CHAPTER 3. MICROBIOLOGY

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I. INTRODUCTION

Terminal Island Treatment Plant (TITP) is a full secondary and partial tertiary wastewater treatment plant that receives and treats an average of 15 million gallons of flow per day (mgd) with a full capacity to treat 30mgd. TITP discharges effluent into the LA Harbor receiving waters. Discharges that may affect the quality of the Harbor receiving waters are regulated under the Porter-Cologne Water Quality Act of 1969, and California Water Code Sections 13000-13999.16. The Title 17 of the California Administrative Code contains effluent regulations. The California State Water Resources Control Board (SWRCB) and nine Regional Water Quality Control Boards (RWQCB) are responsible for enforcing these acts, codes, and regulations to ensure that the quality of California's receiving waters are maintained by issuing National Pollutant Discharge Elimination System (NPDES) permits. The Terminal Island Treatment Plant's NPDES permit mandates indicator bacterial monitoring in the Harbor receiving waters.

In 1993 the Environmental Monitoring Division (EMD) began monitoring the Los Angeles Harbor receiving waters and the Cabrillo Beach shoreline to assess water quality and to mitigate public health risk. One of the major means of measuring water quality for compliance monitoring is the use of indicator bacteria. When large numbers of indicator bacteria are present, it is assumed that there is a greater likelihood of the presence of pathogens. Some indicator bacteria used in monitoring are total and fecal coliforms, *E. coli*, and enterococci. These bacteria, with the exception of *E. coli,* are not usually pathogenic under normal conditions but are abundant in wastewater effluent and are more easily isolated than are pathogens. The SWRCB has promulgated total coliform and fecal coliform limitation standards for recreational Bathing Waters and areas of Shellfish Harvesting (Table 3-1). Limitation standards for enterococci have been recommended and proposed by the Los Angeles County Department of Health Services (LACDHS) and the SWRCB. However, there are no enterococcus limits in the TITP NPDES permit, and the California Ocean Plan has not promulgated enterococcus limits at this time.

From January to May of 2002, the City of Los Angeles conducted a method comparison study for the purpose of replacing the current membrane filtration method (MF) with a faster and less costly and less labor intensive method; the chromogenic substrate method (CS). In October 2002, the RWQCB granted the City of Los Angeles approval to conduct testing for the presence of indicator bacteria at HTP and TITP shorelines using the CS method. The City of Los Angeles' Environmental Monitoring Division began using the CS method for testing shoreline samples in December 2002. This method tests for *E. coli* rather than the historically used fecal coliforms. *E. coli* is a fecal coliform bacteria that is specific to fecal material from humans and other warm-blooded animals. The Environmental Protection Agency (EPA) recommends *E. coli* as the best indicator of health risk from water contact in recreational waters; some states have changed their water quality standards and are monitoring accordingly (Click10, 2003). *E. coli* replaces fecal coliform using a 1:1 ratio with the same recreational and bathing waters and shellfish harvesting standard limitations.

II. MATERIALS AND METHODS

A. SAMPLING LOCATIONS

In 2002, as part of TITP compliance monitoring, the City of Los Angeles', Environmental Monitoring Division (EMD), conducted more than 3,500 indicator bacteria tests at sites within the Cabrillo Beach and the Outer Los Angeles Harbor (Harbor) areas in efforts to help protect public health.

Seven Harbor receiving water stations (Figure 3-1) were collected five times a month and tested for total and fecal coliform bacteria as well as enterococcus. In conjunction with the Water Quality program (see Chapter 4), twelve stations, including HW33 and HW64, (Figure 3-1) were sampled monthly and tested for fecal coliforms only. Cabrillo Beach shoreline samples were collected daily at two stations (Figure 3-1) and analyzed for the three indicator bacteria.

B. METHODOLOGY

All samples were collected in sterile sample bottles with 1 to 2 inches of airspace. Shoreline samples were collected at ankle depth. Harbor samples were collected 0.5 meters below the surface. The samples were analyzed by the membrane filtration method (MF) for total coliforms, fecal coliforms, and enterococci in accordance with Standard Methods (APHA 1992). In December of 2002, with approval of the RWQCB, Cabrillo Beach shoreline samples were analyzed for total coliform and *E. coli* using the chromogenic substrate method (APHA, 1992).

Harbor weekly samples were collected aboard one of the City's monitoring vessels, usually the *Marine Surveyor*, and brought back to the laboratory for analyses four times a month. One weekly monitor and the monthly water quality samples were filtered at sea and initially incubated on board. After the vessel was docked, the MF plates were transferred to laboratory incubators for the remainder of the incubation period.

