ABSTRACT

WELLBORN, LAUREN SUSAN. Drinking Water Quality Assessment and Analysis of Centralized and Decentralized Wastewater Treatment Systems in the Philippines. (Under the direction of Francis de los Reyes III.)

The Philippines is a nation in Southeast Asia seeking to move forward in its development. The centralized and decentralized water and sanitation industries are growing, and a nongovernmental organization, Gawad Kalinga (GK), has taken a leading role to address issues of homelessness and poverty, significant problems throughout the country. This project had two main goals: to assess the effectiveness and sustainability of water and sanitation systems installed in villages built by Gawad Kalinga to help ensure systems installed in future villages are reliable; and to assess the microbial populations in three of the wastewater treatment plants (WWTPs) operated in Metro Manila, with the goal of identifying organisms present in the treatment plants to allow for improved design and operation of future WWTPs.

Water quality was measured at 27 GK sites (from 43 total sources) using Hach portable laboratory equipment and 3M coliform petri plates. The following parameters were measured: pathogens, total coliforms and thermotolerant bacteria (*E. coli*), acidity, alkalinity, arsenic, carbon dioxide, chloride, chlorine dioxide, free and total chlorine, color, total dissolved solids, copper, hardness, iron, manganese, nitrate, nitrite, ammonia, pH, dissolved oxygen, phosphate, sulfate, sulfide, suspended solids, temperature and turbidity.

Results show that 95% of the sites visited tested positive for pathogens, as indicated by the Hach PathoScreen test. Many wells (52%) tested positive for total coliforms. *E. coli* was found in 16% of the wells tested, but was not found in any municipally provided water source. Nitrate levels above the WHO and Philippine national standard of 35 mg/L

were found in 30% of sources measured, and arsenic levels at or above the WHO standard of 50 ppb was found in one site. Interviews with 154 beneficiaries indicated that GK was responsible for a net improvement in water availability and sanitation access for 28% of its residents after they moved to GK villages.

Three WWTPs were sampled for molecular analysis: University of the Philippines (UP), PhilAm, and Makati South. These three plants serve different communities and are designed slightly differently. Terminal restriction fragment length polymorphism (T-RFLP) was performed on samples taken from each of the plants in February, 2008 and again in March (UP) or June (PhilAm, Makati South) to determine changes in the microbial communities over time. Results indicate that although there was a change over time (most significantly for the UP treatment plant), most of the terminal restriction fragments do not match with known fragments, indicating most of the species in the samples are not in the T-RFLP database. Cloning results confirm the presence of potentially novel species, and approximately 64% of species cloned did not match 98% identity with known sequences. Results that could be matched with known species via phylogeny indicate that Betaproteobacteria comprise the largest fraction of microorganisms in the sampled WWTPs, followed by Firmicutes.

The Makati South WWTP had lowest levels of diversity and richness among the three plants, but Manila Water Services, Inc reports that Makati South is the most reliable WWTP among those sampled for effluent water quality.

Drinking Water Quality Assessment and Analysis of Centralized and Decentralized Wastewater Treatment Systems in the Philippines

by
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Biography

Lauren Wellborn was raised in Boone, North Carolina, and began her college career at the University of North Carolina at Chapel Hill studying computer science in 2001. She transferred to North Carolina State University during her sophomore year, and after a few semesters was given the opportunity to spend a summer interning at the Danish Hydraulic Institute in Horsholm, Denmark. During this time, Lauren discovered environmental engineering and soon after decided to change her focus. She entered into the Environmental Engineering Department at NCSU the following fall semester and worked with Francis de los Reyes III and Morton Barlaz as an undergraduate research assistant. After graduating in December 2006, Lauren spent several months traveling through Central and South America, and then began her Masters program in 2008 under the mentorship of Francis de los Reyes III.

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Introduction

The Philippines is located in Southeast Asia and is comprised of 7107 islands occupying approximately 115,000 mi^2, an area slightly larger than Nevada. A brief look at the history of the Philippines aids in understanding the current state of the water environment in the country.

Likely populated via a land bridge from mainland Asia, the Philippines was discovered by the western world in 1521 (by Magellan) and was subsequently ruled by the Spanish until 1898. Independence was finally established in 1898, after a series of revolutions and a history of Filipino dissatisfaction, but the United States annexed the country just six months later as the result of the 1898 Treaty of Paris signed at the conclusion of the Spanish American War. After several more decades of American and then Japanese rule, the Philippines established its independence shortly after WWII. Despite many centuries battling corruption and outside rule, the Philippine national government, established after independence, has continued to disadvantage the country through corruption and the funneling of monies away from government projects. As of 2003, the poverty rate was 30% (2).

According to the United Nations Statistics Division (1), the Philippine population as of 2007 was 88.0 million, and as of 2003, more than 50% of the population relied on groundwater wells for drinking (8). Average water consumption in the Philippines is 120 liters per person per day in urban settings and 60 liters per person per day in rural areas, 80% of which is returned as wastewater (8). Biochemical oxygen demand is generated at approximately 37 g per person per day (8). Indiscriminate disposal of human waste is the leading cause of water quality degradation in urban areas of the Philippines (8). The World Health Organization (5)

reports that as of 2006, 93% of the population had sustainable access to improved drinking water sources[1], and 78% had access to improved sanitation[2], but water and sanitation related diseases remain a significant problem in the country due to poor water quality and hygiene, especially in informal settlements.

A nongovernmental, nonprofit organization, Gawad Kalinga, emerged in response to the under-addressed slum dwelling populations of the Philippines. Gawad Kalinga (GK), meaning "to give care" in Tagalog, is a local organization seeking to establish communities in place of slums. GK relies on donations and volunteers, both local and foreign, and sweat equity of beneficiaries to build communities for the relocation of slum dwellers and the conversion of slums to villages. GK has thus far built more than 200,000 homes in more than 2,000 villages, and has plans of building thousands of additional villages. Each of these communities ideally provides adequate water and wastewater treatment for beneficiaries and a plan for disposing of solid waste. Educational programs accompany relocation to GK villages in an effort to further transform the lives of beneficiaries.

[1] The UN reports that improved drinking water sources include piped water into dwelling, plot or yard; public tap/standpipe; borehole/tube well; protected dug well; protected spring; rainwater collection and bottled water (if a secondary available source is also improved). It does not include unprotected wells, unprotected springs, water provided by carts with small tanks/drums, tanker truck-provided water and bottled water (if secondary source is not an improved source) or surface water taken directly from rivers, ponds, streams, lakes, dams, or irrigation channels (7).

[2] Improved sanitation facilities include flush/pour flush toilets or latrines connected to a sewer, -septic tank, or -pit, ventilated improved pit latrines, pit latrines with a slab or platform of any material that covers the pit entirely, except for the drop hole and composting toilets/latrines. Unimproved facilities include public or shared facilities of an otherwise acceptable type, flush/pour-flush toilets or latrines which discharge directly into an open sewer or ditch, pit latrines without a slab, bucket latrines, hanging toilets or latrines which directly discharge in water bodies or in the open and the practice of open defecation in the bush, field or bodies or water (7). "Access to improved sanitation," therefore, includes facilities that could easily contaminate groundwater, calling into question the sustainability aspect of the improved drinking water sources.

As one of the foremost organizations in the country, GK has the potential to effectively transform the sanitation conditions of beneficiaries by adequately designing wastewater treatment systems and drinking water wells in GK villages, but also has the potential to fail in this regard.

Objectives

The goal of this study was three-fold. First, the study aimed to provide a picture of the current state of the environment in GK villages. This was important because the GK designer team is made up of architects who are not trained in sanitation design. Describing the current state of the installed water and sanitation systems in recently constructed villages allowed the design team to better understand consequences of water and sanitation system designs. Assessment included interviews of beneficiaries about their opinion on access and quality of drinking water and sanitation systems before and after the GK villages were built, water quality measurements, and assessment of septic tank designs.

Second, the study aimed to provide recommendations for improving water and sanitation design practices for improvement of future villages.

Third, the research included a component of sampling and microbial analysis of wastewater treatment plants (WWTPs) in Metro Manila. The country is moving towards improving access to water and sanitation, and a greater understanding of the microbial community present in WWTPs will aid in more efficient design and operation of current and future WWTPs.

This thesis will first explore the water and sanitation situation in the Philippines, examining reasons for the current state of the environment and suggestions for the future. Chapter Two focuses on the microbial community present at WWTPs in Metro Manila, Philippines, based on samples collected from January to June, 2008.

Factors Influencing Sanitation

According to UN Data (1), average gross domestic product per capita in the Philippines is $1639 (2007), and the poorest quintile's share in the national income or consumption, as of 2006, was only 5.6% (7). This indicates a large disparity between the rich and poor in the country, which is highly visible, especially in urban centers such as Manila, where squatter areas comprise a significant portion of the city. In 2006, 43.7% of urban dwellers were slum dwellers, which is reduced from the reported 55% in 1990 (7). The Philippine Midterm Progress Report on the Millennium Development Goals (MDGs) (2), however, reports that numbers of slum dwellers are expected to increase because of rural-urban [job-seeking] migration, population growth and new family formation, and because programs to address the urban poor population are either inadequate or overwhelmed. As a result of adherence to Catholic teaching, many of the urban poor do not practice contraceptive use or family planning, leading to an average population growth of 2%, which will double the population in the next 35 years (2).

