerobes require more sophisticated and expensive techniques (Franzblau et al., 1984).

Recent studies in Puerto Rico have posed coliphages as more specific than E. coli in terms of pollution detection. Toranzos and Gerba (1988) demonstrated that coliphages were only detected in water receiving sewage effluents. The viradel technique (Goyal and Gerba, 1983) was used to concentrate the viruses in one liter water samples. Escherichia coli C3000 (ATCC 15597) was then used for coliphage assessment. Since the assay is specific for a particular strain of E. coli, the problematic tropical background flora are eliminated. The only problem is that if E. coli is able to survive for prolonged periods in tropical waters, the viruses that infect them could do so too. Coliphage assays would only be appropiate in tropical environments if it was demonstrated that only one strain of E. coli is not capable of extraenteral survival (Hazen, 1988). Studies to date (Elías et al., 1988; López et al., 1987; Valdés-Collazo et al., 1987; Hazen et al., 1981; Jiménez et al., 1988; Pérez-Rosas et al., 1984; Carrillo et al., 1985) have shown this to be unlikely, since all tested strains of E. coli were capable of extraenteral survival in tropical waters.

We have already mentioned the phenomena of viable but unculturable bacteria. This has been show to happen with many pathogens in both temperate and tropical waters, which suggests that most of the time, indicators may not be correlated to disease risk (Baker et al., 1983; Colwell et al., 1985; Hazen et al., 1987). Considering this, the best way to assess tropical source water might well be the direct enumeration of the pathogens themselves. Several approaches to pathogen enumeration have been proposed. Among these, immunofluorescent staining have been shown to detect extremely low (10 cells/ml) levels of pathogenic bacteria (Colwell et al., 1985). Cross

reactivity, however, hampers the potential of this approach, even when monoclonal antibodies are used.

Currently, DNA, cDNA, or RNA probes may be the most sensitive method for pathogen detection. DNA probes have been developed for Salmonella sp., and for enterotoxigenic E. coli (Hazen, 1988). The detection of any of these in tropical drinking water would surely point to an imminent human health hazard.

CONCLUSIONS

1. Turbidity is a very important factor affecting water treatment. By associating themselves with particulate matter, bacteria gain protection against chlorine disinfection. Whenever water turbidity increases, special care should be taken in analyzing samples to determine bacterial water quality.
2. Turbidity also affects the performance of the media used for the detection of indicator bacteria. The high levels of background bacteria brought about by high turbidity of both influents and effluents can interfere with the expression of the characteristics normally associated with indicator bacteria on differential media. High turbidity also clogs membrane filters and makes it difficult to observe bacterial growth on fermentation tubes.
3. Coliforms may survive treatment and remain in an unculturable, but viable (injured) state. Injured coliforms cannot be detected using standard methodology. This can result in water being classified as potable when it is not. The only method capable of detecting injured coliforms was membrane filtration using m-T7 agar.
4. Membrane filtration using m-T7 agar recovered less nontarget bacteria than any other fecal coliform method. This method could be used to recover naturally occurring coliforms with higher selectivity in tropical environments.
5. Fecal streptococci are inadequate indicators of water quality in the tropics. Some components of the normal environmental flora are able to give false positive fecal streptococci reactions on standard

media. In addition, further investigation is needed to determine the higher resistance of fecal streptococci to chlorine disinfection and the occurrence of tropical fresh water bacteria capable of growing on culture media containing sodium azide.

6. Although standard water treatment was able to significantly reduce the total bacterial densities present in source water, AODC was able to detect high concentrations of bacteria in drinking water. Further research as to the nature and identity of this background flora is needed.
7. Due to the high numbers of fecal coliform positive bacteria of non-fecal origin, water treatment plants in Puerto Rico are overchlorinating finished drinking water. Thus, not only is the chlorination process treating a health risk, it is creating one by increasing the concentration of trihalomethanes in the water.
8. In terms of bacterial densities, raw water brought in from Cidra Lake to the Guaynabo treatment plant had unique characteristics relative to those of the other plants studied. The possible effects of water travel through such a long pipeline, in terms of bacterial quantity and quality, should be further studied.
9. New, better indicators of tropical drinking water quality are an immediate necessity. The only practical solution to this problem might be the direct isolation of pathogenic microorganisms from drinking water.

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Table 1. **Water Quality Parameter by Site and Water Source. (Measurements were taken between September 24, 1987 and March 7, 1988).**

INFLUENTS

Site	Temperature (°C)	pH	TCl[1] (mg/L)	FCl[2] (mg/L)	Turbidity (NTU)
Bayamón	24.45 ± 0.39	7.76 ± 0.13	0.02 ± 0.02	0.00 ± 0.00	34.03 ± 9.61
Guaynabo	24.00 ± 0.63	7.36 ± 0.17	0.00 ± 0.00	0.00 ± 0.00	19.48 ± 8.77
Villalba	22.50 ± 0.23	7.86 ± 0.18	0.00 ± 0.00	0.00 ± 0.00	60.75 ± 30.00
Yauco	21.46 ± 0.37	7.96 ± 0.10	0.00 ± 0.00	0.00 ± 0.00	6.57 ± 1.89

EFFLUENTS

Site	Temperature (°C)	pH	TCl[1] (mg/L)	FCl[2] (mg/L)	Turbidity (NTU)
Bayamón	25.18 ± 0.30	7.79 ± 0.17	1.13 ± 0.08	0.96 ± 0.06	1.60 ± 0.28
Guaynabo	25.00 ± 0.39	7.20 ± 0.16	1.34 ± 0.17	0.68 ± 0.10	1.19 ± 0.12
Villalba	22.67 ± 0.22	7.57 ± 0.19	2.45 ± 0.15	1.68 ± 0.19	1.99 ± 0.20
Yauco	23.15 ± 0.32	7.73 ± 0.19	1.43 ± 0.09	0.89 ± 0.08	1.12 ± 0.14

All values are mean ± one standard error, 1 = Total chlorine, 2 = Free Chlorine.

Table 2. **Bacteriological Density by Site and Water Source. (Measurements were taken between September 24, 198 and March 7, 1988).**

INFLUENTS

Site	m-Endo (CFU)	m-FC (CFU)	m-T7 (CFU)	m-KF (CFU)	MPN1	MPN2	MPN3
Bayamón	1074 ± 553	190 ± 84	80 ± 46	203 ± 68	133 ± 79	62 ± 30	94 ± 46
Guaynabo	3662 ± 1835	1510 ± 667	439 ± 265	1928 ± 1006	1285 ± 166	969 ± 198	1123 ± 1
Villalba	28475 ± 2030	903 ± 354	146 ± 45	950 ± 471	1068 ± 172	984 ± 177	1129 ± 1
Yauco	1808 ± 859	233 ± 89	127 ± 77	948 ± 333	949 ± 204	564 ± 176	950 ± 20

EFFLUENTS

Site	m-Endo (CFU)	m-FC (CFU)	m-T7 (CFU)	m-KF (CFU)	MPN1	MPN2	MPN3
Bayamón	0.00 ± 0.00	0.00 ± 0.00	1.18 ± 1.18	6.36 ± 5.40	2.00 ± 0.00	2.00 ± 0.00	2.91 ±
Guaynabo	0.25 ± 0.18	0.00 ± 0.00	0.00 ± 0.00	0.17 ± 0.11	2.92 ± 0.92	3.75 ± 1.75	12.7 ±
Villalba	0.75 ± 0.79	2.00 ± 2.00	0.45 ± 0.45	1.92 ± 0.79	2.00 ± 0.00	2.50 ± 0.50	2.00 ±
Yauco	0.62 ± 0.37	0.54 ± 0.37	0.09 ± 0.09	4.31 ± 3.46	2.46 ± 0.46	2.00 ± 0.00	2.46 ±

All values are mean ± one standard error per 100 ml. MPN1 = Total coliform MPN, MPN2 = Fecal coliform MPN, ... = Fecal streptococci MPN

Table 4. **Average Indicator Organism / AODC* Ratios per Viable Count Method Assessed (Expressed as Percentages).**

INFLUENTS

SITE	m-Endo agar	m-FC agar	m-T7 agar	m-KF agar	MPN1	MPN2	MPN3
Bayamón	7.29	2.63	1.21	3.64	2.25	9.24	1.56
Guaynabo	313	128	33.7	108	191	166	189
Villalba	96	15.7	2.39	20.2	19.8	19.6	21.0
Yauco	98	12.9	2.50	66.4	43.0	26.0	68.0

EFFLUENTS

SITE	m-Endo agar	m-FC agar	m-T7 agar	m-KF agar	MPN1	MPN2	MPN3
Bayamón	0.00	0.00	0.07	0.33	0.14	0.14	0.19
Guaynabo	0.00	0.00	0.00	0.04	3.27	4.60	19.0
Villalba	0.02	0.07	0.02	0.06	0.15	0.24	0.15
Yauco	0.05	0.03	0.00	0.38	0.28	0.27	0.28

All values are percent X 10^{-4}, MPN1=Total coliform MPN, MPN2=Fecal coliform MPN, MPN3= Fecal streptococci MPN.

Table 3. Direct Count and Percent Activity by Site and Sample Type (Measurements taken between September 24, 1987 and March 7, 1988)

BAYAMON

Sample Type	AODC* (X 10^7)	Percent Activity**
Influent	7.19 ± 3.67	73.84 ± 4.15
Effluent	1.74 ± 0.44	56.31 ± 3.58

GUAYNABO

Sample Type	AODC* (X 10^7)	Percent Activity**
Influent	4.39 ± 0.76	32.14 ± 6.2
Effluent	0.82 ± 0.41	26.53 ± 5.6

VILLALBA

Sample Type	AODC* (X 10^7)	Percent Activity**
Influent	3.44 ± 0.46	76.38 ± 3.65
Effluent	1.42 ± 0.27	62.69 ± 6.27

YAUCO

Sample Type	AODC* (X 10^7)	Percent Activity**
Influent	5.02 ± 0.96	50.45 ±
Effluent	2.53 ± 0.38	36.58 ±

*Acridine Orange Direct Counts.

**as determined by the number of red fluorescing cells divided by the total number of fluorescent cells and multiplied by 100.