Visual observations were made at each sampling location. Observations at shoreline stations consisted of tallying items of sewage origin (plastic goods - feminine tampon applicators, or rubber goods - rings from male condoms) and non-sewage origin (ocean debris, seaweed, refuse, tar, and dead marine organisms) along a 30-foot reach of shoreline, on both land sides of the station. Other shoreline observations included any unusual odors, particularly those that could be of sewage origin, the volume of flow from storm drains associated with the station, changes in water color due to plankton, and the

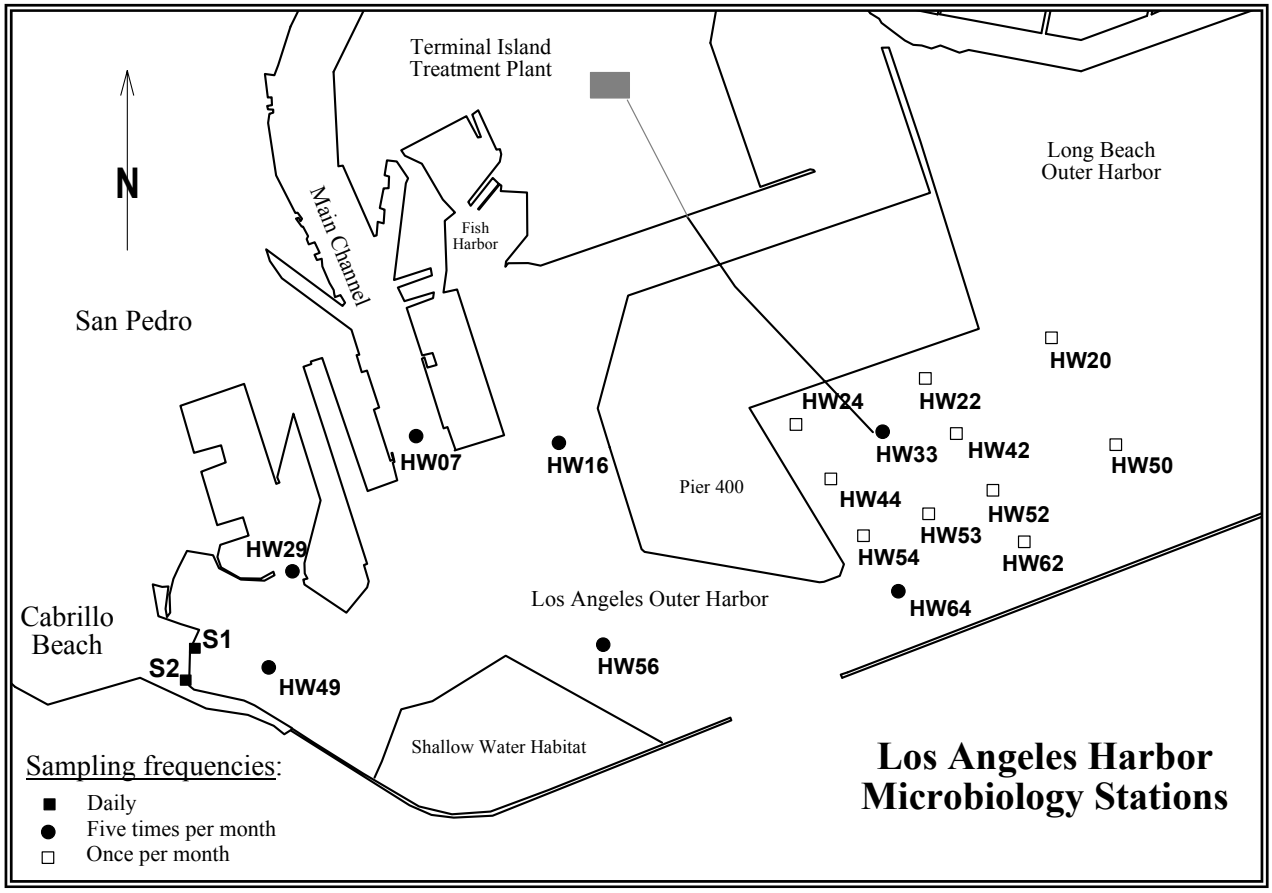

Figure 3-1. Microbiological sampling stations in Los Angeles Harbor.

presence of oil or tar. Additionally, at station S2, observations included water and air temperature, weather, wind direction and speed, wave height, and sea conditions. Harbor observations included water color, odor, air and water temperature, turbidity, and presence of items of sewage and non-sewage origin. Observations of wind, weather, and tidal stage were made every 4 hours on board the harbor vessel. Daily rainfall data were obtained from the National Weather Service for the Los Angeles Civic Center.