Though the Philippines in general is rich in water resources, urban centers crowded with impoverished people rarely allow for adequate supplies of drinking water, wastewater treatment, or solid waste management (9). Many slum areas illegally tap into water lines, potentially compromising the clean supply of water to users downstream, but rarely are slum dwellers able to adequately treat and dispose of their waste products. As a result, some areas become overrun with accumulated human and solid waste, resulting in serious health threats

to those living nearby. Figure 1, below, is a photograph of the Baseco squatter community, located on Manila Bay. Despite the passage of the Ecological Solid Waste Management Act (RA 9003), dump sites such as these are present in many places throughout the country and are host to a variety of chemical and bacterial pollutants, which pose a threat not only in terms of direct contact but also threaten water supplies as pollutants are easily carried to surface water or groundwater.

Figure 1 – Photograph of Baseco on Manila Bay.

Efforts Relating to Water and Sanitation

Addressing water and sanitation problems requires implementation of effective national programs and allocation of sufficient funds to ensure their success. The Philippine national government has implemented many water-related laws, including a Clean Water Act (2002), in an effort to protect the nation's water resources, but enforcement is weak due to many

factors, including inadequate resources, poorly maintained records, and weak cooperation among different agencies and local government units (8). According to the Philippine Midterm Progress Report on the MDGs, the government believes a focus on drilling new wells in waterless areas is important, along with groundwater monitoring systems to determine if the groundwater is being used at sustainable rates (2). Specific focus is on the development of low cost water supply systems (such as hand pumps, gravity fed systems and rainwater catchment), with a general focus on sanitation (2).

The government also stresses the importance of development and construction of low cost sanitation facilities (2). Approximately 60% of the population in the Philippines lives in coastal areas and discharges untreated human waste to these waters, contributing to the degradation of coastal waters and a strain on the ecosystems, and the Philippine Environment Monitor reports that the main source of bacterial groundwater contamination is domestic wastewater (8).

The Philippine government recognizes a decline in the availability of sanitation facilities due to lack of sufficient investment (2), indicating that these concerns are low priorities in terms of spending despite the listing of sanitation and sewerage as a high priority in the Philippines Agenda 21 of 1996 (8). Local governments recognize that more investment needs to be made in infrastructure, but they are constrained by limited budgets and willingness to pay, high investment and operational costs, and space availability in high density urban areas (8).

The national government of the Philippines has historically been fraught with corruption, making problems of the urban poor very difficult to address. The Philippine Midterm Progress Report on the MDGs (2) reports that the Office of the Ombudsman established that "a total of US$48 billion was lost to graft and corruption for the last 20 years and that only

60% of the national budget was actually spent on government projects" (2). Without adequate assistance from the national government, improving the housing or sanitation situation will be difficult.

Centralized and Decentralized Water and Sanitation Systems

Funding allocated by the Philippine government for water and sanitation services are usually lumped together. Because demand for water is very high, 97% of government funds allotted for water and sanitation investments are directed towards supplying drinking water (8).

Drinking water supply in the Philippines is the responsibility of various government units and water utilities (9). Metro Manila is served by two private companies: Maynilad Water Services Inc (MWSI) and Manila Water Company Inc (MWCI), and by several smaller companies serving subdivisions. Water supply in the capital region is mainly from surface water (9), but 86% of municipally supplied drinking water systems throughout the country distribute groundwater (8), and water supplied by all sources in the Philippines is considered unsafe for drinking unless it is treated (9). According to the Greenpeace document "The State of Water in the Philippines," however, 20% of people in the Philippines do not get water from formal sources, and as of 2005, only 44% had direct household water connections (9). Those without household connections access water from wells, springs, communal faucets, and/or from small scale informal providers (9).

Though infrastructure development for sanitation services began over a hundred years ago, the development process was delayed by lack of funding and by the process of privatization, which only began in recent decades (8). As of 2003, only 7% of the Philippines' total population was connected to sewer systems, only 3% of the wastewater produced in Metro Manila was being treated at large-scale treatment plants, and sanitation facilities maintained

on-site rarely produced effluent of acceptable quality (8). Because of the high capital and operational costs of installing centralized systems, decentralized systems are being increasingly explored (8). Technical alternatives with lower costs than those for centralized systems are available, and experiences in other developing nations have proven that piping costs for smaller systems are reduced by 40 to 74%. This has encouraged investments in these systems in the Philippines, but as of 2003, most of these smaller treatment systems were still under construction (8).

Chapter 1: Drinking Water and Sanitation

This Chapter focuses on the drinking water and sanitation situation in the Philippines at large, and within Gawad Kalinga villages specifically. Assessments were conducted from January to July, 2008. Gawad Kalinga was selected for study because of its presence throughout the country, allowing a wide variety of villages to be included in the survey. GK villages number in the thousands and are located in both urban and rural areas, in all types of topography, and are managed locally. This diversity allowed for a more comprehensive study and a broader set of recommendations for design and implementation of drinking water and sanitation systems in future villages.

Figure 2 shows the areas of the Philippines where the site assessments took place. Of the 43 sources measured total, 8 were in Metro Manila, 3 were in Cavite, and 14 were in the Batangas area (for a total of 25 in Luzon, the northernmost region); 11 samples were taken from sites around Davao, two sources were measured from Samal Island, and 5 sources were sampled in Bukidnon (for a total of 18 in Mindanao, the southernmost region).

Figure 2 - GK site sampling locations (Map provided by Google Maps).

Data Collection and Site Assessment

Specific site visits were recommended by GK national staff in Manila and by GK staff in Davao, Mindanao. Project directors were contacted and asked whether the GK site they managed was using on-site groundwater for drinking or if they were experiencing problems with their municipality-provided drinking water source. Sites chosen included those that were using on-site groundwater, sites that were using groundwater treated and distributed by the municipality, sites where the project director thought the residents were satisfied with

their water sources, and sites where the project director thought the residents probably were not satisfied. Despite these efforts, project directors were often incorrect about the source of drinking water on the sites, and were also often mistaken about the residents' satisfaction with the water.

A four-pronged approach was used for each site assessment.

1. Interview with site Project Director.

   This interview was intended to determine basic site information: location, population, history of the site, type and quantity of water supply, septic tank design, and solid waste disposal practices of residents. These interviews were eventually terminated after it became clear that the information that the project directors were providing was not always correct.

2. Interviews with beneficiaries.

   These interviews were used to determine the following with respect to drinking water: whether people were drinking the water supplied onsite or purchasing water from other sources; why residents might purchase their water rather than use their local wells; whether residents treated their water before consumption; the quantity of water used in the homes; whether residents were satisfied with their water; and a comparison of availability of water, quantity used, and source type, before and after their relocation to GK villages.

   Beneficiaries were also asked the following with respect to their septic systems: the septic tank location (to determine their awareness of septic systems and to determine if the tanks are accessible for pumping); their plans for emptying their tanks when necessary; whether or not they are satisfied with their septic tanks; and what their

wastewater disposal methods were prior to relocation.

Finally, beneficiaries were asked how they dispose of their solid waste, including which of the following is utilized: composting, recycling, burning, open dumping, and collection, and which of those disposal methods were employed prior to moving to GK villages.

3. Water Quality Sampling.

   Samples were taken from one or more on-site wells or from the municipally supplied water source on site. Where multiple wells were used for drinking, at least two wells were tested for water quality.

   Water was sampled using two liter plastic containers. The containers were not sterilized or washed with detergent (to avoid introduction of chemicals that may alter the composition of the sample) but were vigorously rinsed with sample water at least three times before the samples were collected. Sample containers were filled to the top with no headspace until testing could begin.

   Bacterial samples were taken directly from the source using a sterilized (via 4 Watt, handheld, long-wave UV lamp by Hach (Loveland, CO); contact time of 2 minutes) syringe and were immediately plated onto 3M Petrifilm Coliform Count Plates (3M, St. Paul, MN). Bacterial samples were incubated for 24 hours whenever possible, but strict temperature control was never available. Sometimes samples were incubated under a laptop at approximately 35˚ C (confirmed by thermometer measurement; 35˚ was the desired temperature), and sometimes samples were incubated using a light bulb (though samples were protected from the light using a

barrier and temperature was unable to be measured), as shown in Figure 3. Because proper incubation was not available, petri plates that came back negative cannot necessarily be viewed with confidence, but samples that came back positive are reliable.

Figure 3 – Makeshift incubator for Petri Plates

The PathoScreen Field Kit (Hach Co., Loveland, CO) was used to determine presence/absence of pathogens including *Salmonella, Proteus, Klebsiella, Citrobacter, Clostridium, Edwardsiella*, and other hydrogen sulfide producing organisms that are associated with fecal contamination. Samples for pathogen analysis were collected directly into sample vials, which were pre-sterilized using a bleach solution. Packets of substrate were emptied into the vials, ensuring they did not touch the rims of the bottles to avoid contamination of the samples.

The following chemical parameters were measured using the Hach CEL/890 Advanced Drinking Water Laboratory (Hach Co.) as directed by the supplier : acidity, alkalinity, arsenic, carbon dioxide, chloride, chlorine dioxide, free and total chlorine, color, total dissolved solids, copper, hardness, iron, manganese, nitrate, nitrite, ammonia, pH, dissolved oxygen, phosphate, sulfate, sulfide, suspended solids, temperature and turbidity.

