

**Puerto Rico Water Resources & Environmental
Research Institute
Annual Technical Report
FY 2013**

Introduction

The Puerto Rico Water Resources and Environmental Research Institute (PRWRERI) is located at the Mayagüez Campus of the University of Puerto Rico (UPRM). The Institute is one of 54 water research centers established throughout the United States and its territories by Act of Congress in 1964 (P.L. 88-379) and presently operating under Section 104 of the Water Research and Development Act of 1984 (P.L. 98-42), as amended.

The Puerto Rico Water Resources Research Institute (PRWRRRI) was established in April 22, 1965, as an integral division of the School of Engineering at the College of Agricultural and Mechanic Arts, the official name of the UPRM at that time. An agreement between the Director of the Office of the Water Resources Research Institute of the Department of the Interior and the University of Puerto Rico at Mayagüez was signed in May 25, 1965. This agreement allowed the Institute to receive funds as part of the Water Resources Act of 1964. In June 1, 1965, the Chancellor of the Mayagüez Campus appointed Dr. Antonio Santiago-Vázquez as the first director. The first annual allotment of funds for fiscal year 1965 was \$52,297.29. Since its inception, the Institute has had eight directors in nine appointment periods as shown in the list below.

- 1 - Dr. Antonio Santiago-Vázquez - 1965 - 1968
- 2 - Eng. Ernesto F. Colón-Cordero - 1968 - 1972
- 3 - Eng. Felix H. Prieto-Hernández - 1972 - 1974
- 4 - Dr. Roberto Vázquez (acting director) - 1974 - 1975
- 5 - Dr. Rafael Ríos-Davila - 1975 - 1980
- 6 - Dr. Rafael Muñoz-Candelario - 1980 - 1986
- 7 - Eng. Luis A. Del Valle - 1987 - 1989
- 8 - Dr. Rafael Muñoz-Candelario - 1989 - 1994
- 9 - Dr. Jorge Rivera-Santos – 1995 - Present

The official name of the Institute was changed in 2005 to Puerto Rico Water Resources and Environmental Research Institute.

The general objectives of the Puerto Rico Water Resources and Environmental Research Institute are (1) to conduct research aimed at resolving local and national water resources problems, (2) to train scientists and engineers through hands-on participation in research, and (3) to facilitate the incorporation of research results in the knowledge base of water resources professionals in Puerto Rico and the U.S. as a whole. To accomplish these objectives, the Institute identifies Puerto Rico's most important water resources research needs, funds the most relevant and meritorious research projects proposed by faculty from island universities, encourages and supports the participation of students in funded projects, and disseminates research results to scientists, engineers, and the general public.

Since its creation, the Institute has sponsored a substantial number of research projects, supported jointly by federal, state, private, and University of Puerto Rico's funds. Through its website, the Institute's work is more

widely known to the Puerto Rican and world communities and, at the same time, provides means of information transfer with regard to the reports produced through the institute's research activities.

The Institute is advised by an External Advisory Committee (EAC) composed of members from water resources related government agencies, both federal and state levels. This committee virtually convenes annually to established research priorities and to evaluate and recommend proposal for funding under the 104-B program. The EAC has representation from the private sector as well. The FY-2013 EAC composition was as follows.

1. Dr. Antonio Santiago Vázquez, Engineering Consulting Firm, former Institute's director.
2. Mr. Pedro Díaz, USGS District Chief, Puerto Rico and Caribbean Office.
3. Eng. Victor Trinidad, US Environmental Protection Agency
4. Eng. Angel Meléndez, PR Environmental Quality Board

During the meetings, members are supported by the Institute's Director and Associate Director.

Due to the close retirement dates of some of the EAC members and recently changes in government directorates, the Institute's Director is engaging in recruiting new members for next fiscal year. New agencies that could participate in the EAC include the PR Department of Natural and Environmental Resources (PRDNER), Federal Emergency Management Agency (FEMA), US Fish and Wildlife Service (FWS), and US Army Corps of Engineers (CoE).

Research Program Introduction

The Institute functions as a highly recognized advisor to the industry and government sectors on water resources and environmental issues. This role translates into multidisciplinary functions and activities that add relevance and impact to the research program the Institute supports. By virtue of the local relevance of its research and the prestige and leadership of the investigators it has supported, the Institute has become the focal point for water - related research in Puerto Rico.

FY-2013 104-B base grant supported two research projects, both continuing projects. The original proposal was approved for three projects, but one of the projects had to be dropout after the original budget was substantially reduced by the USGS Water Resources Research Institutes Program. The project “An Integrated Approach for the Detection of Estrogenic Activity in a Tropical Urban Watershed,” in charge of Dr. Jorge Ortiz, a P.I. from the Río Piedras Campus of the University of Puerto Rico system, was cancelled. This project aimed to assess the presence of estrogenic activity in freshwater environments in Puerto Rico affected by human activities. The P.I. submitted the proposal for the second time in FY2014, with modifications to incorporate some work performed during FY2013, and was approved for continuation.

The other two projects are continuing projects by PIs from our home campus. One of them, “Microbial Source Tracking: The hunt for *E. faecalis* the dominant Enterococci among non-pigmented environmental Enterococci in the water systems of Puerto Rico,” by Dr. Luis Rios from the Department of Biology, had difficulties in achieving substantial progress due to budgetary restrictions. The P.I. estimated that the allocated budget, after the budgetary adjustment requested by the USGS, was not enough to complete the project and decided to wait for additional fund allocation. The P.I. failed to communicate his decision to the Institute’s Director on time to make possible budget adjustments. An explanatory letter from the P.I. is included herein. The P.I. has been granted a time extension to complete the project while additional funds are being identified.

The second active project “Field Demonstration of Removal of MS2 Bacteriophage and *Bacillus subtilis* with a Solar-Powered Engineered Experimental Drum Filtration and Disinfection (SEED) Unit,” by Dr. Sangchul Hwang from the Department of Civil Engineering and Surveying, continued as per proposed achieving significant outcomes. The system of drum units had to be overhauled after almost seven years of operation. Performance details are presented below.

In FY 2013, the PRWRERI continued strengthening its collaboration with the Jobos Bay National Estuarine Research Reserve (JBNERR), located in Aguirre-Salinas, Puerto Rico. This close collaboration is supported by the Memorandum of Understanding signed by both organizations in 1998. JBNEER is undergoing changes in its directorship, however, the Institute’s Director is actively involved in continuing collaboration with the new administration. A short description of each undergoing projects (or ended during FY-2013) is included next.

Costal Training Program (CTP): In this project the PRWRRRI provides knowledge through conferences, seminars, and workshops for professional development and networking for the JBNERR staff and to enhance integration across sectors at the reserve. An Education Coordinator is in charge of this project. This project was renewed during FY-2013 and continues through June 2014. Among the conferences held this year are “Collection and Mounting of Arthropods,” “Birds Banding and Molting Techniques,” “Rainwater Harvesting at Home” and “The use of Bio-filters for Rainwater Potabilization at Home.” The CTP focuses on (a) green design and sustainable principles incorporated in infrastructure planning and/or operational procedures to address climate change and resource protection, (b) socio-economic sustainability and community empowerment geared towards coastal resource protection, and (c) community and ecosystem resilience related to natural disasters.

Research Program Introduction

Implementation of System Wide Monitoring Program (SWMP): A fundamental part of the JBNERR stormwater management program is the collection of abiotic parameters, meteorological and nutrients SWMP data. The PRWRRI was in charge of collecting, organizing, processing, and submitting these data to the Centralized Data Management Office (CDMO). Data is also disseminated to the scientific community including the JBNERR's Research Advisory Committee for program future actions and to the Stewardship Coordinator to direct restoration efforts. This project was modified during the last fiscal year and the PRWRRI continued only supporting the laboratory data analysis with the services of the Virginia Institute of Marine Science. As part of the project, personnel from the PR Department of Natural and Environmental Resources have been trained and have assumed a leading role in data collection for the reserve.

Spatial works for delimiting areas for the boundaries of JBNERR: This work was used to determine the size and boundaries of parcels surrounding the reserve. Construction activities in some of these parcels has negatively impacted the ecosystem of the JBNERR estuary. The field work was finished by August, 2012, but our services in interpreting the results and meetings with the legal division of PRDENR have continued.

Taxonomy Analysis of Zooplankton and Ichthyoplankton at JBNERR: This is a prospective zooplankton taxonomic composition and abundance monitoring program launched by JBNERR administration as part of their System-Wide Monitoring program (SWMP) targeting biotic, abiotic, and land use/habitat change aspects of the Jobos Bay estuary. Sample collection and analysis are been performed since 2011. The project was renewed during FY-2012 with new termination date as August, 2014.

JBNERR Training and Technology Transfer for School Teachers (3TST): This project aims to provide schools from the Municipality of Guayama and Salinas with training, technology transfer, and information on water quality and environmental issues. This is a collaborative effort between PRWRRI, JBNERR, and OPAS (Spanish acronyms for the Organization Pro Environmental Sustainability) to promote education programs among schools in the vicinity of JBNERR.

Geospatial Analysis at JBNEER: Started on November 2012 and continued through FY2013, this project supported the JBNERR programmatic strategic plan by analyzing geospatial environmental data. These data relates to the land use and habitat inventory and looks for areas subjected to possible susceptibility to environmental hazards, specifically, tsunami hazard. This project used GIS tools to conduct a geospatial environmental data analysis regarding tsunami hazard for the JBNERR watershed to be used as a general baseline in case of a natural disaster. The main objective was to show the vulnerability of the habitats and communities around JBNERR to tsunami events.