Quality assurance and quality control measures were performed to verify the validity of the analytical data collected. All aspects that influence the reported data were subjected to established microbiological quality control procedures in accordance with Standard Methods. These included sampling techniques, sample handling and preservation, facilities, personnel, equipment, supplies, media, and analytical test procedures. In addition, duplicate analyses were performed on ten percent of all samples. When quality control results were not within acceptable limits, corrective action was taken. The laboratory also participated in performance evaluation samples provided by an independent vendor accredited by the National Institute of Science and Technology, National Voluntary Laboratory Accreditation Program (NIST, NVLAP). The quality assurance program helped ensure the production of uniformly high quality and defensible data. The California Department of Health Services (CDHS), through their Environmental Laboratory Accreditation Program, certified the EMD microbiology laboratory for 2002.

C. DATA ANALYSIS

Application of most statistical techniques requires the assumption of symmetrical distributions such as the normal curve. Microbial distributions, however, are not symmetrical. Bacterial counts often have a skewed distribution because of many low values and a few high values. For this reason, it was necessary to convert microbiological data from a skewed to a symmetrical (or normal) distribution using a log transformation prior to data analyses.

A geometric mean is statistically the best estimate of central tendency for log-normalized data. For data comparison, geometric means were calculated for each of the three bacterial indicator groups. Additionally, data were divided into periods of wet and dry weather to assess the effects of stormwater run-off on concentrations of indicator bacteria. Regulatory agencies have defined wet weather as the day of rain plus the two subsequent days. As granted by the TITP NPDES permit, data collected within 48 hours following a rain event were not included in compliance calculations for six-month medians and non-compliance with Bathing Water and Shellfish Harvesting limits.

The indicator bacterial counts were submitted in written reports on a weekly, monthly, and annual basis to the RWQCB and EPA. In addition, all indicator bacterial counts were transmitted daily by electronic mail to the LACDHS. This daily communication helped protect the public health by enabling the LACDHS to inform the public of high indicator bacterial counts in recreational waters and post warning signs as warranted by California State Assembly Bill 411 (CDHS, Health and Safety Code, Assembly Bill 411, 1997).

III. RESULTS

A. OUTER HARBOR

Wet weather bacterial counts were higher than those for dry weather for all three indicators and at all LA Harbor weekly stations (Figure 3-2). Station HW29 and HW33 showed the highest bacterial densities for both wet weather and dry weather periods for all three indicators. HW29, located at the entrance of the Cabrillo Marina, showed higher total coliform and enterococcus wet weather counts than HW33, which is located at the outfall (Figure 3-1). Station HW33 had much higher wet and dry fecal coliform counts than all stations. HW16 showed the lowest counts for both wet and dry weather. This station was also one of three stations showing the lowest counts for 2001. There were no observations of materials of sewage origin.

