SEPTIC TANKS
AS A SOURCE OF POLLUTION OF
GROUNDWATER IN THE JOBOS BAY RESERVE

FINAL REPORT

Submitted to the
Department of Natural Resources
Of the Commonwealth of Puerto Rico

by

Graduate School of Public Health
Medical Sciences Campus
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**INVENTORY OF SEPTIC TANKS
AS A SOURCE OF POLLUTION OF
GROUNDWATER IN THE JOBOS BAY RESERVE**

FINAL REPORT

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A. Objectives

The principal objective of this work is to determine the impact of septic tanks on ground water quality near the community of Las Mareas. The water quality in the region is being impacted by nonpoint sources of contamination such as the septic tanks of the community, agricultural activities, solid waste dumps, industrial sources like the PREPA Thermoelectrical Energy plant, and gas stations. These activities affect the water quality and also affect the ecology of the Mar Negro lagoon because the aquifer flows into the lagoon. This community is located near the National Estuarine Reserve of Jobos Bay (JBNERR); which has experienced mortality of black mangrove.

Other objectives of this study were: 1) Determine the chemical impact of nonpoint sources of contamination on the Mar Negro mangrove system ecology. Three wells were selected north of the community of Las Mareas (upstream of the aquifer flow), three piezometers

were bored within the community and one point of sampling stations was established in the Mar Negro Lagoon. 2) Monitor the microbiological water quality using indicator microorganisms of fecal contamination (hermtolerant coliforms and enterococcus) of groundwater upstream and in the community.

B. Study Area

Jobos Bay National Estuarine Research Reserve (JBNERR), originally known as Jobos Bay National Estuarine Sanctuary, was designated in September, 1981 by an agreement between the Department of Natural and Environmental Resources (DNER) of the Commonweal of Puerto Rico and the National Oceanic and Atmospheric Administration (NOAA). This designation established the Jobos Bay as the eleventh interest place of the National Estuarine Research Reserve; according to amendment of the 315 section of the Coastal Zone Management Law.

The Reserve is constituted of a superficial area of 11 square kilometers. The Reserve is located between Guayama and Salinas. The research community known as Las Mareas is located west at Salinas Reserve; it is constituted by approximately 700 residents.

The reserve adjoins to the north the Land Authority of Puerto Rico properties, dedicated to fruits and corn harvest. To the northeast it adjoins PREPA Thermoelectric Energy Plant of Aguirre and the old sugar processing central. To the west it adjoins with Las Mareas community.

The reserve is located in a south coastal plain inside the Subtropical Dry Forest zone. It receives a yearly pluvial rain of 1129 mm. Is maximum precipitation occurs in October with an average of approximately 228.6 mm (9 inches) of rain, it driest month is March with approximately 5.4 mm (1 inches). The reserve temperature mean is 26.55°C. The winds fluctuate between 6 to 7 knots. Groundwater is the principal source of fresh water in the reserve.

The vegetation is composed of four types of mangroves; red mangrove (*Rhizophora mangle*), the white mangrove (*Laguncularia racemosa*), black mangrove (*Avicennia germinans*) and the button mangrove (*Conocarpus erectus*). These mangroves function as sediment traps that delay water movement and trap the suspended materials, gradually raising the ground level and producing organic soil.

The south area of Puerto Rico is a semi-dry zone. The principal source of water to supply the human and agricultural demand is the ground water (Robles, et al., 2003). In this zone the aquifer that is located near the surface that is an unconfined alluvial aquifer, whose water table in some areas is located at one feet of depth. Because of the proximity to the surface the alluvial aquifer is impacted by urban human activity, industrial activity and the agricultural activity of the zone.
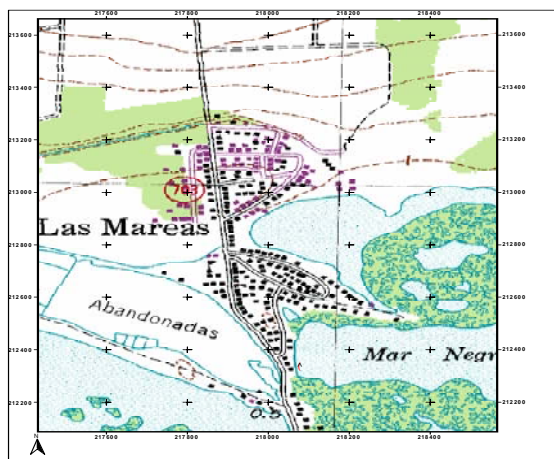
Figure 1: Map of study area

C. Methodology

1. Sampling stations

a. Location of sampling points for groundwater and lagoon samples

The sampling area included the agricultural zone near the community of Las Mareas including the community and the Mar Negro Lagoon located in Salinas, Puerto Rico. Three wells used for agricultural irrigation in farms north of the community (Jaguas West, Jaguas East and Saliche), were selected as sampling points of groundwater not impacted by the community. Inside the community of Las Mareas three piezometers were bored at a depth of 5 feet for the sampling of ground water. There was also one point in the Mar Negro lagoon selected for the sampling of surface water (Fig. 2).
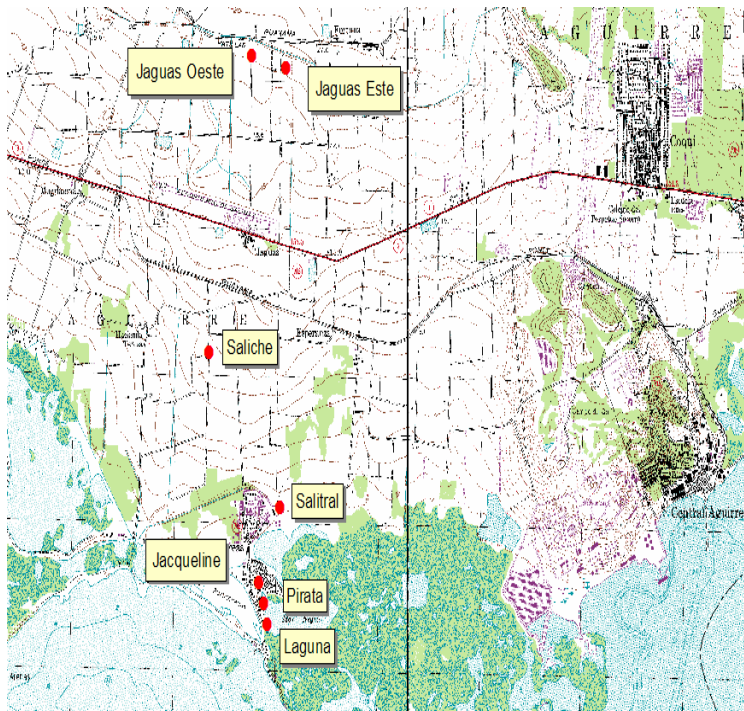
Figure 2: Location of sampling point near Jobos Bay, Salinas

Table 1: Collection of sampling point coordinates for groundwater and lagoon samples

Sampling Points	Coordinates	Elevation (meters above sea level)
Pirata	N 17° 56'45.7'', W 066°15'46.9''	< 1
Jacqueline	N 17° 56'50.6'', W 066°15'48.3''	< 1
Salitral	N 17° 57'06.8'', W 066°15'41.2''	< 1
Saliche (Estate)	N 17° 57'40.9'', W 066°16'04.7''	7-8
Jaguas East	N 17° 58'50.7'', W 066°15'50.3''	18-19
Jaguas West	N 17° 56'41.1'', W 066°15'46.3''	18-19
Lagoon	N 17° 56'41.1'', W 066°15'45.1''	< 1
Estates		
Pollos	N 17° 58'20.1'', W 066°14'09.5''	12-13
Teresa	N 17° 58'16.9'', W 066°16'27.8''	10-11
Aguirre	N 17° 57'56.5'', W 066°15'05.3''	12-13
Burgos	N 17° 58'04.2'', W 066°15'34.8''	11-12

b. Location of sampling points for oyster and water samples

Two sampling sites were selected at Jobos Bay National Estuarine Research Reserve. They were named Las Mareas and Canal Sampling sites. These were located within a mangrove forest area called "Mar Negro", comprising several channels and small lagoons.

Las Mareas is situated near the western boundary of JBNERR near a mangrove lagoon connected to Jobos Bay by a narrow mangrove channel. It is the sampling site farthest away from the ocean within the mangrove system. The small housing community located along the northern border of the lagoon (Las Mareas community) is not connected to the municipal sewer

system, since these facilities are not available in this area. Raw sewage is discharged into septic tanks, which could leach into the lagoon. The Canal sampling site is located at the southern border of the channel that connects this site to Jobos Bay. This location is considered the "control" site since water quality should be less affected by discharges of fecal coliform bacteria from Las Mareas. It is the sampling site closest to the open ocean.

c. Sampling stations description for the groundwater and lagoon sampling points

Station I: **Pirata**

The Pirata station is located near a residence under construction inside Las Mareas community approximately 10 meters from Mar Negro lagoon. Its coordinates and elevation respectively are N 17° 56'45.7'', W 066°15'46.9'' and < 1 m above sea level.

Station II: Salitral

The Salitral station is located inside a community near the Salitral and adjacent to the drainage channel of the estate of Mr. Héctor Vega. Its coordinates and elevation respectively are: N 17° 57'06.8'', W 066°15'41.2'' and < 1 m above sea level.

Station III: Jacqueline

The Jacqueline station is located in the backyard of a house inside the community. This station is adjacent of a house septic tank. Its coordinates and elevation respectively are: N 17° 56'50.6'', W 066°15'48.3'' and < 1 m above sea level.

Station IV: Jaguas West

The station Jaguas West is a groundwater pump for irrigation of the plantain harvest. This property is of Mr. Héctor Vega. This station is located to the north of the Las Mareas

community. Its coordinates and elevation respectively are: N 17° 56'41.1'', W 066°15'46.3''
and 18-19 m above sea level.

Station V: Jaguas East

The station Jaguas East is another groundwater pump for irrigation of the plantain
harvest. This is also property of Mr. Héctor Vega. This station is located to the north of Las
Mareas community. Its coordinates and elevation respectively are: N 17° 58'50.7'', W
066°15'50.3''and 18-19 m above sea level.

Station VI: Saliche

This station is a groundwater pump for the irrigation of the Saliche estate, operated by
Mr. Javier Rivera. This station is located to the north of Las Mareas community. Its coordinates
and elevation respectively are: N 17° 57'40.9'', W 066°16'04.7'' and 7-8 m above sea level.

Station VII: Lagoon

This station is located in the Mar Negro lagoon at the end of fisherman's dock. From this
surface water samples are taken. Its coordinates and elevation respectively are: N 17° 56'41.1'',
W 066°15'45.1''and 0 m above sea level.

2. Field Procedure

The water samples were collected every fifteen (15) days during, April to September
2005 from: the Mar Negro Lagoon, three irrigation wells north of the community (Jaguas West,
Jaguas East and Saliche), and three water monitoring piezometers (iron and inoxidable pipets
with 3mm openings to let the water pass) inside the community of Las Mareas. The piezometers
have a depth of five feet. The piezometers were sunk with the collaboration of the Agricultural
Experimental Station. The water samples in the piezometers were extracted using a vacuum

pump (GE) of 1/6 HP that brings the water to the graduate cylinder located on the ground surface.

All the instrumentation used in the field is previously washed with ethanol to avoid the samples contamination and they are put in the Castle Gravity/Laboratory Sterilizer. During each sampling the following physical parameters of the groundwater were measured: temperature, pH, dissolved oxygen, conductivity and salinity. These physical parameters were measured using the DataSonde® 4 and MiniSonde® Hydrolab Instrument and Horiba Ltd. U-10 water quality checker. The time and date of the sampling were recorded.

3.   Sample Analysis

a. Microbiological analysis using traditional technologies

i. Sampling

The water samples were collected in sterilized 1L plastic bottles and located in an ice chest at around 4° C and transported to the Environmental Health Laboratory located in Sciences Medical Campus at San Juan. The samples were filtered the same collecting day in the Environmental Health Laboratory.

ii. Membrane filtration

To analyze the fecal coliforms and enterococcus parameters, the membrane filtration technique was used. This method is differential and selective; it allows isolating bacteria using different media cultures. The cellulose acetate membrane has a porous size of 0.45 μm, allowing the water to travel easily, trapping the bacteria on the surface. The membrane filtration is one of the most utilized techniques for managing large sample volumes. This technique is not

recommended for conditions where the water presents a lot of turbidity due possible porous obstruction. Afterward, the sample is filtered and transfered to a sterile Petri dish who which has the selective media for the type of bacteria that has to be quantified. Three volumes of each of the samples were filtered: 1 ml, 10 ml and 50 ml. Between filtrates, the funnels are washed with buffer solution. The membrane was grown in two different media to identify the presence of fecal coliforms and enterococcus. Every dilution (1 ml, 10 ml, and 50 ml) of each sampling was transfered to both media cultures for a total of forty-six dishes per sampling.

The selective media culture to identify the presence of enterococcus were *M Enterococcus*. To identify fecal coliforms the *MFC Agar* was used. The confirmatory test for both utilized *Azide Dextrose Broth* and *Lauryl Triptose Broth* (LTB). The purpose of the test is to exclude false positive and false negative results. "This membrane filtration technique is very reproducible, it can be use for large samples of volumes and prove numerical results more rapidly than the multiple tube technique (APHA et al., 1995).

iii. Media cultures and Confirmatory test

The media culture for enterococcus is *M Enterococcus*; its ideal pH is 7.2 ± 0.2. This media culture is prepared by adding 42 g of the powder in 1 L of purify water. The mixture is heated and agitated during one minute to dissolve the powder. This media is not sterilized. A positive test for this media culture test produces intense pink and brown colonies. These dishes are incubated for 48 hr at 35°C. The confirmatory test to identify enterococcus uses *Azide Dextrose Broth*. A positive test for this media culture is the presence of turbidity. *Azide Dextrose Broth* is prepared by dissolving 34.7 g in L of water. The solution is mixed and heated to dissolve the powder. This broth is sterilized for fifteen minutes and incubated for twenty four hours at 35°C. It final pH should be 7.2 ± 0.2.

The media culture for fecal coliforms is *MFC Agar*; its ideal pH is 7.4. The fecal coliforms grow and ferment lactose. A positive test for this media culture is blue colonies due to the fermentation of the stain of the media. The incubation period is 18-24 hr at 45°C. To prepare this media 52 g of the agar is suspended in 1 L of purified water. The suspension is vigorously mixed and boils for 1 minute to dissolve the powder. 10 ml of 1% rosolic acid solution in 0.2 N NaOH is added to the mixture. This media is not sterilized. The instructions for preparing the rosolic acid are to add 0.5 g of the acid in the powder in 50 ml of 0.2 N NaOH. The mixture is agitated and maintained in a sealed container with a black lid in the refrigerator.