Table 5. **Correlation Matrix: Combined Influent Bacteriological Methods and Water Quality**

	MF TC	MF FC	MF MT7	MF FS	MPN TC	MPN FC	MPN FS	AODC	%ACT	TEMP	PH	TURB
MF TC	1.000											
MF FC	0.889***	1.000										
MF MT7	0.708**	0.699**	1.000									
MF FS	0.818**	0.724**	0.552	1.000								
MPN TC	0.828***	0.738**	0.594*	0.698**	1.000							
MPN FC	0.798**	0.732**	0.619*	0.706**	0.884***	1.000						
MPN FS	0.766**	0.687**	0.648*	0.68*	0.888***	0.902***	1.000					
AODC	0.083	0.004	0.042	0.097	0.167	0.127	0.232	1.000				
%ACT.	0.052	0.101	0.128	0.100	0.216	0.117	-0.146	0.345	1.000			
TEMP	0.218	0.185	0.223	0.027	0.233	0.314	0.253	0.124	0.028	1.000		
PH	0.117	0.175	0.049	0.241	0.135	0.147	-0.108	0.206	0.039	0.440	1.000	
TURB	0.064	0.083	0.098	0.140	0.056	0.020	0.011	0.380	0.435	0.090	0.033	1.000

n=44, * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$, MF TC = m-Endo agar, MF FC = m-FC agar, MF MT7 = m-T7 agar, MF FS = m-KF agar, MPN T
Total coliform MPN, MPN FC = Fecal coliform MPN, MPN FS = Fecal streptococci MPN, AODC = Acridine Orange Direct Counts, %ACT = Percent activity, TEMP = Temperature, TURB = Turbidity.

Table 6. Correlation Matrix: Combined Effluent Bacteriological Methods and Water Quality.

	MF TC	MF FC	MF MT7	MF FS	MPN TC	MPN FC	MPN FS	AODC	%ACT	TEMP	PH	TURB	T CL	F CL
MF TC	1.000													
MF FC	0.839***	1.000												
MF MT7	0.022	0.035	1.000											
MF FS	0.306	0.221	0.448	1.000										
MPN TC	0.231	0.215	0.080	0.084	1.000									
MPN FC	-0.077	-0.051	-0.050	-0.052	0.573	1.000								
MPN FS	0.089	0.095	0.382	0.247	0.856*	0.649	1.000							
AODC	0.225	0.117	0.097	0.215	-0.109	-0.279	-0.162	1.000						
%ACT	-0.151	-0.161	0.031	0.163	-0.334	-0.192	-0.247	0.341	1.000					
TEMP	-0.032	-0.066	0.038	-0.107	0.054	0.036	0.115	-0.058	-0.102	1.000				
PH	0.153	0.106	0.106	0.353	0.067	-0.008	0.113	0.069	0.192	-0.262	1.000			
TURB	0.229	0.221	0.540	0.478	0.087	0.247	0.316	0.148	0.277	-0.112	0.195	1.000		
T CL	0.220	0.098	0.041	0.152	-0.136	-0.690	-0.113	0.283	0.446	-0.484	0.024	0.334	1.000	
F CL	0.188	0.121	0.021	0.184	-0.101	-0.036	-0.093	0.268	0.422	-0.567	0.025	0.321	0.995***	

n=46, * = P < 0.05, ** = P < 0.01, *** = P < 0.001, T CL = Total Chlorine, F CL = Free Chlorine, MF TC = m-Endo agar, MF FC = m-FC agar, MT7 = m-T7 agar, MF FS = m-KF agar, MPN TC = Total coliform MPN, MPN FC = Fecal coliform MPN, MPN FS = Fecal streptococci MPN = Acridine Orange Direct Counts, %ACT = Percent activity, TEMP = Temperature, TURB = Turbidity.

Table 7. **Correlation Matrix: Bayamón Influent Bacteriological Methods and Water Quality.**

	MF TC	MF FC	MF MT7	MF FS	MPN TC	MPN FC	MPN FS	AODC	%ACT	TEMP	PH	TURB
MF TC	1.000											
MF FC	0.933***	1.000										
MF MT7	0.742**	0.804**	1.000									
MF FS	0.625*	0.53	0.517	1.000								
MPN TC	0.732**	0.805**	0.739**	0.372	1.000							
MPN FC	0.629*	0.652*	0.503	0.569	0.771**	1.000						
MPN FS	0.616*	0.7*	0.675*	0.316	0.953***	0.823***	1.000					
AODC	0.665*	0.477	0.499	0.472	0.335	0.525	0.373	1.000				
%ACT	0.509	0.31	0.222	0.554	0.152	0.478	0.134	0.623*	1.000			
TEMP	0.875***	0.881***	0.718**	-0.566	0.624*	0.66*	0.601*	0.559*	-0.548	1.000		
PH	0.612*	0.714**	0.471	0.231	0.547	0.555	0.628*	0.354	0.24	0.839***	1.000	
TURB	0.028	0.027	0.089	0.247	0.131	0.264	0.319	0.073	-0.087	-0.036	0.356	1.000

n=11, * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$, MF TC = m-Endo agar, MF FC = m-FC agar, MF MT7 = m-T7 agar, MF FS = m-KF agar, MPN TC = Total coliform MPN, MPN FC = Fecal coliform MPN, MPN FS = Fecal streptococci MPN, AODC = Acridine Orange Direct Counts, %ACT = Percent activity, TEMP = Temperature, TURB = Turbidity.

Table 8. **Correlation Matrix: Bayamón Effluent Bacteriological Methods and Water Quality.**

	MF TC	MF FC	MF MT7	MF FS	MPN TC	MPN FC	MPN FS	AODC	%ACT	TEMP	PH	TURB	TCL	FC
MF TC	1.000													
MF FC	•	1.000												
MF MT7	•	•	1.000											
MF FS	•	•	0.845***	1.000										
MPN TC	•	•	•	•	1.000									
MPN FC	•	•	•	•	•	1.000								
MPN FS	•	•	•	0.845***	•	•	1.000							
AODC	•	•	0.109	0.095	•	•	-0.109	1.000						
%ACT	•	•	0.108	0.108	•	•	0.108	0.431	1.000					
TEMP	•	•	0.056	0.221	•	•	-0.056	0.187	0.252	1.000				
PH	•	•	0.299	0.234	•	•	0.299	0.520	0.080	0.184	1.000			
TURB	•	•	0.952***	0.782**	•	•	0.952***	0.054	0.299	0.138	0.262	1.000		
TCL	•	•	0.600	0.630*	•	•	0.600**	0.231	0.316	0.090	0.008	0.733**	1.000	
FCL	•	•	0.529	0.662*	•	•	0.529	0.415	0.303	0.100	0.137	0.640**	0.927**	

n=11, * = P < 0.05, ** = P < 0.01, *** = P < 0.001, • = Zero values correlation, T CL = Total Chlorine, F CL = Free Chlorine,.MF TC = m-Endo agar, MF FC = m-FC agar, MF MT7 = m-T7 agar, MF FS = m-KF agar, MPN TC = Total coliform MPN, MPN FC = Fecal coliform MPN, MPN FS = Fecal streptococci MPN, AODC = Acridine Orange Direct Counts, %ACT = Percent activity, TEMP = Temperature, TURB = Turbidity. ACT = Percent activity; TEMP = Temperature; PH = pH; TURB = Turbidity.

Table 9. Correlation Matrix: Guaynabo Influent Bacteriological Methods and Water Quality

	MF TC	MF FC	MF MT7	MF FS	MPN TC	MPN FC	MPN FS	AODC	%ACT	TEMP	PH	TURB
MF TC	1.000											
MF FC	0.934***	1.000										
MF MT7	0.387	0.597*	1.000									
MF FS	0.931***	0.796**	0.376	1.000								
MPN TC	0.634*	0.576*	0.204	0.286	1.000							
MPN FC	0.516	0.659*	0.817**	0.291	0.341	1.000						
MPN FS	0.457	0.508	0.634*	0.275	0.602*	0.740**	1.000					
AODC	0.244	0.130	0.553	0.459	0.172	0.460	0.562	1.000				
%ACT	0.473	0.471	0.337	0.259	0.291	0.641*	0.649*	0.184	1.000			
TEMP	0.458	0.605*	0.671*	0.166	0.686*	0.737**	0.687*	0.462	0.169	1.000		
PH	0.204	0.081	0.231	0.080	0.213	0.239	0.050	0.318	0.621**	0.290	1.000	
TURB	0.79*	0.656*	0.015	0.78*	0.254	0.260	0.220	0.618*	0.474	0.030	0.591*	1.000

n=11, * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$, MF TC = m-Endo agar, MF FC = m-FC agar, MF MT7 = m-T7 agar, MF FS = m-KF agar, MPN TC = Total coliform MPN, MPN FC = Fecal coliform MPN, MPN FS = Fecal streptococci MPN, AODC = Acridine Orange Direct Counts, %ACT = Percent activity, TEMP = Temperature, TURB = Turbidity.

Table 10. **Correlation Matrix: Guaynabo Effluent Bacteriological Methods and Water Quality.**

	MF TC	MF FC	MF MT7	MF FS	MPN TC	MPN FC	MPN FS	AODC	%ACT	TEMP	PH	TURB	TCL	FC
MF TC	1.000													
MF FC	•	1.000												
MF MT7	•	•	1.000											
MF FS	0.194	•	•	1.000										
MPN TC	0.131	•	•	-0.135	1.000									
MPN FC	0.131	•	•	-0.135	•	1.000								
MPN FS	0.131	•	•	-0.135	•	•	1.000							
AODC	0.617*	•	•	0.179	0.273	0.273	0.000	1.000						
%ACT	0.258	•	•	0.253	0.249	0.249	0.249	0.182	1.000					
TEMP	0.336	•	•	0.173	0.000	0.000	0.000	0.438	0.340	1.000				
PH	0.000	•	•	0.068	0.233	0.233	0.233	0.342	0.480	-0.370	1.000			
TURB	0.202	•	•	-0.316	0.371	0.371	0.371	0.123	0.407	-0.062	0.528	1.000		
T CL	0.397	•	•	0.087	0.076	0.076	0.076	0.399	0.481	0.289	0.132	0.481	1.000	
F CL	0.383	•	•	0.157	0.010	0.010	0.010	0.403	0.406	0.273	0.118	0.523	0.979**	

n=11, * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$, • = Zero values correlation, T CL = Total Chlorine, F CL = Free Chlorine, MF TC = m-Endo agar, MF FC = m-FC agar, MF MT7 = m-T7 agar, MF FS = m-KF agar, MPN TC = Total coliform MPN, MPN FC = Fecal coliform MPN, MPN FS Fecal streptococci MPN, AODC = Acridine Orange Direct Counts, %ACT = Percent activity, TEMP = Temperature, TURB = Turbidity. ACT = Percent activity; TEMP = Temperature; PH = pH; TURB = Turbidity.