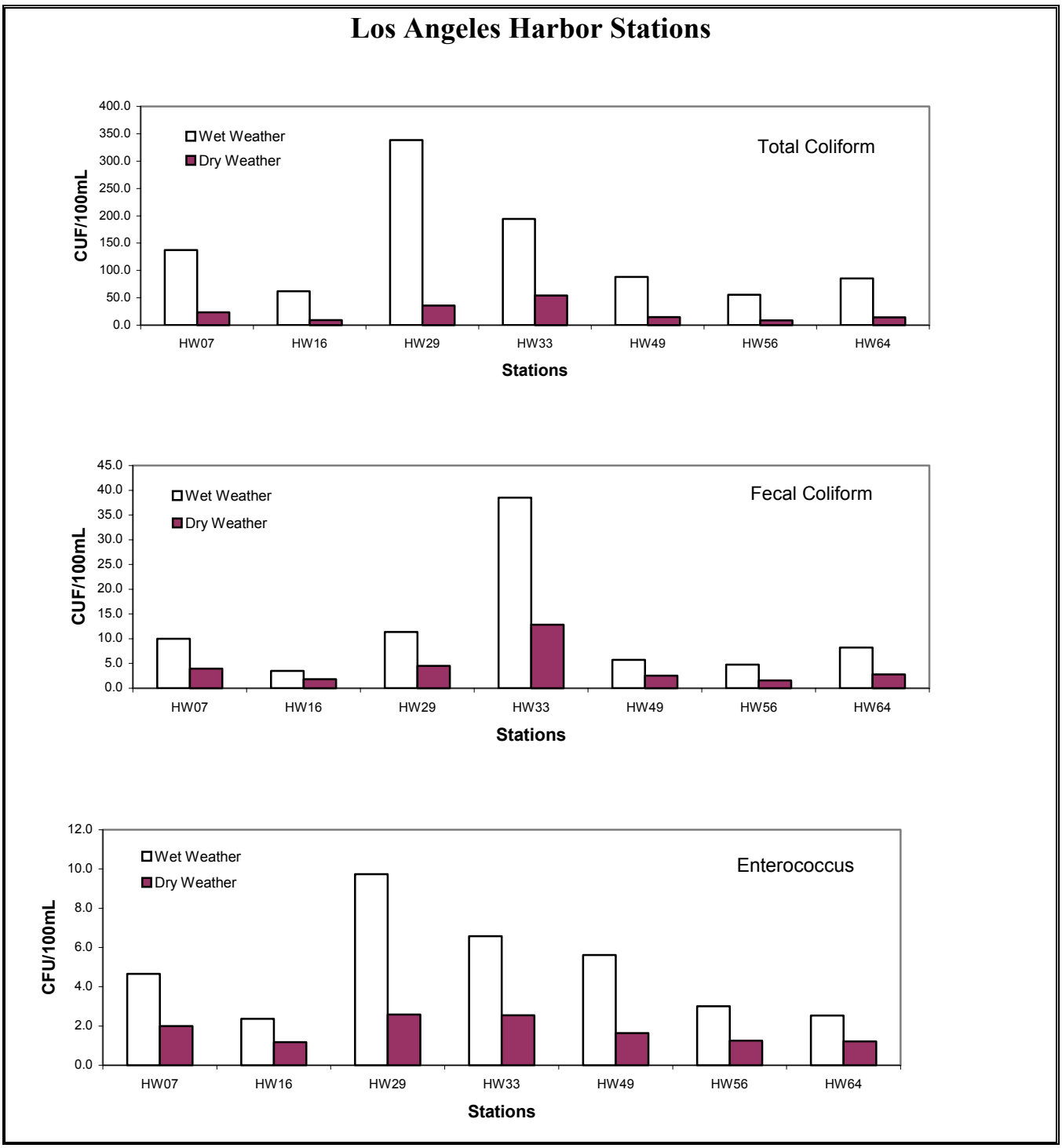

Figure 3-2. Wet and dry weather geometric means at Los Angeles Harbor surface stations, 2002.

B. WATER QUALITY PROGRAM "PLUME TRACKING"

Stations HW24, HW33, and HW44 were the three stations with the highest fecal coliform densities (Figure 3-3). HW24 had the highest fecal coliform counts, followed by HW33 and HW44, respectively. Station HW33 is located at the mouth of the outfall and HW24 and HW44 are nearest the outfall to the west. Stations HW20 and HW50, located farthest east of the outfall, showed the lowest fecal coliform densities. With the exception of HW24, HW33, and HW44 all other plume stations had counts as low as the harbor stations located outside of the outfall discharge area. There were no observations of materials of sewage origin.

