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LAND USE PATTERNS AND FECAL CONTAMINATION OF COASTAL WATERS IN WESTERN PUERTO RICO

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Executive Summary of Research Project

The Department of Environmental Health of the Graduate School of Public Health of the Medical Sciences Campus, University of Puerto Rico (UPR-RCM) conducted this research project on how different patterns of land use affect the microbiological quality of rivers flowing into Mayagüez Bay in Western Puerto Rico. Coastal shellfish growing areas, stream and ocean bathing beaches, and pristine marine sites in the Bay are affected by the discharge of the three study rivers. Satellite imagery was used to study watershed land uses which serve as point and nonpoint sources of pathogens affecting stream and coastal water users. The study rivers drain watersheds of different size and type of human activity (including different human waste treatment and disposal facilities). Land use and land cover in the study watersheds were interpreted, classified and mapped using remotely sensed images from NASA's Landsat Thematic Mapper (TM).

This study found there is a significant relationship between watershed land cover and microbiological water quality of rivers flowing into Mayagüez Bay in Western Puerto Rico. Land covers in the Guanajibo, Añasco, and Yagüez watersheds were classified into forested areas, pastures, agricultural zones and urban areas so as to determine relative contributions to fecal water contamination. The land cover classification was made processing TM images with IDRISI and ERDAS software.

Significantly larger median pathogen indicator densities appear in the Yagüez River (180 PFU/100 mL of coliphages), which drains the watershed with the highest urban density of the three (7.22% of watershed area within high density urban cover, only 5.18% agricultural). The Añasco River showed the lowest median coliphage density near its mouth (18 PFU/100mL), and drains a mostly agricultural watershed (5.18% low density urban, 27.8% agricultural). The Guanajibo River watershed drains a mixed land cover area (7.41% medium density urban, 18.7% agricultural) and shows an intermediate coliphage average

density near its mouth (29 PFU/100 mL).

The densities of the water quality indicators were correlated with stream flow at two study sites (sampling stations) to determine whether they were of point or nonpoint origin. While urban density correlates with pathogen indicator density, no significant statistical correlation was found between stream flow and density of the different microbiological water quality indicators used. This is probably due to raw wastewater discharges coming from sanitary sewer bypasses into study streams during frequent pumping station failures. The direct effect of nonpoint source contamination on microbiological stream water quality cannot be observed under these circumstances. However, high correlations were found between suspended solids concentrations and densities of organisms in sites draining areas with less urban and higher agricultural covers, and with higher sediment yields. This implies that processes of erosion and transport to stream channels of fecally contaminated sediments are occuring in these areas.

The study determined that a significant statistical difference exists between microbiological water quality in coastal water sampling stations receiving discharges from streams with differing land use patterns in their watersheds. Parametric and non-parametric statistics were used for this purpose.

The study also evaluated current and alternative microbiological water quality indicators which would be of use to regulatory and health agencies dealing with water pollution control. Four different fecal indicators were used in this study (total and fecal coliforms, enterococcus and coliphages) to increase the precision of water quality measurements and to compare results. Also, tests were done for the presence of pathogenic parasites to determine the usefulness of the different indicators.

Introduction

Increasing evidence suggests that the presence of fecal contamination indicator bacteria in tropical surface and estuarine waters could be of indigenous nature (Feachem, 1974; Oluwande et al., 1983; Santiago-Mercado et al., 1987; Fujioka et al., 1988; Hazen, 1988; Rivera et al., 1988; Hazen and Toranzos, 1990; Hernández-Delgado et al.,1990, 1991; Hernández-Delgado, 1991; Toranzos, 1991). However, there is a general lack of data regarding how much can land use patterns affect microbiological water quality in the tropical environment.

Indicator bacteria have been isolated in the absence of pathogens. However, pathogens have been also isolated in the absence of indicator bacteria (Thomson, 1981; see reviews by Hazen et al., 1987; Hazen, 1988; Hazen and Toranzos, 1990; Toranzos, 1991). Moreover, fecal coliforms, enterococci and human pathogenic bacteria such as *Salmonella* spp. can replicate under natural conditions in the tropical aquatic environment (López-Torres et al., 1987; Jiménez et al., 1989; Muñiz et al., 1989).