Table 11. **Correlation Matrix: Villalba Influent Bacteriological Methods and Water Quality.**

	MF TC	MF FC	MF MT7	MF FS	MPN TC	MPN FC	MPN FS	AODC	%ACT	TEMP	PH	TURB
MF TC	1.000											
MF FC	0.654*	1.000										
MF MT7	0.739**	0.497	1.000									
MF FS	0.643*	0.331	0.486	1.000								
MPN TC	0.496	0.268	0.296	0.547	1.000							
MPN FC	0.761**	0.689*	0.596*	0.482	0.443	1.000						
MPN FS	0.61*	0.639*	0.76**	0.482	0.127	0.786**	1.000					
AODC	0.138	0.080	0.193	0.330	-0.287	0.419	0.340	1.000				
%ACT	0.051	0.059	0.364	0.008	-0.268	0.271	0.088	0.577*	1.000			
TEMP	0.587*	0.340	0.817**	0.278	0.178	0.504	0.653*	0.449	0.069	1.000		
PH	0.685*	0.554	0.525	0.271	-0.454	0.410	0.452	0.119	0.069	0.532	1.000	
TURB	0.146	0.120	0.384	0.374	-0.057	0.176	0.432	0.239	0.490	0.144	0.073	1.000

n=11, * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$. MF TC = m-Endo agar, MF FC = m-FC agar, MF MT7 = m-T7 agar, MF FS = m-KF agar, MPN TC = Total coliform MPN, MPN FC = Fecal coliform MPN, MPN FS = Fecal streptococci MPN, AODC = Acridine Orange Direct Counts, %ACT = Percent activity, TEMP = Temperature, TURB = Turbidity.

Table 12. **Correlation Matrix: Villalba Effluent Bacteriological Methods and Water Quality.**

	MF TC	MF FC	MF MT7	MF FS	MPN TC	MPN FC	MPN FS	AODC	%ACT	TEMP	PH	TURB	TCL	FC
MF TC	1.000													
MF FC	0.873***	1.000												
MF MT7	0.128	0.091	1.000											
MF FS	0.026	0.237	0.237	1.000										
MPN TC	•	•	•	•	1.000									
MPN FC	0.128	0.091	0.091	0.004	•	1.000								
MPN FS	•	•	•	•	•	•	1.000							
AODC	0.131	0.075	0.054	0.255	•	0.430	•	1.000						
%ACT	0.398	0.430	0.281	0.358	•	0.258	•	0.145	1.000					
TEMP	0.363	0.539	0.135	-0.419	•	0.135	•	0.458	0.100	1.000				
PH	0.248	0.014	0.150	0.414	•	0.033	•	0.264	-0.237	-0.609*	1.000			
TURB	0.277	0.228	0.041	0.081	•	0.588*	•	0.134	-0.246	0.327	0.071	1.000		
TCL	0.335	0.030	0.269	0.447	•	0.508	•	0.244	0.318	-0.355	0.255	-0.403	1.000	
FCL	0.333	0.042	0.333	0.470	•	0.457	•	0.234	0.290	-0.316	0.194	-0.371	0.981***	

n=11, * = P < 0.05, ** = P < 0.01, *** = P < 0.001, • = Zero values correlation, T CL = Total Chlorine, F CL = Free Chlorine, MF TC = m-Endo agar, MF FC = m-FC agar, MF MT7 = m-T7 agar, MF FS = m-KF agar, MPN TC = Total coliform MPN, MPN FC = Fecal coliform MPN, MPN FS Fecal streptococci MPN, AODC = Acridine Orange Direct Counts, %ACT = Percent activity, TEMP = Temperature, TURB = Turbidity. ACT = Percent activity; TEMP = Temperature; PH = pH; TURB = Turbidity.

Table 13. **Correlation Matrix: Yauco Influent Bacteriological Methods and Water Quality.**

	MF TC	MF FC	MF MT7	MF FS	MPN TC	MPN FC	MPN FS	AODC	%ACT	TEMP	PH	TURB
MF TC	1.000											
MF FC	0.923***	1.000										
MF MT7	0.658*	0.616*	1.000									
MF FS	0.972***	0.893***	0.615*	1.000								
MPN TC	0.915***	0.818***	0.678*	0.833***	1.000							
MPN FC	0.885***	0.813***	0.761**	0.807**	0.98***	1.000						
MPN FS	0.782**	0.694*	0.724**	0.723*	0.851***	0.835***	1.000					
AODC	0.411	0.440	0.197	-0.492	0.241	0.136	0.195	1.000				
%ACT	0.029	0.079	0.401	-0.021	0.055	0.191	0.021	0.732**	1.000			
TEMP	0.696*	0.586*	0.239	0.645	0.722**	0.678*	0.728**	0.404	0.026	1.000		
PH	0.499	0.628*	0.278	-0.430	0.669*	0.682*	0.581*	0.18 5	0.123	0.562	1.000	
TURB	0.045	0.204	0.242	0.023	0.004	0.099	0.135	0.558	0.571	0.261	0.227	1.000

n=11, * = P < 0.05, ** = P < 0.01, *** = P < 0.001, MF TC = m-Endo agar, MF FC = m-FC agar, MF MT7 = m-T7 agar, MF FS = m-KF agar, MPN TC = Total coliform MPN, MPN FC = Fecal coliform MPN, MPN FS = Fecal streptococci MPN, AODC = Acridine Orange Direct Counts, %ACT = Percent activity, TEMP = Temperature, TURB = Turbidity.

Table 14. **Correlation Matrix: Yauco Effluent Bacteriological Methods and Water Quality.**

	MF TC	MF FC	MF MT7	MF FS	MPN TC	MPN FC	MPN FS	AODC	%ACT	TEMP	PH	TURB	T CL	F CL
MF TC	1.000													
MF FC	•	1.000												
MF MT7	0.731***	0.731**	1.000											
MF FS	0.903***	0.903***	0.426	1.000										
MPN TC	0.619*	0.619*	0.893***	0.323	1.000									
MPN FC	•	•	•	•	•	1.000								
MPN FS	0.611*	0.611*	0.884***	0.317	•	1.000								
AODC	0.345	0.345	0.420	0.158	0.584*	•	0.589*	1.000						
%ACT	0.255	0.255	0.317	0.154	0.481	•	0.486	0.687**		1.000				
TEMP	0.416	0.416	0.280	0.331	0.263	•	0.261	0.169	0.010	1.000				
PH	0.236	0.236	0.015	0.374	0.043	•	0.044	0.289	0.206	0.207	1.000			
TURB	0.066	0.066	0.246	0.066	0.114	•	0.108	-0.039	0.290	0.273	0.57*	1.000		
T CL	0.342	0.342	0.073	0.402	0.204	•	0.209	-0.326	0.149	0.436	0.187	0.022	1.000	
F CL	0.331	0.331	0.010	0.403	0.119	•	0.123	-0.240	0.029	0.440	0.216	0.039	0.984***	

n=11, * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$, • = Zero values correlation, T CL = Total Chlorine, F CL = Free Chlorine, MF TC = m-Endo agar, MF FC = m-FC agar, MF MT7 = m-T7 agar, MF FS = m-KF agar, MPN TC = Total coliform MPN, MPN FC = Fecal coliform MPN, MPN FS Fecal streptococci MPN, AODC = Acridine Orange Direct Counts, %ACT = Percent activity, TEMP = Temperature, TURB = Turbidity. ACT = Percent activity; TEMP = Temperature; PH = pH; TURB = Turbidity.

Table 15. **Media Performance in the Assessment of Target Bacteria.**

INFLUENTS

	Average Recovery*							Performance†
	Endo	FC	T7	KF	MPN1	MPN2	MPN3	High<----------->Low
Bayamón	422	144	66	143	133	61	94	Endo>FC=T7=KF=MPN1=MPN2=MPN3
Guaynabo	1912	1087	139	558	1256	911	1079	Endo=FC=T7=KF=MPN1=MPN2=MPN3
Villalba	4335	539	99	401	1019	928	1086	Endo>MPN1=MPN2=MPN3>FC=T7=KF
Yauco	1276	217	101	1618	963	479	975	Endo=FC=T7=KF=MPN1=MPN2=MPN3

EFFLUENTS

	Average Recovery*							Performance†
	Endo	FC	T7	KF	MPN1	MPN2	MPN3	High<----------->Low
Bayamón	0	0	1	6	0	1	0	Endo=FC=T7=KF=MPN1=MPN2=MPN3
Guaynabo	0	0	0	0	1	2	12	Endo=FC=T7=KF=MPN1=MPN2=MPN3
Villalba	0	2	0	2	0	2	0	Endo=FC=T7=KF=MPN1=MPN2=MPN3
Yauco	0	0	0	5	2	0	1	Endo=FC=T7=KF=MPN1=MPN2=MPN3

*Average Recovery = Average of the target colonies recovered on each plate, Endo=m-Endo agar, FC=m=FC agar, T7=m-T7 agar, KF=m-KF agar, MPN1=Total coliform MPN, MPN2=Fecal coliform MPN, MPN3=Fecal streptococci MPN

†Performance as measured by Student Newman-Keuls (SNK) Test.

Table 16. **Media Performance in the Assessment of Nontarget Bacteria.**

INFLUENTS

	Average Recovery*							Performance†
	Endo	FC	T7	KF	MPN1	MPN2	MPN3	High<----------->Low
Bayamón	702	54	14	63	72	20	45	Endo>FC=T7=KF=MPN1=MPN2=MPN3
Guaynabo	1923	349	109	87	606	260	136	Endo=FC=T7=KF=MPN1=MPN2=MPN3
Villalba	2208	273	46	174	182	316	228	Endo=FC=T7=KF=MPN1=MPN2=MPN3
Yauco	821	28	25	287	370	67	236	Endo=KF=MPN1>FC=T7=MPN2=MPN3

EFFLUENTS

	Average Recovery*							Performance†
	Endo	FC	T7	KF	MPN1	MPN2	MPN3	High<----------->Low
Bayamón	0	0	1	4	2	2	2	Endo=FC=T7=KF=MPN1=MPN2=MPN3
Guaynabo	0	0	0	0	3	4	14	Endo=FC=T7=KF=MPN1=MPN2=MPN3
Villalba	0	2	0	1	1	2	3	Endo=FC=T7=KF=MPN1=MPN2=MPN3
Yauco	6	4	0	2	1	1	2	Endo=FC=T7=KF=MPN1=MPN2=MPN3

*Average Recovery = Average of the target colonies recovered on each plate, Endo=m-Endo agar, FC=m=FC agar, T7=m-T7 agar, KF=m-KF agar, MPN1=Total coliform MPN, MPN2=Fecal coliform MPN, MPN3=Fecal streptococci MPN
†Performance as measured by Student Newman-Keuls (SNK) Test.