Where possible, samples were analyzed immediately on site, and where that was not possible, they were transported to a more convenient location for testing. Samples were refrigerated between collection and testing whenever possible. Samples of ClO_2, free and total chlorine, and sulfide should have been analyzed immediately but were analyzed later for samples collected from the following sites: Violet Denison (Davao), Robinhood (Bukidnon), and Paradise (Bukidnon). Due to the instability of these compounds, reported measurements may be low. Note that all samples measured for free and total chlorine should have been collected in glass bottles to avoid chlorine chemically reacting with the plastic, but were instead sampled in plastic bottles. Reported chlorine levels may be low as a result.

4. Site Walk-through.

   Sites were visually inspected to determine location of septic tanks with respect to drinking water wells, solid waste management practices, proper maintenance of wells and well spouts, accessibility of septic tanks for pumping, exhaust system placements on septic tanks, canal designs, and other obvious sanitation problems. Residents and

project directors were both asked well depths and septic tank designs in order to confirm design.

The section “Challenges in Data Collection” describes specific lessons learned in the field from a personal perspective.

Results and Implications

Findings – Water

Forty three water sources were sampled for water quality. Of those 43 sources, 31 were untreated wells or springs, 10 were sources provided by the municipality, and 2 were wells being treated on-site. Complete results from the water quality tests are available in Appendix 1 separated by site and source.

Results show that 95% (41 positive / 43 total) of sources sampled tested positive for pathogens in a presence/absence test (indicating presence of *Salmonella, Proteus, Klebsiella, Citrobacter, Clostridium, Edwardsiella*, or other hydrogen sulfide producing organisms that are associated with fecal contamination). The Philippines Environment Monitor (8) reports that the Philippine government's monitoring of groundwater indicates that up to 58% of groundwater sampled is contaminated with coliforms. Sixteen wells sampled in this project (52%) tested positive for total coliforms (the highest concentration is estimated around 115 CFU/mL), and two sites using water provided by the local water district also showed positive for total coliforms (the highest concentration is estimated around 3 CFU/mL). *Escherichia coli* was found in five of the wells or springs (16%), with the highest concentration measured at around 22 CFU/mL. Wells testing positive for *E. coli* were located within a range of less than 5 to 15 meters from the nearest septic tank (though groundwater flow direction could not be determined). No correlation could be made between well depth and presence of *E. coli* due to lack of available information on well depths. *E. coli* was not found in water provided by the local water districts.

Interviews indicate that families are experiencing only 1.2 cases of diarrhea per family per year in GK villages (though this number is an estimation provided by those interviewed), but

the Philippines Environment Monitor states that approximately 31% of illnesses monitored over a 5 year period were caused by water-borne sources (8).

Nitrate levels above the Philippine national standard of 35 mg/L were found in 10 of the 31 untreated, on-site sources, and in 3 of the treated sources (30% of sources measured total), though sites should be retested with a more reliable test. Arsenic at or above the Philippine National standard of 50 ppb was found at one site. Arsenic above the US standard of 10 ppb was found at 9 sites (21%), including sties utilizing both onsite groundwater and municipally supplied groundwater, and sites located in the northern and southern regions of the country. Lowering the arsenic standards in the Philippines would require groundwater treatment for many areas throughout the Philippines.

There was a reported net increase in water availability for 28% of families after they moved to GK villages. At least some family members in 44% of families interviewed were drinking from onsite sources, but only one fifth of those drinking water from onsite sources ever boil the water, even when they are sick, and none of the beneficiaries interviewed use any other treatment method. Approximately 84% of people, including those who treat and buy bottled water for drinking, report that they are satisfied with the water on the sites. Those who were dissatisfied cited inadequate water supply, water access that was inconveniently far away, or the inability to be sure the water was free from contamination. Average water consumption in the Philippines is 120 liters per person per day, though the average person interviewed in a GK village reports drinking 1.4 L of water per day. People are drinking less water in areas where it is necessary to buy treated water for drinking.

Problems and Suggestions – Water

Placement of wells is an important consideration when designing sites, as placement can make wells more or less likely to become contaminated with human waste, agricultural waste, or chemicals transported in rain events. Well placement was not given adequate consideration at any site visited, and in some cases, well placement will likely result in fecal contamination in the future from poorly designed septic tanks located too near the well. Figure 4 is a photograph from GK San Francisco Javier in Batangas. There are three septic tanks (one of which is circled in red) located about 10 feet away from the shallow (10') well on this site. This is an open bottom single chamber septic tank, which could easily contaminate the groundwater. The well tested positive for pathogens and contained 2 CFU/mL total coliforms when tested with Petrifilms.

According to the USEPA, wells should be placed no nearer than 50 feet from the nearest properly designed septic tank or livestock containment, no nearer than 100 ft from compost piles or fertilizer storage, and no nearer than 250 ft from manure stacks and garbage heaps (4). Topography is currently not considered when designing or placing wells, but this should be corrected. Wells should be installed in elevated locations so as to prevent pooling rainwater, which may carry runoff contaminated with coliforms, pathogens or nitrogen, from seeping into wells or shortcutting to the water table via well casings. Many wells were as shallow as 10 ft, allowing for easy contamination from poorly designed, nearby septic systems; wells should be as deep as practically possible, ideally 100 ft or more. Unfortunately, maps depicting topography are not available for most GK sites, and additional funding and manpower would be needed to create maps for use in well placement design.

Figure 4 – GK San Francisco Javier (Batangas). Note the septic tank (circled in red) located too closely to the well.

After installation, wells should be sterilized with a bleach solution (1L bleach: 5L water) to inactivate any bacteria introduced during installation. The 1:5 solution should be poured down the well head, mixed by pumping, and allowed to disinfect for 24 hours. Repeating this procedure occasionally to kill coliforms and other bacteria in the well is good practice.

Many sites' well spouts were very poorly maintained and presented a serious risk of

contamination to collected water. Some spouts had unsanitary additions – old t-shirts, bits of cloth, sections of tires, etc. – used to filter the water or redirect flow into receptacles, as in Figure 5. Other spouts were growing biomass or algae, as in Figure 6, which is a photograph of a well in GK First Calacan (Batangas), which coincided with the highest reported rates of diarrhea of any site visited (12-24 times/person/year). Well spouts must be cleaned occasionally, especially if algae or other biomass is visibly growing on the spout. Filtering materials, likely to be ineffective, should not be added to pump heads; settling should be used instead if necessary to remove suspended particles. If spouts need extensions, they should be carefully cleaned and regularly maintained by the community.

Figure 5 – GK San Francisco Javier (Batangas). Note the spout addition that can harbor bacteria.

Figure 6 – GK First Calacan (Batangas). This well was growing algae and black slimy biomass.

Well placement and appropriate ground cover above the well should also be taken into consideration when building a well. Many wells are centers for washing or bathing in communities, and if this is the case, controlling runoff and ensuring that wash-water does not short-circuit down the well casing are very important. Figure 7 is a photograph of the main drinking water source in GK Shell Libjo in Batangas, above which women conduct their

washing on a daily basis. The water pools above the well and seeps into the ground, percolating to the water table and potentially contaminating their water source.

Figure 7 - GK Shell Libjo (Batangas). Women washing directly beside the well.

Nitrate is another contaminant of concern (especially to small children) in approximately one fourth of the GK wells tested. Many wells in GK sites are located near agricultural plots, and as residents' understanding of appropriate application rates for chemical fertilizers is generally poor, over-application of fertilizers contributes nitrate to groundwater. Figure 8 is a photograph of the well in GK Buhay Nasapa (Batangas), which is placed in an agricultural area. This well is adequately spaced from the nearest septic tank, and the cover over the well

certainly aids in preventing rainwater from collecting nitrates and shortcutting down the well casing, but there is still standing water above the well due to the site's topography. This well did not test positive for nitrate contamination, but this could be because the nitrate test used was ineffective at sites with groundwater also containing chloride, ferric iron, nitrite or water with extreme pH, and this well showed trace levels of chloride, ferric iron and nitrite. This well did test positive for pathogens.

In general, onsite wells and municipal sources (including municipal wells) showed similar levels of nitrates; 29% of onsite wells and 25% of municipally supplied wells were in excess of the standard.

In situations where wells must be placed in agricultural areas, preventing fertilizer from agricultural runoff from entering the well should be a priority, and fertilizer should not be over-applied; whenever possible, organic fertilizer (as from compost) should be used.

Fertilizer based nitrate in conjunction with possible contamination from poorly designed septic tanks is a serious and common problem at many GK sites. While many residents understand the benefits of filtering or boiling their water to remove bacteria, they are not aware of the possible threat from nitrates, which are persistent through soil percolation and difficult to remove at the point of use. Education is critical to ensure that residents understand proper fertilizer use and septic tank and well maintenance. Education of the GK design team is central to improving septic tank design.

Figure 8 - GK Buhay Nasapa (Batangas). This well is located in an agricultural area.

Assessment of Decentralized Wastewater Systems

Gawad Kalinga is responsible for improving sanitation of 28% of its residents since they moved to GK villages, but 13% report an increase of diarrhea since moving to GK villages (for a net improvement of 15%). Several residents who experienced a reduction in cases of diarrhea attributed the change to education and sanitation conditions provided by GK.