Hardina and Fujioka (1991) isolated also naturally-occurring indicator bacteria from tropical soils. The fact that indicator bacteria appear to be indigenous and that they can grow in the aquatic environment may lead us to misinterpret the real microbiological quality of surface waters in tropical countries. Therefore, coliphages have been suggested as an alternate indicator of fecal contamination (Hilton and Stotzky, 1973; Dutka et al., 1987; Haavelar et al., 1991). Coliphages have been associated to both remote and recent fecal contamination in tropical surface (Hernandez-Delgado et al.,1990, 1991) and marine waters (Toranzos and Hernández-Delgado, 1992), and do not replicate under natural conditions (Hernández-Delgado, 1991; Toranzos and Hernández-Delgado, in press).

Objectives

1) To determine whether significant statistical differences exist between microbiological water quality in streams draining watersheds with different land cover patterns.

2) To compare the use of indicator bacteria and coliphages as indicators of human fecal contamination and their correlation to recent human fecal contamination of waters.

Methodology

Water quality study strategy:

Field observations complemented remote sensing findings and provided most of the water quality data. Total and Fecal Coliforms, Enterococci, and Coliphages indicators were used for this purpose. After an initial assessment, four stream sites and four coastal study sites near the study river mouths and near a wastewater ocean outfall in the Bay were selected for detailed study. The latter are representative of coastal areas receiving flow from rivers with variably disturbed watersheds, and are marked as stations E1 through E4 in Appendix A. The resulting data was analyzed using simple statistical tools to evaluate differences in densities of microbiological water quality parameters between streams draining watersheds with different land covers. Other water quality parameters (dissolved oxygen, conductivity, pH) were measured in the field.

A survey of stream microbiological water quality delivered by the three main streams flowing into the Bay was carried out starting in August of 1994. The mouths of the Yagüez, Añasco, and Guanajibo rivers and a site next to a U.S.G.S. permanent flow measuring station also in the Guanajibo river were sampled for fecal indicators and for the pathogens Giardia Lamblia and Cryptosporidium in a first phase of the project. Sampling was carried out once a month for fecal indicators, Giardia and Cryptosporidium. The latter two were analyzed by the Calcium Carbonate Precipitation technique, followed by Immunofluorescent staining, in this first stage. These samples were preserved for later Polymer Chain Reaction technique (PCR) analysis for pathogens. A total of eight samples were taken monthly at the different stations in the study rivers (4), one hundred meters off their mouths in the Bay (3), and at the sewage outfall (1). In addition, a composite sample was taken from the Mayagüez Bay in the first phase of the project.

Monitoring stations description:

Sampling of the three rivers that flow in to the Mayagüez Bay was done beginning August 26, 1994. The Guanajibo river was monitored at two stations. The upstream station is located at Road P.R. # 100, where the U.S.G.S. flow station lies near Hormigueros. The downstream station is located at the river mouth below Road P.R. # 102, near Punta Guanajibo. A mixture of pasture and medium density urban areas lie near both stations.

The Yagüez river station is located at Road P.R. # 2, near the Darlington Building in Mayagüez. The sampling station in this watershed is located within the high density urban area, the third largest in Puerto Rico.

The Añasco river station is located at Road P.R. # 2, near an old iron bridge and the entrance of Añasco. The sampling station itself is located near sugar cane fields, abundant in the coastal flood plain in that watershed. The station is near the river's mouth.

Sampling strategy:

Sampling of the Guanajibo, Yagüez, and Añasco rivers and Mayagüez Bay was carried out monthly. The samples were taken from the top of bridges, using a sampling bottle. For each station four samples were aseptically collected using sterile 1 L polypropylene plastic bottles and kept on ice until assayed generally within 24 h. These samples were analyzed for total solids, total and fecal coliforms, enterococcus, coliphages and Giardia and Cryptosporidium. Also physical and chemical measurements were taken.

Sample collection and measurements at the stream stations was carried out by research assistants from UPR-RCM and UPR Rio Piedras Campus (UPR-RP) using portable equipment purchased for this project. Research assistants and faculty from the Department of Environmental Health at UPR-RCM and the Microbiology Department at UPR-RP analyzed the microbiological water samples. Analytical procedures for suspended solids were carried out at UPR-Mayagüez's (UPR-RUM) labs.