Table 17. **Specificity and Selectivity of Viable Count Methods in the Assessment of Influents.**

PARAMETER	METHODS						
	m-[illegible]do ag	m-FC agar	m-T7 agar	F	MPN1	MPN2	MPN3
Presumptive Target	17	37	20		26	30	15
Verified Target	10	26	15		12	19	11
Presumptive nontarget	24	22	10	4	15	28	39
Verified nontarget	14	16	8		9	20	28
Specificity Indexes (%):							
False Positive Errors	41	30	25		54	37	27
False Negative Errors	50	19	12	3	33	29	45
Selectivity Index (%)	41	63	[illegible]	3	63	52	29

MPN1=Total coliform MPN, MPN2=[illegible] MPN3 [illegible]ptococ[illegible]

Table 18. **Specificity and Selectivity of Viable Count Methods in the Assesment of Effluents.**

PARAMETER	METHODS						
	m-Endo agar	m-FC agar	m-T7 agar	m-KF agar	MPN1	MPN2	MPN3
Presumptive target	20	25	19	15	21	31	15
Verfified target	7	13	12	10	6	14	9
Presumptive nontarget	17	19	16	7	7	35	4
Verified nontarget	5	8	10	4	3	15	2
Specificity Indexes (%)							
False positive error	65	24	37	33	71	55	40
False negative error	63	46	33	23	40	59	18
Selectivity index (%)	54	57	54	68	75	47	79

MPN1=Total coliform MPN, MPN2=Fecal coliform MPN, MPN3=Fecal streptococci MPN.

Table 19. **Bacterial Species Isolated from Influent Waters.**

Number of Species	Times Isolated	% Times Isolated	% Recovery on Fecal Coliform Media		
			m-FC	m-T7	MPN (EC Broth)
Escherichia coli	73	42.7	30.1	42.7	27.4
Enterobacter sp.	27	15.8	63.0	37.0	37.0
Aeromonas hydrophyla	9	5.3	22.2	44.5	35.3
Unidentified*	15	8.8	26.7	26.7	46.7
Vibrio mimicus	2	1.2	50.0	50.0	-
Pseudomonas sp.	4	2.3	25.0	75.0	-
Klebsiella pneumoniae	12	7.0	20.0	80.0	-
Citrobacter freundii	21	12.3	29.0	35.7	-
Achromobacter sp.	1	0.6	-	100.0	-
Shigella dysenteriae	3	1.8	-	100.0	-
Proteus vulgaris	2	1.2	-	100.0	-
Serratia odorifera	4	2.3	100.0	-	

TOTAL = 170

*Unidentifiable by the API-20E System for the identification of enterobacteriaceae (Analytab)

Table 20. **Bacterial Species Isolated from Chlorinated Effluents.**

Number of Species	Times Isolated	% Times Isolated	% Recovery on Fecal Coliform Media		
			m-FC	m-T7	MPN (EC Broth)
Escherichia coli	22	61.1	27.3	50.0	22.7
Klebsiella pneumoniae	3	8.3	33.4	66.6	-
Pseudomonas sp.	6	16.7	94.4	5.6	-
Unidentified*	3	8.3	66.6	33.4	-
Citrobacter freundii	2	5.6	100.0	-	-
Enterobacter cloacae	5	13.9	100.0	-	-

TOTAL = 41

*Unidentifiable by the API-20E System for the identification of enterobacteriaceae (Analytab)

Figure 1. Study Sites.

1 = La Plata Water Treatment Plant (Bayamón)

2 = Los Filtros Treatment Plant (Guaynabo)

3 = Villalba Urban System Plant (Villalba)

4 = Ranchera Ward Treatment Plant (Yauco)

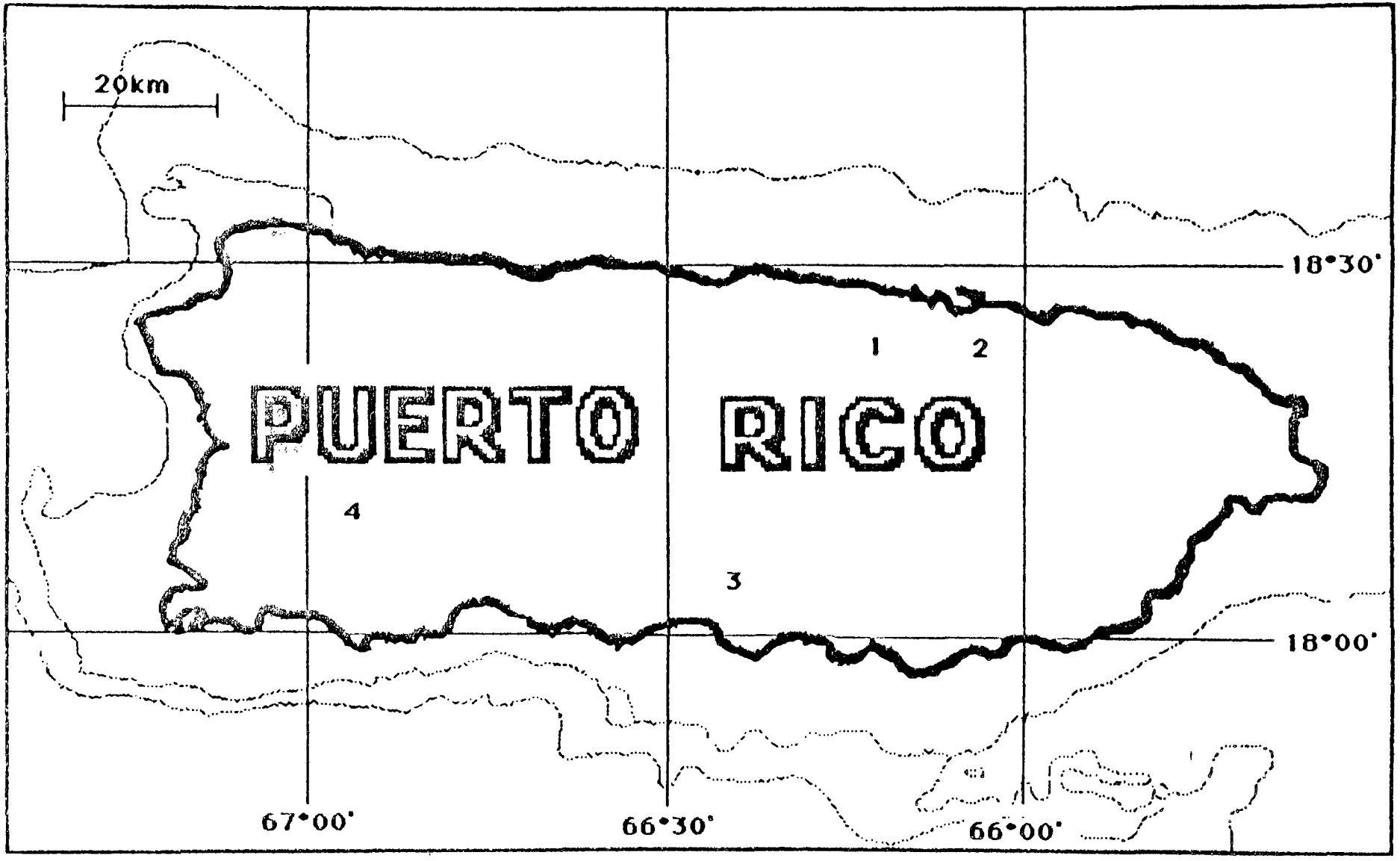

20km
18°30'
1
2
PUERTO RICO
4
3
18°00'
67°00'
66°30'
66°00'

Figure 2. Total Coliform Densities found in Bayamón Influent Waters (September 24, 1987 to March 7, 1988).