Sites visited had the following types of sanitation systems: 60% of residents interviewed were using tanks made of a single chamber with an open bottom; 11% of residents

interviewed were using a two chamber tank, with an open bottom second chamber; 15% of residents interviewed were using tanks that are completely sealed with seemingly no drainage. There was one site using cesspools (pits with lids), and one site installing an anaerobic baffled reactor (ABR). Of all sites visited, only the one with the ABR is a legal system according to the Philippine Plumbing Code (3).

While all residents interviewed knew the location of their septic tanks, 25% of those interviewed did not have any plan for emptying their septic tanks, and only 40% planned to pump their tanks when they become full. Twenty percent of residents plan to build new tanks when the tank reaches capacity, either because they are unfamiliar with pumping or because they believe pumping/cleanout costs more than building a new tank. The remainder of residents interviewed have various other plans, including punching holes in the sides of their existing tanks to drain the solids.

Problems and Suggestions – Wastewater

Single chamber, open bottom septic tanks allow liquid from the tank to percolate downwards through the solids, where it is not properly treated, to join the water table below. Figure 9 shows an open bottom single chamber septic tank under construction at GK Nazareno (Samal Island, Mindanao). This design is dangerous to groundwater supplies because the wastewater percolates through the solids layer, with a greater probability of contaminating the water table with harmful bacteria and nitrates. At a minimum, tanks should be designed with two chambers, the second of which is to be used for leaching. This two-chamber design provides a clarification step, such that at least a portion of the contaminants are maintained in the tank for removal via pumping or cleanout, allowing water that rejoins groundwater to be of a higher quality. A two chamber tank with one chamber for leaching is a vast improvement over current designs at most GK sites, but it is still illegal according to Philippine Plumbing

Code, which requires secondary treatment such as reedbed systems, anaerobic baffled reactors or leaching fields (3).

Figure 9 - GK Nazareno (Samal Island, Mindanao) with open bottom single chamber septic tank.

According to the Philippine plumbing code, effluent from all septic tanks must lead to secondary treatment or a leach field. Properly designed drainage fields, however, were absent in all sites visited. Septic tanks at GK San Juan in Batangas, for instance, are built completely sealed but with no drainage field or secondary treatment. These tanks are installed between rows of houses, so that when they fill up, there will be no other options for

waste disposal except repairing the tanks, which may be prohibitively expensive to the residents. The twenty houses under construction at GK San Juan, all with this configuration, will experience significant problems with their wastewater systems and potentially their drinking water in the future.

Exhaust pipes to vent gases produced in septic tanks were often absent or poorly designed. The anaerobic wastewater decomposition process produces methane, carbon dioxide, and hydrogen sulfide, which must be vented to prevent the gases from forcing their way from the septic tank back up the incoming pipes and into the homes. Exhaust pipes on many of the tanks at many of the sites were absent. These gases can present a serious hazard in the home as methane is explosive and many of the kitchens, located adjacent to the bathrooms, use open flame. Some exhaust pipes are in place but extend to just below the roof of the house, venting the gases inside. Instead, exhaust pipes should extend through the roof. Figure 10 is a photograph of GK Assumption (Batangas), where residents were annoyed with the smell of the septic gases venting into their homes, so they plugged the pipes and covered them with plastic. This will cause methane gas build up inside the tanks. If the pipes were extended through the roofs, this would not be an issue.

Figure 10 - GK Assumption (Batangas). Note the covered exhaust pipes extending to just below the roofline.

In order for septic tanks to function properly, they must be pumped/desludged approximately every five years. Septic tanks at many sites, however, are inaccessible to desludging trucks, as in GK Banyuhay, in Figure 11. Parts of this site are inaccessible to trucks because the site lacks roads in some areas or the roads are not sufficiently wide to allow the passage of a truck. Though some residents would prefer to desludge their tanks when necessary, tanks are sometimes inaccessible even to manual desludging when they are located under the homes. Other residents' tanks were covered by up to a foot of soil with no manholes installed in the tanks, making desludging especially difficult (as in GK Buhay Nasapa in Batangas). As a

result of septic tank inaccessibility combined with a lack of education about septic systems, many of the residents interviewed (40%) plan to build new tanks when theirs fill, but there is not always adequate space even for this option. At GK OKS in Batangas, residents are having problems with their septic systems and are using bathrooms in neighbors' homes as a solution. This solution is unsustainable and could be avoided using appropriate tank placement and site planning. Most of the GK sites visited have been built in the last five years (since 2003), so problems with septic systems have not yet been fully realized by GK designers or residents.

Figure 11 - GK Banyuhay (Manila). Parts of this site are inaccessible to desludging trucks.

Many residents reported that they plan to build new tanks when theirs fill. This result reflects the lack of knowledge of most residents on septic tank operation. Building new septic tanks has very negative environmental consequences (e.g., digging new tanks in the vicinity will expose people to a variety of harmful pathogens and bacteria; the new plumbing will likely not follow proper plumbing code, which could result in further unintentional groundwater contamination). In reality, pumping costs significantly less than building a new tank. Manila Water Inc. provided a quote of 803 Php (approximately US$16) for pumping, though the expense may increase in more rural areas. The best way to ensure that tanks are pumped regularly and that everyone's tanks are pumped is to schedule pumping for the entire village on the same day once every 5 years. In that case the price is likely to be improved by economies of scale. Arranging this pumping schedule should be a concern of someone directly involved in the site (such as the project director), and ideally in the hands of residents.

Teaching the residents how to use their septic systems is very important. A well designed system can be easily rendered useless by residents who do not understand it. Residents should be educated on what is appropriate to flush down their toilets and should understand that they must pump their septic tanks, not build new ones. They should be informed of whom to call, the costs of desludging, how often to desludge, and the purpose of the ventilation pipes leaving their septic tanks. Project directors should follow up with these issues.

Many of the sites visited utilize canals for sink, bathing, washing, or rainwater runoff. Most sites visited have open canals in front of the houses. This necessitates stepping over the canals to enter the houses, and also means there is exposed, often stagnant graywater (because of insufficient flow or blockage) in canals in front of the homes. The residents of

these homes usually wash and bathe behind their houses where there are no canals, leaving the excess graywater to run off to the surrounding land. This runoff compromises foundations of homes downhill and provides a breeding ground for mosquitoes. Figure 12 is a photograph of GK Malaybalay (Mindanao), where washing and bathing water run off to the landscape and make land behind the houses marshy. This scenario is common in GK villages and could easily be solved by relocating the canals from the front to the back of the houses.

Since most villages are set up with two rows of back-to-back houses, these two rows of houses must build two canals which are not maximally effective since they do not catch all of the household runoff. It makes more sense to instead build one canal between the two rows behind the houses to catch the water used for washing and bathing. This will not only improve the appearance of the fronts and backs of the houses, but will lower the cost of village construction.

Figure 12 - GK Malaybalay (Mindanao). Note that washing and bathing water run off to the landscape.

Several sites also had poorly designed canals. In addition, canals at all sites eventually ended at the GK property line, which means neighbors receive untreated graywater from GK sites. This is socially irresponsible and sets a bad example to neighboring communities. Water from canals should be treated before release. This can be accomplished in a variety of ways,

for example, by routing the water through circles of banana trees or other types of biological treatment (such as reedbed systems). Ensuring that the water does not run the path of least resistance into neighbor's lots is also important. Canals should be continued until village water reaches a reasonable deposit location (i.e., surface water or unused land). Figure 13 illustrates the canals at GK Brookside (Manila). The canals effectively divert water from the GK site, but the runoff moves untreated into neighbors' lots.

When water from GK sites is not effectively captured in canals and is instead allowed to runoff to the surrounding land, it degrades the neighboring property and creates breeding grounds for mosquitoes and other disease vectors.

Figure 13 - GK Brookside (Manila). Site runoff moves untreated into neighbors' lots.

Challenges in Data Collection

This section seeks to enumerate specific lessons learned in the field from a personal perspective, and will hopefully be helpful to others seeking to do field work in a similar capacity.

The language barrier was a constant challenge in the interviewing process. Most project directors spoke English, and if not, there was always a resident available who could translate. By the end of my six months, I could usually recognize answers and determine if the question or response had been translated correctly. Questions were always asked the same way in an attempt to remove bias, but may not have always been translated the same way. Project directors were sometimes uninformed about their sites, and sometimes beneficiaries were also uninformed about well depths and septic tank designs. Only confident responses were analyzed in the results, and it is possible that the residents or project directors were wrong, making the results unreliable. There were no site plans available for analysis.

Project directors always accompanied me to the sites and were often under time pressure, so generally only eight beneficiaries were interviewed at each site. In some cases, site populations were smaller than 8 families, and in that case only one family or a small group was interviewed. Otherwise, each beneficiary interviewed represented a different household. Usually beneficiaries interviewed were women, but gender was noted in the results and is not expected to present a bias.

Water quality sampling presented a continuous challenge throughout the data collection period. The Hach portable laboratory equipment, though excellent in its versatility and thoroughness, weighed approximately 40 lbs and was difficult to transport long distances,

making commuting difficult. The kit also used many harmful or toxic chemicals, so extra care had to be taken in transporting the kit to ensure that no accidental harm was caused to passersby. Other people would frequently and generously try to take the case to help carry it, but I was reluctant to allow their assistance in an effort to not expose them to harmful chemicals. This sometimes presented a minor cultural barrier. Water sample analysis was also time consuming - approximately 2-3 hours was required per source, so analysis on site was possible only occasionally depending on time availability of the project director. When onsite analysis was impossible, samples were transported to a more convenient location and analyzed there. Occasionally multiple wells were sampled in one day, and analysis all in the same day was impossible. When possible, samples were refrigerated during the lag time. Several tests in the analysis produced toxic chemicals, and there was no appropriate disposal method available, so despite significant efforts to find a better disposal method, waste was either spread over an unused portion of grass or dumped down the drain with excess flush water where wastewater treatment facilities were available.