Simultaneously, microbiological water quality distribution in the Bay was studied using samples taken aboard UPR-RUM's Department of Marine

Sciences's watercraft. The 42-feet boat R/V Sultana was used for sampling the Mayagüez and Añasco Bay stations. A Global Positioning System (Trimble Transpak II GPS) was used to locate the stations. The water samples were taken from the water column to a depth range from 0-3 feet by means of a submersible pump (Appendix B).

All water samples were collected and preserved according to methods established in the "Standard Methods for the Examination of Water and Wastewater" (APHA, 1992). Temperature and salinity were measured at each station by means of an instrument installed in the boat (TEMPSAL model 541, Interocean Systems). Suspended solids concentration was determined for each sample site using 47 mm polycarbonate membranes. These analyses were performed according to APHA, 1992.

After the first stage of the project, representative sampling stations were selected in the Bay near the streams' discharges. Sampling for Giardia and Cryptosporidium was discontinued at the stream stations after the first weeks of the project due to the low densities found. Higher volumes of sample water need to be processed to find measurable quantities of the pathogens themselves, as was done in the Bay stations. A portable filter and a water pump was used in the sampling boat to capture the cysts of the parasites from ocean water (Appendix B). Samples were taken monthly, also gathering discharge and water quality data produced by USGS stations in the watershed.

Microbiological analyses:

Indicator bacteria were assayed by the membrane filtration technique using m-Endo Agar for the isolation of total coliforms (TC), and m-FC for the isolation of fecal coliforms (FC). Concentrations are expressed in colony forming units per 100 mL (CFU/100 mL). Representative colonies were confirmed according to standard methods (APHA, 1985). Phage assays were done according to the method of Hernández-Delgado et al. (1991), which is a modification of the direct plaque assay of Grabow and Coubrough (1986). Briefly, a 100 mL sample of water was mixed with 100 mL of Trypticase Soy Agar (2X concentration) and the host bacteria *Escherichia coli* C3000 (ATCC 15597), then poured into 10 sterile petri dishes and incubated at 35.5 C for 12 h. Concentrations are expressed in plaque forming units per 100 mL (PFU/100 mL).

In a future stage of this study, the PCR technique will be used for the detection of Giardia and Cryptosporidium, in all negative samples. This will be done to increase the sensitivity of the immunofluorescent technique and to possibly detect the presence of Giardia and Cryptosporidium not recognized by commercially available antibodies. The PCR analysis procedure is undergoing calibration at UPR-RP for this purpose.

Environmental parameters:

Physical - chemical water quality parameters were measured during sampling. These included: stream level, temperature, pH, dissolved oxygen, salinity, conductivity, and turbidity. These parameters were analyzed with a Hach One pH meter model P/N 43800-00, a Hanna Dissolved Oxygen Portable meter model HI-8043, and a Hanna portable Conductivity meter HI 933.

Coordination with the United States Geological Survey (USGS) was made for orientation visits by them and project technicians to the USGS sampling stations located in the Guanajibo, Añasco and Yagüez river. They also provided training of students on remote use of the USGS hydrological analysis programs and database. The Civil and Electrical Engineering Departments of UPR-RUM provided computer terminals connected to the USGS central computers for this purpose. The USGS was also contracted for construction of an automatic sampling machine on the Guanajibo river at San German (SIGMA type).

Remote sensing strategy:

This project combined remote sensing, Geographical Information Systems (GIS), and field observations to determine land covers in the study watersheds in the West and Southwest of Puerto Rico. Remote sensing techniques were used to classify types of land cover of different potential impact on surface water quality. It was also used to identify important nodes in the rivers and streams where field measurements should be made. Arrangements were made to obtain, read and interpret remote images from a 1985 Landsat Thematic Mapper (TM) mission. The data was obtained from the Laboratory for Applied Remote Sensing and Image Processing (LARSIP) of UPR-RUM. A remote sensing technician was employed for image analysis. NASA equipment available at UPR-RCM was used for remotely sensed and GIS data input and analysis. An ERDAS image processing system and digitizers in a remote sensing laboratory at UPR-RP were also used.

Land cover classification of images of the study's watersheds was carried out after importing them into the IDRISI format and georeferencing. The Guanajibo, Añasco, and Yagüez watersheds were classified to compare forested areas, pastures, agricultural zones and urban areas so as to determine their contribution to fecal water contamination.