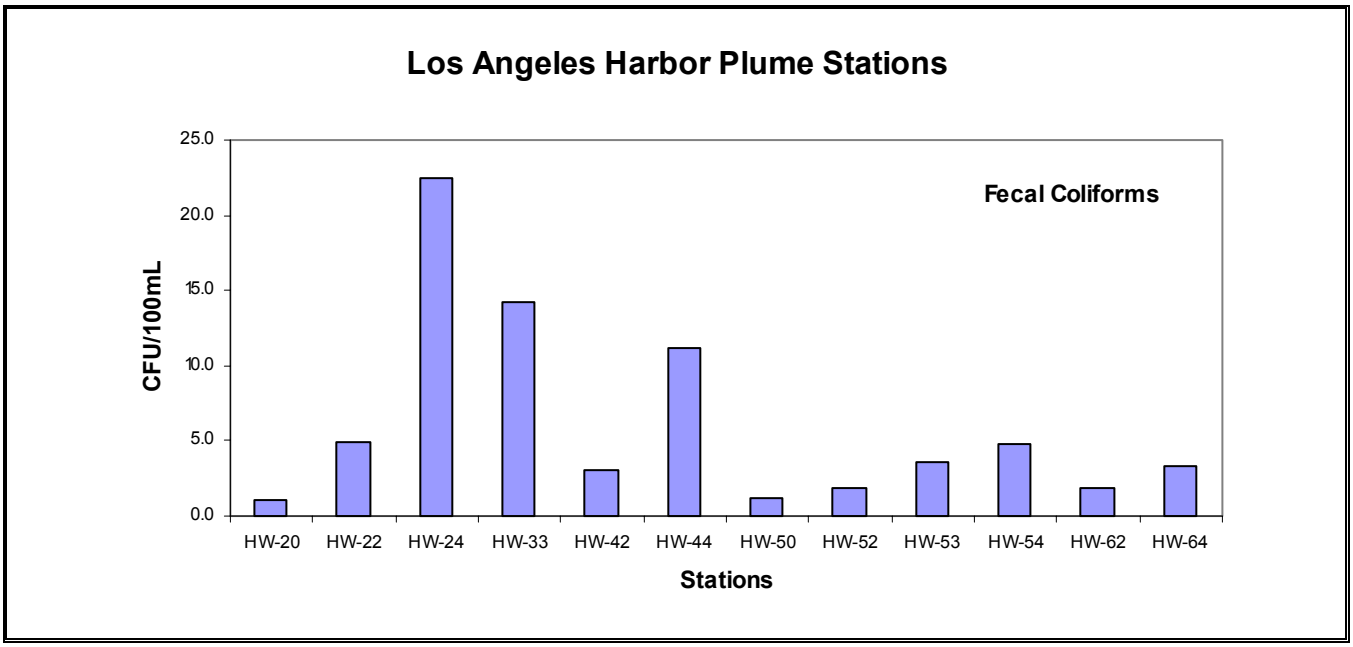

Figure 3-3. Geometric means for fecal coliform bacteria at Los Angeles Harbor water quality plume tracking stations, 2002.

C. CABRILLO BEACH

January, November, and December had the highest rainfall amounts and, consequently, had the highest bacterial densities (Figure 3-4). This was seen at both Cabrillo Beach stations, S1 and S2. There was no rainfall in February, July, August, or September. Dry weather bacterial counts were lower than wet weather counts with a few exceptions, such as fecal coliforms at S1 in March and June and enterococcus at S1 in June and at S2 in May. Station S2 showed higher counts for all indicators for both wet and dry weather months than did S1. Total coliform counts between S1 and S2 were fairly comparable, while the biggest difference in indicator counts for the two stations was seen for fecal coliforms and enterococcus. There were no observations of materials of sewage origin at either Cabrillo Beach station.

The number of non-compliance incidents per year at S1 and S2 decreased when compared to 2001 data (CLA, EMD 2002) and, subsequently showed an increase in percent compliance (Table 3-1). Station S1 met all threshold limits with the exception of two, both of which were greater than 50% in compliance. The highest incidence of non-compliance was at S2. While showing a reduction in non-compliance

when compared to 2001 data, S2 exceeded 50% of the Bathing Water and Shellfish threshold limits, with a compliance range of 8% to 100%. Station S2 was 100% in compliance with 3 of 6 threshold limits compared with a compliance of 1 of 6 limits for 2001 and none for 2000 (CLA, EMD 2000).