Bacterial samples were always taken directly from the source, but in the case of Petrifilm samples, incubation at 35°C was required for 24 hours after sampling. There was never a reliable incubator available, but when my laptop was available, I stored the samples under it, where heat from the battery acted as incubator, and though exact incubation temperature is impossible to say, it was likely around 35°C. Some samples were incubated using a bag taped around a light bulb. Samples were padded from the light with a number of napkins to prevent light interference. Occasionally no incubator was available or able to be improvised. Incubation did not seem to influence growth significantly as the average temperature of the sites I visited was approximately 27°C.

Some of the tests in the Hach kit showed significant interference from other chemicals. Unfortunately, the test for nitrate-nitrogen was among them. Any amount of iron or nitrite interfered with the results, which, for the same sample, often varied between 0 mg/L NO_3-N and 35 mg/L NO_3-N (the upper limit in measuring capacity of the instrument). I was able to confirm that the equipment was reliable in the absence of interferences by using a sodium nitrate standard and testing it using the kit, which provided extremely accurate results. More tests should be done to determine true levels, which appear to often exceed WHO and Philippine national standards of 50 mg NO_3/L (11.3 mg/L NO_3-N).

Implications and Suggestions for Further Research

Continued monitoring of groundwater at GK sites and throughout the country is critical to ensuring people have safe drinking water. A more reliable nitrate test should be used in the future, as the Hach test provided gave unreliable results when used to test water containing chloride, ferric iron, nitrite or water with extreme pH (6). Monitoring nitrate in the groundwater provides a good indicator of groundwater contamination by fertilizers or human waste.

Since GK is one of the organizations making the most headway in improving the lives of those in the poorest areas of the Philippines, the findings from this study will ideally be used to broadly improve design and construction practices, and to help prevent current problems from occurring in future villages. The findings of this study will potentially impact the lives of thousands more in the Philippines as GK plans to build more villages in the next few years.

To help ensure that the goal of improving water supply and sanitation design becomes a reality, a follow-up study is recommended. Continued involvement in Gawad Kalinga is the

most effective means of ensuring villages are built to protect the water-environment and architects are sufficiently educated about water and sanitation design and planning.

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Chapter 2: Molecular Analysis of Centralized WWTPs in Metro Manila

Introduction

Domestic wastewater is comprised mostly of undigested food remnants, including an array of organic compounds and bacterial cells (4). Industrial wastewater can also include heavy metals and organic compounds that do not occur naturally. Bacteria are present in fecal matter in very high numbers (10^9 cells/gram fecal matter), both in healthy individuals, where the bacteria are mostly harmless, and in individuals carrying harmful bacteria, viruses, and intestinal parasites (4). Wastewater must be treated to destroy the harmful organisms in the waste before the water can be sent to receiving bodies, and though disinfection is part of the wastewater treatment process, WWTPs are not designed with this as the primary aim. Instead, treatment plants are designed to remove organic and inorganic compounds such that the wastewater can be safely unloaded into a receiving body without destroying the self-purification properties of that receiving body (4,10). Untreated wastewater sent to surface water will cause serious and potentially irreversible changes in the ecology of the receiving body of water. Organic compounds serve as substrates for bacterial growth, and this growth requires oxygen, which will deplete the water of oxygen needed for plant and animal life, leading to the subsequent death of animal and plant life in the water. Nitrogen and phosphorus present in wastewater stimulate the growth of algae and cyanobacteria, which often are accompanied by toxin production and stagnation of the water, a process known as eutrophication (4).

Societies have practiced wastewater treatment for thousands of years, but modern wastewater treatment originated in England during the industrial revolution in response to rapid urbanization and subsequent health problems, including typhoid and cholera, resulting from untreated human waste (4,10). These systems operate under the principle that microbes

metabolize organic and inorganic compounds for growth, thus removing these compounds, which would also act as substrates in the natural environment. This process creates a microbial community in the wastewater which can then be settled out and separated from the wastewater in a process known as clarification.

Environmental engineers use microbial communities in combination with physical processes as tools to degrade organic and inorganic compounds in wastewater. The activated sludge process involves the introduction of wastewater to an established microbial community in a completely mixed basin (aerated mechanically), resulting in 1500-2500 mg/L of suspended solids (10). The design of the aeration basin allows for appropriate contact time (also known as hydraulic retention time, typically 4-8 hours) before the wastewater flows to a clarification step from which some of the settled solids are sent back to the completely mixed basin and the rest of which are wasted. Clarified water moves on to disinfection and the effluent is then sent to receiving bodies of water. The solids recycle is central to the activated sludge process, as it helps maintain the microbial community in the waste treatment process and prevents slow-growing organisms from being washed out during the clarification step. The solids recycle also controls the F/M ratio (further explained below). See Figure 14 for a schematic of the activated sludge process.

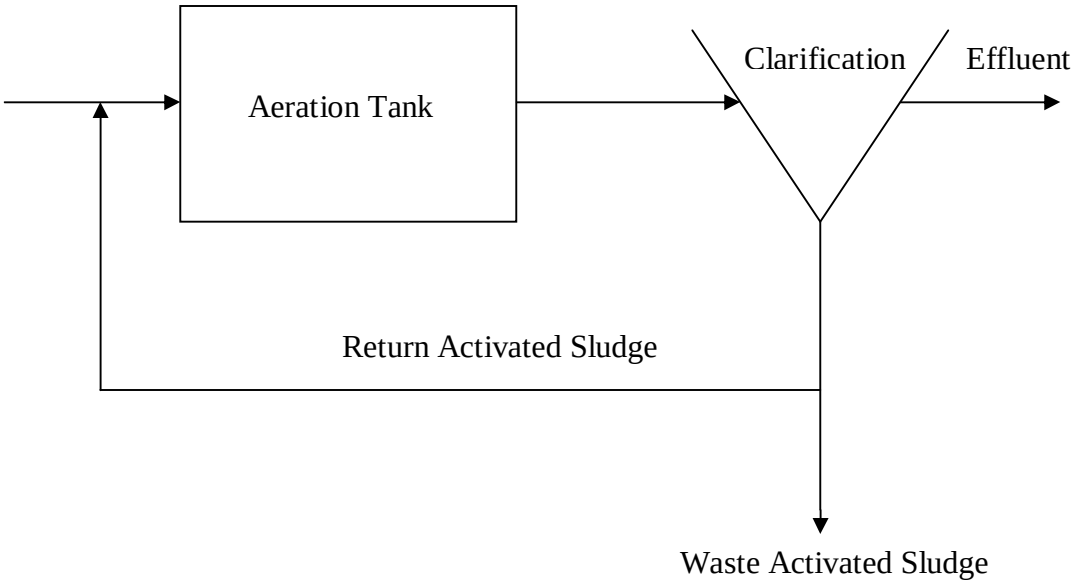

Figure 14 - Schematic of Conventional Activated Sludge Process

Whether or not a compound is metabolized in the activated sludge process depends on whether or not organisms capable of degrading that compound are present within the system. Since microorganisms are responsible for the removal of biochemical oxygen demand and the nutrients nitrogen and phosphorus, it is essential to understand the substrate and nutrient removal efficiencies of these organisms in order to design and operate treatment plants and meet regulations.

The following parameters are used for monitoring and control of activated sludge WWTPs.

a) Mixed liquor suspended solids (MLSS) and mixed liquor volatile suspended solids (MLVSS)

   The term “mixed liquor” refers to the content of the aeration tank. MLSS measures

the concentration of inorganic and organic material in suspension in the mixed liquor, including microorganisms, and is typically maintained around 2500 mg/L. MLVSS is considered the organic component of MLSS, normally comprising approximately 80% of the MLSS. MLVSS includes nonmicrobial organic matter as well as live and dead organisms and cellular debris, and thus does not necessarily correlate with the active portion of the biomass (10). MLSS and MLVSS parameters are functions of influent loading, volume, and solids retention time (4).

b) Sludge loading, also known as food to microorganism (F/M) ratio:
The F/M ratio is an indication of the organic load in the activated sludge system and is calculated as follows:

$F/M = (BOD_5)/(\text{hydraulic retention time (days)} * MLSS)$
Units: g BOD_5/(day*gMLSS)

A low F/M ratio indicates that less substrate is available for biomass production, resulting in reduced biomass production per unit of organic loading and an overall reduced volume of sludge requiring disposal. As solids handling comprises approximately 50% of operation costs of a WWTP (11), reducing solids production reduces operational costs significantly (7,10). Floc quality is generally improved with lower F/M ratios, because maintaining low F/M ratios requires solids retention times to be high enough that there is time for flocs to form. Maintaining a high F/M ratio, however, results in high MLSS, which in turn increases the oxygen requirements because of the increased demand for oxygen in aerobic digestion of sludge, which adds expense.