The classification was made combining TM bands 4,3,2 which provide information on water boundaries definition, coastal wetland and flooded areas, as well as vegetated zones. Using this combination one may obtain results similar to traditional color IR aerial photography. This creates differences in color of vegetation areas in the image based on reflectance. A red color gradient is showed on those pictures in which high vegetated areas appear as dark red and low vegetated areas appear as light red. After an unsupervised classification was completed, a supervised one was carried out using aerial photography and different images as references. Field visits to the watersheds were also used for ground truthing.

When the images were rectified, a composite image was created with the IDRISI module "Composit". This composite image of three bands from the image were used to create land cover classification by the unsupervised method using the module "Cluster". To generate the clustered image ten clusters were specified since there are about ten broad land cover classes in the region.

Using the differences in spectral reflectance and field trips, the land cover classification was generated by techniques such as reclassification, windowing, cut and paste and overlaying rasterized polygons defining homogeneous clusters based on reflectance. Once the land cover classification was generated, the IDRISI module called AREA was used to generate the land cover area values in tables for every land cover class by sub-basins.

A Geographic Information System (GIS) approach was used to develop an integrated basin-wide land cover data base for the study area. NASA-financed equipment available at UPR-RCM was used for image data input and analysis. This includes an IDRISI image processing system and a digitizer located at the Department of Environmental Health.

Results

Remote sensing analysis:

Quantification of land cover areas was carried out using image analysis software (IDRISI and ERDAS packages). Appendices C1 to C3 present classified Landsat TM images of the study watershed areas. A supervised classification of the Landsat images indicated the predominant watershed land covers upstream of the study's sampling stations shown in Table 1. Natural color compositions of images of the study watersheds are shown in Appendices C4 to C6. Land use clasifications were based on the same images.

The Añasco watershed is the least urbanized, most agriculturally developed (mainly

Table 1. Land cover areas in study watersheds upstream of sampling stations (km.^{2})

River	Guanajibo upstream[a]	Guanajibo downstream[b]	Yagüez	Añasco
Total	397.37	422.28	37.96	467.34
Forest	273.96	289.25	33.18	254.75
Forest		3.05	-	-
Rural		-	-	19.61

Pastures-agricultural	74.91	80.50	1.97	129.88
Urban or residential	28.31	31.29	2.74	24.22
Shadow	18.41	19.45	0.07	19.61
Clouds	1.78	1.79	0	37.71
Water	0	0	0	1.17

[a] Sampling station located at Road P.R. # 100, near Hormigueros.
[b] Sampling station located at Road P.R. # 102, near Punta Guanajibo.

sugarcane fields in coastal flood plains), and largest of the three. Even its 24.22 km.^{2} of what was classified as urban cover include 19.61 km^2 of rural residential neighborhoods, so that the impact of urban runoff on this river would be expected to be the lowest of the three. The watershed consists mainly of mountain steep terrain. Its major land uses are: sugar cane agriculture located downstream in the watershed coastal valleys; forest, grass and spots of artisan agriculture (including coffee and bananas) upstream of the watershed and in the river margins. It has low density urban areas across the roads upstream of the watershed. These urban areas increase in density and size as the river flows downstream of the watershed. The Añasco township urban area in this watershed is found near its mouth.

The Yagüez river is restrained in a relative small watershed. The most relevant aspect of this watershed is the urban area of Mayagüez city. There are rural areas toward the PR-105 and PR-106 roads. Forest, grass and artisan agriculture land uses are present along the watershed, with forest being the most predominant use. The Yagüez watershed is the smallest and most densely urbanized watershed, so that the impact of high density urban storm water runoff on the correspondingly low flow stream would be expected to be the largest.

Like the Añasco watershed, the Guanajibo river watershed presents a mountaineous steep topography. It has some high density urban areas in San German and Hormigueros towns. The most prodominat features in term of land uses are forest, urban and agricultural areas, while the high density urban areas are found in the alluvial valleys. The Guanajibo watershed contains two urban centers upstream of the sampling stations. Table 2 shows it is an intermediate watershed in terms of percentage of urban area, agricultural development, and population density upstream of sampling stations. Statistics not included in Table 2 include percentage forest areas in the study watersheds, which are Guanajibo (68% forest), Yaguez (87%), and Añasco (54%).