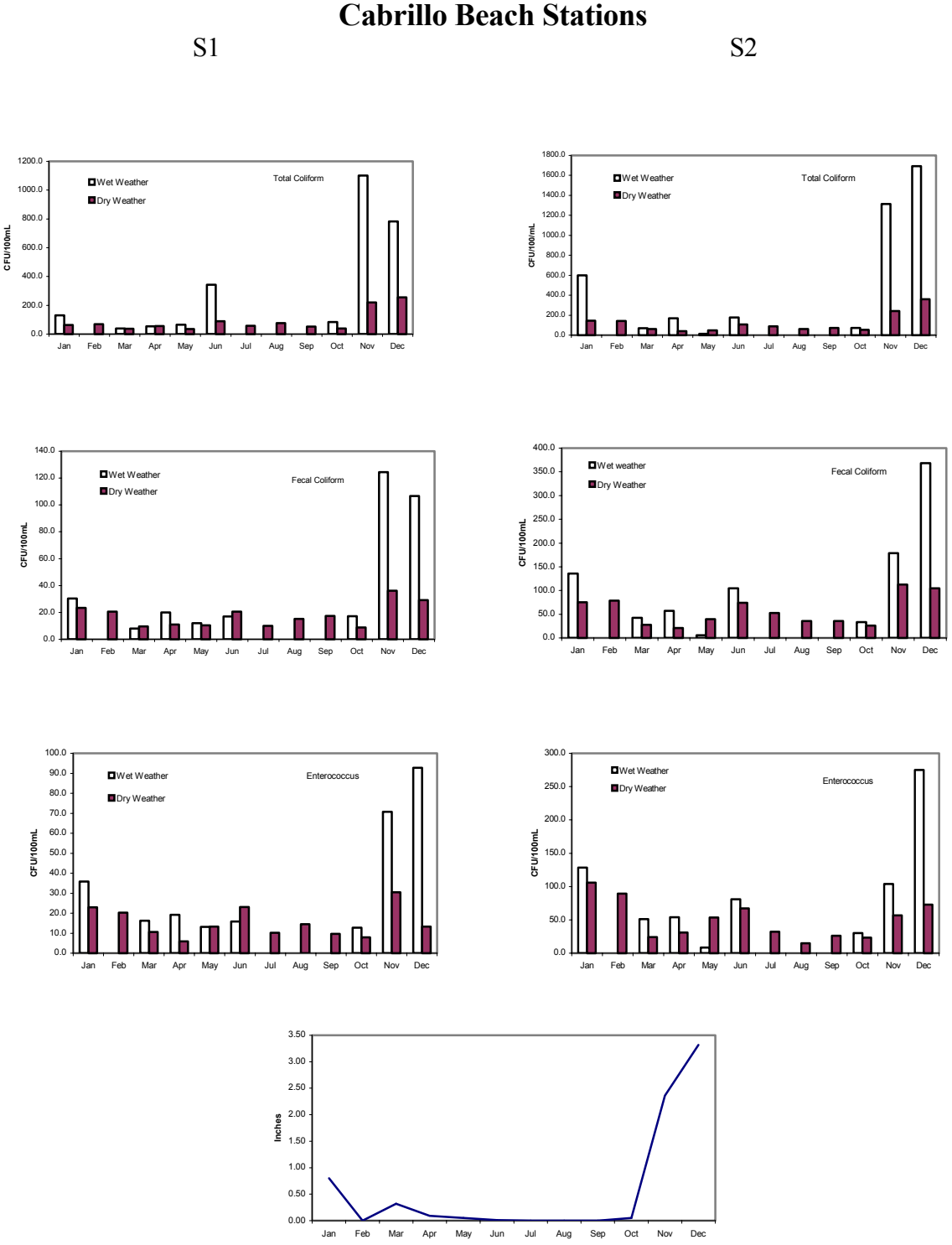

Figure 3-4. Monthly wet and dry weather geometric means for indicator bacteria at Cabrillo Beach shoreline stations and rainfall data, 2002.

Visual observations at the two shoreline stations (Table 3-2) were made a total of 730 times (2 stations x 365 sample days). During these visual observations, no occurrences of materials of sewage origin were observed.

Table 3-1. Number of dry weather* daily non-compliance and percent compliance for NPDES Bathing Water and Shellfish Harvesting coliform limits at Cabrillo Beach shoreline stations.

Station		Total Coliform Limits				Fecal Coliform Limits	
		(1) verified >10,000	(2) >20% >1,000	(3) Median >70	(4) >10% >230	(5) >200 30-Day	(6) >400 60-Day
S1	# Non-compliance/Yr	0	0	4	5	0	0
	% Compliance	100%	100%	67%	58%	100%	100%
S2	# Non-compliance/Yr	0	0	8	11	0	5
	% Compliance	100%	100%	33%	8%	100%	58%

* Dry weather excludes the day of rain and subsequent 48 hours
(1) Total coliform exceeding 10,000 CFU/100 mL when verified within 48 hrs. (calculated daily) – Bathing Water Limit
(2) >20% total coliform exceeding 1000 CFU100 mL in 30 days (calculated monthly) – Bathing Water Limit
(3) median exceeding 70 CFU/100 mL in 30 days (calculated monthly) -- Shellfish Harvesting Limit
(4) >10% total coliform exceeding 230 CFU/100 mL in 30 days (calculated monthly) -- Shellfish Harvesting Limit
(5) Fecal coliform geometric mean exceeding 200 CFU/100 mL in 30 days (calculated monthly) – Bathing Water Limit
(6) Fecal coliform >10% exceeding 400 CFU/100 mL in 60 days (calculated monthly) – Bathing Water Limit

Table 3-2. Number of daily occurrences and percent compliance with all materials of sewage origin at Cabrillo Beach shoreline stations.

Station	Grease Particles	Suspended Solid	Rubber Goods	Plastic Goods	Odor	Total Non-compliance
S1	0	0	0	0	0	0
S2	0	0	0	0	0	0
# Non-compliance	0	0	0	0	0	0

V. DISCUSSION

The vast majority of samples collected in the Harbor showed good water quality during the dry weather periods of 2002, including Cabrillo Beach station, S1. Although station S1, had 2 of 6 limits not meeting compliance limits 100%, these limits were still >50% in compliance. The one exception was Cabrillo Beach shoreline station S2 where three of six NPDES Bathing Water and Shellfish Harvesting limits were not met 100% of the time. However, data does show that the wastewater discharge from the TITP outfall is not the cause of contamination at station S2.