The F/M ratio is controlled by the sludge wasting rate; a higher wastage rate will

decrease sludge age and increase F/M ratio. Conventional plants operate with ratios of 0.1 to 0.4 g BOD5/day/g MLSS (4).

c) Solids retention time (SRT)

Solids retention time is also known as mean cell residence time (MCRT) or sludge age. While hydraulic retention time (HRT) may be on the order of a few hours, SRT normally ranges from 5-15 days, depending on the season (10). SRT is considered the central parameter in activated sludge design, but many plants still do not precisely control this parameter. SRT is calculated as follows:

SRT = total amount of sludge solids in the system/rate of loss of sludge from the system

d) Other factors influencing performance

Temperature, oxygen supply, and aeration period can all affect growth rates and can select for different organisms. Though the microbial community in activated sludge systems is constantly in flux, any time the microbial community changes significantly, plant performance may also change.

When considering the microbiology in activated sludge, it is also important to remember that the mixed liquor is arranged into flocs, which are heterogeneous by nature. Flocs are complex structures which provide microenvironments for organisms such that organisms that thrive within a floc might not survive outside of it. Spatial location within the floc determines an organism's access to substrate, oxygen and other important nutrients, allows for protection from toxins in the system, and also determines whether or not the organism will be retained in the system after clarification (4). Floc organization also determines settling ability of the activated sludge during clarification. Settling is important for retaining organisms within the system, so proper floc formation is of central importance in ensuring

appropriate operation of WWTPs. Understanding the organisms present in the flocs and which organisms cause problems with settling aid in determining how WWTPs are designed and operated.

Microbial Populations in WWTPs

Despite the fact that activated sludge WWTPs have been in operation since the early part of the twentieth century, still very little is known about the specific microbial populations present in activated sludge (4). This is likely due to a variety of reasons: because the plants have been largely designed and operated by engineers who until recently designed the plants as chemical rather than biological processes, because the microbial communities are so complex as to ward off even microbial ecologists (4), and because, until recently, methods employed for the analysis of activated sludge samples were unable to accurately characterize the community (7). In any case, understanding microbial communities present in WWTPs is of vital importance to ensure these systems operate efficiently.

Microbial Ecology

There has been a recent surge of studies that attempt to correlate microbial diversity with ecosystem functionality and stability (14). Stability can refer to three things: resistance (ability to resist effect of a disturbance), resilience (the ability to recover after the introduction of a disturbance) and constancy (the ability to maintain the same microbial community through time) (15).

There are three contradicting hypotheses relating community diversity with ecosystem stability. The first is that increasing community diversity also increases ecosystem stability, because diverse communities are less susceptible to invasion by exotic species and better able to cope with toxins; research supports this hypothesis (15,18,19). The second

hypothesis is that increasing species diversity reduces the equilibrium of species within the system and leads to lower local stability, as a shock to the system will select for organisms that are present and resilient, and thus that species will assume dominance; research supports this hypothesis as well (15). Research also supports a third hypothesis that functional group diversity (16) and/or functional group composition are responsible for system stability (16,17). Research supporting each of these hypotheses was performed on terrestrial and microcosm ecosystems.

Only recently have studies been published that attempt to correlate microbial diversity with system stability in biological WWTPs. Research in this area also produces contradictory results. Several studies confirm that increasing system diversity increases system stability: Von Cantein et. al (20) report that biofilms with a higher diversity of species are more resilient to toxins than those with lower diversity; Eichner et. al (21) report that introducing genetically engineered organisms for degrading specific toxic compounds increased the resilience of bioreactors.

McCann (22) attempts to explain the increased stability associated with increased species diversity as one of two mechanisms. First, systems with high species diversity are more likely to contain species capable of coping with a variety of toxins, such that when a toxin is introduced, some species within the population will survive the introduction. A second theory is that system stability could also be attributed to functional redundancy, that is, that various species within the system are capable of performing the same function, and that when one species is selected out of the system, other species are able to compensate (22). This model is particularly useful when viewing activated sludge WWTPs, because in the activated sludge basin, most organisms present are aerobic heterotrophs seeking organic matter. In this scenario, maintaining high diversity within the reactor allows for many species to fill the same niche. These two concepts combined are known as the insurance hypothesis for

biodiversity (22).

Other studies performed on biological WWTP reactors indicate that increased species diversity does not lead to greater system stability (27, 28, 29), particularly in methanogenic bioreactors.

In total, these studies indicate that some microbial communities in WWTPs, particularly in those sections of WWTPs with aerobic processes, are more stable with respect to resiliency with increasing diversity, while in other microbial communities also found in WWTPs, increased diversity may not increase (or may even decrease) system stability.

Identifying the microbial community structure at any WWTP is helpful in terms of characterizing the plant and recognizing when changes to the microbial community have taken place. Acknowledgement of changes in the microbial ecology of a treatment plant allows for better understanding of the unit processes and their operation or failure, and allows for recognition of the likelihood that toxins have been introduced into the system. It may also help in identifying problems which cause bulking and foaming.

Molecular Methods

Microbial populations in activated sludge were historically studied by cultivation, but cultivation techniques have been criticized for their inability to accurately characterize the microbial community in terms of dominant and/or important species relating to treatment plant function or malfunction (4, 6, 7). Because of inherent biases associated with culturing organisms, cultivation methods have been replaced by molecular methods that permit the identification of organisms without the use of isolation and cultivation (4, 6, 7, 9).

To appropriately infer relationships among diverse species, it is necessary to consider genes that have been conserved throughout evolutionary history, such as those that define ribosomal RNA (rRNA). The 16S gene, made up of 1542 nucleotides and located on the small unit of the ribosome, is the most conserved gene in all cells, meaning portions of rRNA from distally-related organisms are still remarkably similar (4). The 16S gene has hypervariable regions, where sequences have diverged evolutionarily, but also has highly conserved regions, which are extremely similar among all species (4). Because of this similarity, sequences of different organisms can be aligned and the differences measured to illustrate evolutionary relatedness among species (4).

It should still be noted, however, that the use of non-culture-based methods including polymerase chain reaction (PCR) techniques is also not without its limitations. Bias may still be present because of biases or inefficiencies in DNA extraction or PCR amplification.

Population Breakdown

Many efforts have been made to characterize microbial populations in activated sludge. Many studies performed using 16S rRNA molecular techniques to determine the microbial populations present in wastewater treatment reactors in the United States and Europe have determined that β-Proteobacteria make up the most significant portion of microorganisms found in activated sludge (1,5, 6, 7, 8, 10), but because many of the organisms found in clone libraries have not been cultured, details of the organisms found remain largely unknown.

Figure 15 shows the microbial community structures found in two surveys, one from WWTPs in the United States, and one from WWTPs in Europe. The community structures shown represent averages of many samples taken in many different WWTPs (7 foaming in the US, 9 nonfoaming in the US, 8 nonfoaming in Europe), including full-scale WWTPs and several configurations of lab-scale reactors (1,2). Two of these studies show that β-

Proteobacteria comprise the majority (approximately 24%) of the microbial population present in foaming WWTPs in the US and the plants sampled in Europe, while in non-foaming plants in the US, α-Proteobacteria and β-Proteobacteria are both dominant, each at approximately 24% of the total microbial population (1,2).