The confirmation test for fecal coliforms is accomplished with LTB. LTB is a media culture which has lactose; a positive test will produce turbidity and gas. This media is incubated for 24 hr at 35°C. Its preparation is accomplished adding 35.6 g of the powder in 1 L of water. It is mixed and heated to dissolve the powder. The media culture is poured in to little essay tub which have invert fermentation vials (Durham tubes).The media is sterilized during 15 minutes at 121°C.

iv. Bacteria Quantification

The colonies are counted directly in each dish. The equation to calculate the bacteria concentration is realized by this formula:

$$CFU \text{ (colony forming units)}/100ml = \frac{\text{coliform colonies counted}}{\text{ml sample filtered}} \times 100$$

v. Description of Microbiological Parameters

- **Thermotolerants Coliforms (Fecal)**

  - Aerobic or facultative anaerobic
  - Negative Gram
  - Constituted by two genres: *Escherichia, Klebsiella*
  - Non-spore formers

> ➤ Lactose fermenters with the production of gas and acid at 44.5°C
> ➤ Culture media: **MFC**
> ➤ Confirmation test: **LTB**
> ➤ Microorganism comes from the gastrointestinal tract from warm blood animals.

- **Enterococci**

> ➤ Positive Gram bacterias
> ➤ Morphology:coccus
> ➤ Present in the gastrointestinal tract of warm blood animals
> ➤ Culture media: **m Enterococcus**
> ➤ Confirmation test: **Azide Dextrose Broth**
> ➤ Example: *Enterococcus faecalis*

vi. Laboratory tasks performed during microbiological analysis:

- Media culture preparation

- Solution preparation

- Membrane filtration

- Culture dishes quantification

- Instrumentation calibration

- Materials cleaning

- Data analysis

b. Microbial Source Tracking PCR-Based Methodology

Several library independent Microbial Source Tracking methods have been developed to rapidly determine the source of fecal contamination. In this study, a *Bacteroides* 16S rDNA PCR-based method was used to test for the presence of specific groups of fecal contaminants. Assays specific for human (HF) and general *Bacteroides-Prevotella* (GB) were used to screen water samples from septic tanks in six locations in Salinas, Puerto Rico. These organisms are frequently used as source identifiers because they compose a majority of the fecal microbiota in

humans, are anaerobic, and exhibit host-specific differences between different animal groups (Dick, et al., 2005).

Water samples were taken in Las Mareas community, located in the municipality of Salinas, situated at the southeastern coast of the island. This community uses septic tanks to dispose of used waters. The sites were depicted as: Salitral, Pirata, Laguna, Jacqueline, and Saliche. Water samples from piezometers near the septic tanks were collected in sterile containers, and were preserved in ice until they arrived at the laboratory.

Various concentrations (10, 20, 50, and 100mL) of water samples were filtered using polycarbonate membranes. Once filtered, they were stored in autoclaved 2ml centrifuge tubes, and stored at -20 °C overnight. The samples were sent the next day to the Environmental Protection Agency in Cincinnati, OH for processing. The MoBio Fecal DNA kit (MoBio Labs, Inc.) was used to obtain genomic DNA according to manufacturer's instructions.

DNA obtained from all the sites was amplified using *Bacteroides-Prevotella* primers (Table 2). Each 25µl PCR mixture contained 10X *Ex Taq* buffer, deoxynucleoside triphosphates (DNTPs) at a concentration of 2.5mM each, primers at a concentration of 25pM each and 0.626U of *Ex Taq* (TaKaRa, Inc.). The thermal cycler programs were as follow: initial denaturing at 94°C for 2 min, 35 cycles at 94° for 1 min, 1 min for each annealing temperature for the primers (Table 2), and 72°C for 1.5 min, followed by a final extension at 72°C for 7 min. Electrophoresis was performed by preparing 1% agarose gels stained with GelStar (Cambrex, Inc.).

Table 2: Primers used for the study

Primer[a]	Sequence	Target	Annealing temp (°C)	Reference
Bac32F	AACGCTAGCTACAGGCTT	*Bacteroides-Prevotella*	53	[b]Bernhard, et al., 2000
Bac708R	CAATCGGAGTTCTTCGTC	*Bacteroides-Prevotella*		[b]Bernhard, et al., 2000
HF134F	GCCGTCTACTCTTGGCC	HF10	61	[a]Bernhard, et al., 2000
HF183 F	ATCATGAGTTCACATGTCCG	HF8 cluster, HF74	59	[a]Bernhard, et al., 2000

[a] All forward primers were paired with Bac708R.

c. Microbiology of oyster and water samples

i. Sampling

Twelve oysters *(Crassostrea rhizophorae)* were hand picked from Red Mangrove *(Rhizophora mangle)* roots at each station. All *C. rhizophorae* were immediately placed in sterile 0.5 L plastic bags. These were rapidly placed inside a cooler with ice and transported to the laboratory at the University of Puerto Rico at Humacao. Water samples were taken from each station, in triplicate, using sterile Whirl-Pak plastic bags.

ii. Weight determinations

Oysters were opened using a stainless steel oyster knife. The soft tissue from each *C. rhizophorae* was transfered to a pre-tared sterile 50mL Falcon® graduated centrifuge plastic tube using a stainless steel tweezer. Oyster soft tissue weight determinations were performed using a Denver Instrument model APX1502 toploading balance (linearity ± 0.02g).

Table 3: Soft tissue weight determination (in grams) from oysters sampled at Las Mareas and Canal sampling sites.

Sample #	Las Mareas	Canal
1	0.84	1.01
2	1.21	1.36
3	1.84	0.87
4	1.39	1.35
5	0.88	0.78
6	0.74	0.88

iii. Tissue homogenization

Each tube containing the oyster soft tissue was filled to 20mL with sterile 0.5% peptone. Contents were homogenized for approximately one minute using a Tissue Tearor Model 985-370 (Biospec Products, Inc.) variable speed tissue homogenizer. The tip of the homogenizer was thoroughly cleaned with 70% ethanol prior to each homogenization.

iv. Filtration

A 0.1mL aliquot of each homogenate was filtered in an all-glass Sartorius filter holder through a 47 mm diameter, 0.4 μM pore size Poretics Polycarbonate membrane (Osmonics, Inc.). The filter holder was sterilized with 70% ethanol prior to each filtration.

A 10mL aliquot of each water sample was filtered, as described above, in order to perform bacterial analyses in seawater. All membranes were aseptically folded using stainless steel tweezers and each placed in sterile 1.5mL microcentrifuge tubes. The tubes were placed in a refrigerator and shipped to EPA laboratories at Cinncinnati, Ohio for DNA analyses of fecal coliform bacteria.

d. Organic Chemical Analysis

i. Sampling

Duplicates samples were collected using a one-liter amber colored glass bottles with Teflon-lined caps (pre-washed with detergent and hot tap water, then rinsed with distilled and de-ionized water, and dried in an oven at 400° C for 1 h). The water samples were placed in an ice chest at around 4° C and transferred to the Agricultural Experimentation Station Pesticide Laboratory at Río Piedras on the same collecting day. The samples were stored at 4° C in a refrigerator from the time of collection until extraction, which was done the next day after collection.

ii. Filtration

All water samples were first filtered through a Whatman GB/F filter of 45 mm, then through a Nylon membrane filter (0.45μm) before chemical analysis for the purpose of removing suspenders solids.

iii. Organic anthropogenic compound extraction and analysis

Organic compounds were extracted by the SPE-disk method outlined by Mersie et al., (2002). A 1-L water sample was passed through a pre conditioned Empore C18 disk and re-extracted in 5 ml of ethyl acetate. Analyses were performed by gas chromatography/mass spectrometry (Perkin Elmer GC/MS Autosystem-TurboMass) by using a 30m x 0.25 mm x 0.1μm film thickness DB-5 capillary column with the following operating conditions: a temperature program of three min at 70°C, then increasing 10 C/min to 250 C and holding for three min; three min solvent delay on MS and helium carrier gas at 1.0 ml/min flow rate. An injection of 1mL/min in an injection port set in splitless mode at 250°C was used. The mass spectrometer detector was set at total ion mode with a range 50 to 450 amu. Compound

identification was based on the retention times and molecular spectral fragmentation by using a Wiley mass spectrum's library.

iv. Laboratory task to perform during organic compounds analysis

- Standards solutions preparation

- Calibration curve preparation

- Water samples filtration

- Fortification and extraction of the samples with C_{18}

- Samples preparation to be analyzed by GC/MS

- Instrument calibration

- Samples analysis by GC/MS

- Materials cleaning

D. Other tasks performed

The piezometer well boring was carried out March 11, 2005. The following weeks the piezometers were visited for cleaning and preparation for sampling. After consulting with the USGS the conclusion was reached that the extracted water was from the acquifer.

The first ocular visit was done to evaluate the septic tanks conditions in Las Mareas community November 4, 2004. The first visit attendance included: Dr. Jose Norat-Principal Investigator, Dr. Hernando Mattei- Co-Investigator, Dr. Rafael Dávila- Consultant, Yamil Toro-Consultant, Raúl Santini- Department of Natural and Environmental Resources contact, Eva María Rivera Hernández- Graduate Student, Research Assistant, Kaura Jaramillo Suárez-Graduate Student, Research Assistant and Jaqueline Vázquez- Las Mareas Community Leader.

Also the reserve was visited with the company of the JBNERR personal to determine the sampling locations for the oyster tissues.

As part of the arrangement there was a meeting with Dr. José Dumas of the Agricultural Experimental Station Thursday 14 of October 2004. In this meeting the following topics were discussed:

- Dr. Dumas pointed out the map of the locations where the Agricultural Experimental Station dug the piezometers for a previous work to collect samples from groundwater.

- The Agricultural Experimental Station personnel showed interest in collaborating in the process of well boring to collect groundwater samples in this project.

- The piezometer well boring was carried out to collect groundwater samples. (approximately 5 ft. of depth)

- As part of the collaboration of the study it was agreed to train the students Eva María Rivera and Kaura Jaramillo Suárez on using organic chemical analyzing instrumentation.

As part of this arrangement letters were written to the Director of the laboratories (Pesticide and Central laboratory), Mrs. Nilsa Acín, to get an authorization for the students to work in those laboratory facilities. The Agricultural Experimental Station is part of the Mayagüez Campus of the University of Puerto Rico. In January 2005 the authorization was received for the students to work at the Pesticides laboratory in the Agricultural Experimental Station.

As a part of the preliminary phase information was collected about the terrain conditions and quality of the groundwater in the areas adjoining the community. On April 1, 2005 with the collaboration of the Land Authority, sampling was carried out in the deep wells of the Esperanza, Aguirre, Teresa, Saliche and Burgos' estates near the community. Surface water of the lagoon

was also measured for pH, conductivity, temperature, dissolved oxygen, nitrate, nitrite, chlorine and turbidity.

A literature review was carried out about septic tanks operation, impact of septic tank pollution, microorganisms in the water, and organic anthropogenic compounds in water bodies.

Another component of this project was the construction a survey questionnaire to measure the perceptions and attitudes of the community residents towards the construction, operation, maintenance and impact of the septic tanks in environmental health, to be considered in possible problem solutions. This component of the project was directed by Dr. Hernando Mattei and consulted with Dr. Rafael Dávila.

This component involved the following tasks in this project:

- Preparation of the questionnaire, specifically on the residential septic tanks and their impact on groundwater pollution in the Jobos Bay Estuarine Reserve.
- Inspection of the septic tanks of Las Mareas sector. This inspection was realized in selected septic tanks with a communitarian leader.
- Measurement of perceptions and attitudes toward water pollution by septic tanks.

Dr. Mattei met with Dr. Rafael Dávila Thursday 7 of October; in this meeting they discussed the following aspects:

- Questionnaire model for the surveys of knowledge, perception and attitude towards the septic tanks of Las Mareas residents.
- Education campaign to the community about the septic tanks.
- Physical inspection of the septic tanks.
- Septic tanks evaluation in the Las Mareas community.

The draft of the questionnaire was worked. It was revised to reach a final version, and then it was submitted to the IRB office to be administrated to the community.

Flow conditions of groundwater within the community were studied.

This task involved the use of a stain test for the detection of septic tank filtration on March 4, 2005. Mr. Alfredo Casta Vélez (National director of Environmental Health) was contacted for the authorization of the environmental health personnel of Ponce to carry out the stain test. These tests were performed by Mr. Jorge Rivera (plumbing inspector) of the Environmental Health Department with the help of the students Eva María Rivera and Kaura Jaramillo. Afterwards, on Monday, 7 of March Mr. Jorge Rivera and Dr José Norat visited the septic tanks to look at results. Very slow flow was observed, as stain had not filtered into the ground in significant amounts after several days of test.

E. Results and Discussion

1. Results

a. Physical – Chemical Parameters Results

An ascending tendency was observed in the concentrations of, ammonium and in the level of salinity and pH from the irrigation wells upstream to the lagoon of Mar Negro downstream (Table 4).   These results point to effluents from the community of Las Mareas as responsible for higher levels of ammonium and other inorganic contaminants present in groundwater and surface water in the sampling zone.

Table 4: Average of physical chemical parameters of the sampling zones

Station	*pH	n	*Temp. (ºC)	n	*NH$_4^+$ (mg/L – N)	n	*NO$_3^-$ (mg/L – N)	n	*Turb. (NTUs)	n	**OD mg/L	n
Piezometers	7.24	24	28.85	24	24.36	15	7.19	15	241.66	24	2.34	12
Lagoon	7.48	9	28.93	9	97.028	5	24.514	5	272	8	4.76	6
Irrigation Wells	7.12	22	27.87	22	0.711	10	7.31	10	36	17	4.62	17

*Hydrolab Instrument, **Horiba Instrument

i. Physical – chemical results (Hydrolab instrument)

Table 5: Physical –Chemical parameters station I: Pirata

Date	pH	Sp Cond. (mS/cm)	Temp. (ºC)	DO Saturation (%)	NH$_4^+$ (mg/L–N)	NO$_3^-$ (mg/L –N)	Cl$^-$ (mg/L)	Turb. (NTUs)	Depths of water table (inch)
31-May-05	7.50	7.86	29.61	26.10	20.59	11.32	195.50	101	41.90
8-Jun-05	7.55	3.73	28.26	19.00	25.04	10.69	62.42	>1000	▲
13-Jun-05	7.48	7.43	29.04	38.50	19.71	7.81	160.00	235	24.00
21-Jun-05	7.45	5.32	29.50	38.60	24.43	11.63	470.20	>1000	23.30
28-Jun-05	7.50	7.87	28.89	98.50	21.32	11.08	371.45	>1000	28.30
12-Jul-05	6.85	4.59	29.48	29.00	▲	▲	9158.50	>1000	47.50
19-Jul-05	6.90	7.75	30.20	93.80	▲	▲	▲	>1000	43.50
12-Aug-05	7.20	1.31	30.80	18.80	▲	▲	▲	>1000	39.50
Average	7.30	5.73	29.47	45.30	22.22	10.50	1736.34	168	36.90
STDEV	0.29	2.42	0.78	32.30	2.38	1.55	3639.11	94	10.10
N	8	8	8	8	5	5	6	8	7