A. OUTER HARBOR

Wet weather counts were consistently higher than dry weather counts as has been seen in previous monitoring years (CLA, EMD 1994 - 2001). Increased storm drain flow and surface runoff to the area during rain events are prominent sources of contamination. Stations HW29 and HW33 registered the highest bacterial geometric means of all harbor stations for wet and dry weather periods. Station HW29, which had higher total coliform and enterococcus counts than HW33, is located at the mouth of the Cabrillo Marina. It is subject to influences from activities within the marina and any storm drains associated with it. Station HW33 is located at the mouth of the TITP outfall and showed the highest fecal counts for wet and dry periods. Given that the presence of fecal bacteria maybe an indication of sewage contamination, and also given its location, it is not surprising that Station HW33 reflected the presence of the TITP effluent. However, due to quick dispersal of the plume (CLA, EMD 2002), as evidenced by the lower counts seen at the majority other LA Harbor monitoring stations, with the exception of HW29, the TITP discharge has a small to moderate impact on the receiving waters at large.

B. WASTEWATER DISCHARGE "PLUME TRACKING"

Stations HW33, HW24, and HW44 had the highest fecal coliform counts of all water quality monitoring sites. Since the completion of Pier 400, HW24 has consistently shown the highest geometric means, even greater than HW33, located at the mouth of the outfall. This indicates that the discharge flows northwest into the corner of Pier 400 (Figure 3-1), also giving rise to higher counts at S44 just west of HW33. To further corroborate this, “Probability estimates obtained from salinity anomaly measurements show that the wastewater field is most frequently located in the northwestern portion of the discharge area” (CLA, EMD 2002). Because of their locations these stations may not be as exposed to harbor currents and flows as are other stations and waters may remain stagnant longer than at other plume monitoring sites. All other water quality stations, with the exception of HW33, had means <10 cfu/100 ml, suggesting low to insignificant impact of the TITP discharge on the surrounding waters. Because of plume dispersal, low indicator bacteria counts throughout the Harbor, and the higher counts seen at the Cabrillo Beach shoreline, there is no evidence of impact of the plume on the Cabrillo Beach bathing waters.

Although the TITP effluent itself is not tested for indicator bacteria, current TITP treatment processes and the low counts at the mouth of the outfall point to low bacterial content. Terminal Island Treatment Plant is a full secondary and partial tertiary treatment facility with one third of it’s effluent contributed by the AWTF facility. Water produced by the AWTF was tested from October to December 2002. The geometric mean was < 2.2 MPN/100 mL for total coliform and *E. coli* bacteria.

This further supports the contention that the outfall has small to moderate impact on the Harbor waters and the Cabrillo Beach shoreline.

C. CABRILLO BEACH

Station S1 had lower wet and dry weather bacterial counts and higher percent compliance with NPDES Bathing Water and Shellfish Harvesting standards when compared to its companion station S2. Station S1 is located at the boat launch and is sampled at the 2-feet drop of the launch ramp. It is adjacent to a restroom, an L-shaped jetty, and a parking lot that is frequented by launch users as well as visitors to the beach area and the Cabrillo Marine Aquarium. The L-shaped jetty limits the exposure of the site to harbor currents thereby reducing potential contamination from the harbor area outside the jetty. As indicator bacteria counts at the immediate TITP discharge area (HW33) are lower than counts at S1 and considering the low counts at stations HW49 and HW56, both just outside the Cabrillo Beach shoreline area, it is unlikely that any impact from the TITP effluent would be in evidence or detectable at this site. Additionally, the floor of the restroom and the adjacent sidewalk are hosed down daily, and while restroom wash is directed to the sewer, the sidewalk runoff flows across the boat launch area and drains to S1. During rain events, runoff from the parking lot, boat launch, and jetty are contributors of pollution. More, immediate potential sources of contamination or pollution to the area are runoff from the jetty and parking lot.

Station S2, located at Cabrillo's sandy beach, had indicator bacteria counts higher than S1 for both wet and dry weather periods. This site has consistently exceeded the majority of NPDES Bathing Water and Shellfish Harvesting standards. As in the case of S1, S2 also had indicator counts higher than HW33 (Figure 3-1) and as the indicator counts decreased with distance from the outfall, it is unlikely that the source of bacterial contamination at S2 is the TITP discharge. It is more probable that the source of bacterial contamination and the cause of NPDES exceedances at S2 are local. Total coliform counts between S1 and S2 were comparable, but fecal coliform and enterococcus counts for S2 were more than double those for S1. This may be indicative of a source of sewage or, more likely, animal pollution at S2.

Because of poor water quality and excessive NPDES exceedances at the Cabrillo Beach shoreline, in 1999 the Los Angeles City Council requested the Bureau of Sanitation, in cooperation with various other agencies, to report on the water quality at Cabrillo Beach, identify potential problems, and suggest mitigation measures to improve water quality. The data generated from two such studies in 1999 (CLA EMD) suggested that the source(s) of the bacterial contamination was not of sewage origin or from storm drains (CLA, unpublished). In addition, it was found that the bird sanctuary area adjacent to the beach, Cabrillo Marina, Fishing Pier, bait barges, and the TITP outfall discharge were not the source(s) of the bacterial contamination.