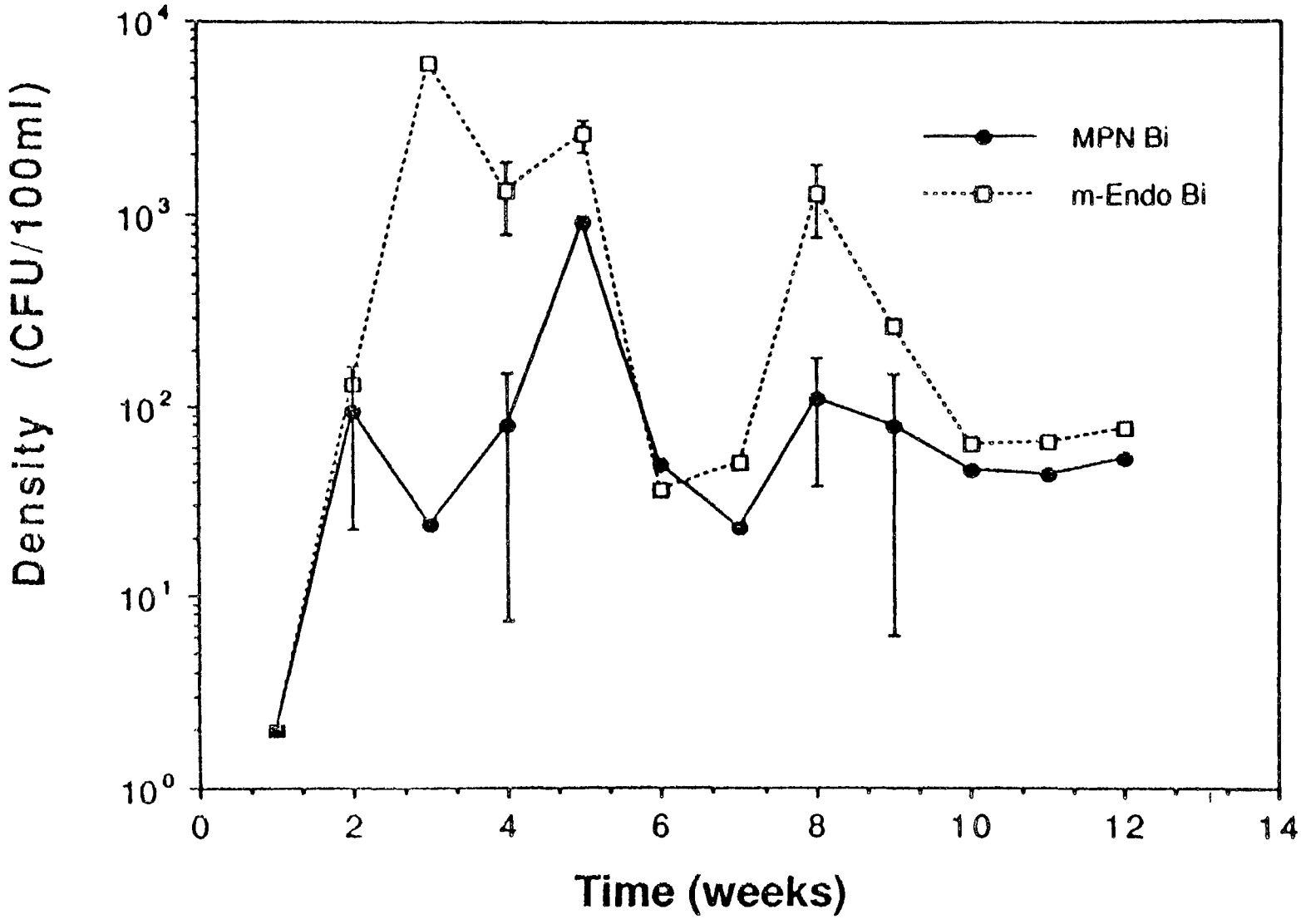

Density (CFU/100ml)
10^4
10^3
10^2
10^1
10^0
0
2
4
6
8
10
12
14
Time (weeks)
MPN Bi
m-Endo Bi

Figure 3. Total Coliform Densities in Guaynabo Influent Waters (September 24, 1987 to March 7, 1988).

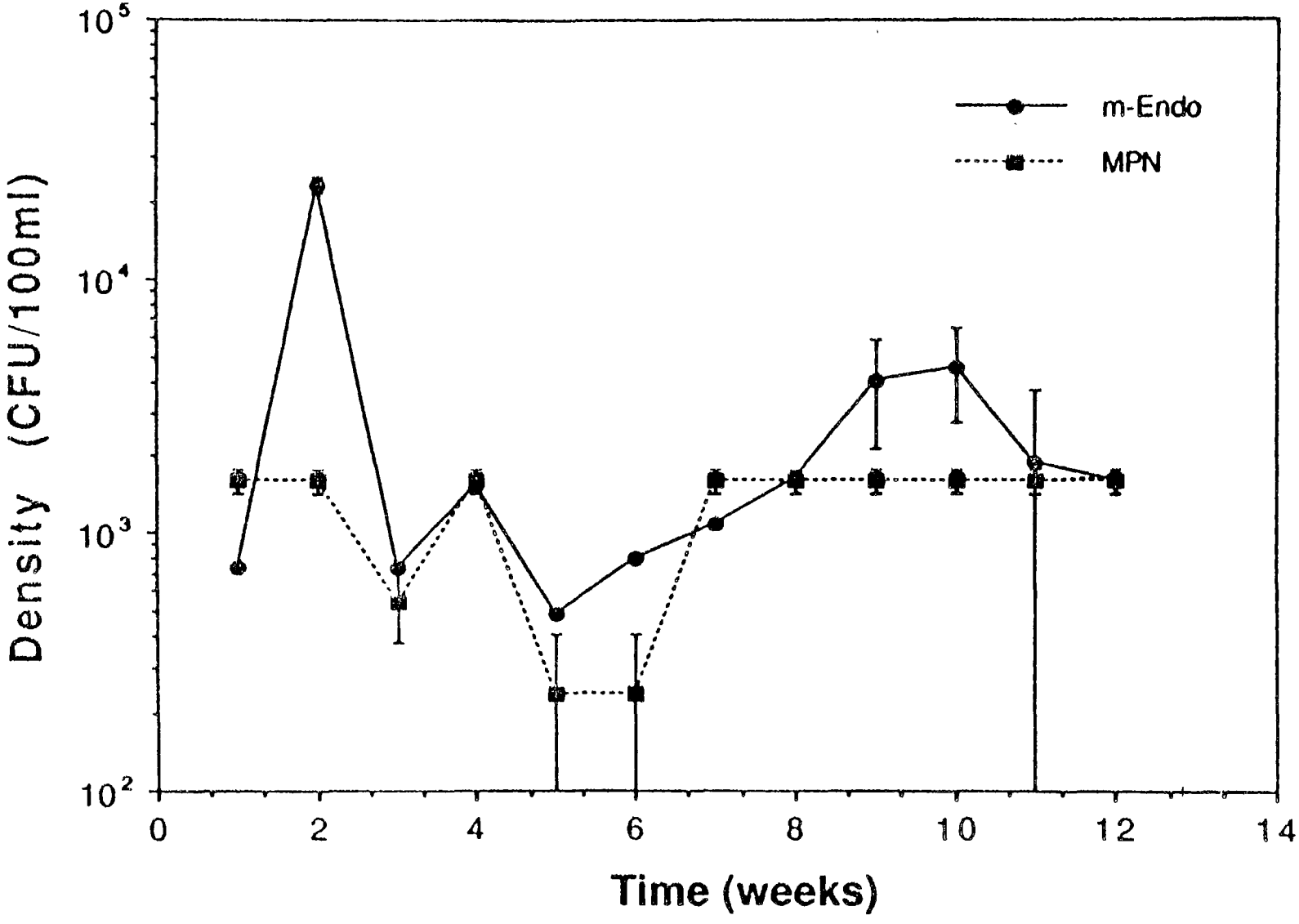

m-Endo
MPN
Density (CFU/100ml)
10^5
10^4
10^3
10^2
0
2
4
6
8
10
12
14
Time (weeks)

Figure 4. Total Coliform Densities in Yauco Influent Waters (September 24, 1987 to March 7, 1988).

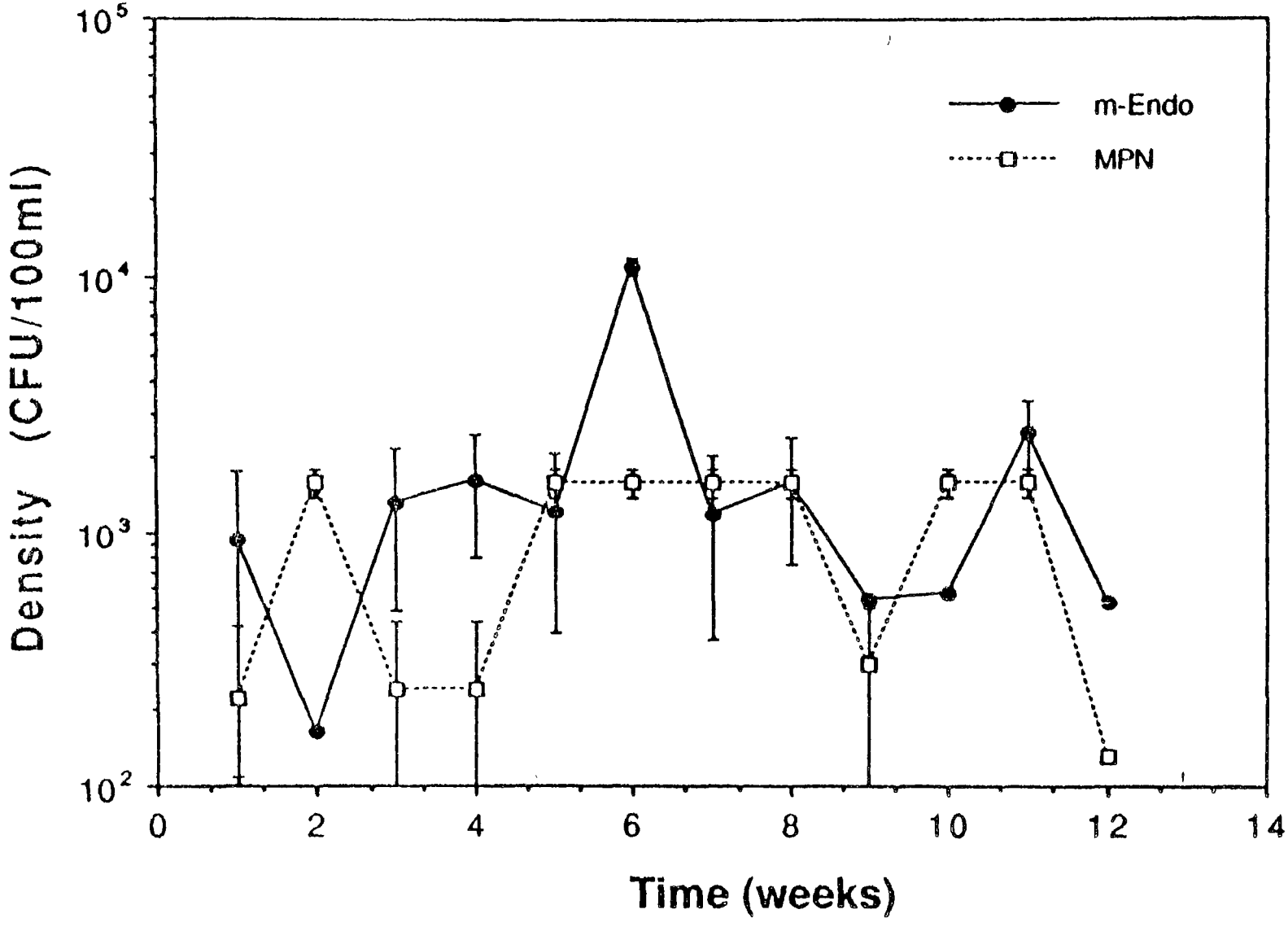

m-Endo
MPN
Density (CFU/100ml)
10^5
10^4
10^3
10^2
0
2
4
6
8
10
12
14
Time (weeks)

Figure 5. Total Coliform Densities in Villalba Influent Water (September 24, 1987 to March 7, 1988).