▲ Not detected

Table 6: Physical – Chemical parameters station II: Jacqueline

Date	pH	Sp Cond. (mS/cm)	Temp. (ºC)	DO Saturation (%)	NH$_4^+$ (mg/L–N)	NO$_3^-$ (mg/L –N)	Cl$^-$ (mg/L)	Turb. (NTUs)	Depths of water table (inch)
31-May-05	7.78	0.77	28.74	76.20	14.76	1.24	187.20	0	20.30
8-Jun-05	7.48	5.40	24.87	44.80	28.89	3.50	45.46	>1000	▲
13-Jun-05	7.58	3.55	27.42	>500.00	20.00	1.90	72.93	0	16.00
21-Jun-05	7.72	2.00	29.07	97.10	19.04	1.12	177.65	>1000	16.20
28-Jun-05	7.63	1.28	28.40	318.90	22.00	2.09	162.65	358	16.20
12-Jul-05	6.91	5.63	28.57	24.40	▲	▲	▲	>1000	9.20
19-Jul-05	7.15	2.74	30.65	60.80	▲	▲	▲	>1000	18.40
12-Aug-05	7.26	0.04	28.70	26.90	▲	▲	▲	814	6.20
Average	7.44	2.67	28.30	92.70	20.94	1.97	129.18	293	14.60
STDEV	0.30	2.07	1.65	103.10	5.17	0.95	65.21	386	5.10
N	8	8	8	8	5	5	5	8	7

▲ Not detected

Table 7: Physical –Chemical parameters station III: Salitral

Date	pH	Sp Cond. (mS/cm)	Temp. (ºC)	DO Saturation (%)	NH$_4^+$ (mg/L–N)	NO$_3^-$ (mg/L –N)	Cl$^-$ (mg/L)	Turb. (NTUs)	Depths of water table (inch)
31-May-05	7.52	24.60	28.65	81.20	28.81	10.53	16707.00	0	▲
8-Jun-05	6.98	26.50	29.25	30.70	31.55	9.88	216.50	>1000	▲
13-Jun-05	6.90	26.40	28.02	35.90	33.39	8.47	440.50	920	21.00
21-Jun-05	7.17	24.20	29.54	62.60	29.94	8.81	1069.60	0	23.00
28-Jun-05	7.12	22.40	27.28	156.30	25.98	7.98	1326.00	135	23.00
12-Jul-05	6.64	19.80	28.61	21.30	▲	▲	▲	>1000	7.00
19-Jul-05	6.88	21.50	29.45	62.10	▲	▲	▲	>1000	4.60
12-Aug-05	6.80	17.43	29.65	39.60	▲	▲	▲	>1000	10.50
Average	7.00	22.85	28.80	61.20	29.93	9.13	129.18	264	14.90
STDEV	0.27	3.20	0.84	43.20	2.81	1.05	65.21	442	8.40
N	8	8	8	8	5	5	5	8	6

▲ Not detected

Table 8: Physical –Chemical parameters station IV: Lagoon

Date	pH	Sp Cond. (mS/cm)	Temp (ºC)	DO Saturation (%)	NH$_4^+$ (mg/L– N)	NO$_3^-$ (mg/L –N)	Cl$^-$ (mg/L)	Turb. (NTUs)	Depths of water table (inch)
31-May-05	7.68	58.40	30.70	206.00	113.26	22.16	2290.00	0	0
8-Jun-05	7.67	50.00	27.73	122.10	83.59	22.80	2132.00	531	0
13-Jun-05	7.50	53.40	26.69	160.80	85.82	22.82	1108.20	0	0
21-Jun-05	7.67	57.80	29.58	293.10	92.04	26.95	2084.30	0	0
28-Jun-05	7.84	46.00	28.68	293.60	110.43	27.84	1916.30	1198	0
12-Jul-05	7.44	55.60	29.99	212.00	▲	▲	▲	0	0
19-Jul-05	7.31	56.90	29.56	183.60	▲	▲	▲	0	0
5-Aug-05	7.11	54.10	28.06	184.90	▲	▲	▲	▲	0
12-Aug-05	7.14	56.60	29.44	198.00	▲	▲	▲	444	0
Average	7.48	54.31	28.93	206.00	97.03	24.51	1906.10	272	0
STDEV	0.26	4.05	1.26	56.30	13.91	2.66	465.54	435	0
n	9	9	9	9	5	5	5	8	9

▲ Not detected

Table 9: Physical –Chemical parameters station V: Jaguas West

Date	pH	Sp Cond. (mS/cm)	Temp. (ºC)	DO Saturation (%)	NH$_4^+$ (mg/L–N)	NO$_3^-$ (mg/L –N)	Cl$^-$ (mg/L)	Turb. (NTUs)
8-Jun-05	7.20	0.75	27.80	500.0	0.80	5.87	5.95	482
13-Jun-05	7.25	0.75	27.60	77.30	0.59	6.88	13.67	0
21-Jun-05	7.40	0.74	27.92	500.00	0.65	5.35	23.38	0
28-Jun-05	7.28	0.75	27.89	125.20	0.69	6.96	31.90	0
12-Jul-05	6.85	0.79	25.68	77.70	▲	▲	9595.50	0
19-Jul-05	6.80	0.76	28.09	80.70	▲	▲	10824.50	0
5-Aug-05	6.89	0.74	27.77	93.20	▲	▲	1920.00	0
12-Aug-05	6.90	0.75	28.33	132.50	▲	▲	▲	▲
Average	7.07	0.75	27.63	198.30	0.68	6.26	3202.13	69
STDEV	0.23	0.02	0.82	187.40	0.098	0.78	4850.36	182
N	8	8	8	8	4	4	7	7

▲ Not detected

Table 10: Physical –Chemical parameters station VI: Jaguas East

Date	pH	Sp Cond. (mS/cm)	Temp. (ºC)	DO Saturation (%)	NH$_4^+$ (mg/L– N)	NO$_3^-$ (mg/L – N)	Cl$^-$ (mg/L)	Turb. (NTUs)
8-Jun-05	7.10	0.75	27.76	79.90	0.67	6.54	3.28	0
13-Jun-05	7.27	0.74	27.68	500.00	0.56	6.64	11.07	0
28-Jun-05	7.28	0.78	28.43	108.60	0.62	11.34	28.15	0
12-Jul-05	6.74	0.75	27.56	79.40	▲	▲	930.10	0
19-Jul-05	7.00	0.75	28.23	85.10	▲	▲	11555.00	187
5-Aug-05	6.90	0.74	27.84	74.10	▲	▲	▲	▲
12-Aug-05	6.87	0.77	28.25	87.80	▲	▲	▲	▲
Average	7.02	0.75	27.96	145.00	0.68	8.17	2505.52	37
STDEV	0.21	0.01	0.34	156.90	0.08	2.74	5074.35	84
N	7	7	7	7	3	3	5	5

▲ Not detected

Table 11: Physical –Chemical parameters station VII: Irrigation Channel

Date	pH	Sp Cond. (mS/cm)	Temp. (ºC)	DO Saturation (%)	NH$_4^+$ (mg/L – N)	NO$_3^-$ (mg/L – N)	Cl$^-$ (mg/L)	Turb. (NTUs)
31-May-05	8.28	3.69	32.02	118.40	3.87	1.32	70.97	>1000
21-Jun-05	7.98	3.63	30.17	500.00	3.78	1.21	113.09	>1000
Average	8.13	3.66	31.10	309.20	3.82	1.27	92.03	>1000
STDEV	0.21	0.04	1.31	269.80	0.07	0.08	29.78	0
N	2	2	2	2	2	2	2	2

▲ Not detected

Table 12: Physical –Chemical parameters station VIII: Saliche

Date	pH	Sp Cond. (mS/cm)	Temp. (ºC)	DO Saturation (%)	NH$_4^+$ (mg/L – N)	NO$_3^-$ (mg/L – N)	Cl$^-$ (mg/L)	Turb. (NTUs)
31-May-05	7.69	0.83	28.46	500.00	0.75	6.19	22.30	0
21-Jun-05	7.66	0.81	27.96	111.20	0.81	4.67	21.47	0
28-Jun-05	7.53	0.91	27.87	103.70	0.77	11.63	130.35	0
12-Jul-05	7.15	0.89	27.65	69.50	▲	▲	1521.50	▲
19-Jul-05	6.96	0.90	28.04	108.30	▲	▲	10649.50	0
5-Aug-05	7.01	0.92	28.14	140.70	▲	▲	▲	▲
12-Aug-035	6.93	0.83	28.18	97.20	▲	▲	▲	11
Average	7.27	0.87	28.04	161.50	0.77	7.50	2469.02	2
STDEV	0.34	0.05	0.26	150.70	0.028	3.66	4616.94	5
N	7	7	7	7	3	3	5	5

▲ Not detected

ii. Physical-chemical parameters results (Horiba instrument)

Table 13: Physical –Chemical parameters station I: Pirata

Station I: Pirata						
Date	Temperature (°C)	pH	Conductivity (mS/cm)	Oxygen Dissolved (mg/L)	Salinity (%)	Turbidity (NTU)
21-Jun-05	30.00	7.30	8.00	1.70	0.40	78.00
28-Jun-05	30.00	7.70	8.50	1.25	0.40	28.00
12-Jul-05	30.50	7.53	7.79	0.93	0.42	21.50
19-Jul-05	30.35	7.20	10.45	0.28	0.59	▲
12-Aug-05	37.15	7.64	7.62	0.43	0.41	50.00
Average	31.60	7.47	8.47	0.92	0.44	42.50
STDEV	3.11	0.22	1.16	0.59	0.08	25.52
n	5	5	5	5	5	5

▲ Not detected

Table 14: Physical –Chemical parameters station II: Jacqueline

Station II: Jaqueline						
Date	Temperature (°C)	pH	Conductivity (mS/cm)	Oxygen Dissolved (mg/L)	Salinity (%)	Turbidity (NTU)
21-Jun-05	29.00	7.60	2.30	3.20	0.10	110.00
28-Jun-05	39.00	7.70	3.70	3.65	0.02	88.00
12-Jul-05	29.05	7.58	5.99	2.08	0.32	94.00
19-Jul-05	29.55	7.40	3.73	2.45	0.19	▲
12-Aug-05	29.85	7.62	3.65	3.30	0.18	95.50
Average	31.29	7.58	3.87	2.93	0.16	97.33
STDEV	4.32	0.11	1.33	0.65	0.11	9.33
n	5	5	5	5	5	5

▲ Not detected

Table 15: Physical –Chemical parameters station III: Salitral

Station III: Salitral						
Date	**Temperature (°C)**	**pH**	**Conductivity (mS/cm)**	**Oxygen Dissolved (mg/L)**	**Salinity (%)**	**Turbidity (NTU)**
21-Jun-05	29.00	7.00	26.00	3.80	1.60	26.00
28-Jun-05	28.00	7.30	24.00	3.05	1.40	18.00
12-Jul-05	29.05	7.30	21.30	1.47	1.29	14.00
19-Jul-05	30.00	7.30	28.00	3.85	1.70	▲
12-Aug-05	29.45	7.23	18.65	1.81	1.11	103.00
Average	29.10	7.23	23.59	2.80	1.42	19.33
STDEV	0.73	0.13	3.71	1.11	0.24	42.13
n	5	5	5	5	5	5

▲ Not detected

Table 16: Physical –Chemical parameters station IV: Lagoon

Station IV: Lagoon						
Date	**Temperature (°C)**	**pH**	**Conductivity (mS/cm)**	**Oxygen Dissolved (mg/L)**	**Salinity (%)**	**Turbidity (NTU)**
21-Jun-05	30.00	7.90	31.00	4.40	2.00	0.00
28-Jun-05	29.00	8.15	32.00	4.35	2.00	8.00
12-Jul-05	29.35	8.29	30.55	5.11	1.91	2.00
19-Jul-05	29.95	7.67	37.20	5.12	2.37	▲
5-Aug-05	28.00	7.51	0.97	4.73	1.81	1.00
12-Aug-05	29.35	7.68	30.30	3.78	1.89	1.00
Average	29.28	7.86	27.00	4.58	2.00	2.75
STDEV	0.73	0.30	13.01	0.51	0.20	3.21
n	5	5	5	5	5	5

▲ Not detected

Table 17: Physical –Chemical parameters station V: Jaguas West

Station V: Jaguas West						
Date	Temperature (°C)	pH	Conductivity (mS/cm)	Oxygen Dissolved (mg/L)	Salinity (%)	Turbidity (NTU)
21-Jun-05	27.00	7.20	0.76	5.10	0.00	0.00
28-Jun-05	28.00	7.40	0.84	4.50	0.00	18.00
12-Jul-05	27.55	7.50	0.77	4.63	0.03	10.50
19-Jul-05	27.80	7.17	1.08	4.81	0.04	10.00
5-Aug-05	27.55	6.97	0.75	4.53	0.03	12.00
12-Aug-05	28.10	7.22	0.75	6.18	0.03	12.50
Average	27.67	7.24	0.82	4.96	0.02	10.50
STDEV	0.40	0.18	0.13	0.64	0.02	5.88
n	5	5	5	5	5	5

▲ Not detected

Table 18: Physical –Chemical parameters station V: Jaguas East

Station VI: Jaguas East						
Date	Temperature (°C)	pH	Conductivity (mS/cm)	Oxygen Dissolved (mg/L)	Salinity (%)	Turbidity (NTU)
28-Jun-05	28.00	7.30	0.90	4.15	0.00	13.00
12-Jul-05	27.55	7.54	0.76	4.79	0.30	0.00
19-Jul-05	28.00	7.20	1.10	4.90	0.00	▲
5-Aug-05	27.60	7.09	0.74	4.49	0.03	2.00
12-Aug-05	27.95	7.18	0.77	4.89	0.03	1.00
Average	27.82	7.26	0.85	4.64	0.07	4.00
STDEV	0.23	0.17	0.15	0.32	0.13	6.06
n	5	5	5	5	5	5

▲ Not detected

Table 19: Physical –Chemical parameters station VII: Irrigation channel

Station VII: Irrigation channel						
Date	Temperature (°C)	pH	Conductivity (mS/cm)	Disolved Oxygen (mg/L)	Salinity (%)	Turbidity NTUs
21-jun-05	30	8.10	3.90	5.20	0.20	30.00

▲ Not detected

Table 20: Physical –Chemical parameters station VIII: Saliche

Station VIII: Saliche						
Date	Temperature (°C)	pH	Conductivity (mS/cm)	Oxygen Dissolved (mg/L)	Salinity (%)	Turbidity (NTU)
21-Jun-05	27.00	7.60	1.00	5.30	0.00	0.00
28-Jun-05	27.50	7.60	1.10	4.95	0.00	8.00
12-Jul-05	27.75	7.85	1.03	4.68	0.40	0.00
19-Jul-05	28.00	7.20	1.30	3.45	0.10	▲
5-Aug-05	28.00	7.16	0.97	3.54	0.04	1.00
12-Aug-05	28.00	7.24	0.91	3.00	0.04	0.00
Average	27.71	7.44	1.05	4.15	0.10	1.80
STDEV	0.40	0.28	0.14	0.94	0.15	3.49
n	6	6	6	6	6	6

▲ Not detected

b. Microbiological Results

Table 21: Average concentration of microorganisms in sampling sites

Sampling Site	Thermotolerant Coliforms (CFU/100ml)	Enterococcus (CFU/100ml)
Piezometers (n=25)	28.89	432.41
Lagoon (n=11)	233.32	66.95
Irrigation Wells (n=25)	45.19	136.41