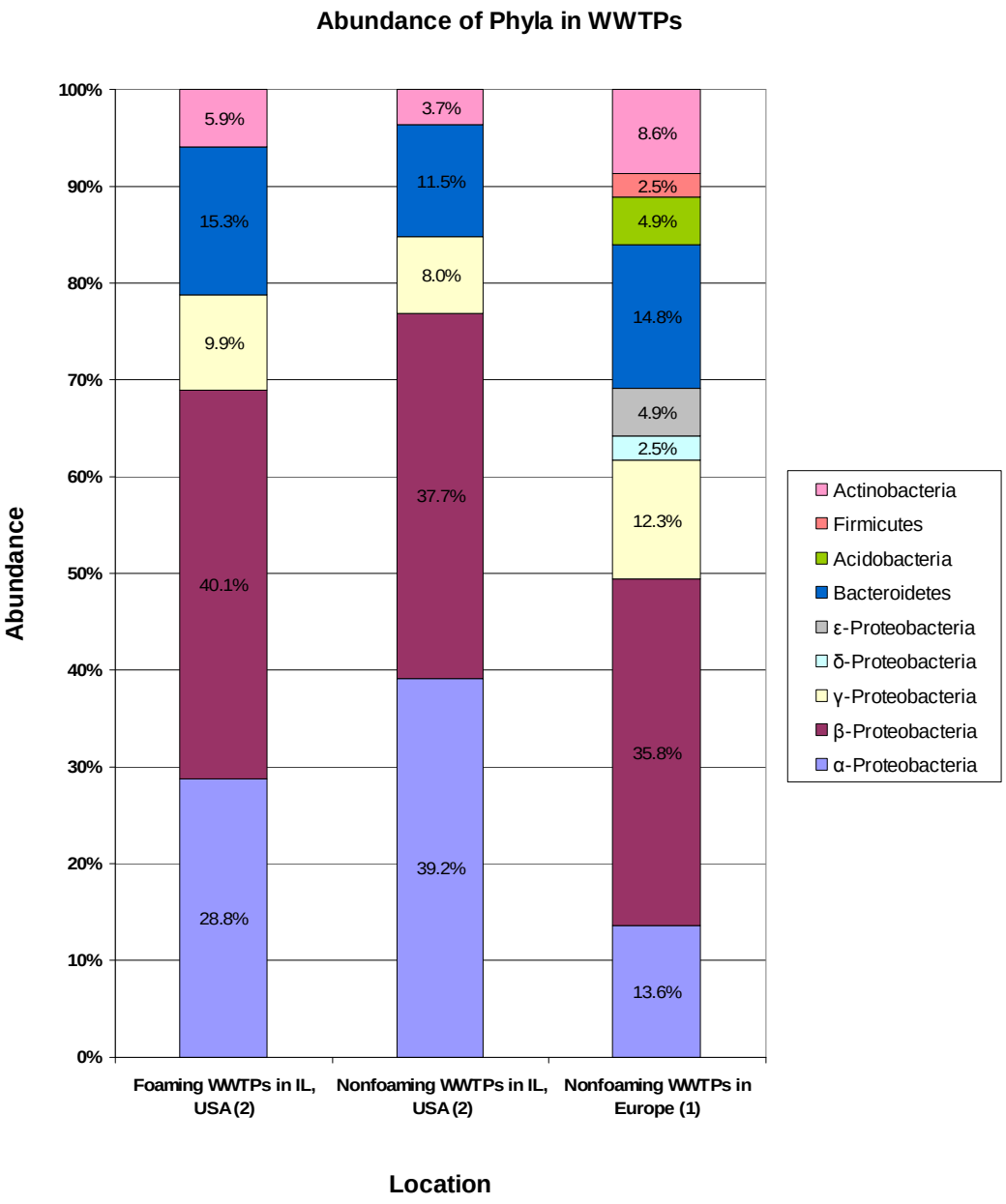

Figure 15 - Bacterial Communities in WWTPs (1, 2)

Studies that reported the use of molecular methods to characterize the microbial populations in WWTPs located in tropical countries were not found.

Sampling of WWTPs

This project was designed to analyze the bacterial populations present in WWTPs in Metro Manila, Philippines, with the goal of comparing the microbial community structure of tropical WWTPs to those found in temperate countries. This study aims to further the knowledge base used for the design and operation of WWTPs in tropical climates.

Manila Water Company, Inc. (MWCI), a private concessionaire that took over the sewerage functions of the government run Metropolitan Waterworks and Sewerage System, operates some 40 WWTPs in Metro Manila (3). The data reported here for the chosen WWTPs are from MWCI, collected as part of their normal testing. The WWTPs rely on central lab test results for parameters such as BOD_5 and solids. These tests are not performed daily, which means adjustments to the operation of the plants cannot be made quickly, and that effluent quality may fall outside of standards for many days before it can be corrected. MWCI also reports that operators are apparently more interested in effluent characteristics than in the processes themselves, indicating a lack of understanding of the principles of the unit treatment processes and their effect on effluent quality. Only pH and SV_{30} (30 min settled volume, measured using the Imhoff cone) can be measured on site, but no database of this information is maintained and pH meters are rarely, if ever, calibrated. COD and SS cannot be measured onsite. Dissolved oxygen (DO) measurements are not performed (though a handheld measurement device is available at some plants), and there are no direct flow measurement devices in the treatment plants, meaning flows must always be estimated and are therefore not strictly controlled.

MWCI is interested in measuring flows for influent, effluent, return and wasted activated

sludge, SV_{30}, MLSS, MLVSS, DO, pH, influent and effluent water quality parameters required by law (including BOD), alkalinity and nutrient (nitrogen and phosphorus) loadings. Acquiring the equipment to perform these tests and educating operators and managers on how to correctly perform the tests will probably take several more years; these data are not available for most of the plants from the sampling period (January to June, 2008).

Three WWTPs were chosen for this study: Makati South (MKS), Phil-Am (PHM), and University of the Philippines (UP). Their locations are indicated in Figure 16 and Figure 17.

Figure 16 - Location of WWTPs sampled (Map provided by Google Maps)

Figure 17 - Location of WWTPs in Metro Manila (Map provided by Google Maps).

The straight-line distances between the plants are as follows: Makati South (MKS) to Phil-Am (PHM) 8.3 miles, PHM to University of the Philippines (UP) 1.6 miles, UP to MKS 9.1 miles (11).

These three plants, Makati South, UP, and Phil-AM, were specifically chosen for sampling

and analysis from among the approximately 40 WWTPs operated by Manila Water Company, Inc. The Makati South WWTP was chosen because it is the largest treatment plant in the country with a 10.5 MGD capacity. Phil-Am WWTP was chosen because it utilizes a unique air lift coarse bubble diffusion aeration system. The UP WWTP was chosen because it serves the University of the Philippines campus and thus has different influent characteristics than the other two plants, which serve residential areas. Schematics and details of the plants are described below in as much detail as Manila Water could provide.

University of the Philippines Wastewater Treatment Plant

The UP WWTP has a 1.8 MGD capacity and serves approximately the green areas within the red circle in Figure 18.

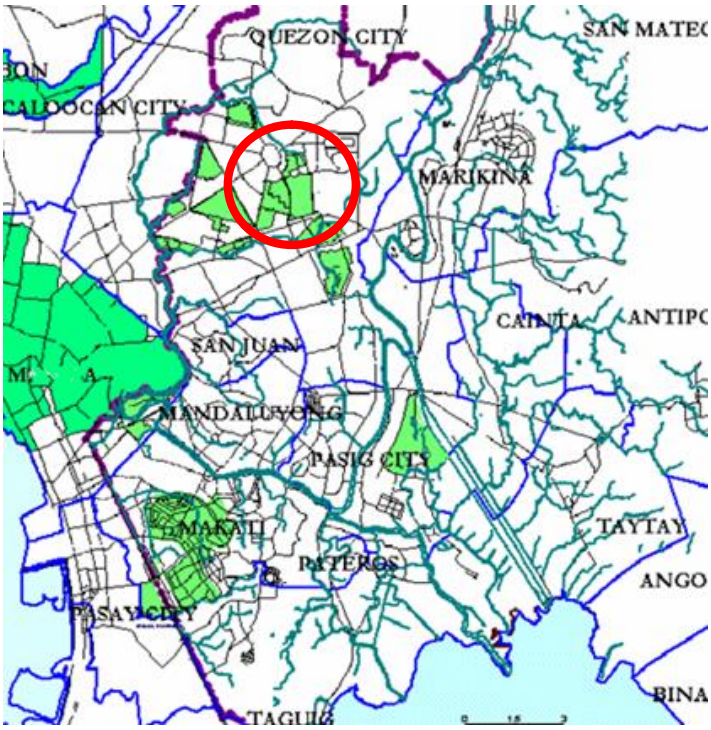

Figure 18 –The green areas inside the circle are served by UP WWTP (3).

Figure 19 and Figure 20 below show the progression of wastewater and solids through the UP WWTP.

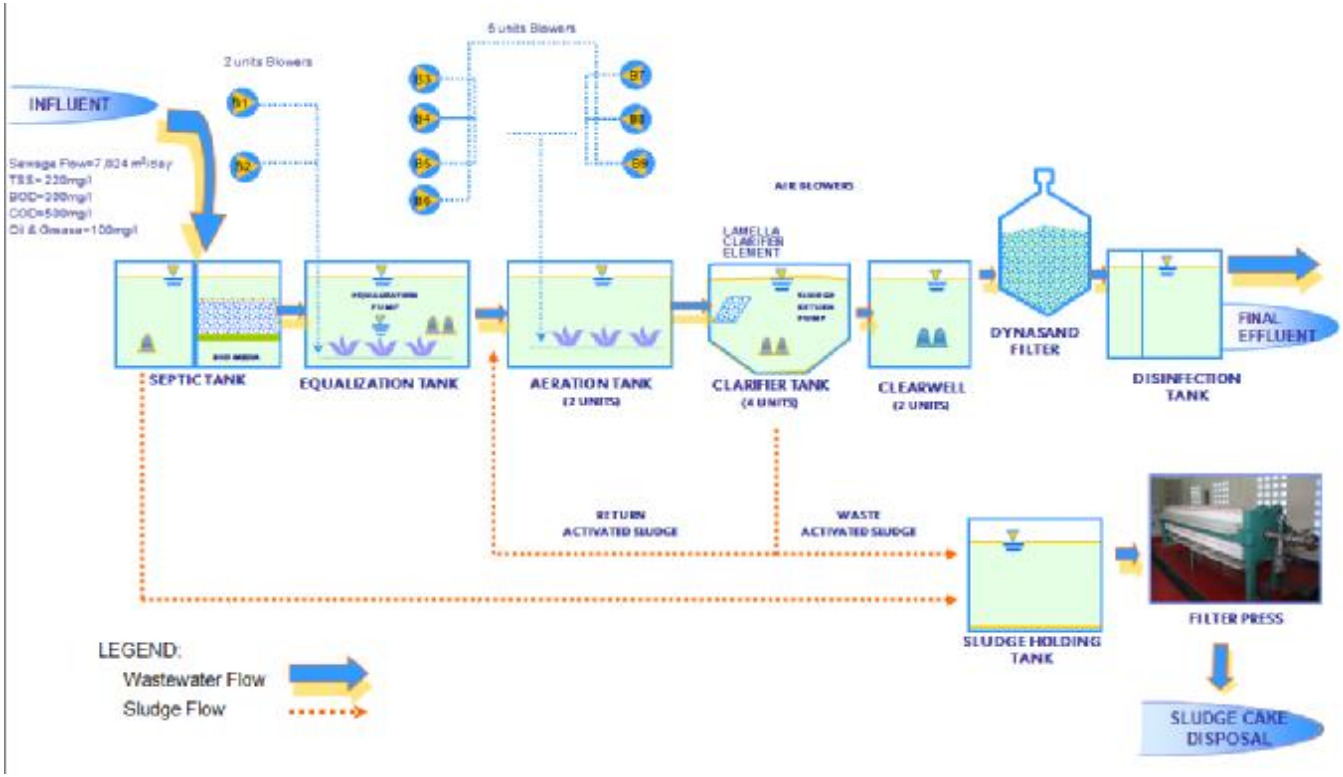

Figure 19 - Detailed schematic of UP WWTP (3).