Table 2. Watershed statistics upstream of study stream sampling stations

River	Total watershed areas (square kilometers)	Average stream flow (cubic feet per second)	Population density (p/km.2) (1990)[c]	Percent urban area (%)	Percent agricultural area (%)
Guanajibo upstream	397.37	121.23[a]	308	7.12	18.85
Guanajibo downstream	422.28	121.23[a]	308	7.41	18.66
Yagüez	37.96	6.45[b]	1184	7.22	5.18
Añasco	467.34	203.08[a]	128	5.18	27.80

[a] Average stream flow of Guanajibo river based on historic USGS records for station PR100. Average stream flow of Añasco river based on historic USGS records for station upstream of PR2.
[b] Average stream flow of Yagüez based on manual measurements during hydrologic years 1993-1994 (USGS).
[c] Based on U.S. Census Bureau.

Microbiological analysis for stream sampling stations:

Results of water quality analyses for the four microbiological parameters are shown below. In Tables 3 to 6, significant differences appear in median pathogen indicator densities between the study stream sampling stations. Median densities of all pathogen indicators in the

Table 3. Total Coliform indicator statistics in sampling stations of Rivers Guanajibo, Yagüez and Añasco between August 1994 and December 1996 (CPU per 100mL)

Sampling Station	n	Median	Standard Deviation	Maximum
Añasco River (Road PR # 2)	24	11197	54500	251000
Guanajibo River Mouth (Road PR # 102)	17	7200	16447	67220

Guanajibo River Upstream (Road PR #100)	23	4663	20321	76550
Yagüez River (Road PR # 2)	18	57500	73798	311000

Table 4. Fecal Coliform indicator statistics for sampling stations of Rivers Guanajibo, Yagüez and Añasco between August 1994 and December 1996 (CPU per 100mL)

Sampling Station	n	Median	Standard Deviation	Maximum
Añasco River (Road PR # 2)	25	2100	28146	128000
Guanajibo River Mouth (Road PR # 102)	21	1850	19776	90000
Guanajibo River Upstream (Road PR #100)	26	1722	12447	46050
Yagüez River (Road PR # 2)	23	18400	30277	97000

Yagüez River are the largest of the four stream stations. They are between five and twelve times the densities of the lowest density stations. Even though sanitary sewers in the city of Mayagüez are connected to a regional wastewater treatment plant that discharges into an ocean outfall, frequent failures of sewage pumping stations produce raw sewage discharges into the Yagüez river. This might explain the high densities of fecal indicator organisms in that river, over and above the magnitudes expected from urban runoff. As could be observed in Table 2, the Yagüez watershed does not differ significantly from Guanajibo and Añasco watersheds in terms of percentage of urbanized watershed area. In fact, total urban area in Yagüez is less than one tenth

Table 5. Enterococcus indicator statistics for sampling stations of Rivers Guanajibo, Yagüez and Añasco between August 1994 and December 1996 (CPU per 100mL)

Sampling Station	n	Median	Standard Deviation	Maximum
Añasco River (Road PR # 2)	25	2650	14730	65000
Guanajibo River Mouth (Road PR # 102)	22	1947	11341	37000
Guanajibo River Upstream (Road PR #100)	26	1489	12028	57000
Yagüez River (Road PR # 2)	25	8750	13536	47000

Table 6. Coliphage indicator statistics for sampling stations of Rivers Guanajibo, Yagüez and Añasco between August 1994 and December 1996 (PFU per 100mL)

Sampling Station	n	Median	Standard Deviation	Maximum
Añasco River (Road PR # 2)	25	18	223	912
Guanajibo River Mouth (Road PR # 102)	21	30	400	1705
Guanajibo River Upstream (Road PR #100)	24	29	166	615
Yagüez River (Road PR # 2)	25	180	524	1666

of total urban area in the other study watersheds, where lower median densities of organisms were found. The Añasco station showed the lowest median density of coliphages, which previous studies have demonstrated are better indicators of recent human fecal contamination. As discussed previously, this watershed contains the lowest urban center population of the three.

Additional analyses were done to find the correlation between stream flow and densities of organisms in the study streams. Table 7 presents the results of the linear regression analysis conducted on these data at the Guanajibo upstream and the Añasco sampling stations.