Collaboration with other federal and state agencies has resulted in various externally funded projects. The Puerto Rico Department of Natural and Environmental Resources (PRDNER) provided funds for the development of "Hydrologic and Hydraulic Studies Guidelines." This project consists in the evaluation of current engineering practices for conducting hydrologic and hydraulic studies in the Island. The new guidelines will include two manuals, namely, "Hydrologic and Hydraulic Study Guidelines: the Technical Manual" and "Hydrologic and Hydraulic Study Guidelines: the Practice Handbook." In addition, this project includes the development of guidelines to sand and gravel extraction operation in rivers in Puerto Rico. A distinct guidelines manual will be produced for this portion of the project. The result of this project will influence the DNER's decision making process related to the approval of new housing developments and other type of projects that affect natural water bodies.

Establishment of a demonstration field in salt tolerant vegetative materials as conservation buffers in salt flats: The Natural Resources Conservation Service (NRCS) of the Department of Agriculture supported this project started in October 2011 with a duration of three years. This project compares the effectiveness of natural salt flat vegetative species as sediment detention barriers in coastal areas assisting Caribbean NRCS in the development of technical information needed for implementation of USDA conservation programs. The

Research Program Introduction

objectives of this project are: 1) Create a demonstration and technology transfer program at the project site for farmers, government personnel and visitors to the Boquerón Wildlife Refuge (BWR) in Cabo Rojo, Puerto Rico, 2) Apply, implement and monitor a demonstration project using recommended salt flat species for Puerto Rico as vegetative erosion protection method at the coastal area of BWR, 3) Generate guidelines and recommendations for application of this conservation technology in coastal areas based on the plant effectiveness as soil erosion protection and plants adaptation, and 4) To reduce the impact of nutrients coming from the Boquerón Wildlife Refuge and the Lajas Valley Irrigation canal on the coastal zone during flood conditions. The project is a Conservation Innovation Grant.

Local flood Mitigation at San Carlos Community in Dorado, Puerto Rico: The purpose of this study is to propose alternatives to mitigate flooding in the San Carlos Community, located at Higuillar Ward in the Municipality of Dorado, Puerto Rico. The community is continuously threatened by severe flooding caused by stormwater runoff from adjacent areas and over flooding of the existing stormwater system. Tasks in this project include: 1) Survey data required for the Hydrologic/Hydraulic study. This survey provides the necessary field information to meet the project objective, 2) Hydrological Study to determine the amount of water from the different watersheds contributing runoff to the community. Discharges for different design events are estimated. It includes a historical analysis to understand the cumulative effect of urban development through time. This analysis help in determining future impacts on the hydrology of the valley, 3) The Hydraulic Study provides the necessary information to dimension structures that help reduce the effects of runoff on the community as well as the estimation of water levels in existing drainage canals and pipelines, using the estimated rainfall amounts in the hydrological study, 4) Analysis of Alternatives - Mitigation alternatives will be discussed with staff of the Municipality of Dorado for consideration and implementation. The alternatives may include, but are not limited to: retention ponds, improvements to existing channels, new channels, and a possible storm sewer system within the community. The study will include measures within and outside the community. This project allows student participation in all stages and the incorporation of non-traditional techniques such as risk and reliability analysis.

Vegetative Filter Design for Overland Flow at JBNEER: - Mar Negro wetland, which is located within the JBNEER's boundaries, is one the biggest mangrove forest in the area. The north side of the Reserve has been used for agricultural activities since the Spanish Colonial times. Since 1993 the mangrove started to diminish and its mortality was promoted by antropogenic activities such as deforestation, domestic sewage discharging to the mangrove lagoon, and change of hydrologic patterns caused by urban developments and agricultural practices. In 1995 the Jobos Bay Reserve implemented a mangrove and wetland restoration program involving the monitoring of mangrove communities. This project consisted of two steps: 1) Study of the hydrologic and hydraulic conditions existing at the north side of JBNERR and 2) The hydraulic design of channels to re-direct irrigation and rain runoff to a parcel of land where existing vegetation can manage the dispersed overland channel discharges before reaching Mar Negro. The design includes an innovative system of lateral weirs which distributes water uniformly along the parcel. This water distribution system controls surface runoff from irrigation and rainfall, reduces surface erosion and improves the quality of overland flows discharging into Jobos Bay.

Field Demonstration of Removal of MS2 Bacteriophage and Bacillus subtilis with a Solar-Powered Engineered Experimental Drum Filtration and Disinfection (SEED) Unit

Basic Information

Title:	Field Demonstration of Removal of MS2 Bacteriophage and Bacillus subtilis with a Solar-Powered Engineered Experimental Drum Filtration and Disinfection (SEED) Unit
Project Number:	2013PR158B
Start Date:	3/1/2013
End Date:	2/28/2014
Funding Source:	104B
Congressional District:	
Research Category:	Water Quality
Focus Category:	Water Quality, Water Supply, Treatment
Descriptors:	Filtration, small systems, bacteria
Principal Investigators:	Sangchul Hwang

Publications

There are no publications.

Final Report (FY2013)

Table of Contents

Student Training.....	2
Results Dissemination.....	2
Field Source Water Quality Monitoring	2
<i>B. subtilis</i> Sporulation.....	11
<i>B. subtilis</i> Removal by Filtration	15
<i>B. subtilis</i> Removal by Disinfection	17
<i>B. subtilis</i> Removal by Filtration Followed by Disinfection.....	19
Regeneration of the Spent Filters.....	20
On-going Research.....	21
Acknowledgements.....	22
References.....	22

May 28, 2014

Student Training

A graduate student, Margaret Hernandez had completed major lab-scale experiment. She is now working on the preparation of the demonstration of the field SEED unit. An undergraduate student, Jose Illas (Biology) had supported both the lab- and field- scale experiment with Margaret Hernandez.

The PI (S. Hwang) has trained the students as follows:

1. Construction of three different types of sand filters,
2. Microscopic analysis of sporulation of *B. subtilis*, and
3. Maintenance of the field SEED unit.

Margaret Hernández attended the 2013 NWRI Clarke Prize Conference and Award Ceremony in Newport Beach, CA on November 15, 2013. She participated in the talks brought by leading scientists and experts in the area of water sustainability.

Results Dissemination

Results were presented as follows:

- Hernandez M., Illas J., Hwang S., “Inactivation of *Bacillus subtilis* by a sequential filtration and disinfection for rural community water supply.” 247th ACS National Meeting, Dallas, TX, Mar 16-20, 2014.

Field Source Water Quality Monitoring

Source water for the SEED unit has been monitored for its biochemical characteristics. Table 1 shows the average values from the duplicate analysis for each water quality parameter.

Table 1. Biochemical quality of the field source water.

Date	pH	Conductivity	Turbidity	TDS	TOC	Temperature	<i>E. coli</i>	Total coli	<i>B. subtilis</i>
28-Aug-12	8.54	265	0.85	156	12.5	27.8	5		
6-Sep-12	8.56	308	0.51	207.5	18.05	24.6	6	800	
13-Sep-12	8.49	333	0.55	235.5	19.55	24.6	3	180	
25-Sep-12	8.58	281	0.74	200	2.9	24.4	11	2860	
1-Oct-12	8.63	276		195.5		25.2			
9-Oct-12	8.64	293	0.565	209.5	1.5	24.7	11		
16-Oct-12	8.62	247	1.205	175	2.7	25.7	11	1050	
25-Oct-12	8.69	298	0.785	212.5	3.5	24.55	16	630	1070
12-Nov-12	8.71	281	0.685	177	1.1	24.7	19	710	1100
4-Dec-12	8.74	245	1.11	173.5	8.25	23.7	31	1320	900
18-Dec-12	8.72	320	0.68	226.5	1.65	22	1	21	1280
22-Jan-13	8.66	325	1.23	231.5	0.85	21.65	1	31	105
29-Jan-13	8.64	340	0.72	240.5	0.3	22.35	0	11	110
12-Mar-13	8.43	361	0.55	258.5	0.55	23.9	5	240	120
9-Apr-13	8.43	352	0.395	253	3.35	23.85	0	750	280
23-Apr-13	8.45	286	0.38	202.5	2.5	22.55	17	1290	260
27-Aug-13	8.46	305	1.875	220.5	15.65	26	13	1090	370
10-Sep-13	8.61	242	1.14	172	1.2	23.8	50	1180	210
24-Sep-13	8.60	319	0.8	228	1.6	24.5	16	870	220
8-Oct-13	8.72	323	0.55	228.5	1.6	24.8	15	420	150
17-Oct-13	8.72	336	0.6	238	1.7	25.05	12	200	160
22-Oct-13	8.72	323	0.55	228.5	1.6	24.8	15	1980	150
5-Nov-13	8.71	230	2.45	175.5	2.2	24.1	36	1560	10200
26-Nov-13	8.72	323	0.55	228.5	1.6	24.8	15	800	790
16-Dec-13	8.69	329	0.52	234	1.25	23.35	17	570	770

The values of pH ranged from 8.43 in March 2013 to 8.74 in December 2012 with a mean of 8.617 and standard deviation of 0.102 (Figure 1). Statistics show that the median pH 8.64 was greater than the mean pH, making the distribution asymmetric with increasing values from April until December and then decreasing. This made the distribution to skew to the left.