The highest level of enterococcus bacterial was found in the groundwater of the community of Las Mareas (Fig. 11). This could be because of the high concentration of septic tanks that exist in the community. Not as expected the concentrations of thermotolerant coliform were low in the community. The thermotolerant coliforms are weak competitive and are usually eliminated by competition and predation (Atlas & Bartha, 2002). Factors such as pH, temperature, solar irradiation, predation, osmotic stress, nutrient deficiencies, particulate levels, turbidity, oxygen concentrations and microbial community composition affect bacteria inactivation (Noble, et al., 2004). The presences of natural substances could be inhibiting their resistance in the groundwater. These agents could be phenols, ammonium compounds, ethylene and sulfur compounds (Prescott et al. 2002). The reason of finding higher concentrations of enterococcus is that they are more resistant to stress conditions than thermotolerant coliforms (Payment et al. 2003). The densities of enterococcus and fecal coliforms were high in the lagoon. These densities exceed the water quality standard of marine surfacel water for primary contact of "Junta de Calidad Ambiental" (35 CFU/100ml for enterococcus and 200 CFU/100ml for thermotolerant coliform). The lagoon is being impacted by the groundwater contamination, surface run-off, human and animals activities and the septic tanks of the community. These

densities of microorganisms potentially represent a risk to human health by direct contact and by the consumption products like oysters. The concentration of enterococcus in the irrigation wells were also high. Farmers in the area confirmed the utilization of chicken excrement for the fertilization of their crops, which could be impacting the water quality of the groundwater.

i. Groundwater and lagoon microbiological results

Table 22: Average density of thermotolerant coliforms and enterococcus at Pirata station
Pirata

Thermotolerant Coliforms	Enterococcus	Date
0	16.33	13-Apr-05
0	8	31-May-05
0	10.50	8-Jun-05
0	10.50	13-Jun-05
5	2	21-Jun-05
0	4	28-Jun-05
0	0	12-Jul-05
0	0	19-Jul-05
0	0	12-Aug-05
0	0	24-Aug-05
0.50	**5.13**	**Average**

Table 23: Average density of thermotolerant coliforms and enterococcus at Jacqueline station
Jacqueline

Thermotolerant Coliforms	Enterococcus	Date
0	7.66	13-Apr-05
0	451	31-May-05
0	822	8-Jun-05
0	826	13-Jun-05
0	80	21-Jun-05
30	438	28-Jun-05
4	726.50	12-Jul-05
8.5	60	19-Jul-05
433.33	230	12-Aug-05
52.87	**404.57**	**Average**

Table 24: Average density of thermotolerant coliforms and enterococcus of Lagoon station

Lagoon

Thermotolerant Coliforms	Enterococcus	Date
0	0	13-Apr-05
45	33	31-May-05
1643	347	8-Jun-05
17	6.50	13-Jun-05
5	36	21-Jun-05
7.50	47	28-Jun-05
431	19	12-Jul-05
2	10	19-Jul-05
8	46	5-Aug-05
394.66	170	12-Aug-05
13.33	22	24-Aug-05
233.31	**66.95**	**Average**

Table 25: Average density of thermotolerant coliforms and enterococcus of Salitral station

Salitral

Thermotolerant Coliforms	Enterococcus	Date
0	0	13-Apr-05
10	3758	31-May-05
0	2425	8-Jun-05
16.50	493.50	13-Jun-05
40	1456	21-Jun-05
30	706	28-Jun-05
145	842	12-Jul-05
0	48	19-Jul-05
0	646.66	12-Aug-05
26.83	**1152.79**	**Average**

Table 26: Average density of thermotolerant coliforms and enterococcus of Jaguas East station

Table 27: Average density of thermotolerant coliforms and enterococcus of Jaguas West station

Table 26: Average density of thermotolerant coliforms and enterococcus of Jaguas East station
Jaguas East

Thermotolerant Coliforms	Enterococcus	Date
1	770	8-Jun-05
0	0.50	13-Jun-05
0	0	28-Jun-05
0	1	12-Jul-05
4	9	19-Jul-05
83	12.66	5-Aug-05
0	0	12-Aug-05
0.66	0	24-Aug-05
11.08	**99.14**	**Average**

Table 27: Average density of thermotolerant coliforms and enterococcus of Jaguas West station
Jaguas West

Thermotolerant Coliforms	Enterococcus	Date
89	443	8-Jun-05
172.50	227	13-Jun-05
0	31	21-Jun-05
1.50	384	28-Jun-05
0	395.50	12-Jul-05
107	195	19-Jul-05
16	86.66	5-Aug-05
109	165.66	12-Aug-05
21.33	346	24-Aug-05
57.37	**252.65**	**Average**

Table 28: Average density of thermotolerant coliforms and enterococcus of Saliche station

Saliche

Thermotolerant Coliforms	Enterococcus	Date
5.50	2	31-May-05
3	4	21-Jun-05
184	45	28-Jun-05
13.50	225	12-Jul-05
60	50	19-Jul-05
130	5.33	5-Aug-05
10	6.66	12-Aug-05
118.60	5.33	24-Aug-05
65.58	**42.92**	**Average**

ii. Oyster microbiological results

Sampling date: July 21, 2005

Table 29: Most Probable Number (MPN) confirmed test density estimates of fecal coliform bacteria in oyster and water samples at Jobos Bay Reserve.

Sample Oyster	number of positive tubes in each dilution				MPN 5 tube combination of positives	MPN result in table	grams in 2 mL sample	MPN per gram
	Undiluted	1:10	1:100	1:1000				
Control site, homogenate 1	1	0	0	0	1-0-0	2	0.144	0.288
Control site, homogenate 2	0	0	0	0	0-0-0	<1.8	0.226	<0.407
Las Mareas, homogenate 1	5	2	0	0	5-2-0	49	0.127	6.223
Las Mareas, homogenate 2	5	1	0	0	5-1-0	33	0.119	3.927

Water	Not diluted	1:10	1:100	1:1000	MPN 5 tube combination of positives	MPN result in table	mL in Sample	MPN per mL
Control site, water							2	<0.9
Las Mareas, water	5	5	1	0	5-1-0	33	2	16.5
positive control	5	5	5	5	5-5-5	1600	0.144	230.4

Table 30: Density of Enterococci bacteria in oyster and water samples at Jobos Bay Reserve, determined by membrane filtration.

Sample* Oyster	mL filtered	grams filtered	CFU	CFU per gram
Control site, homogenate 1 A	0.1	0.0072	0	0
Control site, homogenate 1 B	0.01	0.00072	0	0
Control site, homogenate 2 A	0.1	0.011	0	0
Control site, homogenate 2 B	0.01	0.0011	0	0
Las Mareas, homogenate 1 A	0.1	0.0064	1	156
Las Mareas, homogenate 1 B	0.1	0.0064	2	313
Las Mareas, homogenate 1 C	0.01	0.00064	0	0
Las Mareas, homogenate 2 A	0.1	0.006	2	333
Las Mareas, homogenate 2 B	0.01	0.0006	0	0

Sample* Water	mL filtered	grams filtered	CFU	CFU per mL
Control site, A	10	N/A	0	0
Control site, B	1	N/A	0	0
Control site, C	0.1	N/A	0	0
Las Mareas, A	10	N/A	102	10.2
Las Mareas, B	1	N/A	23	23
Las Mareas, C	0.1	N/A	2	20

positive controls	mL filtered	grams filtered	CFU
A	0.1	0.0072	TMTC
B	0.1	0.0072	TMTC
C	0.1	0.0072	TMTC

TMTC, too much to count

A, B and C indicate triplicate samples in water or oyster homogenate.

Sampling date: August 23, 2005

Table 31: Most Probable Number (MPN) confirmed test density estimates of fecal coliform bacteria in oyster and water samples at JBNERR.

Sample Oyster	number of positive tubes in each dilution				MPN 5 tube combination of positives	MPN result in table	grams in 2 mL sample	MPN per gram
	Not diluted	1:10	1:100	1:1000				
Control site, homogenate 1	0	0	0	0	0-0-0	<1.18	0.156	< 0.184
Control site, homogenate 2	0	0	0	0	0-0-0	<1.18	0.132	< 0.156
Las Mareas, homogenate 1	5	0	0	0	5-0-0	23	0.112	2.576
Las Mareas, homogenate 2	0	0	0	0	0-0-0	<1.18	0.08	< 0.094

Water	Not diluted	1:10	1:100	1:1000	MPN 5 tube Combination of positives	MPN result in table	mL in sample	MPN per mL
Control site, water	0	0	0	0	0-0-0	<1.18	2	< 2.36
Las Mareas, water	2	2	0	0	2-2-0	9.3	2	18.6
positive control	5	ND**	ND	ND	ND	ND	ND	ND

**; ND, not determined

Table 32: Density of Enterococci bacteria in oyster and water samples at JBNERR, determined by membrane filtration.

Sample* Oyster	mL filtered	grams filtered	CFU	CFU per gram
Control site, homogenate 1 A	0.1	0.0078	0	0
Control site, homogenate 1 B	0.1	0.0078	0	0
Control site, homogenate 1 C	0.1	0.0078	0	0
Control site, homogenate 2 A	0.1	0.0066	0	0
Control site, homogenate 2 B	0.1	0.0066	0	0
Control site, homogenate 2 C	0.1	0.0066	0	0
Las Mareas, homogenate 1 A	0.1	0.0056	0	0
Las Mareas, homogenate 1 B	0.1	0.0056	0	0
Las Mareas, homogenate 1 C	0.1	0.0056	0	0
Las Mareas, homogenate 2 A	0.1	0.0040	19	4750
Las Mareas, homogenate 2 B	0.1	0.0040	41	10250
Las Mareas, homogenate 2 C	0.1	0.0040	6	1500

	mL filtered	grams filtered	CFU	CFU per mL
Water				
Control site, A	10	N/A	0	0
Control site, B	1	N/A	0	0
Control site, C	0.1	N/A	0	0
Las Mareas, A	10	N/A	0	0
Las Mareas, B	1	N/A	0	0
Las Mareas, C	0.1	N/A	0	0

positive controls	mL filtered	grams filtered	CFU
A	0.1	0.0072	TMTC
B	0.1	0.0072	TMTC
C	0.1	0.0072	TMTC

TMTC, too much to count

A, B and C indicate triplicate samples in water or oyster homogenate.

c. Organic Chemical Results

There was a higher frecuency of detection of organic compounds in the piezometers than in the irrigation wells. The community of Las Mareas and the Mar Negro lagoon belong to the estuarine zone of Jobos Bay. The soil of this zone has high organic material concentrations and this facilitates the accumulation of a high quantity of organic and inorganic compounds in groundwater from natural flow of anthropogenic sources. The anthropogenic organic compounds were detected with high frequency in the piezometer samples the community of Las Mareas and the Mar Negro lagoon in comparison with the irrigation wells (Table 33).

Table 33: Resume of Organic Compounds found in groundwater simples in the community of Las Mareas and the near farms.

Organic Chemical Compounds	Frecuency(%)*	
	Irrigation Wells	Community of Las Mareas
Phenol	0	14
2-phenoxyethanol	0	7
Benzothiazole	7	40
m-tert-butylphenol	0	14
1(3H)-isobenzofuranone	0	27
Chloroxylenol	0	20
2,4-bis(1,1-dimethylethylphenol)	0	14
2-(1-phenylethyl)-phenol	0	14
1,3,5-triazine	0	7
2-(1,1-dimethylethyl)-phenol	14	40
2,4,6-tris(1,1-dimethylethyl)-phenol	14	34
2-propyldecan-1-ol	7	7
1,4-benzenediol	0	14
1-Cyclohexene	0	7
2,2-methylenebis(6-(1,1-dimethylethyl)-phenol	40	60
2,4-bis(1-phenylethyl)-phenol	0	14
2,4-bis(1-methyl-1-phenylethyl)-phenol	0	7
2,4-bis(dimethylbenzyl)-6-t-butylphenol	0	7

*
The frequency of organic compounds was calculated with n =15

d. Microbial Source Tracking PCR-Based Methodology results

The amount of DNA found for each sample was considerable (Table 34). To account for the presence or absence of fecal microorganisms in the samples we performed the Polymerase Chain Reaction (PCR). This essay intends to amplify DNA from an organism, in this case Bacteroides species to detect if they were present in the samples taken. The DNA can be amplified using primers, which are specific DNA sequences that pair to a sample of DNA extracted from the sample, and thus, amplifies it, if both sequences (the primer and the extracted DNA) compliment each other.

It is important to note that no signal was found for the primers used when the Polymerase Chain Reaction (PCR) was performed. This implies that *Bacteroides* species may not be present in the samples. This could have resulted because there was some kind of inhibition for the Polymerase Chain Reaction to perform. An example of this could be nutrients or chemicals available in the samples that might have reacted with the reagents used for PCR, thus inhibiting the amplification. Other possible explanation for this could be the fact that *Bacteroides* species do not survive much once they encounter aerobic conditions in the environment. Another cause would be that other microorganisms are competing against these species.

Table 34: DNA obtained from Jobos water samples after extractions

Sample May 31, 2005	Amount of DNA obtained (ng/µL)
Salitral	25.44
Pirata	29.47
Lagoon	25.73
Jacqueline	23.75
Saliche AT	27.64
Saliche DREN	23.76

As a recommendation, there should be more samplings to determine if these primers show specificity to assess fecal contamination.

When the shellfish were analyzed in June 1 2005, with these primers, they showed no signals either. This could be due to the reasons stated above, or the methods of diluting the samples. However, these primers have proven to give positive results in surface waters ([b]Bernhard & Field, 2000).