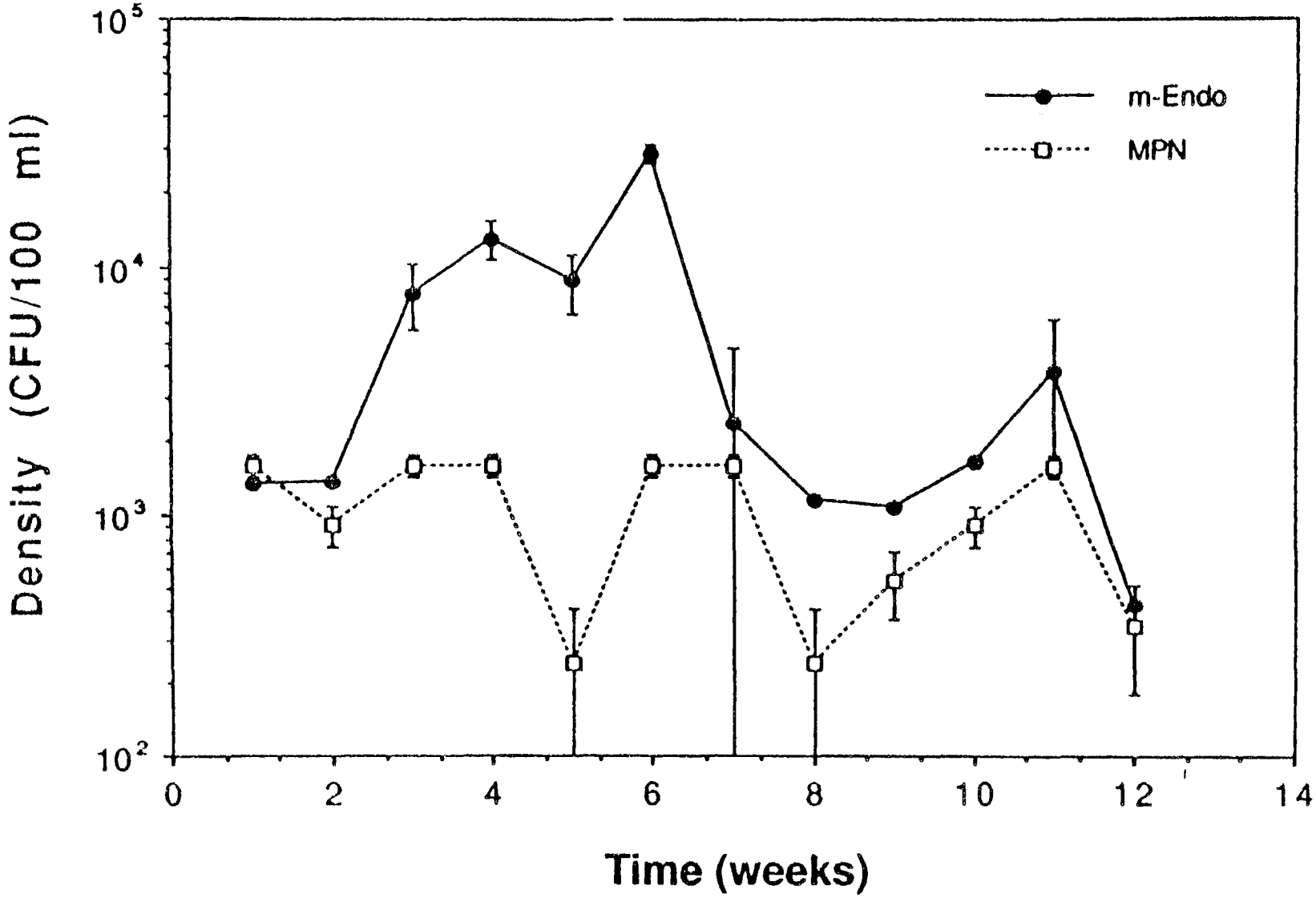

Density (CFU/100 ml)
10^5
10^4
10^3
10^2
0
2
4
6
8
10
12
14
Time (weeks)
m-Endo
MPN

Figure 6. Fecal Coliform Levels in Bayamón Influent Waters (September 24, 1987 to March 7, 1988).

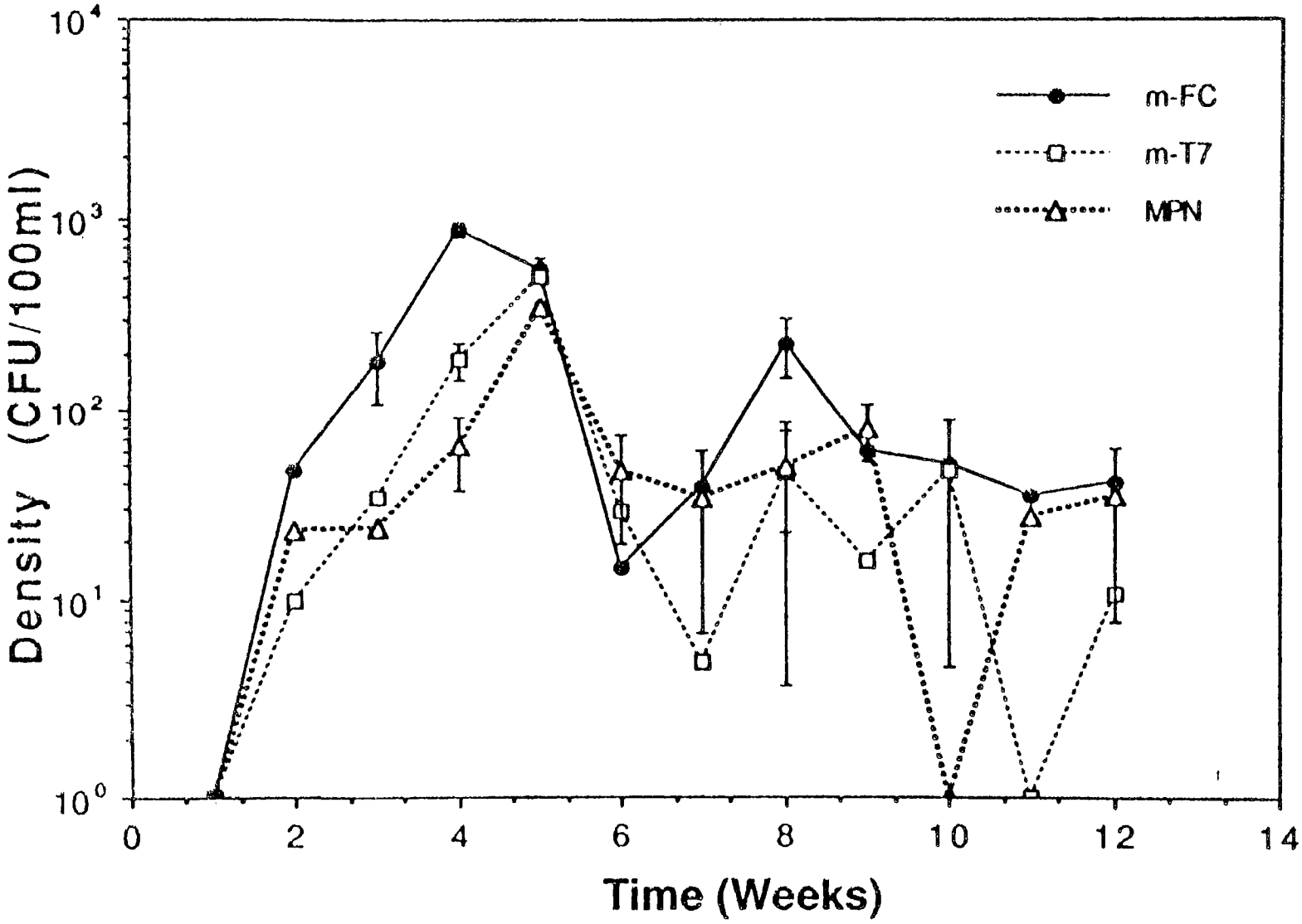

m-FC
m-T7
MPN
Density (CFU/100ml)
10^4
10^3
10^2
10^1
10^0
0
2
4
6
8
10
12
14
Time (Weeks)

Figure 7. Fecal Coliform Densities in Gu[illegible] fluent W[illegible] (September 24, 1987 to March 7, 1988).

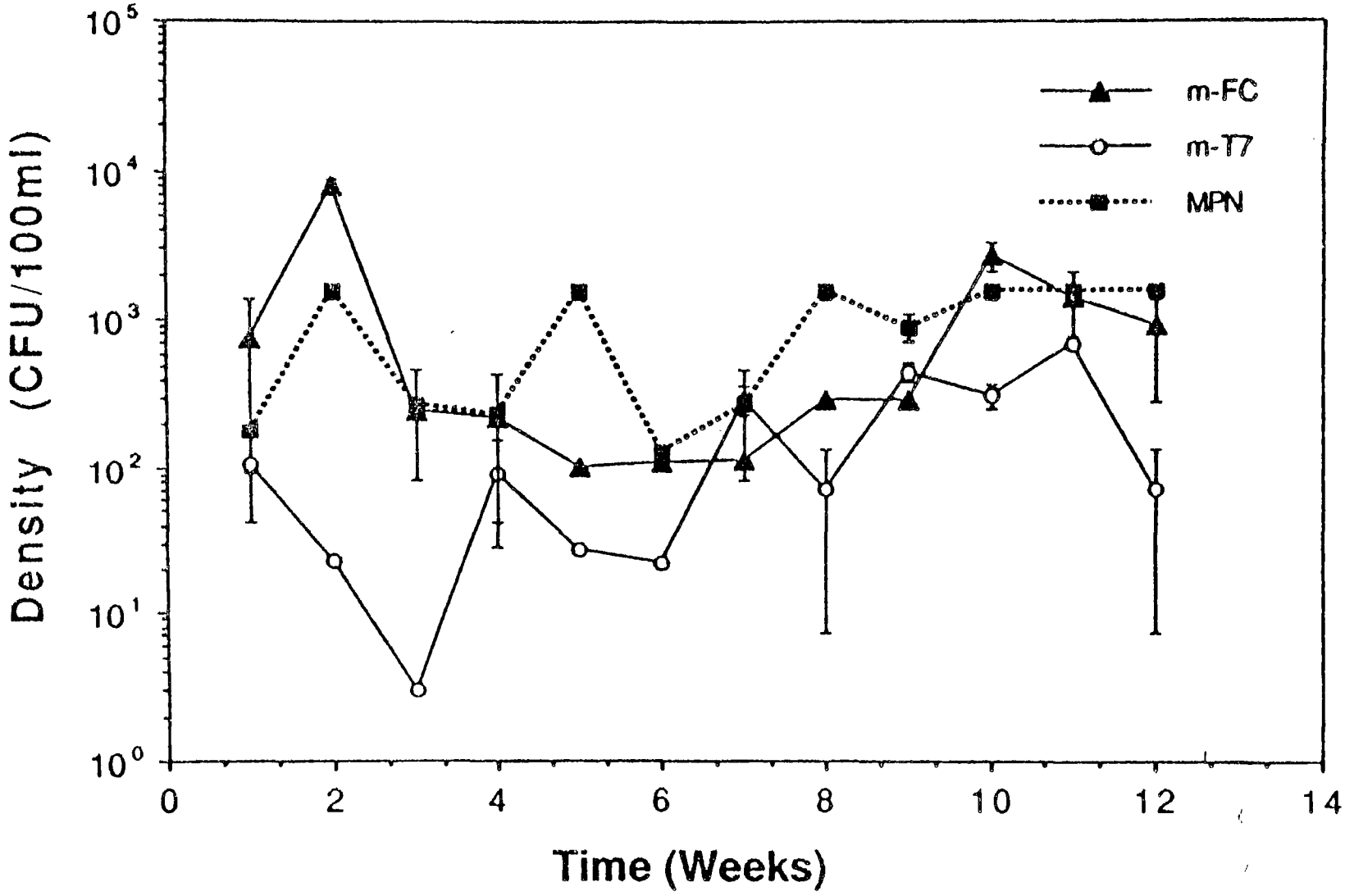

Density (CFU/100ml)
10^5
10^4
10^3
10^2
10^1
10^0
0
2
4
6
8
10
12
14
Time (Weeks)
m-FC
m-T7
MPN