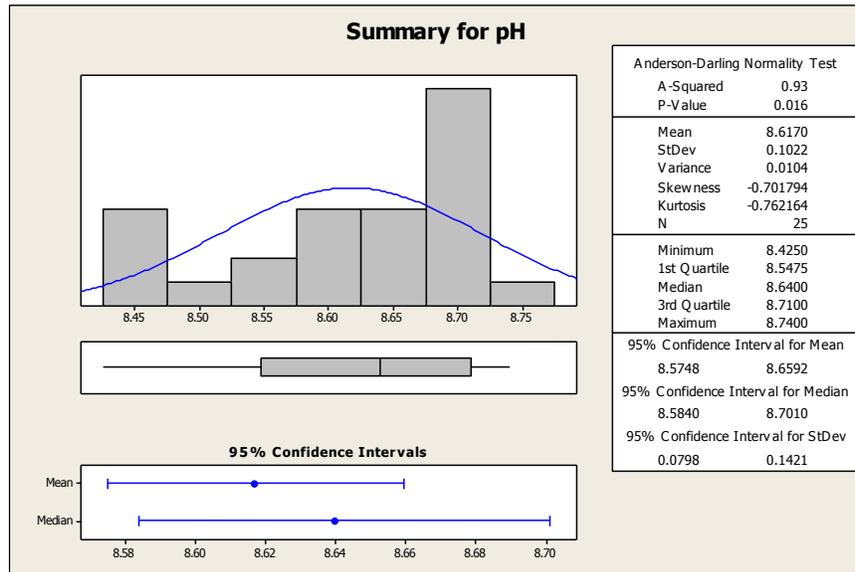


Figure 1 Summary statistics for pH of the field source water.

Conductivity ranged from 230 in November 2013 to 361 $\mu\text{S}/\text{cm}$ in March 2013 (Figure 2). The mean and standard deviation were 301.4 and 36.07 $\mu\text{S}/\text{cm}$. The mean conductivity (301.4 $\mu\text{S}/\text{cm}$) was lower than the median conductivity (307.5 $\mu\text{S}/\text{cm}$). For this reason, the distribution of the conductivity data was asymmetric with high values during January until April and was skewed to the left.

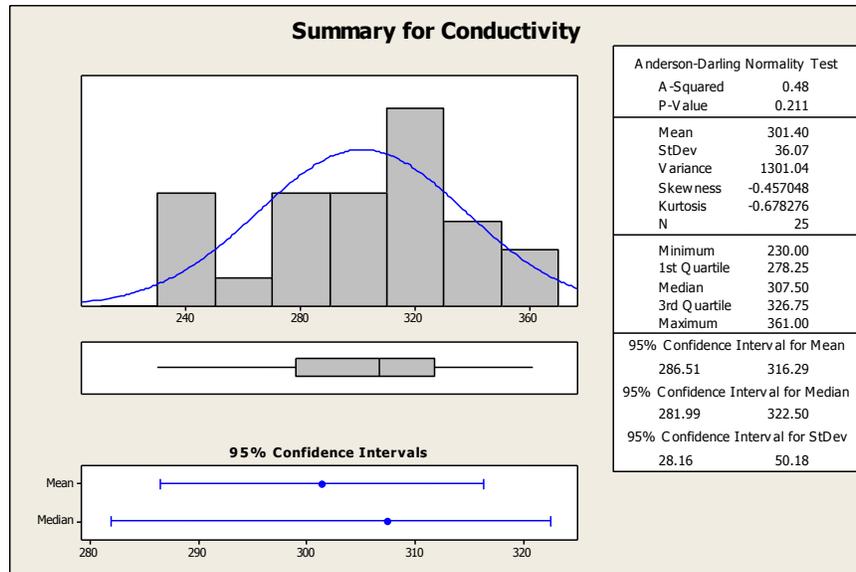


Figure 2 Summary statistics for conductivity of the field source water.

In terms of turbidity (Figure 3), the values ranged from 0.38 in April 2013 to 2.45 NTU in November 2013. Distribution of the data was asymmetric and right skewed since the mean was greater than the median of 0.6825 NTU. Even though turbidity values did not exceed 5 NTU in any measurement during the monitoring period, only 75 % of samples were below 1 NTU. Therefore, turbidity of the field source water did not meet the Surface Water Treatment Rule (SWTR) requirements.

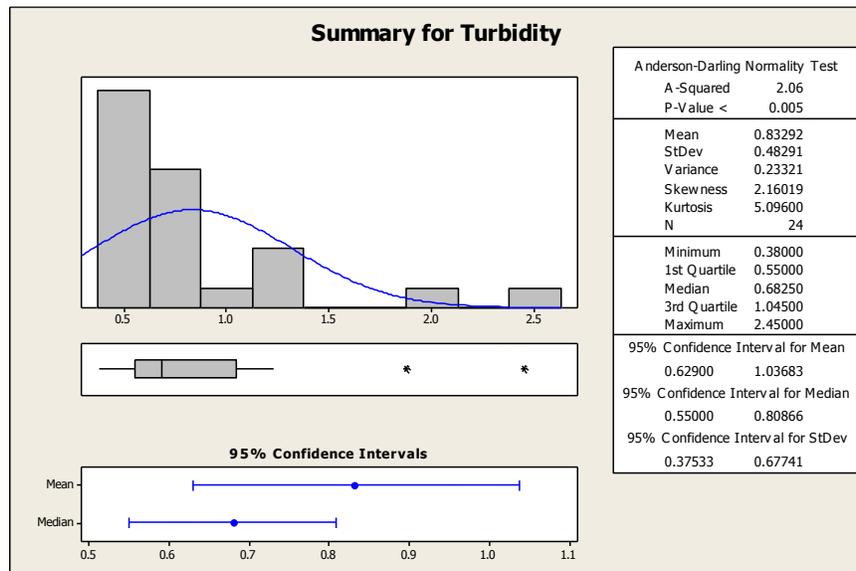


Figure 3 Summary statistics for turbidity of the field source water.

The total dissolved solid (TDS) concentrations were between 156 in August 2012 and 258.5 ppm in March 2013 (Figure 4). The mean and standard deviation were 212.32 and 27.97 ppm, respectively. The distribution of the data was asymmetric and skewed to the left with the highest values during December to March. None of the TDS measurements was greater than 600 ppm. Therefore, it can be classified as good quality water in terms of palatability of the water (WHO, 2011). Due to its low concentration of TDS, the source water does not present a matter of concern for health, scaling in distribution lines neither in house appliance.

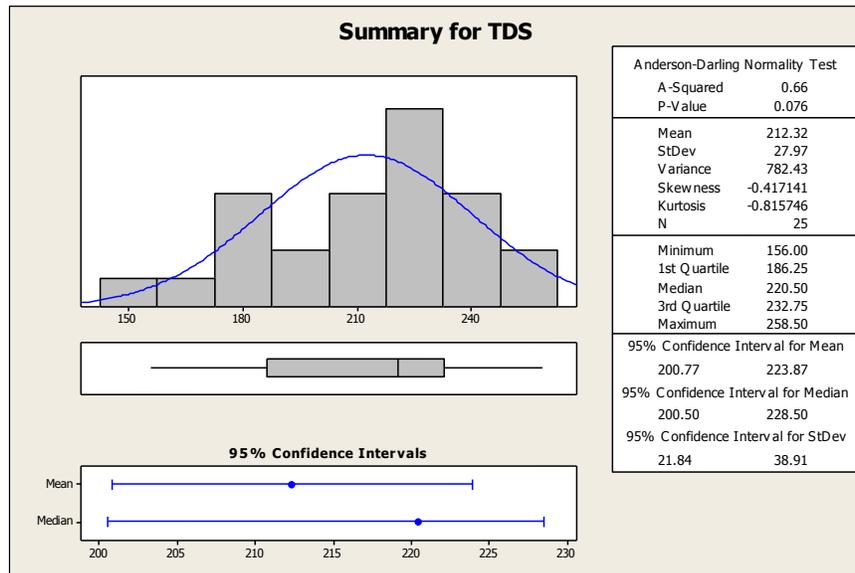


Figure 4 Summary statistics for TDS of the field source water.

TOC concentrations fell into the ranges between 0.3 in January 2013 and 19.55 mg/L in September 2012 (Figure 5). The mean and standard deviation were 4.4854 and 5.7788 mg/L, respectively. Due to inequality of the mean and median values, data distribution was asymmetrical and right skewed. TOC concentrations from September 2013 to December 2013 were mostly constant at ~1.6 mg/L.

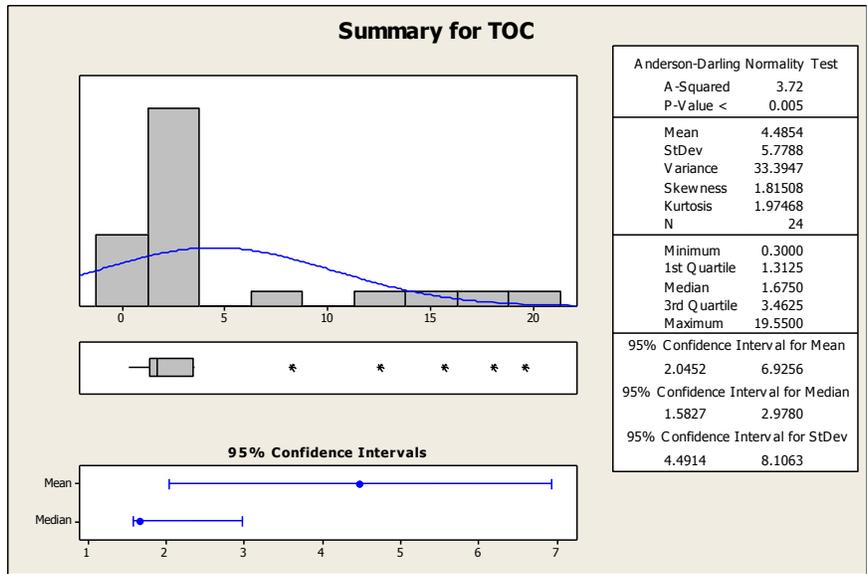


Figure 5 Summary statistics for TOC of the field source water.