These were the only two study stream sampling stations for which continuous streamflow data was available from the U.S. Geological Survey. Small linear correlations were found
between stream flow and densities of organisms, indicating either that most of the variability in microbiological densities in these stations is due to factors other than variation in storm water flow or that grab samples taken outside peak storm flows do not capture the bulk of organisms carried by surface runoff during the first few minutes of peak storm Stream flow data was
obtained from external sources (USGS), which periodically processes continuous flow data

Table 7. Pearson correlation analysis between microbiological water quality parameters and stream flow at the Guanajibo and Añasco Rivers, August 1994 - April 1996 (n=18)

Parameter	Total* Coliforms	Fecal* Coliforms	Enterococcus	Coliphages**
Stream Flow ***				
Guanajibo upstream	R=0.1844	R=0.0974	R=0.0695	R=0.2878
Añasco Rd. #2	R=0.1785	R=0.0800	R=0.2053	R=0.2712

*CFU/100 mL.
**PFU/100 mL.
***cfs (cubic feet/second)

registered by permanently stationed flowmeters.

In Table 8, results of linear regression analyses conducted on suspended solids and microbiological data from Guanajibo, Yagüez and Añasco Rivers are presented. Suspended

Table 8. Pearson correlation between microbiological water quality and suspended solids at the Guanajibo, Añasco and Yagüez Rivers, August 1994 - April 1996 (n=18)

Parameter	Total* Coliforms	Fecal* Coliforms	Enterococcus	Coliphages**
Suspended solids ***				
Guanajibo upstream	R=0.3104	R=0.3195	R=0.3737	R=0.5536
Añasco Rd. #2	R=0.6332	R=0.7021	R=0.6133	R=0.4110
Yagüez Rd. #2	R=0.0005	R=0.0148	R=0.2044	R=0.0835
Guanajibo downstream	R=0.0218	R=0.5756	R=0.3402	R=0.0112

*CFU/100 mL.
**PFU/100 mL.
***mg/l (milligrams/liter)

solids samples were grabbed simultaneously with microbiological samples and analyzed by the study team. As was the case with stream flow, linear regression analyses suggests that not all variability in microbiological parameters can be associated with variations in the concentration of suspended solids in the water column (in which case R^2 would be =1.0). However, correlations with density of organisms are larger for suspended solids than for stream flow at the Guanajibo upstream and Añasco sampling stations, as described in Table 7, for all microbiological parameters. This suggests that microbiological densities upstream of these sites increase appreciably with increases in suspended solids concentrations, most probably due to contaminated sediments carried by storm water runoff.

Correlations between suspended solids and organisms at the Guanajibo downstream and Yagüez stations were not as high, indicating to the possible direct raw wastewater discharge origin of the high levels of fecal organisms at these stations. Raw wastewater discharges contain high densities of organisms which are not correlated with suspended solids or stream flow since they are not carried by storm water runoff. The implication is that the probable origin of most fecal contamination upstream of these two stations is not nonpoint sources of water pollution such as overflowing septic tanks from rural residential communities or feces of farm animals or wildlife deposited on the soil. The most important source of fecal contamination seems to be the semi constant discharges of raw sewage into streams from poorly operated urban sanitary sewer systems.

As can be observed in Table 9 in conjunction with Table 8, correlation coefficients of suspended solids with densities of organisms were higher in stations where median suspended solids concentrations were higher.

Table 9. Median suspended solids at study stream sampling stations (mg/l), August 1994 - April 1996 (n=18)

Sampling station	Suspended Solids
Guanajibo upstream	31.16
Añasco	78.31
Guanajibo downstream	20.18
Yagüez	18.97

The implication is that organisms are carried into some of the study streams by storm water runoff along with sediments in some (probably rural) parts of the watersheds, representing an important contribution of microbiological contaminants. In other stations, the importance of sediments as sources of microbiological contaminants is less than that of direct raw wastewater discharges from bypasses of non functioning urban sanitary sewer pumping stations.

Microbiological analysis for Mayagüez Bay sampling stations:

Table 10 presents average results of microbiological water quality analysis for the Bay. Significant differences appear in pathogen indicator densities between the study sampling stations in the Bay.