2. Graphics

pH average per sampling station (Hydrolab)
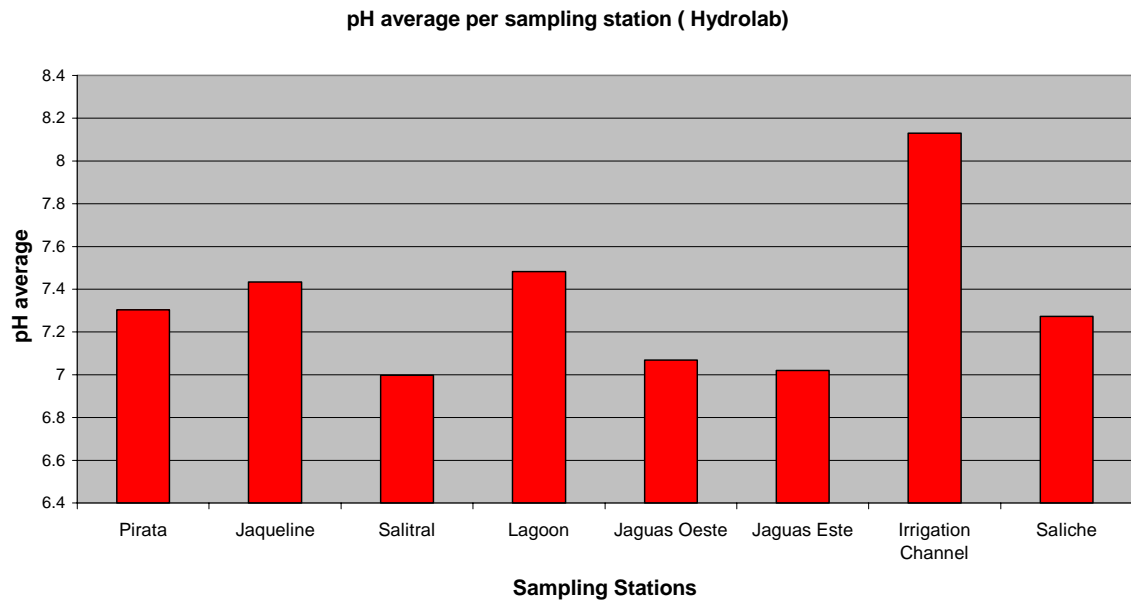
Figure 3: pH average per sampling station

pH average per station (Horiba)
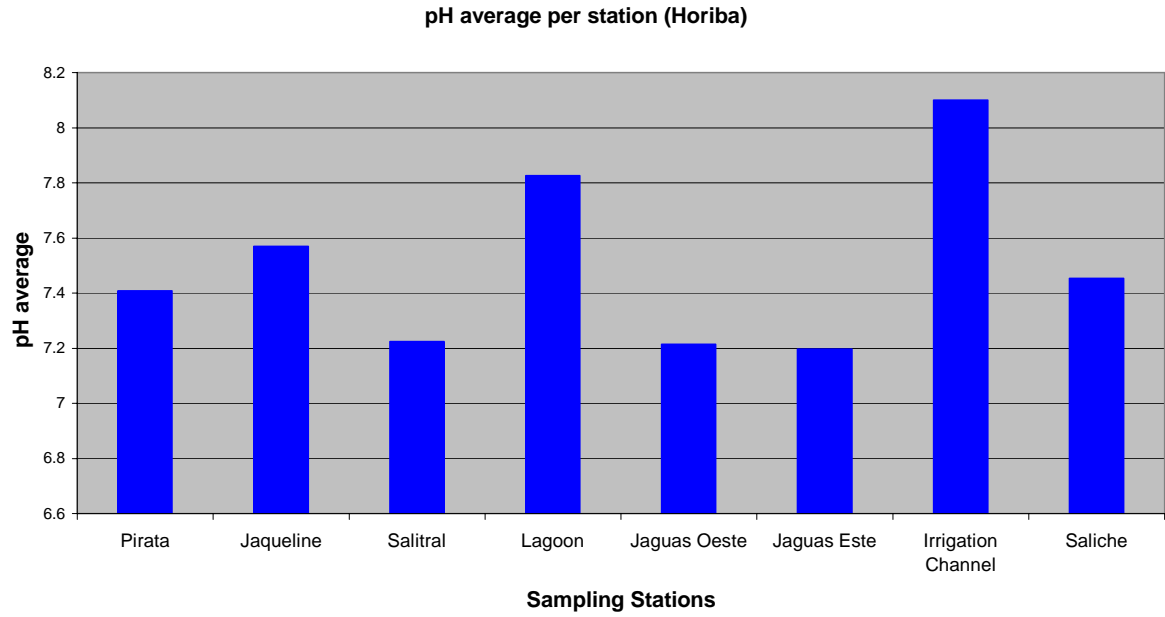
Figure 4: pH average per station

Conductivity average per station (Hydrolab)
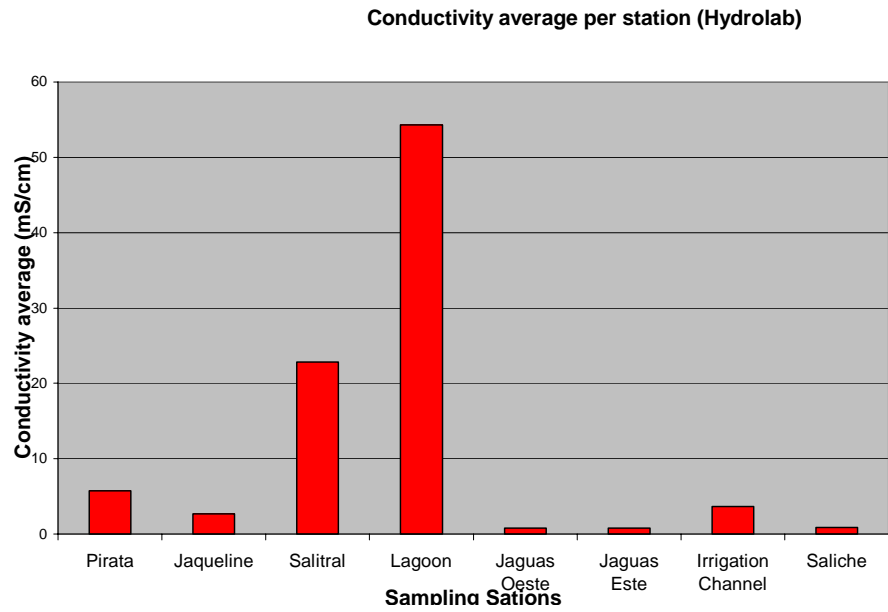
Figure 5: Conductivity average per station (Hydrolab)

Average Conductivity per station (Horiba)
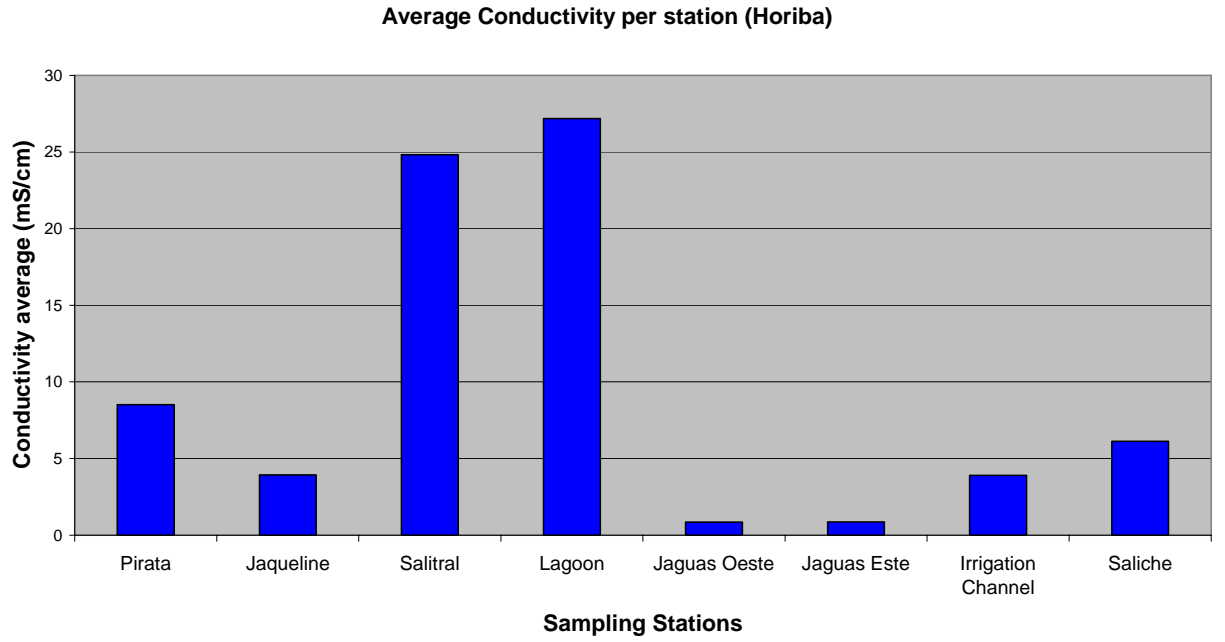
Figure 6: Average Conductivity per station (Horiba)

Average of dissolved oxigen (Hydrolab)
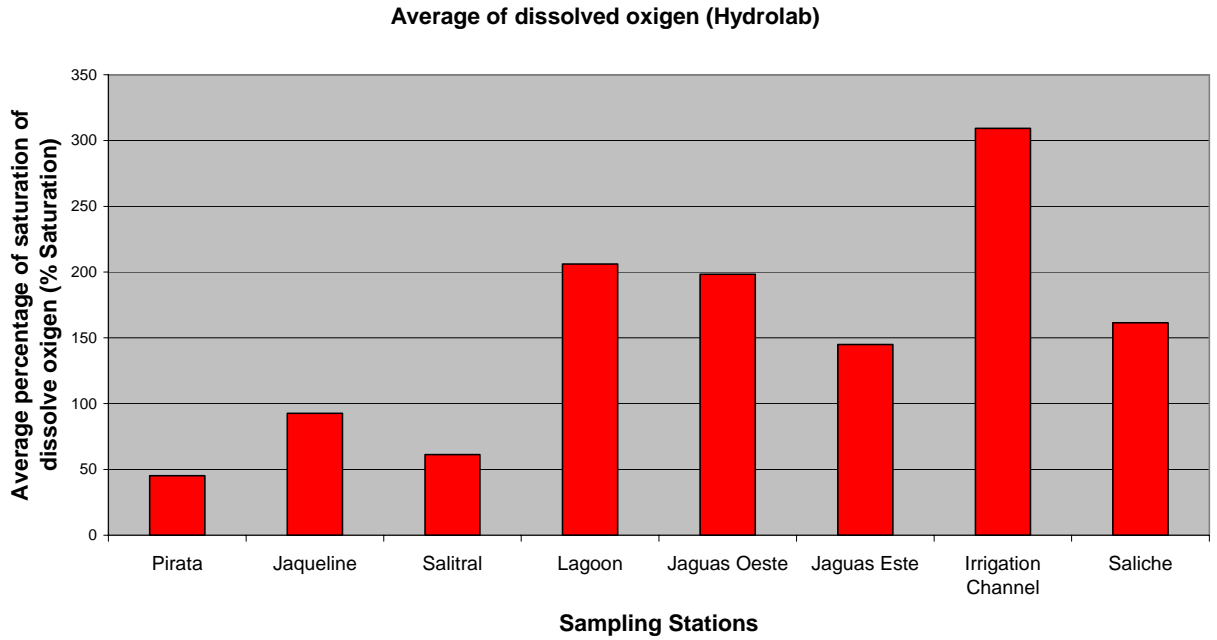
Figure 7: Average of dissolved oxygen (Hydrolab)

Average of dissolve oxigen (Horiba)
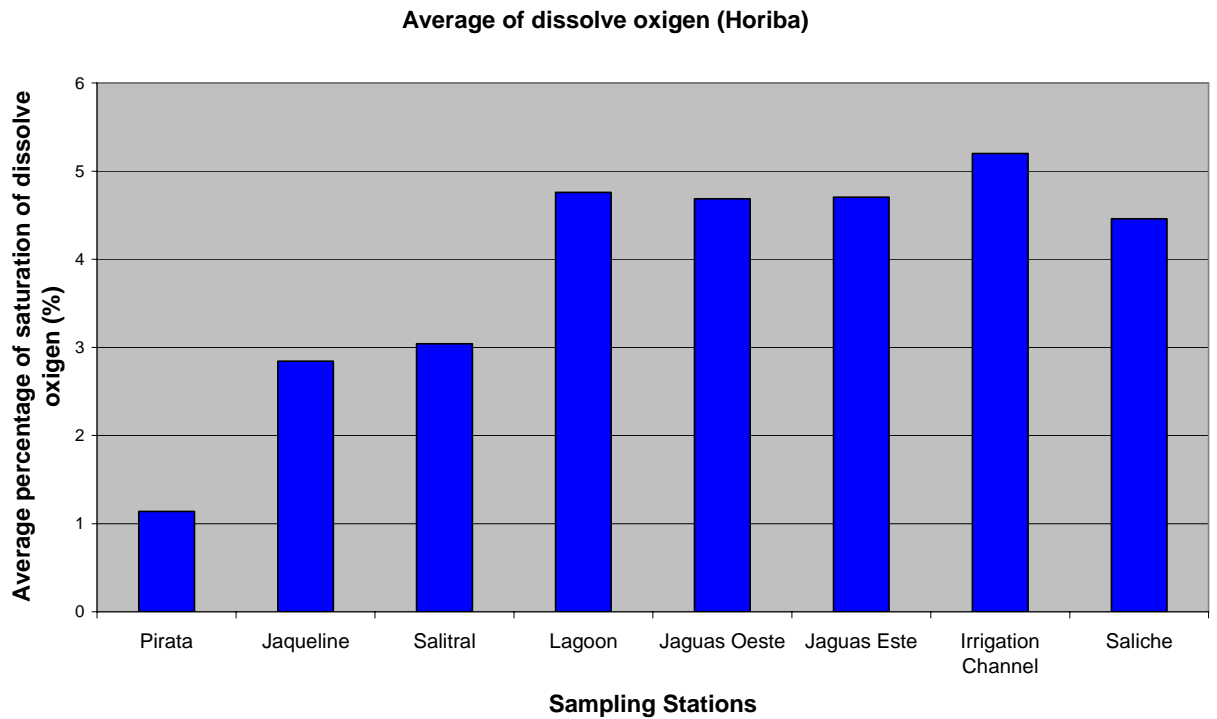
Figure 8: Average of dissolve oxygen (Horiba)

NH₄ average per station (Hydrolab)
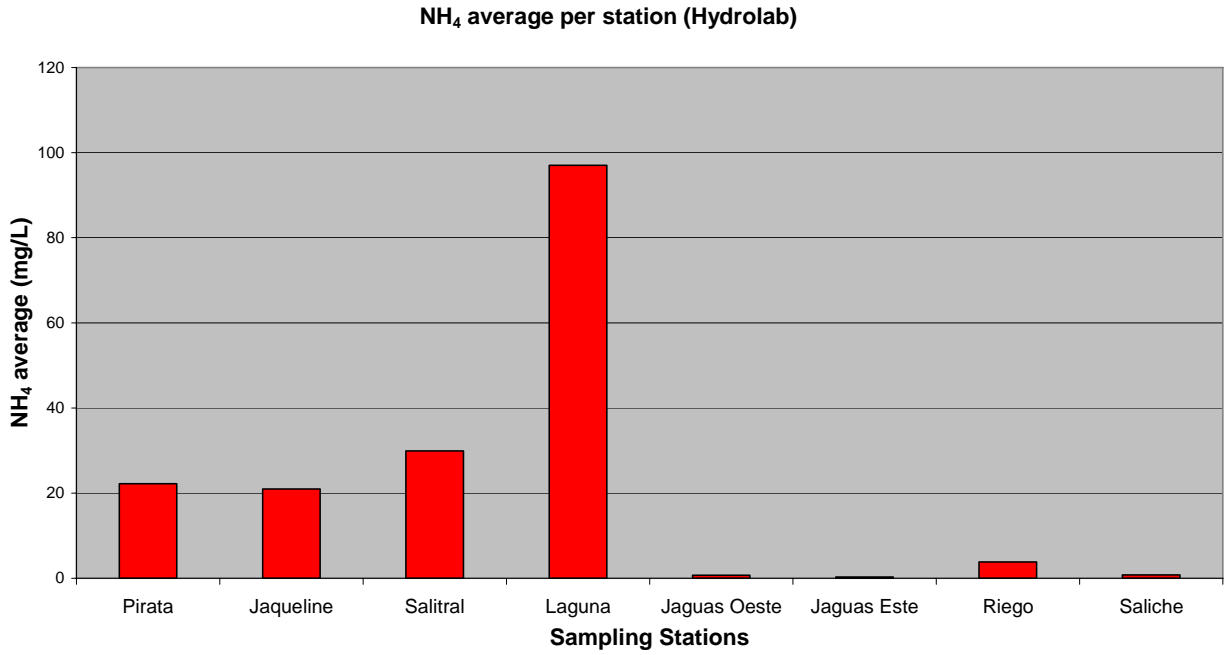
Figure 9: NH₄⁺ average per station (Hydrolab)