Temperature during the monitoring period ranged from 21.65 °C in January 2013 to 27.8°C in August 2012 (Figure 6). These values represented the seasonal variations in winter and summer in Puerto Rico. The mean and standard deviation were 24.298 and 1.306°C, respectively. Data distribution was almost symmetrical with the lowest values during the winter period, from the middle of December to the end of January.

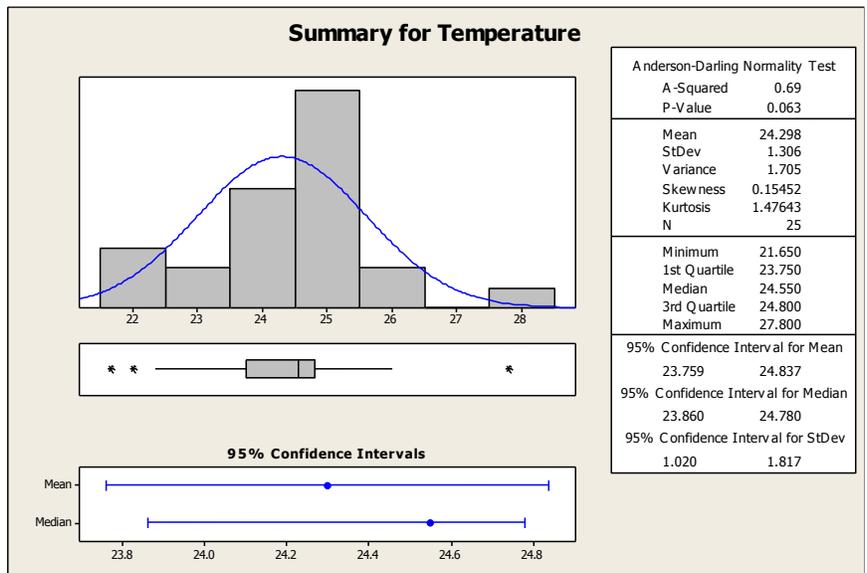


Figure 6 Summary statistics for temperature of the field source water.

The numbers of *E. coli* varied at a minimum of 0 CFU/100 mL in January and a maximum of 50 CFU/100 mL in September 2013 (Figure 7). The mean and standard deviation were 13.58 and 11.847 CFU/100 mL, respectively. Data distribution was asymmetrical with the lowest *E. coli* number during the winter season and skewed to the right. According to the SWTR, 12 out of the 14 samples are in acute Maximum Contaminant Level (MCL) violation if water resource is not treated appropriately.

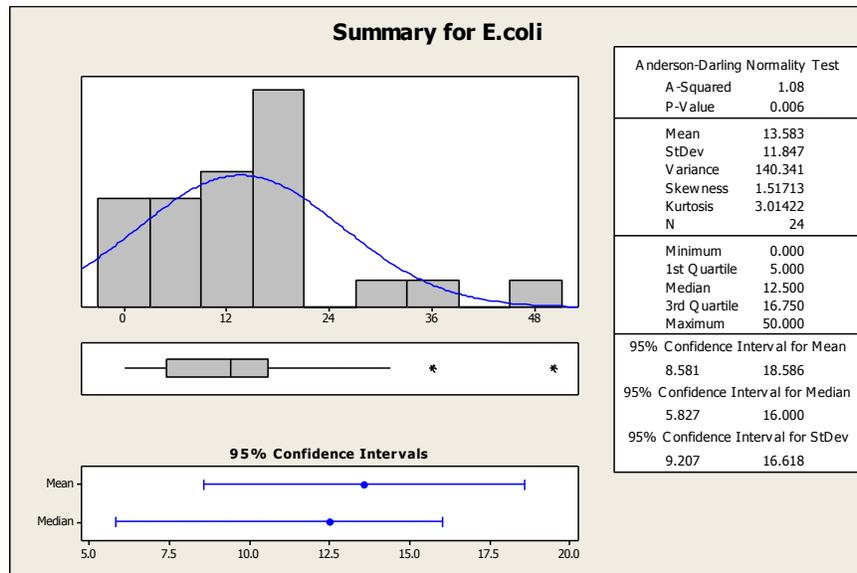


Figure 7 Summary statistics for *E. coli* of the field source water.

The numbers of total coliforms (TC) fluctuated significantly (Figure 8). The minimum number of TC was 11 CFU/100 mL in January 2013, whereas the maximum number of 2,080 CFU/100 mL was measured in September 2012. The distribution of the data was asymmetrical and right skewed. The mean value and standard deviation were 843.77 ± 693.28 CFU/100 mL. All samples were TC positive and their corresponding measurement of *E. coli* was also positive, making the source water to be in acute MCL violation if no further treatment were to be performed.

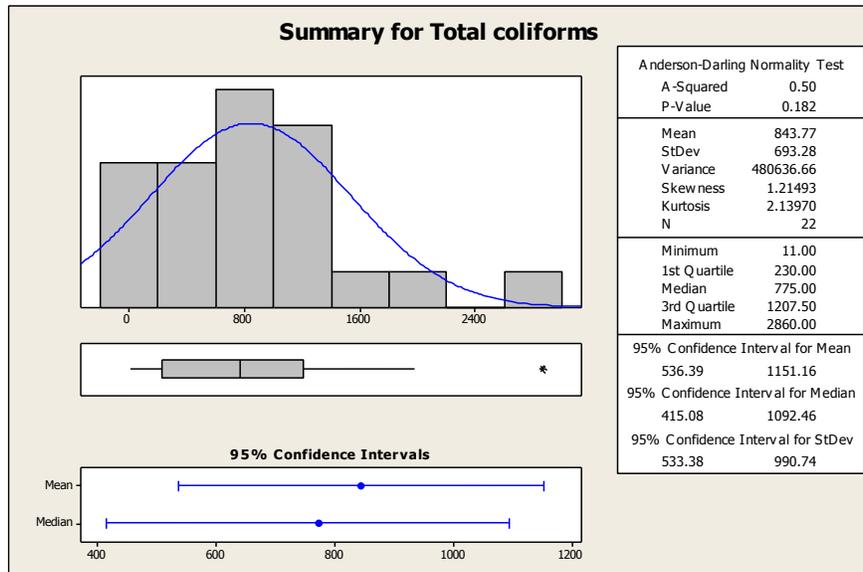


Figure 8 Summary statistics of total coliforms of the field source water.

The numbers of *B. subtilis* was found in the range from 105 CFU/100 mL in January 2013 to 10,200 CFU/100 mL in November 2013, with November and December having the highest numbers (Figure 9). The data had an asymmetric, right skewed distribution. The mean and standard deviation were found to be $1,013.6 \pm 2,327$ CFU/100 mL.

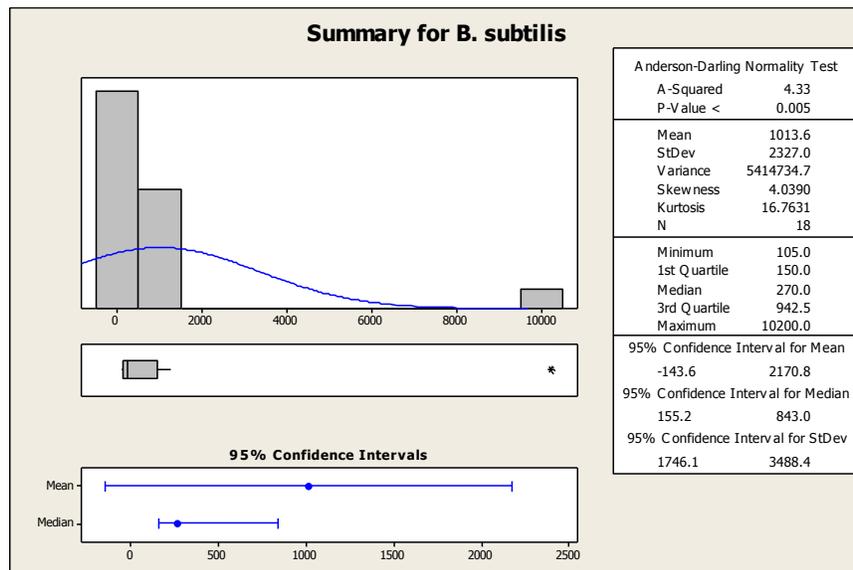


Figure 9 Summary statistics for *B. subtilis* of the field source water.

A strong relationship between conductivity and TDS was observed according to the Pearson correlation coefficient and P-values (Table 2). In addition, the covariance between the water quality parameters was also calculated with the Minitab 16.1 software. Table 3 presents the output of this statistical analysis.