Figure 8. Fecal Coliform Densities in Villalba Influent Waters (September 24, 1987 to March 7, 1988).

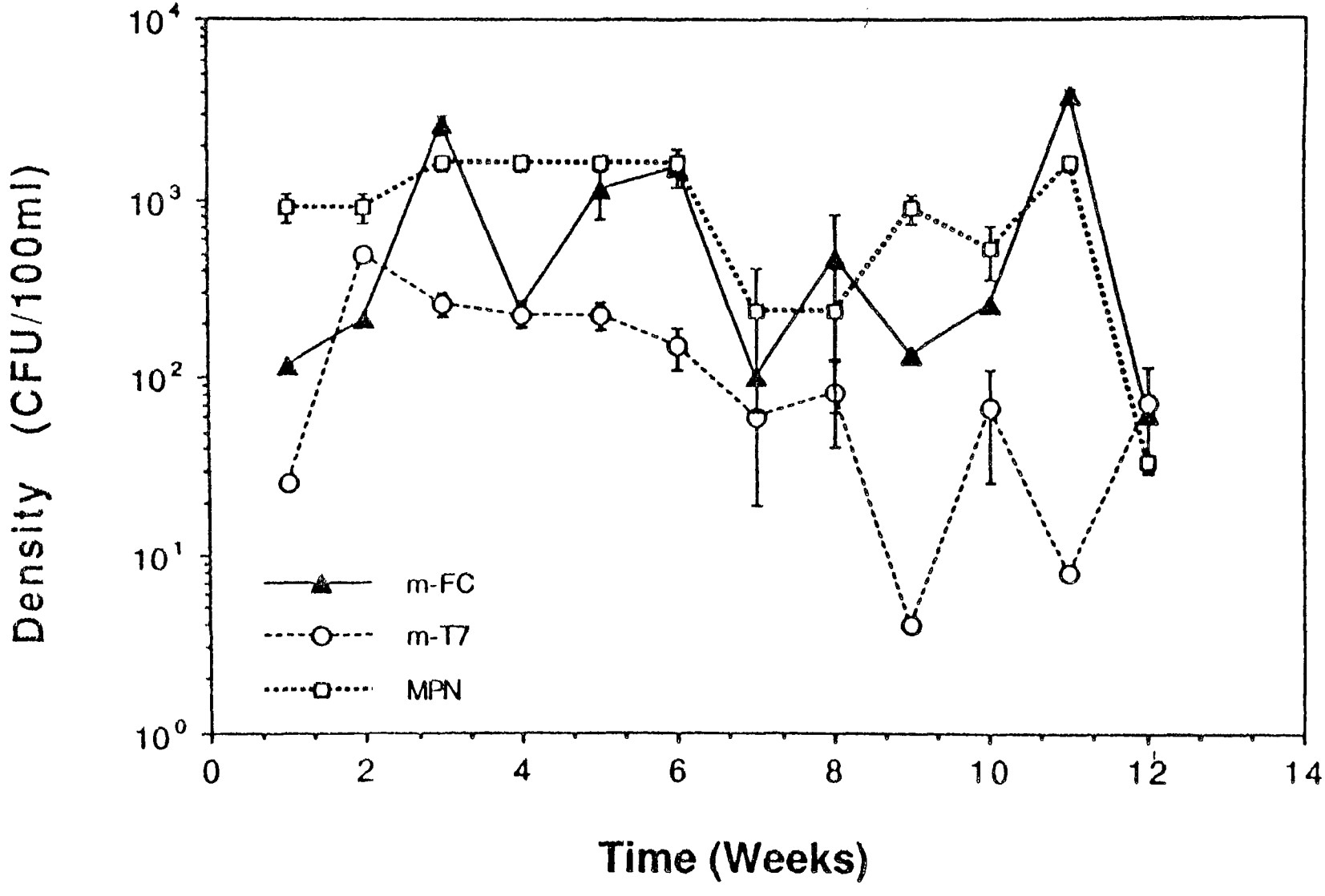

Density (CFU/100ml)
10^4
10^3
10^2
10^1
10^0
0
2
4
6
8
10
12
14
m-FC
m-T7
MPN
Time (Weeks)

Figure 9. Fecal Coliform Densities in Yauco Influent Waters (September 24, 1987 to March 7, 1988).

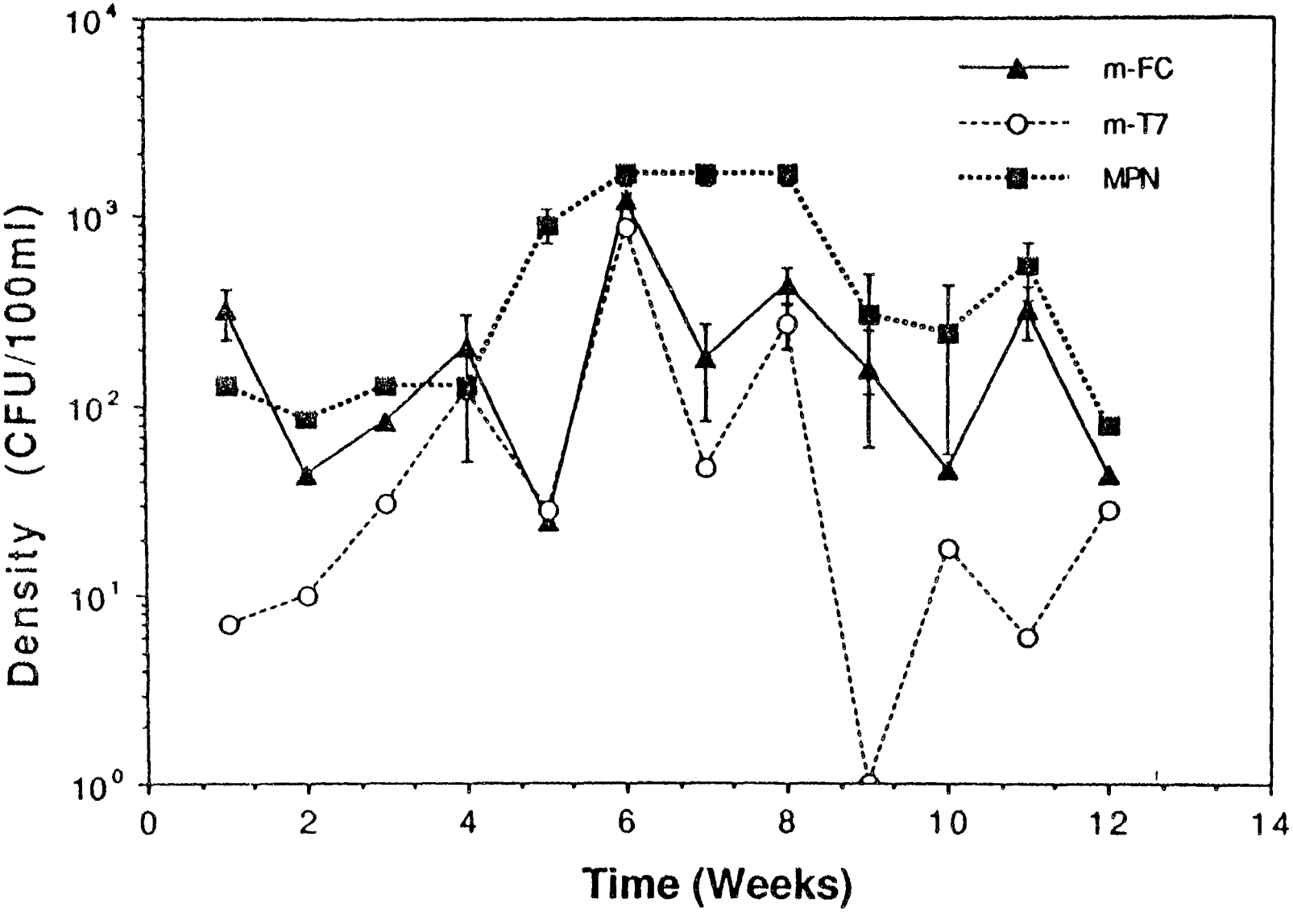

m-FC
m-T7
MPN
Density (CFU/100ml)
10^4
10^3
10^2
10^1
10^0
0
2
4
6
8
10
12
14
Time (Weeks)

Figure 10. Fecal Streptococci Levels in Villalba Effluent Waters (September 24, 1987 to March 7, 1988).