NO₃ average per station (Hydrolab)
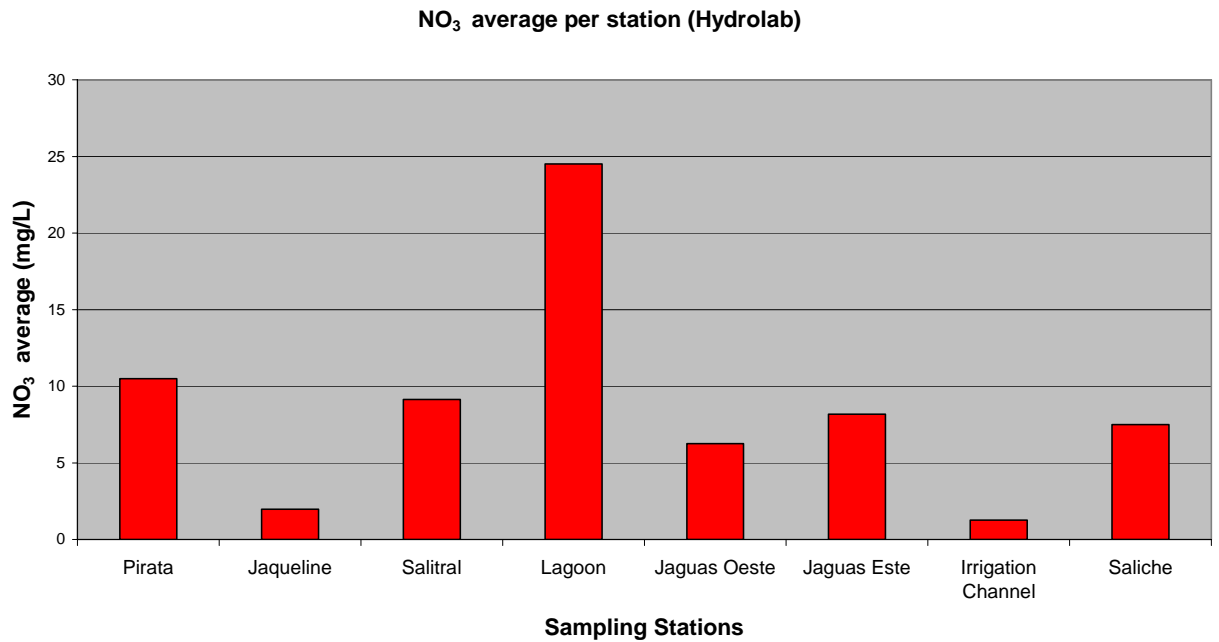
Figure 10: NH₃⁺ average per station (Hydrolab)
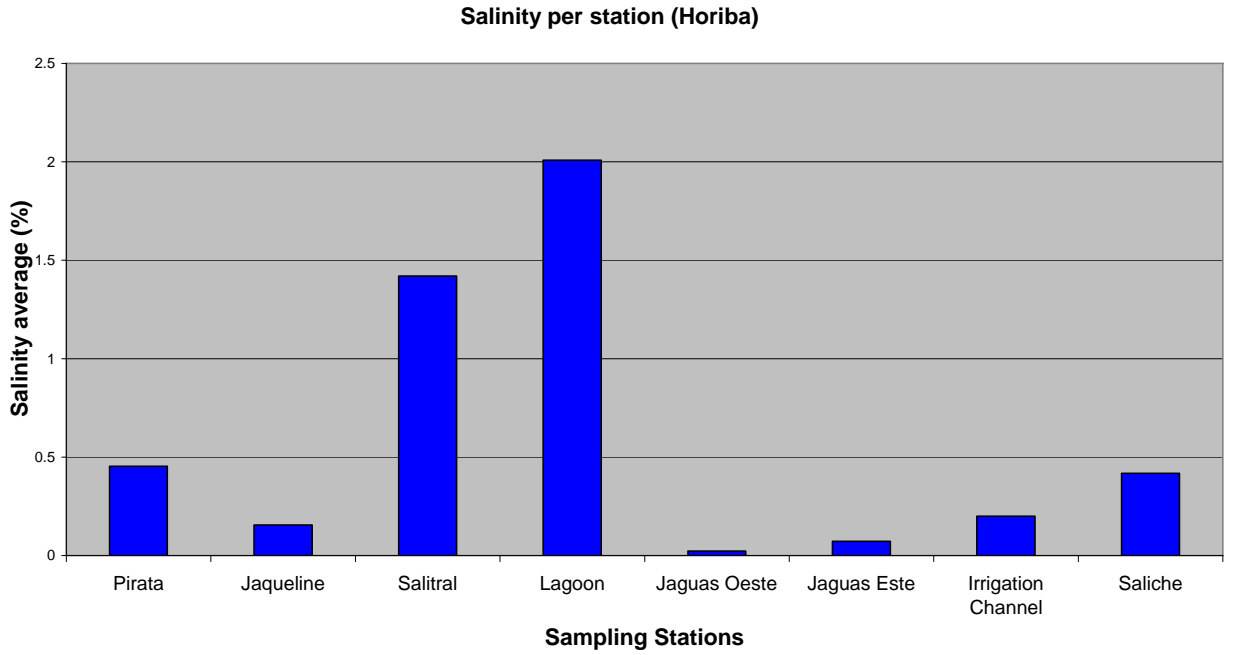
Figure 11: Salinity per station (Horiba)
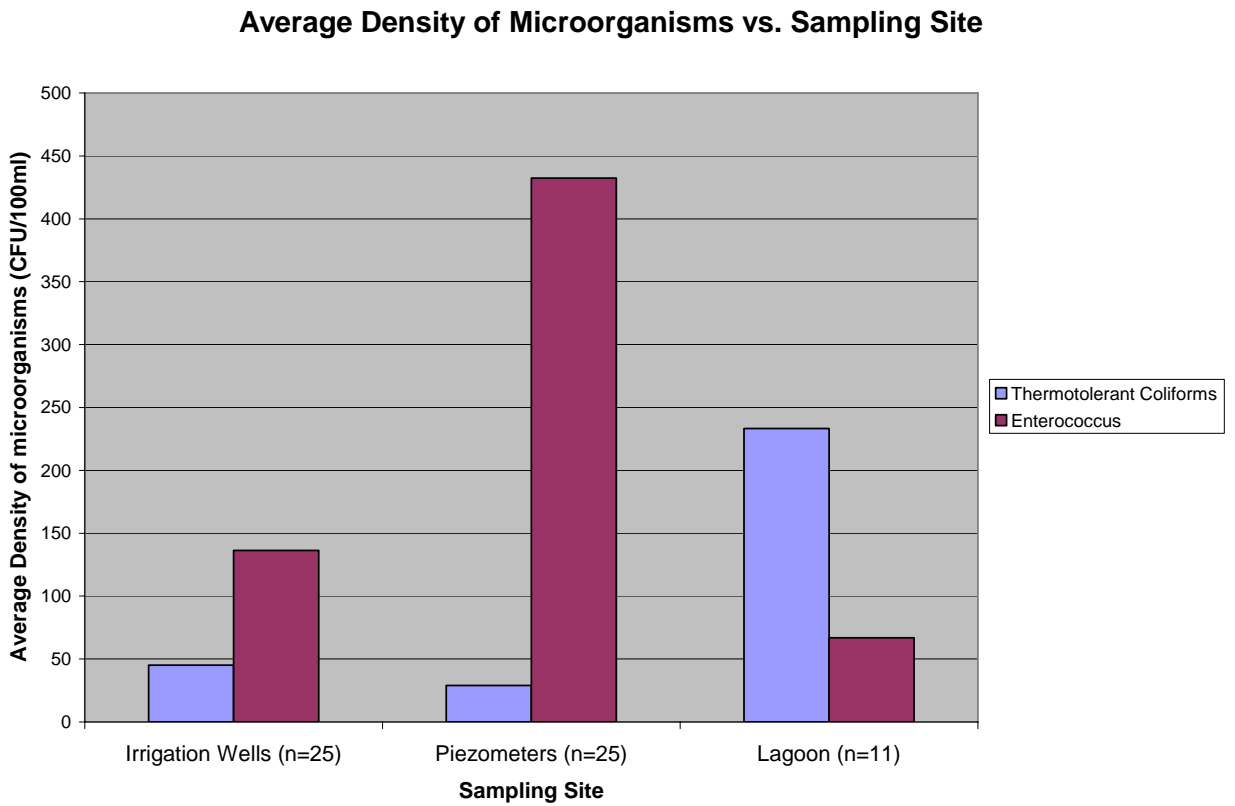
Figure 12: Average density of Microorganisms vs. Sampling Site
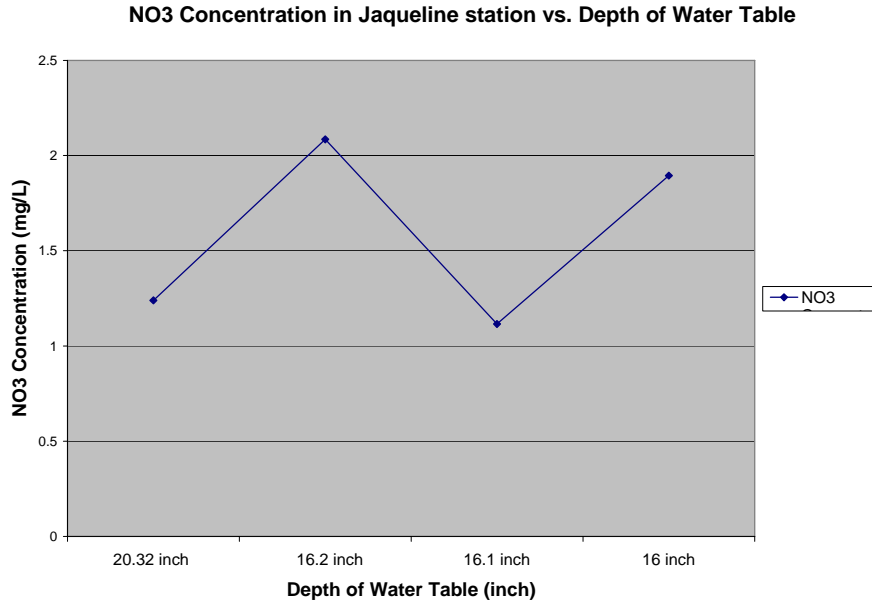
Figure 13: NO$_3^-$ Concentration in Jacqueline station vs. Depth of Water Table
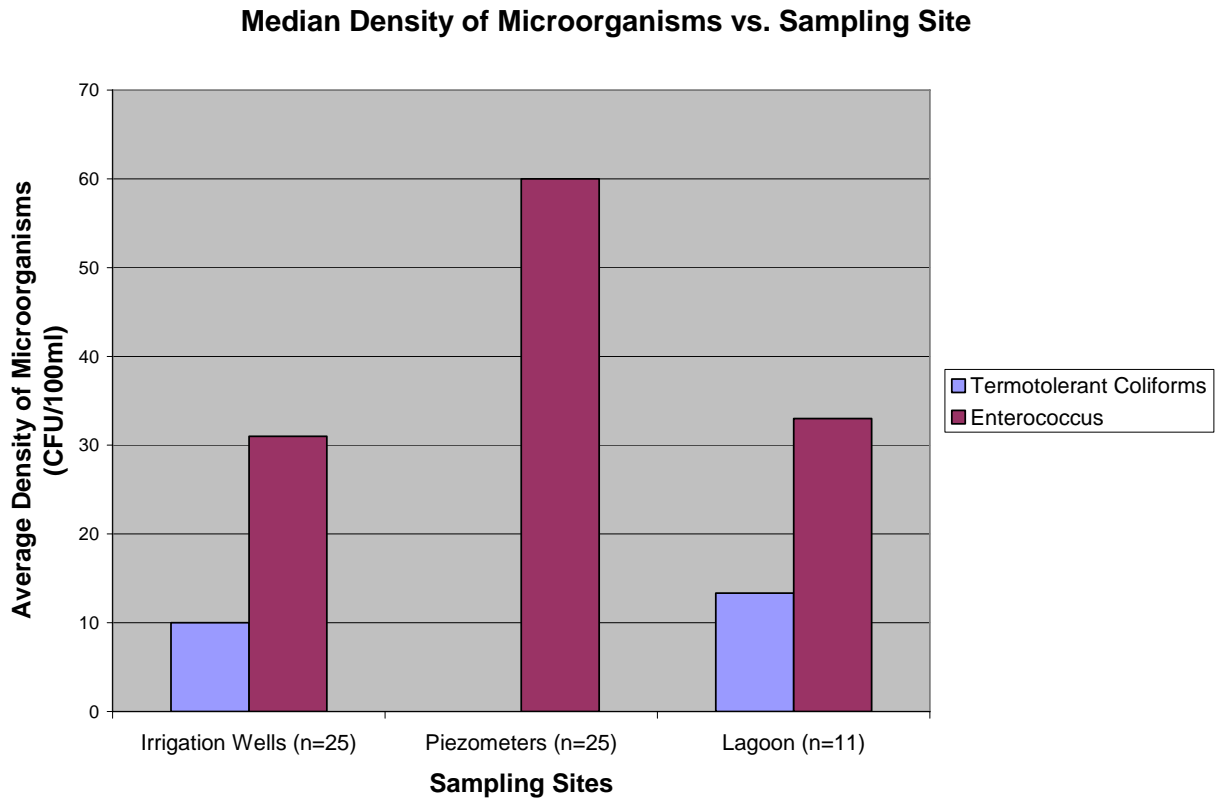
Figure 14: Median Density of Microorganisms vs. Sampling Site