Table 2 Pearson correlation and P-values of the water quality parameters monitored.

	pH	Conductivity	Turbidity	TDS	TOC	Temperature	<i>E. coli</i>	Total coliforms
Conductivity	-0.216							
	0.3							
Turbidity	0.103	-0.581						
	0.633	0.003						
TDS	-0.188	0.953	-0.438					
	0.369	0	0.032					
TOC	-0.418	-0.063	0.101	-0.147	0.433			
	0.042	0.768	0.639	0.492	0.035			
Temperature	-0.128	-0.277	0.113	-0.391				
	0.542	0.181	0.6	0.053				
<i>E. coli</i>	0.312	-0.692	0.451	-0.57	-0.186	0.052		
	0.138	0	0.027	0.004	-0.385	0.809		
Total coliforms	-0.021	-0.551	0.236	-0.507	-0.008	0.322	0.437	
	0.927	0.008	0.29	0.016	0.973	0.144	0.042	
<i>B. subtilis</i>	0.232	-0.556	0.72	-0.45	-0.021	0.035	0.401	0.341
	0.355	0.017	0.001	0.061	0.934	0.89	0.099	0.167
Cell contents:	Pearson correlation							
	P-value							

Table 1 Covariance among water quality parameters.

	pH	Conductivity	Turbidity	TDS	TOC	Temperature	<i>E. coli</i>	Total coliforms	<i>B. subtilis</i>
pH	0								
Conductivity	-0.8	1301							
Turbidity	0	-10.2	0.2						
TDS	-0.5	961.4	-6	782.4					
TOC	-0.3	-13.4	0.3	-24.1	33.4				
Temperature	0	-13	0.1	-14.3	3.3	1.7			
<i>E. coli</i>	0.4	-298.7	2.6	-191.3	-12.7	0.8	140.3		
Total coliforms	-1.5	-14179	82.1	-9403.1	-31	251.5	3704.4	480636.7	
<i>B. subtilis</i>	61.1	-48646.3	903.5	-28457	-179.7	94.6	12171.6	447467.1	5414734.7

The statistical results showed a positive relation between conductivity and TDS. The Pearson correlation, covariance and P-value for these two parameters were 0.953, 961.4 and 0.000, respectively. In terms of the bacteriological parameters, *B. subtilis* was highly positively correlated with turbidity, having the Pearson correlation, covariance and P-values of 0.720, 903.5 and 0.001, respectively. In contrast, *B. subtilis* was highly negatively correlated with conductivity, with the Pearson correlation, covariance and P-value of -0.556, -48646.3, and 0.017, respectively. *B. subtilis* may be positively correlated with *E. coli* as indicated by the Pearson correlation of 0.401 and the covariance of 12171.6. However, the P-value (0.099) was greater than 0.005 indicating a weak evidence. In terms of *E. coli*, the Pearson correlation and the covariance were 0.437 and 3704.4, respectively, indicating that it may be positively correlated to the presence of TC. The smaller P-value (<0.05) implied a strong evidence of this correlation.

***B. subtilis* Sporulation**

B. subtilis sporulation was visualized at four different durations of heat treatment (Figure 10). The results showed that the sizes of *B. subtilis* spores increased with increasing time. Batch experiments with varying doses of Cl₂ were performed to visualize the effect of the disinfectant concentration on the sporulation (Figure 11). The sizes of spores increased with increasing Cl₂ dose and temperature.

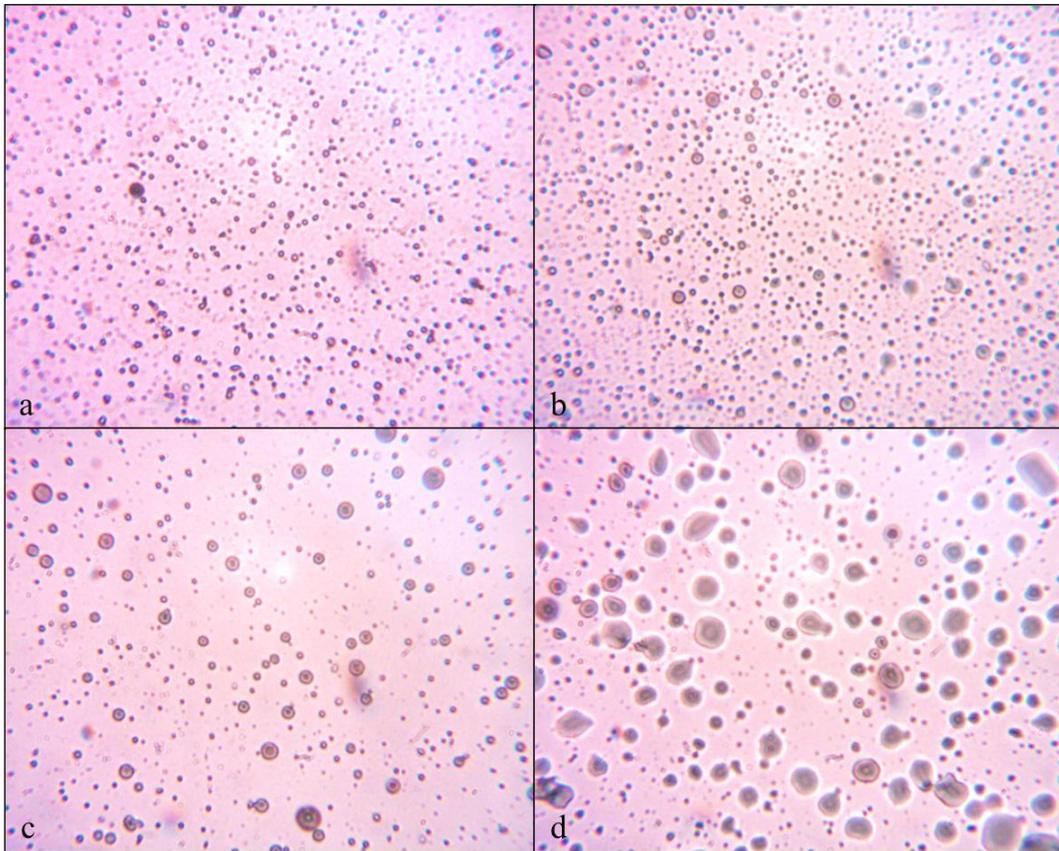


Figure 10 *B. subtilis* spores at (a) 3 minutes, (b) 10 minutes, (c) 15 minutes and (d) 30 minutes of water heat treatment (x100 magnification).

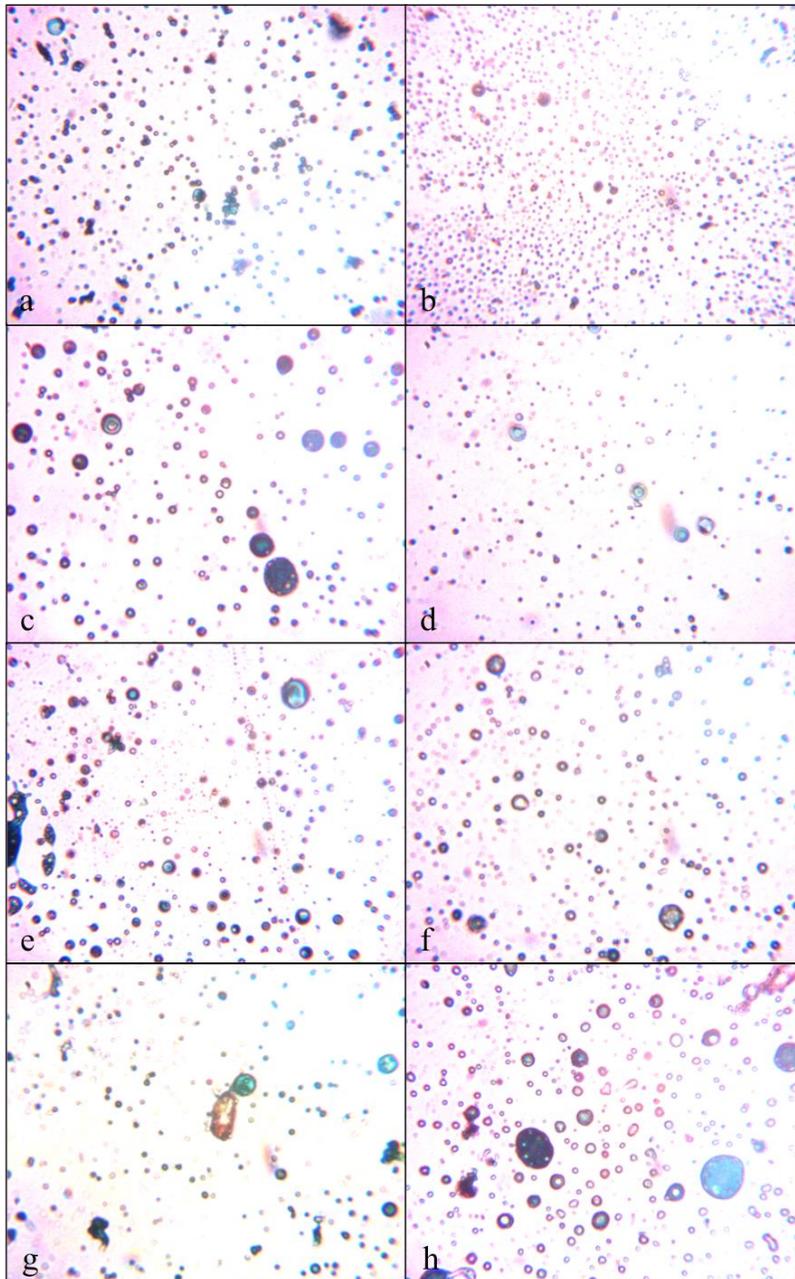


Figure 11 *B. subtilis* spores after 30 minutes contact times at initial Cl_2 concentrations of (a) 0 mg/L after hot treatment, (b) 0 mg/L after cold treatment, (c) 2 mg/L after hot treatment, (d) 2 mg/L after cold treatment, (e) 5 mg/L after hot treatment, (f) 5 mg/L after cold treatment, (g) 10 mg/L after hot treatment and (h) 10 mg/L after cold treatment (x100 magnification).