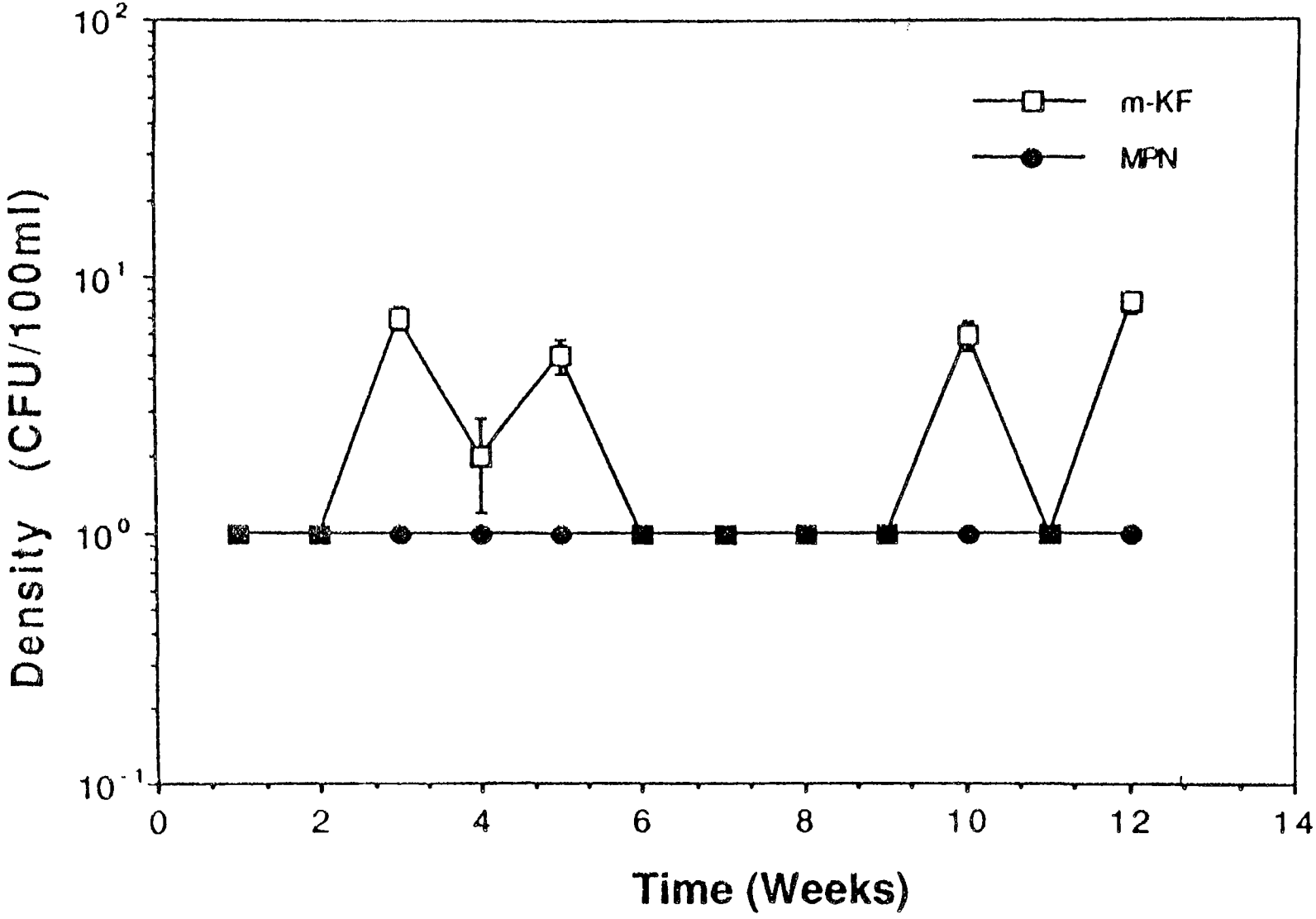
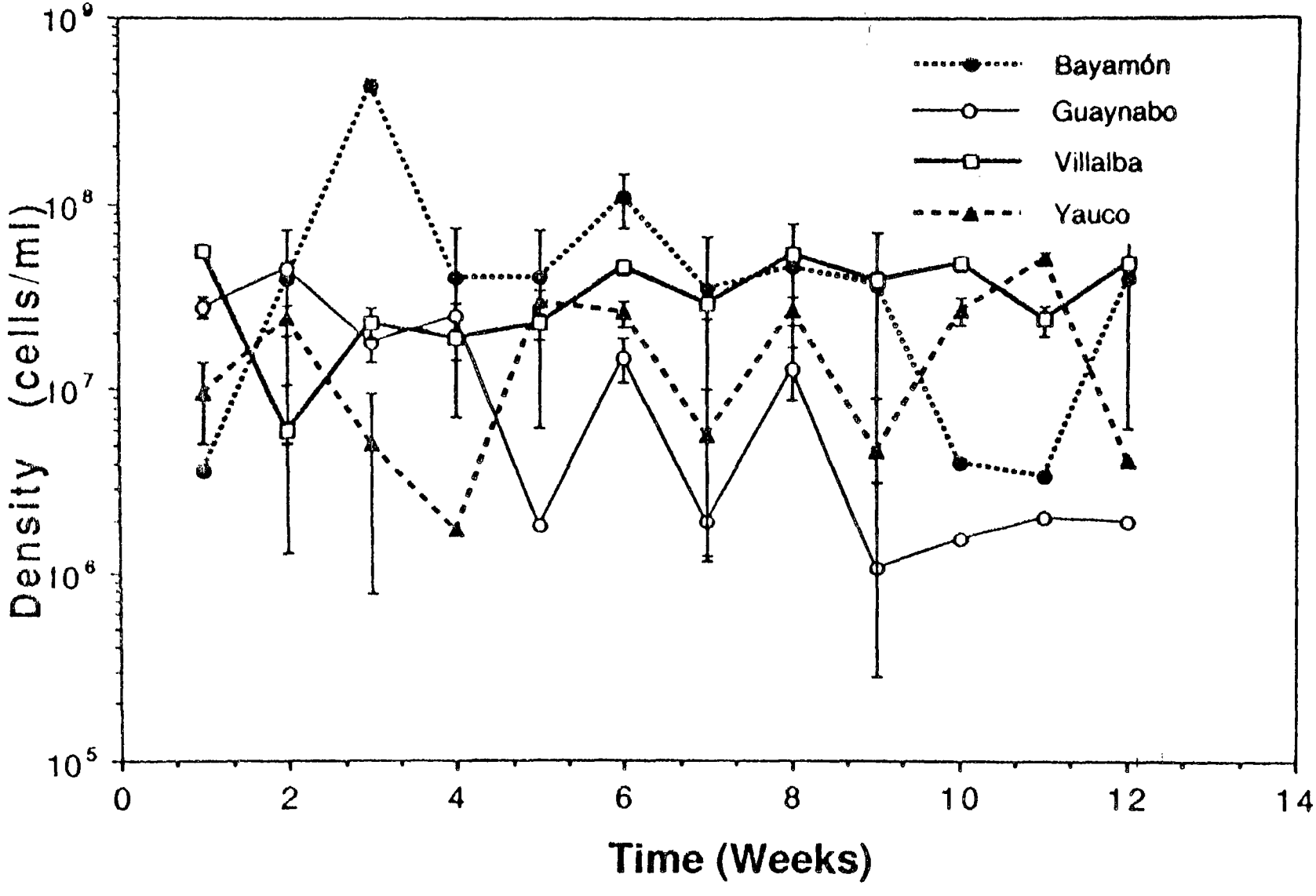

m-KF
MPN
Density (CFU/100ml)
10^2
10^1
10^0
10^{-1}
0
2
4
6
8
10
12
14
Time (Weeks)

Figure 11. Influent AODC* Densities at all Sites (September 24, 1987 to March 7, 1988). *Acridine Orange Direct Counts.


Bayamón
Guaynabo
Villalba
Yauco
Density (cells/ml)
10^9
10^8
10^7
10^6
10^5
0
2
4
6
8
10
12
14
Time (Weeks)

APPENDIX I

ANOVA of Influent Water Quality Parameters by Site.

Parameter	F	df	P
Temperature	9.00	3 , 40	<0.001*
pH	3.68	3 , 40	<0.05*
Turbidity	14.58	3 , 40	<0.001*
Total Chlorine	1.00	3 , 40	>0.05
Free Chlorine	1.00	3 , 40	>0.05

*Significant

APPENDIX II

ANOVA of Effluent Water Quality Parameters by Site.

Parameter	F	df	P
Temperature	24.25	3 , 40	<0.001*
pH	2.28	3 , 40	>0.05
Turbidity	4.24	3 , 40	<0.05*
Total chlorine	16.63	3 , 40	<0.001*
Free chlorine	27.04	3 , 40	<0.001*

*Significant

APPENDIX III

ANOVA of Influent Viable Count Methods by Site.

Method	F	df	P
MF-Total coliforms	5.08	3 , 40	<0.05*
MF-Fecal coliforms (m-FC)	4.75	3 , 40	<0.05*
MF-Fecal coliforms (m-T7)	1.56	3 , 40	>0.05
MF-Fecal streptococci	2.82	3 , 40	>0.05
MPN-Total coliforms	11.39	3 , 40	<<0.001*
MPN-Fecal coliforms	15.68	3 , 40	<<0.001*
MPN-Fecal streptococci	16.57	3 , 40	<<0.001*

*Significant

APPENDIX IV

ANOVA of Effluent Viable Count Results by Site.

Method	F	df	P
MF-Total coliforms	1.01	3 , 40	>0.05
MF-Fecal coliforms (m-FC)	0.89	3 , 40	>0.05
MF-Fecal coliforms (m-T7)	0.51	3 , 40	>0.05
MF-Fecal streptococci	0.84	3 , 40	>0.05
MPN-Total coliforms	1.43	3 , 40	>0.05
MPN-Fecal coliforms	0.69	3 , 40	>0.05
MPN-Fecal streptococci	0.80	3 , 40	>0.05

*Significant

APPENDIX V

ANOVA of Methods; Pooling Sites.

INFLUENTS

Method	F	df	P
MF - m-endo agar	6.870	3, 44	<0.001*
MF - m-FC agar	7.220	3, 44	<0.0005*
MF - m-T7 agar	2.840	3, 30	>0.05
MF - m-KF agar	0.003	3, 44	>0.25
MPN - total coliforms	52.75	3, 44	<0.0005*
MPN - fecal coliforms	66.100	3, 44	<0.0005*
MPN - fecal streptococci	46.600	3, 44	<0.0005*

EFFLUENTS

Method	F	df	P
MF - m-endo agar	0.057	3, 44	>0.25
MF - m-FC agar	1.370	3, 44	>0.25
MF - m-T7 agar	0.930	3, 30	>0.25
MF - m-KF agar	0.545	3, 44	>0.25
MPN - total coliforms	21.690	3, 44	<0.0005*
MPN - fecal coliforms	11.310	3, 44	<0.0005*
MPN - fecal streptococci	2.760	3, 44	<0.05

*significant