Average Density of Termotolerant Coliforms vs. Sampling Station
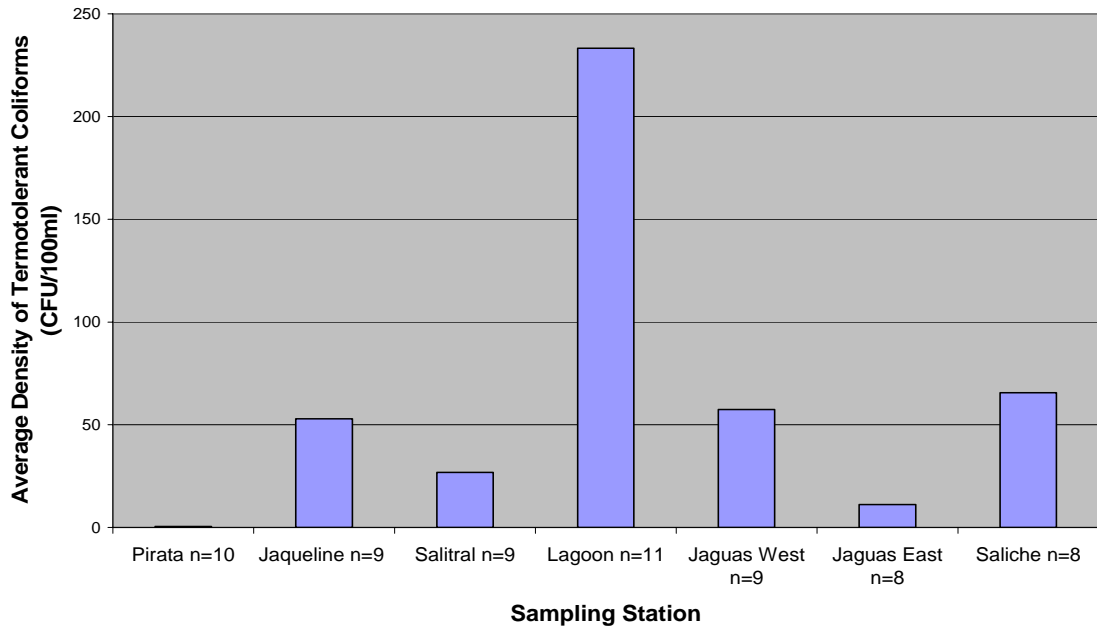
Figure 15: Average Density of Thermotolerant Coliforms vs. Sampling Station

Average Density of Enterococcus vs. Sampling Station
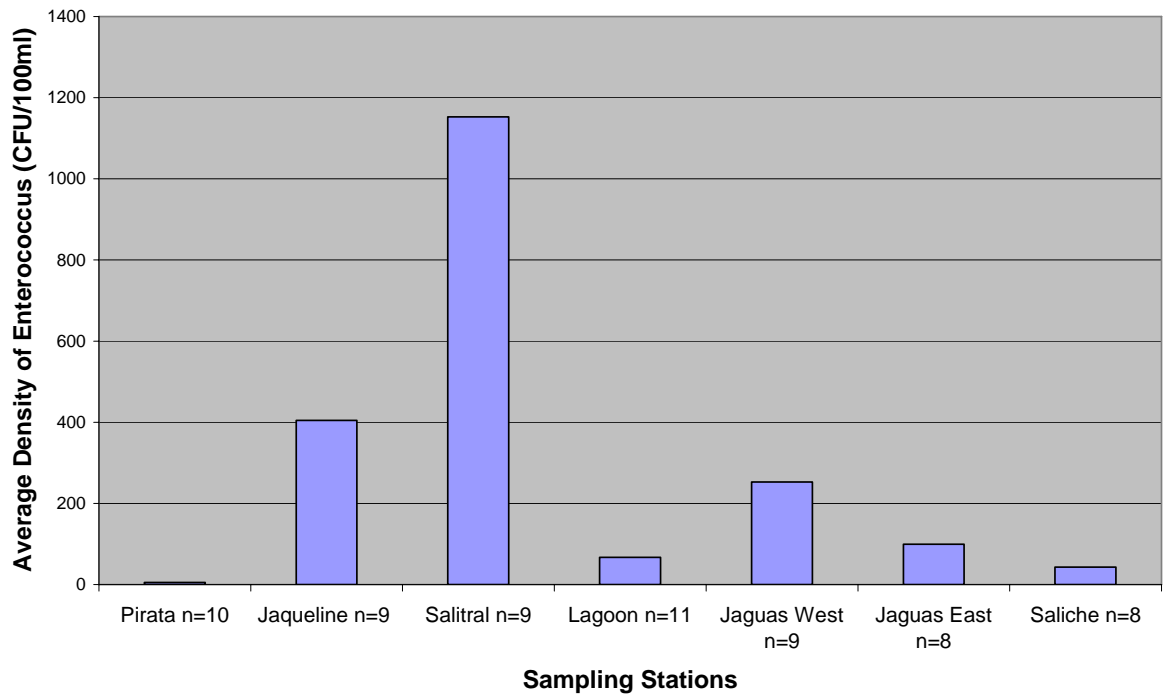
Figure 16: Average density of Enterococcus vs. Sampling Station

Median Density of Termotolerant Coliforms vs. Sampling Station
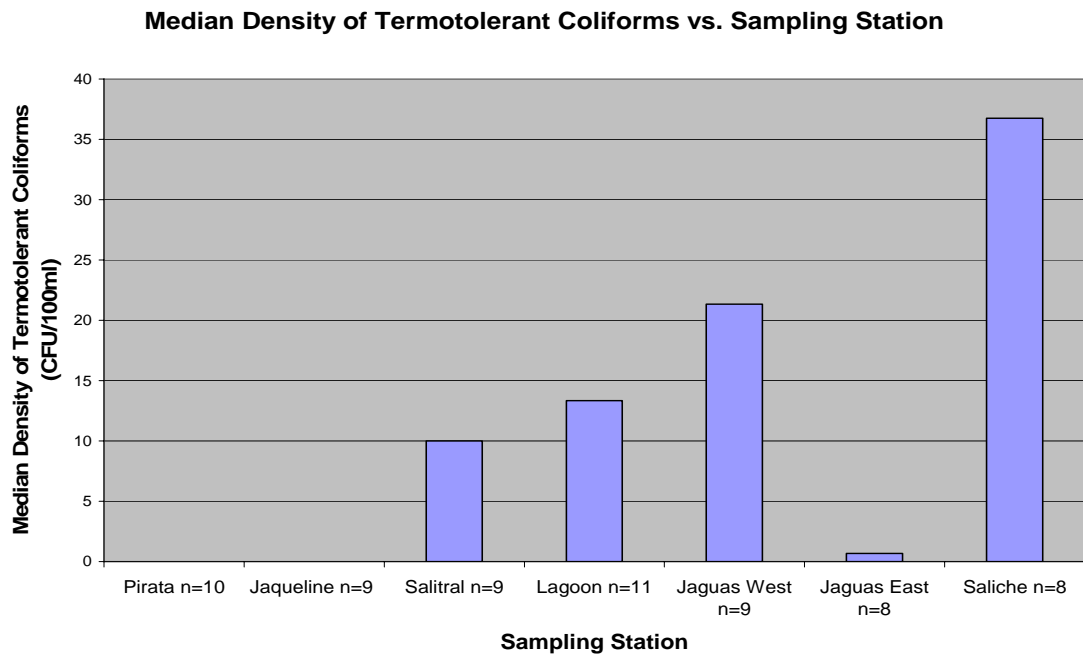
Figure 17: Median Density of Thermotolerant Coliforms vs. Sampling Station

Median Density of Enterococcus vs. Sampling Station
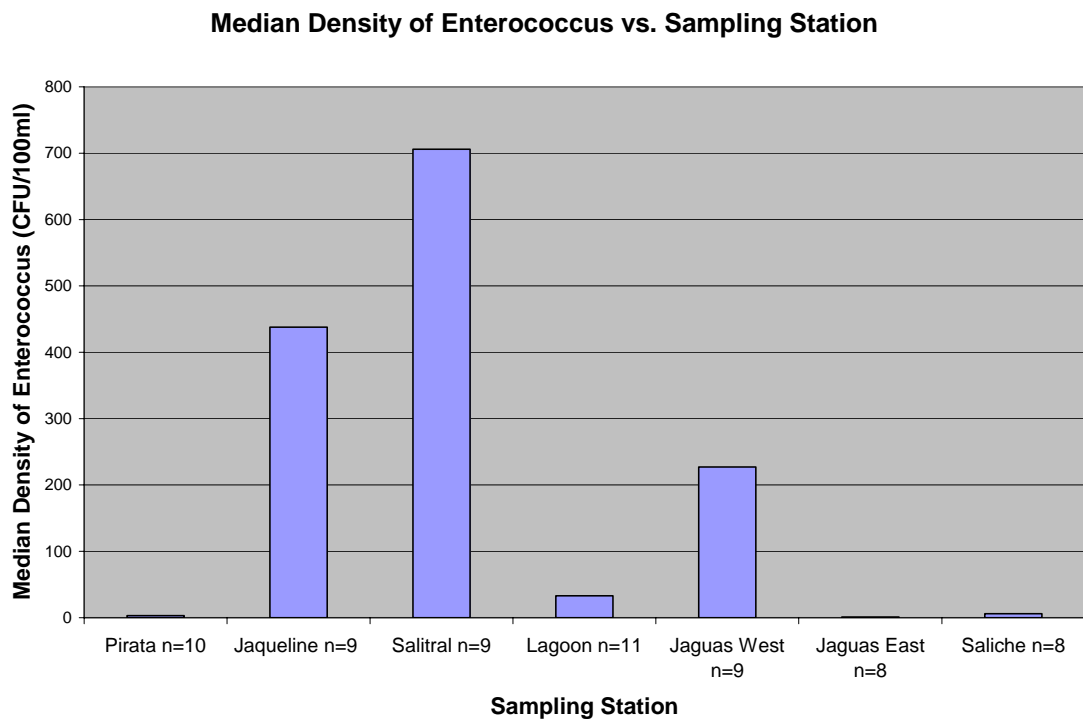
Figure 18: Median Density of Enterococcus vs. Sampling Station

Figure 19: Median Concentration of Inorganic Compounds vs. Sampling Station

F. Conclusions

The results of this study indicate a higher chemical and microbiological contamination of groundwater in the community of Las Mareas compared with the irrigation wells. Significant levels of fecal and organic chemical contamination were found in the lagoon. This situation presents a possible environmental health risk by through primary contact or by consumption of oysters. Fecal indicator organisms were found in oyster tissue from lagoon samples. Some of the possible reasons for this are the poor infrastructure of the zone for the disposition of waste waters and the high water table of the aquifer.

We can not conclude that septic tanks increase nitrate concentration in ground water significantly. Irrigating well showed appreciable concentrations of nitrates (average = 7.31 mg/L -N) upstream of the community. Previous studies have showed nitrate pollution in wells of this region. The Mar Negro lagoon did show high concentration of nitrates, possible due also to surface run-off.

There were significant differences in ammonium concentration between the three study areas. Piezometer concentrations were higher than in irrigation wells upstream. Influence of septic tanks leachate on ammonium concentration in groundwater in Las Mareas community is suspected.

Frequency of detection of anthropogenic organic chemicals was higher in Las Mareas groundwater than in irrigation wells. This point towards an effect of septic tanks leachates on groundwater organic chemical concentration.

Fecal coliforms were not detected in several samples of groundwater at Piezometers stations. It is suspected that inhibition process may be occurring. Existing literature points to several factors that inhibit coliform growth like: interaction between dissolved nutrient, organic

matter, antibiotics, lyses, heavy metals, competition for nutrients with marine bacteria, predation by protozoa, algal toxins, degradation of bacterial cell wall by protozoa, seasonal variations, bactericidal action of seawater, temperature and the physicochemical nature of the marine environment (Faust et al., 1975) that could affect survival of thermotolerant coliforms. Enterococcus proved to be a better indicator of fecal pollution of groundwater in this study because it was detected in all the stations and was resistant to adverse conditions.

Considering all parameters studied, septic tanks leachate from Las Mareas community has a significant impact on ground water quality and a potential impact on environmental health.

G. References

Atlas, R. M. & Bartha, R. (2002). *Ecología microbiana y Microbiología ambiental*. Madrid: PEARSON EDUCACION, S.A.

APHA, American Public Health Association, American Water Works Association, Water Environment Federation, (1995).*Standard Methods for the Examination of Water and Wastewater* 19th Edition. Washington, DC: AMERICAN PUBLIC HEALTH ASSOCIATION.

[a]Bernhard, A. E. and K. G. Field. (2000). *A PCR assay to discriminate human and ruminant feces on the basis of host differences in Bacteriodes-Prevotella genes encoding 16S rRNA*. Applied and Environmental Microbiology. 66: pp 4571-4574

[b]Bernhard, A. E. and K. G. Field. (2000). *Identification of nonpoint sources of fecal pollution in coastal waters by using host-specific 16S ribosomal DNA genetic markers from fecal anaerobes.* Applied and Environmental Microbiology. 66: pp 1587-1594

Dick, L. K., Bernhard, A. E., Brodeur, T.J., Santo Domingo, J. W., Simpson, J. M., Walters, S. P. and K. G. Field**.** (2005). *Host distributions of uncultivated fecal Bacteroidales bacteria reveal genetic markers for fecal source identification.* Applied and Environmental Microbiology. 71: pp 3184-3191

Mersie, W.C., C. Clegg, R.D. Wauchope, J.A. Dumas, R.B. Leidy, M.B. Riley, R.W. Young, J.D. Mattice and T.C. Muller. 2002. Interlaboratory comparison of pesticide recovery from water using solid phase extraction disks and gas chromatography. Journal of AOAC International 85(6):1324-1330.

Noble, R.T., Lee, I.M. & Schiff, K. C. (2004). Inactivation of indicador micro-organisms from various sources of faecal contamination in seawater and freshwater. *Journal of Applied Microbiology.* Num. 96, 464-472.

Payment, P., Waite, M. & Dufour, A. (2003). Assessing Microbial Safety of Drinking Water – Improving Approaches and Methods. *OECD, WHO*. 291 pages

Prescott, L.M., Harley, J.P., Klein, D.A. (2002).Microbiologia. España: McGRAW-HILL-INTERAMER

Robles, P.O., González, C.M., Laboy, E.N. and Capella, J. (2003). Jobos Bay Estuarine Profile, A Nacional Estuarine Research Reserve: NOAA.

Appendix A:

Questionnaire

N°	Pregunta

1   **¿Cuántos dormitorios tiene su vivienda?**

Especifique _____

No sabe 8

No responde 9

2   **¿Sabe usted quién construyó el pozo séptico de su vivienda?**

Yo 1

Otra persona 2

¿Quién? _____

No sabe 8

No responde 9

3   **¿Qué tamaño tiene su pozo séptico?**

Largo _____

Ancho _____

Profundidad _____

No sabe 8

No responde 9

4   **¿Sabe usted cuántos compartimientos tiene su pozo séptico?**

Uno 1

Dos 2

Más de dos 2

No sabe 8

No responde 9

5   **¿Cada cuánto tiempo inspeccionan el pozo?**

Especifique _____

Nunca se ha hecho 1

Cuando los baños no bajan 2

No sabe 8

No responde 9

6   **¿Alguna vez ha vaciado el Pozo?**

Sí 1

No 2

No sabe 8

No responde 9

7   **¿Cada cuanto tiempo lo vacía?**

Especifique _____

Cuando lo noto lleno 1

Nunca 2

No sabe 8

No responde 9

8   ¿Quién lo vacía?

Municipio..................... 1

Yo 2

Otra persona 3

      Especifique _____

No sabe 8

No responde 9

9   ¿Está sellado completamente su pozo séptico contra filtraciones? (Respiradero y tapa o un hueco de inspección no cuentan como abierto)

Sí, está sellado 1

No está sellado 2

No sabe 8

No responde 9

10   ¿Alguna vez se ha desbordado el pozo?