Figure 12 and Figure 13 show that the sizes of spores increased with increasing times with the Cl_2 concentrations at 10 and 20 mg/L, supporting the experiment performed without disinfectant (Figure 10). No significant difference in size was noticed between the two Cl_2 doses. Quantitatively, the numbers of *B. subtilis* decreased with decreasing Cl_2 dose.

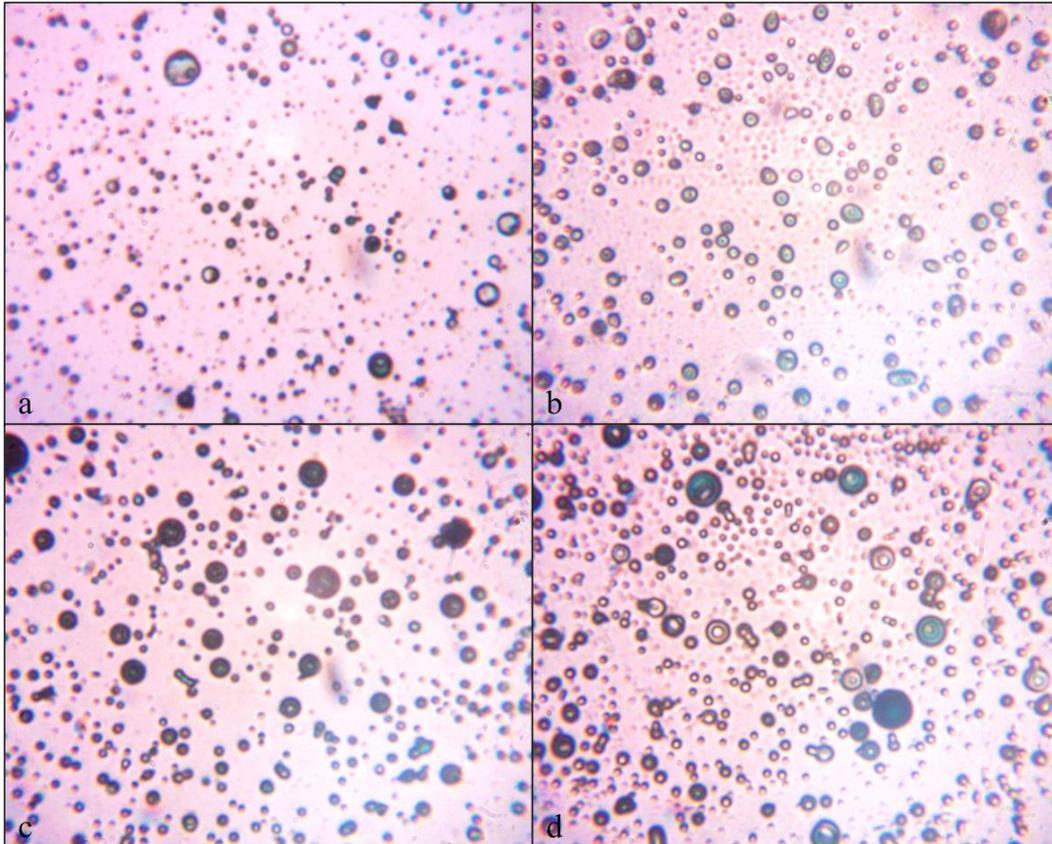


Figure 12 *B. subtilis* spores after 30 minutes contact time of 10 mg/L Cl_2 initial concentration at (a) 3 minutes, (b) 10 minutes, (c) 15 minutes and (d) 30 minutes of water heat treatment (x100 magnification).

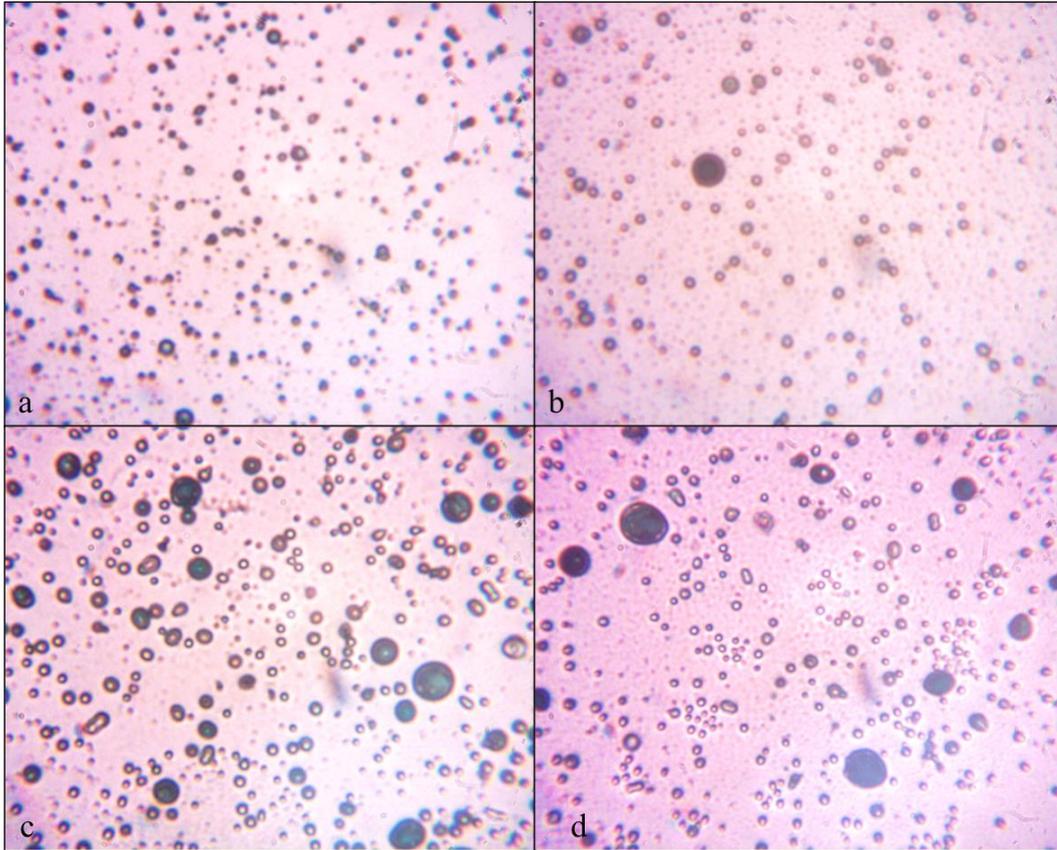


Figure 13 *B. subtilis* spores after 30 minutes contact time of 20mg/L Cl₂ initial concentration at (a) 3 minutes, (b) 10 minutes, (c) 15 minutes and (d) 30 minutes of water heat treatment (x100 magnification).

***B. subtilis* Removal by Filtration**

Three different types of sand filters were run at the sampling times determined by the tracer studies. *B. subtilis* removal by the first filter system (2 sand filters in series) run for more than 10 pore volumes equivalent (PV_e) fluctuated from the negative -948% to the maximum 98% removal. The negative removal, i.e., increased bacteria numbers, may be due to the entrapment of the bacteria inside the filters and the subsequent release from the filters (Figure 14).

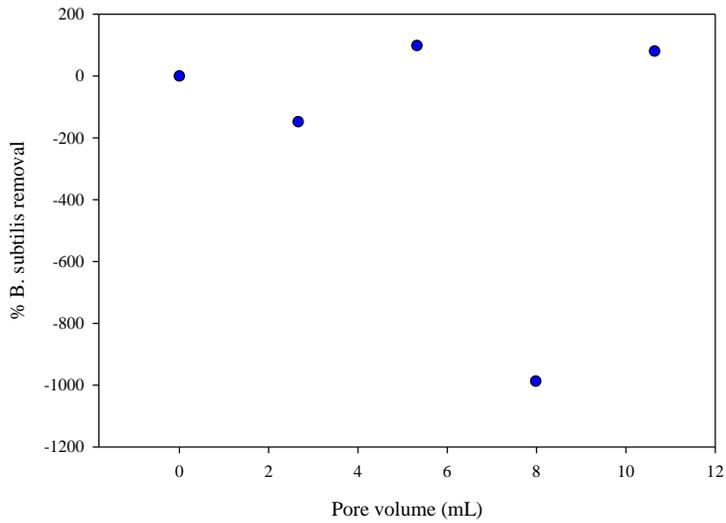


Figure 14 *B. subtilis* removal from filtration of System 1.

Results from the System 2 (GAC incorporated sand filter) are shown in Figure 15. *B. subtilis* percent removal in all sampling times were negative. This behavior may be due to the propensity of the bacteria to attach to the GAC. A similar result was obtained for the removal of *E. coli*, MS2 bacteriophage and anaerobic spores by the GAC filters (Hijnen et al., 2010), although the study showed 1.3-2.7 log removals of *Giardia* and *Cryptosporidium*.