Even though GIS analysis showed the Yagüez River watershed is much smaller than the others (Table 1) and discharges much smaller stream flows into the Bay (Table 2), its effect on microbiological water quality in the Bay is of comparable scale one hundred meters off its mouth. In terms of densities of fecal coliforms, it produces almost the same effect as the Añasco River does the same distance from its mouth, even though the latter has more than thirty times the

Table 10. Average densities of microbiological indicators in Mayagüez Bay sampling stations off mouths of Rivers Guanajibo, Yagüez and Añasco and above Ocean Outfall between August 1994 and June 1996 (per 100mL).[c]

Sampling Station	Total Coliforms (CPU)	Fecal Coliforms (CPU)	Enterococcus (CFU)	Coliphages (PFU)
Off Guanajibo River Mouth	1.25×10^4	2.66×10^3	3.33×10^3	73
Off Yagüez River Mouth	5.91×10^3	1.44×10^3	1.14×10^3	18
Off Añasco River Mouth	9.24×10^3	1.43×10^3	2.69×10^3	33
Above Submarine Ocean Outfall (PRASA)[a]	9.01×10^3	4.25×10^3	485	190
Composite sample [b]	1.94×10^3	94	183	0

[a] dates of Ocean Outfall samples were between 1994 and 27 May 1996.
[b] dates of Composite samples were between 1994 and February 1995.
[c] n varies between 20 and 22 among stations and parameters

average flow of the Yagüez. This indicator is used for water quality standards by the government
of Puerto Rico, as discussed in the next section.

This and other indicators show that the study stream with the most effect on the Bay's water quality is the Guanajibo. This can be explained by its larger flow than the Yagüez and more intense urban development in its watershed than the Añasco. Coliphages and enterococcus, being better indicators of human fecal contamination, particularly in the marine environment, reiterate this observation in Table 10.

Environmental health implications of microbiological analysis for Mayagüez Bay:

Three of the four marine stations (near Guanajibo and Yagüez river mouths, and PRASA outfall) are located in waters classified as SC water body by the Water Quality Standards Regulations of the Environmental Quality Board (EQB) of Puerto Rico. Class SC waters are coastal waters for indirect human contact, such as, fishing and boating, and for propagation and preservation of desirable species (EQB, 1990). Bay station located near Añasco river mouth at Añasco Bay is within waters classified as an SB water body by the EQB Regulations, that is, coastal and estuarine waters intended for use in primary and secondary contact recreation, and preservation and propagation of desirable species (EQB, 1990). This area may be the most critical in terms of health hazards to bathers.

Bay station near Añasco river mouth is the only station located in SB class waters. The standard of fecal coliforms for these waters is 200 cfu/100mL. In this station the fecal coliform standard is exceeded in 75 percent of the samples taken (twelve of sixteen samples exceed the FC standard). The average of fecal coliform density in this station is 1,433 cfu/100mL, which also exceeds the established fecal coliforms standard of 200 cfu/100mL.

At the bay station located near the Guanajibo river mouth the total coliforms water quality standard of 10,000 cfu/100mL is exceeded in five of sixteen samples taken. The total coliform average for this station is 12,508 cfu/100mL, which also exceeds the established total coliform standard. The water quality standard for fecal coliforms (2,000 cfu/100mL) in these waters is also exceed in five samples taken at this station. The average density of fecal coliforms for this station is 2,664 cfu/100mL.

At the bay station located near Yagüez river mouth, the total and fecal coliforms standards (10,000 cfu/100mL and 2,000cfu/100mL, respectively) are exceeded in four of sixteen samples. The total and fecal coliforms averages for this station is 5,909 cfu/100mL and 1,436 cfu/100mL, respectively. The total and fecal coliforms standards are also exceed at station located at the PRASA outfall in the bay. The fecal coliform average for this station is 4,248 col/100mL.

Although the Puerto Rico Environmental Quality Board (EQB) Water Quality Standards Regulation does not establish a standard for enterococcus, the U.S. Environmental Protection Agency designated an enterococcus criteria for marine recreational waters of 35 cfu/100mL (EPA, 1994). In a three year study at the New York City beaches, Cabelli *et al.* (1983)found a high correlation between the enterococcus density and the incidence of gastrointestinal symptoms in bathers. Other organisms studied by Cabelli *et al.* (1983) was E. coli, Klebsiella, and total and fecal coliforms among others. The study identified enterococcus as the best indicator organism for marine waters. Based on the enterococcus water quality criteria, the Mayagüez and Añasco bays marine waters exceed acceptable limits, and could represent a public health hazard if they areas used for recreational purposes. The average density of enterococcus at Mayagüez and Añasco Bays range from 485 cfu/100mL at station near PRASA outfall to 3,333 cfu/100mL at the station near Guanajibo river mouth. At the Añasco station the average density of enterococcus was 2,686 cfu/100mL.