One of the potential sources of pollution identified by the study was the bird population inhabiting Cabrillo Beach area. In July 2000, the City Council's Environmental Quality and Waste Management Committee recommended that the Department of Recreation and Parks construct an anti-bird structure over part of the inner Cabrillo sandy beach. The results of the effectiveness of this structure in reducing bacterial levels at Cabrillo Beach were released in January 2003:

> "In summary the bird exclusion structure was effective at reducing the number of birds and bacterial contamination at the beach in front of the structure. The frequency of California State AB 411 bathing water standards exceedances was reduced (especially for the total coliform and the fecal coliform to total coliform ratio), but not eliminated,

indicating that the fecal contamination of the swimming beach was not fully mitigated by the presence of the bird exclusion structure…."

As a supplement to the anti-bird structure the following measures were also implemented to reduce occurrences of bird fecal matter negatively impacting water quality, while not significantly impacting the bird population:

- Public Awareness Education: Educational material and/or signs were developed to educate beach visitors as to the appropriate disposal of trash, which can be a breeding ground for bacteria as well as an attraction to birds. In addition, the importance of not feeding birds and wildlife will be included.

- Additional trash containers: Trash cans in the City portion of the beach area were provided with covers. Lids on trash cans prevent birds from feeding from the cans, as well as preventing trash from being blown from the trashcans onto the beach and adjacent areas.

The investigation of potential bacterial sources of pollution is still ongoing. Investigation includes further research into the abandoned sanitary sewer outfall pipe and additional bird deterrent measures, as well as current circulation. Possible solutions suggested include relocation of the boat ramp, shortening of the groin between S1 and S2, an opening in the breakwater, an underwater conduit from outer to inner Cabrillo Beach, a pumping system to enhance circulation, deepening the bathymetry, and implementing an aeration system (CLA, Cabrillo Beach Progress Meeting Summary, 2003).

In conclusion, the effectiveness of the above measures in improving the beach water quality has reduced the number of Bathing Water and Shell Fish Harvesting non-compliances from 45 (CLA, EMD 1999), to 40 (CLA, EMD 2000), to 37 (CLA, EMD 2001), and to 24 in 2002. The Bureau of Sanitation will continue to monitor the water quality at the Cabrillo Beach swim area daily. This water quality monitoring will assist in determining the effectiveness of the programs implemented to address bird fecal impacts on water quality at Cabrillo Beach. As additional information is gathered, remedial measures may need to be modified and/or supplemented.

LITERATURE CITED

APHA. See American Public Health Association.

American Public Health Association. 1992. Standard methods for the examination of water and wastewater, 18th ed. Amercian Public Health Association, Washington, DC, pp. 9-1 to 9-115.

CLA, EMD. 2000. See City of Los Angeles, Environmental Monitoring Division

CLA, EMD. 2001. See City of Los Angeles, Environmental Monitoring Division

CLA, EMD. 2002. See City of Los Angeles, Environmental Monitoring Division

City of Los Angeles. 2003. Cabrillo Beach Progress Meeting Summary (16 April 2003)

City of Los Angeles. Environmental Monitoring Division 2000. Marine Monitoring in the Los Angeles Harbor: Annual Assessment Report for the period January through December, 1999. Report submitted to EPA and RWQCB (Los Angeles). Department of Public Works, Bureau of Sanitation, Terminal Island Treatment Plant, San Pedro, California.

City of Los Angeles, Environmental Monitoring Division. 2001. Marine Monitoring in the Los Angeles Harbor: Annual Report for the period January through December, 2000. Report submitted to EPA and RWQCB (Los Angeles). Department of Public Works, Bureau of Sanitation, Terminal Island Treatment Plant, San Pedro, California.

City of Los Angeles, Environmental Monitoring Division. 2002. Marine Monitoring in the Los Angeles Harbor: Annual Report for the period January through December, 2001. Report submitted to EPA and RWQCB (Los Angeles). Department of Public Works, Bureau of Sanitation, Terminal Island Treatment Plant, San Pedro, California.

City of Los Angeles, Environmental Monitoring Division. Unpublished. Marine Monitoring in the Los Angeles Harbor: Annual Report for the period January through December, 2002. Report submitted to EPA and RWQCB (Los Angeles). Department of Public Works, Bureau of Sanitation, Terminal Island Treatment Plant, San Pedro, California.

Click10.Com. "What Are Fecal Bacteria Are Why Are They Important?" 2003. http://www.click10.com/news/2032825/detail.html (25 June 2003)