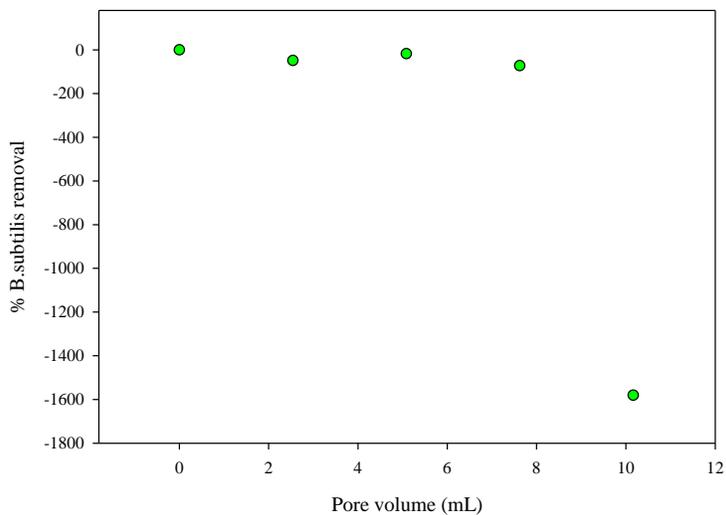


Figure 15 *B. subtilis* removal from filtration of System 2.

The System 3 filter incorporating iron oxide coated sand had the best *B. subtilis* removal of all the filters (Figure 16). Effluent from the System 3 has iron concentrations lower than 0.3 mg/L, the maximum concentration of the Secondary MCL.

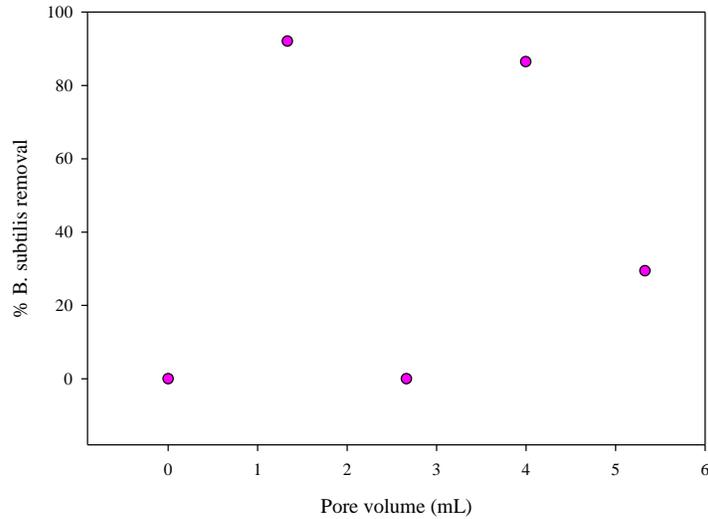


Figure 16 *B. subtilis* removal from filtration of System 3.

The target of this study was for the optimization of small water treatment system for a rural community with scarce economical resources. IOC sand preparation in a large scale by a rural community is not practical, economical, or feasible. Hence, the System 1 (two sand filters in series), which showed the second best *B. subtilis* removal, was chosen for further assessment in combination with the disinfection process.

***B. subtilis* Removal by Disinfection**

Tracer studies were conducted with the constructed disinfection basins to determine the sampling times. Results are shown in Figure 17. The trend of tracer breakthrough was fitted to the three-parameter sigmoidal regression with the following equation:

$$f = \frac{a}{1 + e^{-\frac{x-x_0}{b}}}$$

Table 2 provides the coefficients of the aforementioned equation. Their respective P-values (<0.0001) as well as R² (0.98) provide confidence of the analysis.

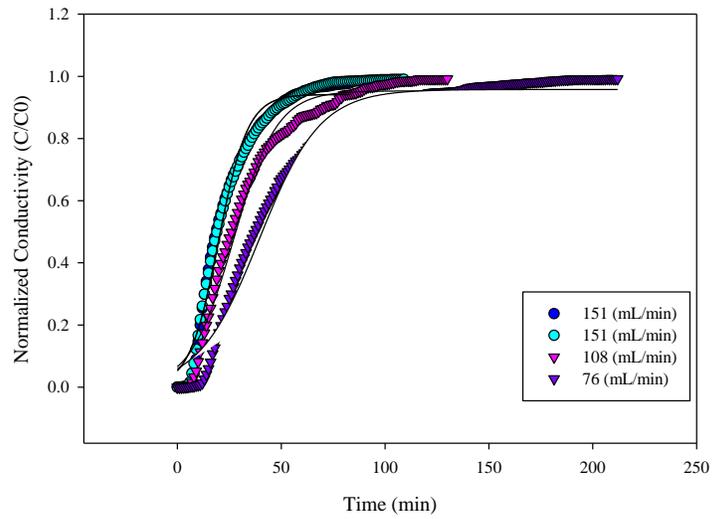


Figure 17 Tracer study results of disinfection chamber.

Table 2 Equation parameters and statistical results from tracer study of disinfection chamber.

Flowrate (mL/min)		Coefficient	Std. Error	P	R ²
151	a	0.9417	0.0075	<0.0001	0.9807
	b	7.0336	0.3194	<0.0001	
	x ₀	19.823	0.3566	<0.0001	
151 (2)	a	0.9417	0.0075	<0.0001	0.9807
	b	7.0336	0.3194	<0.0001	
	x ₀	19.823	0.3566	<0.0001	
108	a	0.9533	0.0054	<0.0001	0.981
	b	10.4117	0.3624	<0.0001	
	x ₀	27.0918	0.3976	<0.0001	
76	a	0.9577	0.0037	<0.0001	0.9822
	b	14.583	0.3941	<0.0001	
	x ₀	39.5584	0.4339	<0.0001	

B. subtilis removal and inactivation by disinfection was performed obtaining high percent removals greater than 92% (Figure 18). More than 20 CT's were required to remove *B. subtilis* greater than 99%.

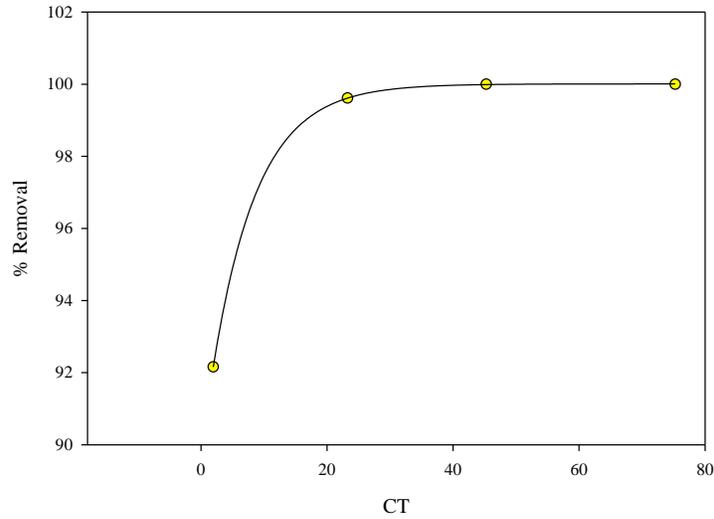


Figure 18 *B. subtilis* removal from disinfection.

***B. subtilis* Removal by Filtration Followed by Disinfection**

A treatment train of filtration followed by disinfection was performed for $> 500 PV_e$ for removal of *B. subtilis* and to evaluate the filters service life (Figure 19). The filters reached an apparent saturation after $200 PV_e$. However, after 400 pore volumes the removal began to increase again.

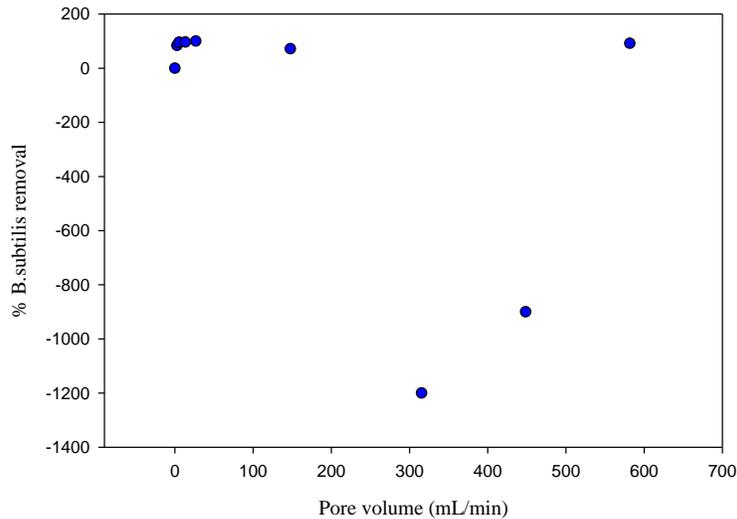


Figure 19 *B. subtilis* removal with a treatment train. Removals achieved after filtration.

Overall *B. subtilis* removals by the treatment train are shown in **Figure 20**. Similar to the *B. subtilis* removals after the filters, the removal increased up to a 50% removal until 11,224 mL of waters were treated and then decreased to 0%. Afterwards, *B. subtilis* removals increased to >95%.