A bathing public beach (Balneario de Añasco) is located to the north of Añasco bay station. Currents in Mayagüez and Añasco bays are essentially parallel to shore, reversing direction according to the ebb and flood of tides (ENSR, 1992). Studies have demonstrated that the net flow of current is in a northerly direction, with periodic reversals to the south (Colón (1970), (1971) and Metcalf & Eddy (1985) cited by ENSR, 1992). Additional studies of the dispersion of pathogen organisms by currents to the areas most used for recreational purposes are needed. The use of some areas of Mayagüez and Añasco Bays for recreational purposes could represent a health hazard problem to persons who have direct contact with these contaminated waters.

Conclusions

This study showed that significant differences in microbiological water quality exist between streams draining watersheds with different land use patterns. A watershed with a high density urban development, Yagüez, showed much higher stream densities of microbial indicators near its river mouth than less densely developed watersheds with similar area percentage of urban - residential cover. The coupling of data from images, Census Bureau, and field measurements helped to determine that the most probable source of microbiological contaminants near urban centers of this region is the discharge of raw water from malfunctioning urban sewers. On the other hand, this study showed that more agricultural - rural areas such as the Añasco watershed suffer from higher degrees of erosion and sediment loadings in streams which carry microbiological contaminants.

Although high levels of microbial pollution were found in all study streams, coliphage densities correlated more closely with population of watersheds, suggesting they are indeed better indicators of recent human fecal water contamination in tropical climates.

The use of GIS - Remote Sensing techniques served to quantify land covers on a watershed scale with a level of precision and speed unattainable by visual photogrametric means. This permitted making comparisons between watersheds that allowed a better scientific understanding of water pollution processes.

Student Achievements

The Medical Sciences Campus conducts research continuously in basic and applied sciences related to human health. The project has increased the amount of student participants in NASA related fields, improving their skill in scientific and environmental research. Research assistants practiced laboratory procedures for measuring the following physical, chemical and microbiological parameters:

- Physical and chemical parameters;
  Total Suspended Solids, Temperature, pH, turbidity, and conductivity.
- Microbiological parameters;
  Fecal coliforms, Fecal Enterococci, Coliphages, Giardia and Cryptosporidium.

Acquisition of computer software and hardware available for student training in image analysis has been made, as well as of laboratory materials and sampling equipment. A scientific paper with students as co-authors was presented in the Summer of 1996 relating land use and water quality in these watersheds.

Relevance to NASA Strategic Enterprises

This project involves applied research in Earth System Science. This is supported by the NASA Office of Mission to Planet Earth.

Benefits to Society

The research project is directly related to the continuing effort to develop and employ Geographic Information System and Remote Sensing in research and enhancement of government's capability to analyze and develop solutions to the needs of the population.

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Appendix A

Location of Study Area and Sampling Stations at Mayagüez Bay

Appendix B
Sampling Equipment for Collecting Giardia and Cryptosporidium Samples

Appendix C1
Guanajibo River Watershed Land Uses

Appendix C2
Yagüez River Watershed Land Uses

Appendix C3
Añasco River Watershed Land Uses

Appendix C4
Image of Guanajibo River Watershed - Natural Color Composition

Appendix C5
Image of Yagüez River Watershed - Natural Color Composition

Appendix C6
Image of Añasco River Watershed - Natural Color Composition

Table __. Average densities of microbiological indicators in sampling stations of Rivers Guanajibo, Yagüez and Añasco between August 1994 and June 1996 (per 100mL)

Sampling Station	Total Coliforms (CPU)	Fecal Coliforms (CPU)	Enterococcus (CFU)	Colifaphages (PFU)
Añasco River (Road PR # 2)	3.67×10^4	1.23×10^4	9.68×10^3	63
Guanajibo River Mouth (Road PR # 102)	6.81×10^3	8.38×10^3	6.79×10^3	67
Guanajibo River Upstream (Road PR #100)	1.10×10^4	5.56×10^3	6.87×10^3	93
Yagüez River (Road PR # 2)	8.76×10^4	3.26×10^4	1.43×10^4	508