Sí 1

No 2

No sabe 8

No responde 9

11 ¿Cree usted que su pozo séptico está contaminando el agua subterránea o la laguna?

Sí 1

No 2

No sabe 8

No responde 9

12 ¿Cuánto está contaminando?

preguntar a Norat si se deja esta pregunta !!!!!!

Poco 1

Regular 2

Mucho 3

No sabe 8

No reponde 9

13 ¿Tiene animales domésticos en su casa?

Sí 1

No 2

No sabe 8

No responde 9

14 ¿Usa Ud. abono en su patio?

Sí 1

No 2

No sabe 8

No responde 9

15 ¿Cuántas libras usa al año?

Especifique _____

No sabe 8

No responde 9

16 ¿Qué aguas llegan a su pozo séptico?

Inodoro 1

Fregadero 2

Lavadora 3

Duchas 4

Lavamanos 5

Limpieza de pescado 6

Otro 7

Especifique _____

No sabe 8

No responde 9

17   ¿Qué productos de limpieza usted utiliza?

Desinfectantes................. 1

Jabones 2

Otros 3

Especifique _____

No sabe 8

No responde 9

18   ¿Qué hace con las pinturas que no usa?

Las boto en el zafacón 1

Las guardo 2

Las regalo 3

Las echo en el pozo séptico ... 4

Las echo en la laguna 5

Las boto en el patio 6

Otro 7

No sabe 8

No responde 9

19   ¿Dónde lavan las brochas y los rolos con pintura?

Fregadero 1

Pluma de afuera 2

Las boto 3

Otro 4

No sabe 8

No responde 9

20   ¿Cambia usted el aceite del carro?

Sí 1

No 2

No sabe 8

No responde 9

21   ¿Qué hace con el aceite usado del carro?

Lo meto en una botella 1

Lo boto en el patio 2

Lo tiro al agua 3

Lo echo al pozo séptico 4

Otro 5

No sabe 8

No responde 9

22 **¿Tiene usted una lancha?**

Sí 1

No 2

No sabe 8

No responde 9

23 **¿Qué mantenimiento le da en la casa?**

echarle gasolina 1

cambiarle el aceite 2

No sabe 8

No responde 9

24 **¿Pesca?**

Sí 1

No 2

No sabe 8

No responde 9

25   ¿Dónde limpia el pescado que coge?

    No responde 9

26   ¿Ha visto manchas en la laguna que parezcan ser de aceite?

    Sí 1

    No 2

    No sabe 8

    No responde 9

27   ¿Cuán grave usted considera que es el problema del desbordamiento de los pozos sépticos en la comunidad?

    Mucho 1

    Bastante 2

    Regular 3

    Poco 4

    Nada 5

    No sabe 8

    No responde 9

28 Comparado con otros problemas de la comunidad ¿cuán importante es el problema de los pozos sépticos?

Muy importante 1

Bastante 2

Regular 3

Poco 4

Nada 5

No sabe 8

No responde 9

29 ¿Cree usted que el problema de los pozos sépticos puede estar causando problemas de salud en su casa?

Sí 1

No 2

No sabe 8

No responde 9

30 ¿Alguna vez ha recibido orientación sobre pozos sépticos?

Sí 1

No 2

No sabe 8

No responde 9

31   ¿Le gustaría recibir orientación?

Sí 1

No 2

No sabe 8

No responde 9

32   ¿De qué forma le gustaría recibir la orientación?

Película 1

Panfleto 2

Visitas al hogar 3

Sistemas demostrativos 4

Otra 5

Especifique _____

No sabe 8

No responde 9

33   ¿Qué día y hora de la semana es más conveniente para asistir a una charla de orientación?

Entrevistador: INDIQUE EN ORDEN DE PRIORIDAD

Día _____

Hora _____

No sabe 8

No responde 9

34 ¿Cuánto dinero estaría dispuesto a pagar para mejorar su pozo séptico para mejorar la calidad del ambiente?

Especifique _____

No sabe 8

No responde 9

35 ¿Cuánto dinero estaría dispuesto a pagar al mes por tener el servicio de alcantarillado sanitario?

Especifique _____

No sabe 8

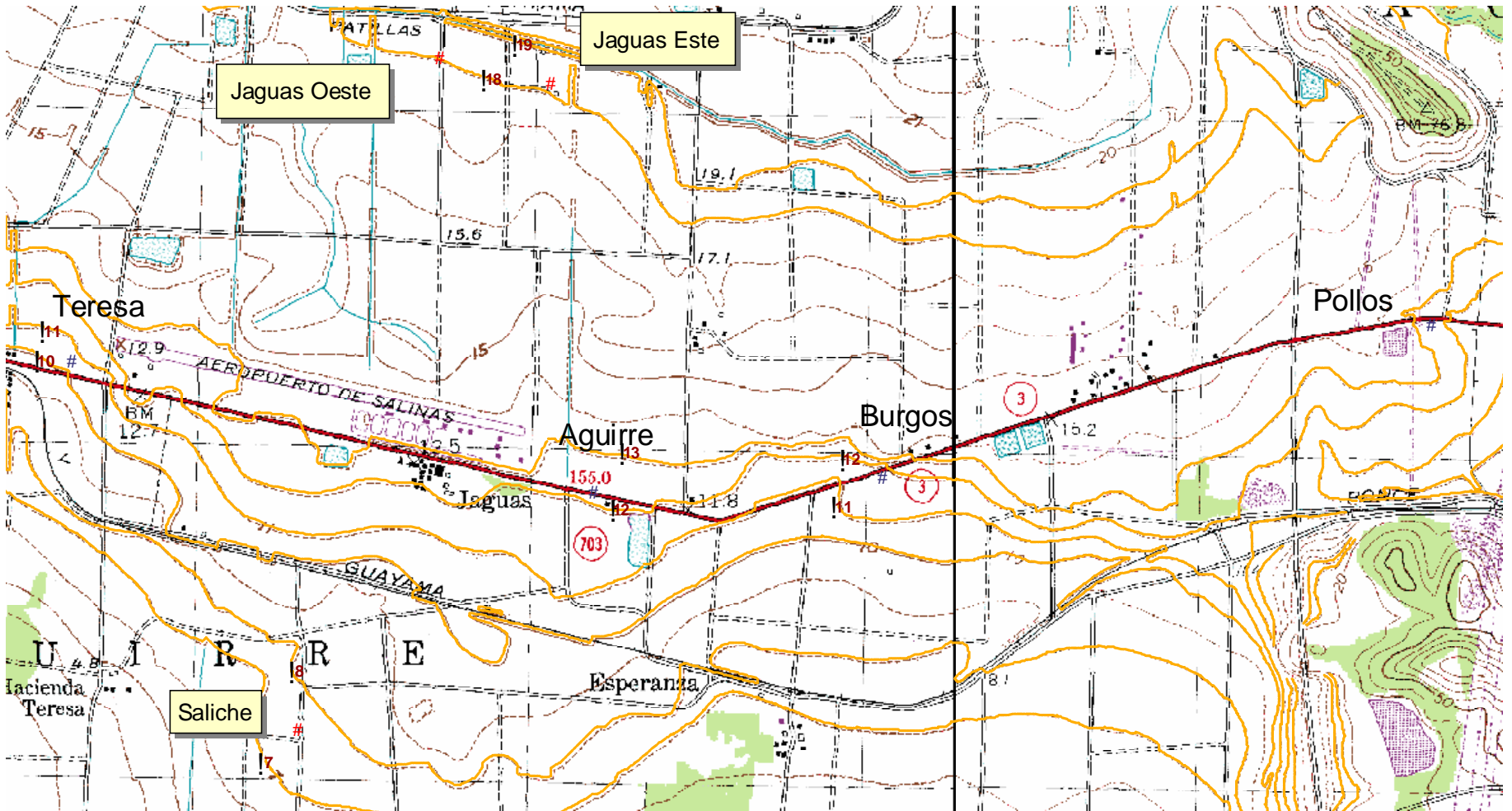No responde 9

Appendix B:

Precipitation

Total precipitation

January	Precip total in	Febuary	Precip total in	March	Precip total in	April	Precip total in	May	Precip total in	June	Precip total in
28	0	28	0	28	0	28	0	28	0	28	0
29	0	1	0	29	0	29	0	29	0	29	0
30	1.27	2	0	30	0	30	12.7	30	0	30	11.684 / 0.508
31	0	3	0	31	0	3	0	31	6.604	3	0
4	0.254	4	1.778	4	0	4	0	4	0	4	0
5	0	5	0.762	5	0	5	0.762	5	0	5	0
6	0.254	6	0	6	0	6	0	6	0	6	0
7	0	7	3.81	7	0.254	7	0	7	0	7	55.88
8	0	8	0.254	8	0	8	0	8	0	8	7.112
9	0	9	0	9	0	9	0	9	3.81	9	2.794
10	0	10	0	10	0	10	0	10	10.16	10	0
11	0	11	0	11	0	11	0	11	0	11	14.732
12	0	12	0.508	12	0	12	2.032	12	0	12	0.254
13	0	13	0	13	0	13	0	13	7.62	13	4.318
14	1.27	14	0	14	0	14	0	14	2.032	14	0.508
15	0.762	15	0	15	0	15	0	15	0	15	0
16	0	16	0	16	0	16	0	16	2.794	16	0
17	0	17	0	17	0	17	0	17	48.006	17	11.938
18	11.176	18	0	18	0	18	0	18	94.488	18	2.032
19	0.254	19	0	19	0	19	0.254	19	29.972	19	0
20	2.794	20	0	20	0	20	1.778	20	2.286	20	0.254
21	0.254	21	0	21	0	21	6.096	21	0.762	21	0
22	0	22	0	22	0	22	4.826	22	0	22	1.27
23	0	23	0	23	0.254	23	2.286	23	0.254	23	5.588
24	0	24	0	24	0	24	8.636	24	0	24	2.794
25	0	25	0	25	0	25	0.254	25	0.762	25	3.048
26	0	26	0	26	0	26	0	26	7.62	26	5.08
27	0	27	0	27	0	27	0	27	0	27	0

July	30	Pecip total in	August	30	Precip total in	September	30	Precip total in
	31			31				
1		5.842	1			1		
2		0	2			2		
3		0	3			3		
4		0	4			4		
5		3.81	5			5		
6		8.128	6			6		
7		30.48	7			7		
8		1.27	8			8		
9		1.016	9			9		
10		7.366	10			10		
11		5.334	11			11		
12		5.842	12			12		
13		5.588	13			13		
14		0.254	14			14		
15		1.016	15			15		
16		0	16			16		
17		0	17			17		
18		2.032	18			18		
19		0	19			19		
20		21.59	20			20		
21		52.07	21			21		
22			22			22		
23			23			23		
24			24			24		
25			25			25		
26			26			26		
27			27			27		
28			28			28		
29			29			29		

Appendix C:

Maps of Study Area

Topographic Map with Location of Sampling Stations at Irrigation Wells

Topographic Map with Location of Sampling Stations in Las Mareas

Las Mareas

Salinas

Abandonadas

Mar Negro

Salitral

Jacqueline

Pirata

Laguna
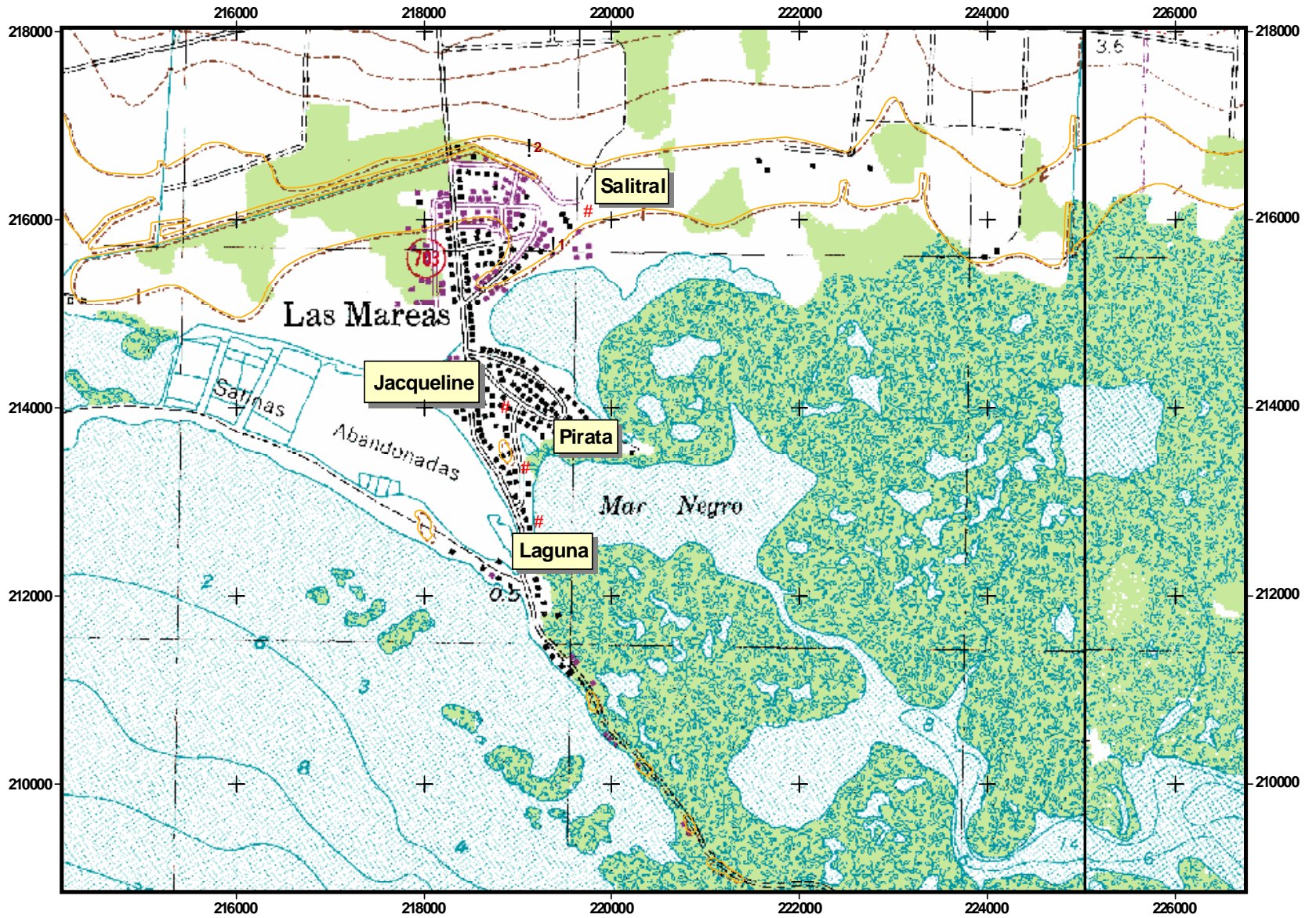
Overview of Study Area with Elevation Contours (in meters)

Elevation Contours (in meters) and Sampling Stations in Las Mareas

Elevation Contours (in meters) and Irrigation Wells Sampling Stations