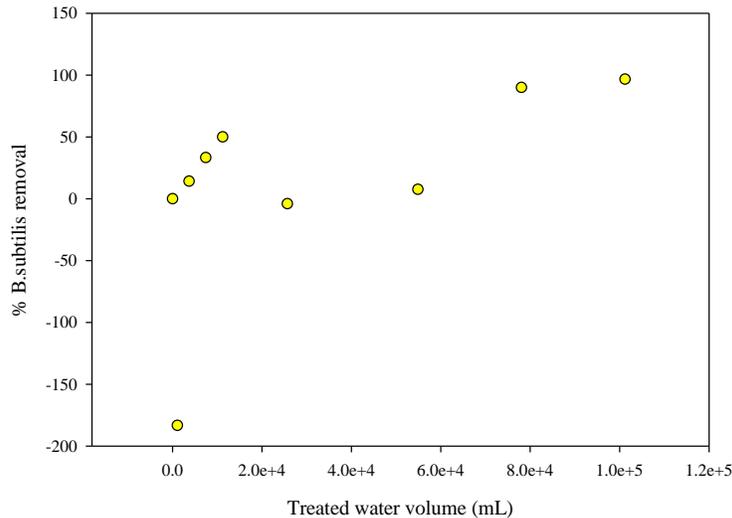


Figure 20 *B. subtilis* removal with a treatment train. Overall removals achieved after disinfection.

Regeneration of the Spent Filters

Spent filters with bacteria were regenerated by flushing the filters in series with the treated final effluent which had a free residual chlorine concentrations at ~5 mg/L. **Figure 21** shows free chlorine concentration profiles after the first sand filter (P1) and the second sand filter (P2). As shown, >80 PV_e of treated effluent water were needed to regenerate the sand filters.

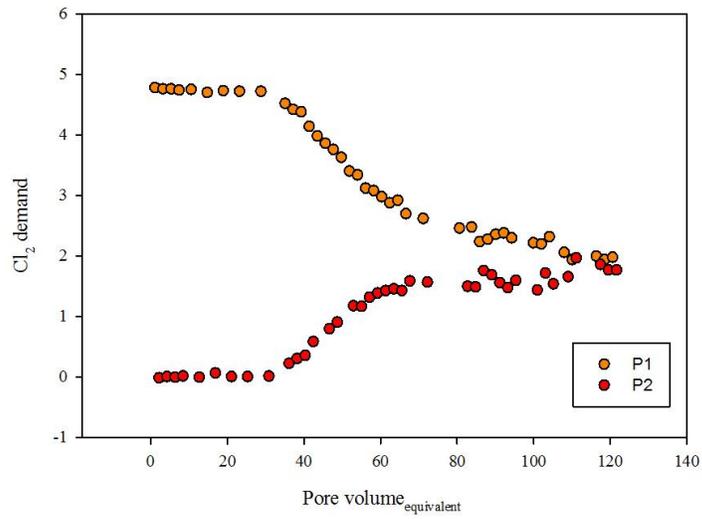


Figure 21 Cl₂ demand during regeneration of spent filters.

On-going Research

- MS2 removal by the lab-scale treatment train of sand filters and disinfection chambers.
- Replacement of the SEED units with plastic drums due to weathering.



Photo 1 The SEED unit at field.

Acknowledgements

This investigation was supported, in part, by the USGS 104B Program. Administrative support from the Puerto Rico Water Resources and Environmental Research Institute is appreciated.

References

Hijnen, W.A.M., Suylen, G.M.H., Bahlman, J.A., Brouwer-Hanzans, A., Medema, G.J. (2010). GAC adsorption filters as barriers for viruses, bacteria and protozoan (oo)cysts in water treatment. *Water Research* 44, 1224-1234.

WHO. (2011). *Guidelines for drinking-water quality*. World Health Organization. 4th edition.

The Total Microbial Community Structure and Enterococci Population Dynamics During a “recent fecal contamination event” in Seawater samples of Puerto Rico

Basic Information

Title:	The Total Microbial Community Structure and Enterococci Population Dynamics During a “recent fecal contamination event” in Seawater samples of Puerto Rico
Project Number:	2013PR160B
Start Date:	3/1/2013
End Date:	2/28/2014
Funding Source:	104B
Congressional District:	
Research Category:	Water Quality
Focus Category:	Water Quality, Non Point Pollution, Ecology
Descriptors:	Enterococci, fecal pollution
Principal Investigators:	Luis A Rios-Hernandez

Publications

There are no publications.

This project did not achieve the expected performance due to budgetary concerns. The original budget approved by March 1, 2013, was substantially reduced after the USGS announced a budget cut of more than 40%. The P.I. estimated that the assigned budget after the reduction was not enough to complete and achieve the proposed objectives of the project. The P.I. failed to communicate his conclusion and decision to the Director in a timely manner to evaluate possible alternatives to the budgetary issue. After an explanatory letter submitted by the P.I in May, 2014 (included herein), and a request for additional time, the Director approved a time extension while additional funding alternatives are considered and approval from the USGS Water Resources Research Institutes Program Director is obtained. Possible additional funds could come from leftover from other 104B projects and University funds.



May 28, 2014

Dr. Jorge Rivera Santos

Director

Puerto Rico Water Resources and Environmental Research Institute

Dear Dr. Jorge Rivera Santos:

On May 21, 2014 I received a communication stating that I should submit my final report to you in order for you to submit a report to USGS, the granting agency. In the communication I was told that the deadline was May 28, 2014. In order to comply with this communication I am writing this explanatory letter to you explaining my reasons for not using any of the funds assigned to my project **“The Microbial Population dynamics and total community structure during a “recent fecal contamination event” in seawater samples of Puerto Rico”** during the last year. As you know, I have been very responsible with the documentation, funds management and reporting in previous years. This pass year a few factors came to interplay such that I was morally obligated to choose not to use the funds and attempt to do the propose work. The first limitation, and the most important was, the final budget approved. I remember submitting my budget totaling \$20,000 (which was de maximum per year in the call for proposal), which was lowered to \$15,040 (on January 11, 2013) and later reduced to \$10,713 (on April 3, 2014). The sequencing materials alone to do this research were more than the total budget (see quote attached; \$11,612.50). The second limitation was the lack of a graduate student to take over the project. It is almost impossible to recruit a graduate student without financial support, especially during the summer months which was eliminated from the budget. The third limitation was that the sequencing facility was asking a service charge of \$2,560.00 for sample handling and sequencing service. It was obvious to me that my budget had a deficit so large that I could not overcome it and still fulfill my scientific commitment.

I do have to admit that it took me a long time to come to the realization that the proposed work as it was described in the proposal could not be done with the money allocated and without a graduate student. I was reluctant to give up and I try to figure out a way of make it work but any possible solution to our limitations would make me compromise my honesty

UNIVERSIDAD DE PUERTO RICO
RECINTO UNIVERSITARIO DE MAYAGÜEZ
DEPARTAMENTO DE BIOLOGIA
P.O. BOX 9012
MAYAGÜEZ, P. R. 00681-9012
TEL. 787-832-4040, EXT. 3900
FAX: 787-834-3673



UNIVERSITY OF PUERTO RICO
MAYAGÜEZ CAMPUS
DEPARTMENT OF BIOLOGY
P.O. BOX 9012
MAYAGÜEZ, P. R. 00681-9012
TEL. 787-832-4040, EXT. 3900
FAX: 787-834-3673



biol@uprm.edu
www.biology.uprm.edu

and scientific integrity. Since this is unacceptable, I decided to not use the funds since I could not fulfill the scientific commitment as stated in the proposal or limited to a smaller version without losing the scientific relevance. I do believe that with more funds (for materials, student support and sequencing services) and perhaps a rollover of the current assignment I could fulfill the scientific commitment this coming year.

I believe in this project and I would like to take this opportunity to request an extension of this project one more year if additional funding is available that will allow me to fulfill the commitments of this research successfully.

I will like to thank you for the support in the past and also apologize for not communicating this problem soon after the budget cut. Like I said, I was reluctant to give up because the research project that I proposed was a significant part to understand recent fecal contamination events in marine recreational waters. I strongly believe that this proposed work could benefit society and could change what we know about fecal contamination in marine tropical waters.

If you have any questions, I will be more than happy to meet with you and discuss this further; again thanks for the opportunity and I hope that my decision does not affect the PRWRERI.

A handwritten signature in black ink, appearing to be 'L. Ríos', written in a cursive style.

Dr. Luis A. Ríos Hernández
Associate Professor
Biology Department

Information Transfer Program Introduction

Meetings, seminars, technical reports, and a web site are used by the Institute to keep the water resources community and general public informed about advances in research. Approximately once every three or four years, the Institute organizes a major conference on water-related research in Puerto Rico and the Caribbean Islands, in collaboration with other island institutes (USVI, Hawaii, Guam, and the Micronesia) and professional organizations in the region. All these activities facilitate the translation of research sponsored by the Institute into practical applications of direct benefit to industry, government, and the general public. In 2011, the last conference held, the Puerto Rico Water Resources and Environmental Research Institute joined the Hawaii Water Resources Research Center, the Virgin Islands Water Resources Research Center, and the Environmental Research Institute of the Western Pacific in Guam to organize the conference titled “Water Resource Sustainability Issues on Tropical Islands.” Next conference is being planned for FY 2015. Other seminar and workshops have been offered as part of the various educational and technology transfer projects in collaboration with JBNERR. Some of this workshops were mentioned above.

During FY2013 none projects were funded from 104B program.

USGS Summer Intern Program

None.

Student Support					
Category	Section 104 Base Grant	Section 104 NCGP Award	NIWR-USGS Internship	Supplemental Awards	Total
Undergraduate	2	0	0	0	2
Masters	1	0	0	0	1
Ph.D.	0	0	0	0	0
Post-Doc.	0	0	0	0	0
Total	3	0	0	0	3

Notable Awards and Achievements

None during FY2013.

Publications from Prior Years