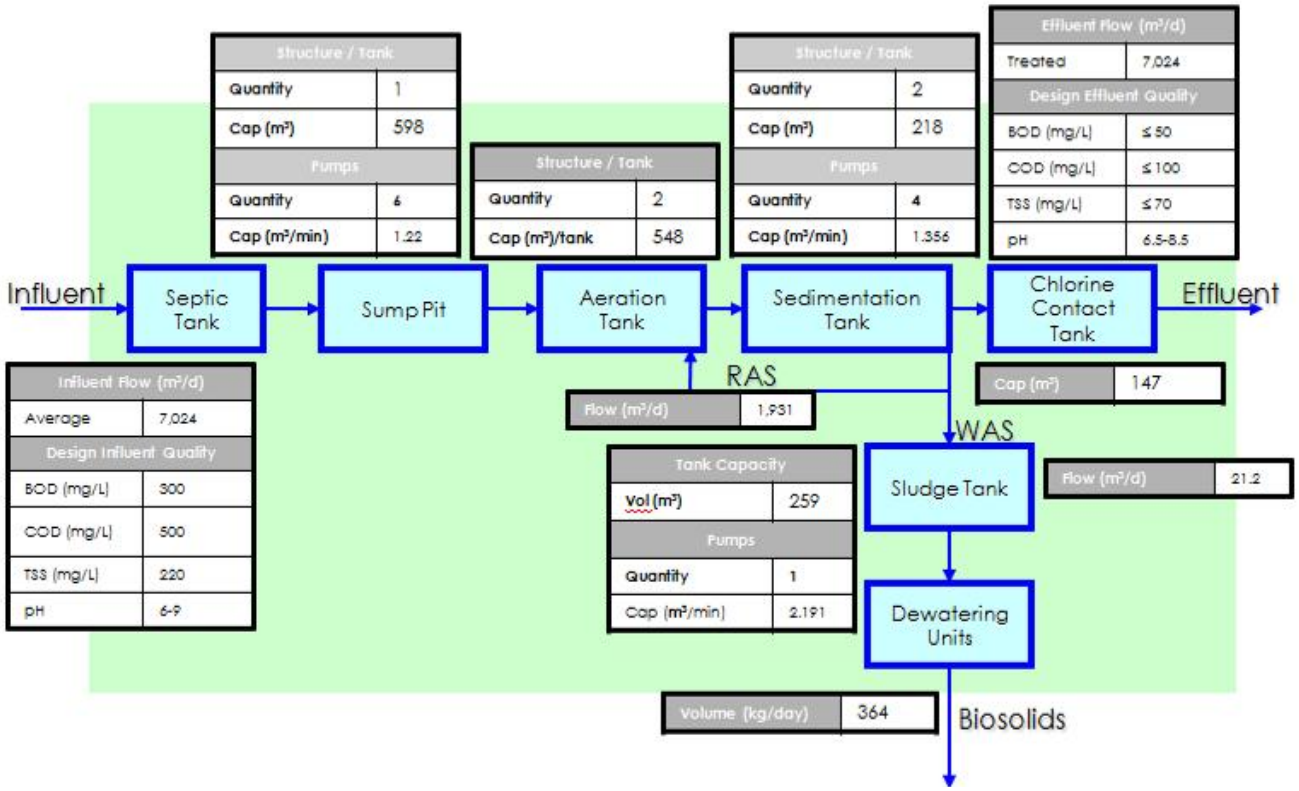

Figure 20 – Parameters used to design UP WWTP (3).

Influent enters into a septic tank, which acts as both a settling chamber and a chamber for the unmixed, anaerobic digestion of solids. The wastewater then flows through biomedia and moves to an equalization tank, which is a mixed chamber which helps control the flow rates throughout the plant. From there, the flow continues to the aeration tank, and then to the clarifier. At this stage, solids are separated from the liquid stream by settling, and the solids stream is divided: some portion of the solids is recycled back to the aeration tank and the remainder of the solids is wasted. The wasted solids move to a sludge holding tank and then are sent to a filter press which makes sludge cake for disposal. After clarification, the liquid stream progresses to a clearwell, which acts as a hydraulic control to regulate flow leaving the clarifier before it is pumped to an upflow Dynasand filter. The Dynasand filter is a sand filter capable of treating water to remove algae, turbidity and microorganisms (13). The effluent is finally sent to a disinfection tank before release into surface water.

Manila Water reports that WWTPs with septic tanks commonly experience odor problems, but did not specify whether the UP WWTP has these problems. No odors were observed during sampling.

Figure 20 illustrates the parameters used to design the UP WWTP.
Table 1 - Design and Measured Values (April to December, 2008) for UP WWTPbelow provides measured values from April to December, 2008. Note that these values are significantly lower than design values.

Table 1 - Design and Measured Values (April to December, 2008) for UP WWTP (3)

Influent	**units**	**Design Value**	**Measured Value**
Flow Rate	m^3/d	7024	4002
BOD	mg/L	300	64
COD	mg/L	500	84
TSS	mg/L	220	75
Effluent			
BOD	mg/L	≤ 50	7
COD	mg/L	≤ 100	16
TSS	mg/L	≤ 70	8

The UP WWTP receives low BOD in the influent (approximately 60 mg/L). To maintain a good F/M ratio, the plant operates with a low MLSS (normally around 1150 mg/L, though sometimes as low as 800 mg/L, compared with the typical 2500 mg/L). A low influent BOD requires the maintenance of low MLSS.

If the total tank volume of 1174 m^3 and 80% of that is utilized, then reactor volume is essentially 940 m^3.

HRT is calculated then as follows:

$$\text{HRT} = \text{Volume/Flow rate}$$

$$\text{HRT} = 940\ m^3 / 4000\ (m^3/d)$$

$$HRT = 0.23 \text{ days } (5.6 \text{ hours})$$

F/M ratio then is calculated as follows:

$$F/M = BOD/(HRT*MLSS)$$

$$F/M = 64 \text{ mg/L} / (0.23 \text{ d} * 1150 \text{ mg/L})$$

$$F/M = 0.24 \text{ d}^{-1}$$

This correlates with the reported values of F/M ratio (0.2-0.5 d^{-1}).

Maintaining MLSS at 800-1100 mg/L, however, means that the air supply (fine bubble diffusers), which operates continuously, often provides more oxygen than the microorganisms require. The over-supply of oxygen explains the high DO concentrations, sometimes reaching as much as 5 mg/L (though typically concentrations in WWTPs are on the order of 2 mg/L). Reducing air flow would save economic resources while simultaneously maintain the oxygen concentrations at levels typical in WWTPs, perhaps changing the microbial community and producing more reliable effluent. This could be done by reducing the number of blowers in operation at any time, or by replacing the blowers with a more flexible air supply.

Table 2 below summarizes operational data available for UP WWTP.

Table 2 - Available operational data for UP WWTP

		Reported (3)			Calculated
		low	*high*	*average*	
HRT	hr	not reported	not reported	-	5.6
SRT	d	5	12	-	-
MLSS	mg/L	800	1600	1150	-
F/M Ratio	d-1	0.1	0.6	0.3	0.24

PhilAm Wastewater Treatment Plant

The PhilAm WWTP has a 0.5 MGD capacity and serves approximately the green areas within the red circle in Figure 21.

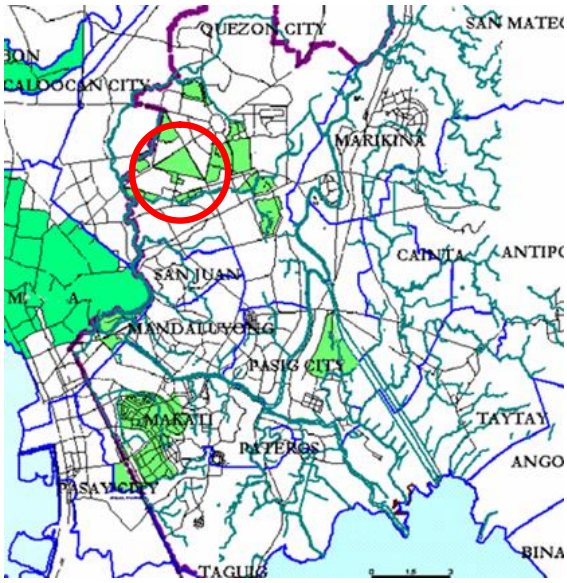

Figure 21 - The green areas inside the circle are served by PhilAm WWTP (3).

Figure 22 below shows the progression of wastewater and solids through the PhilAm WWTP.

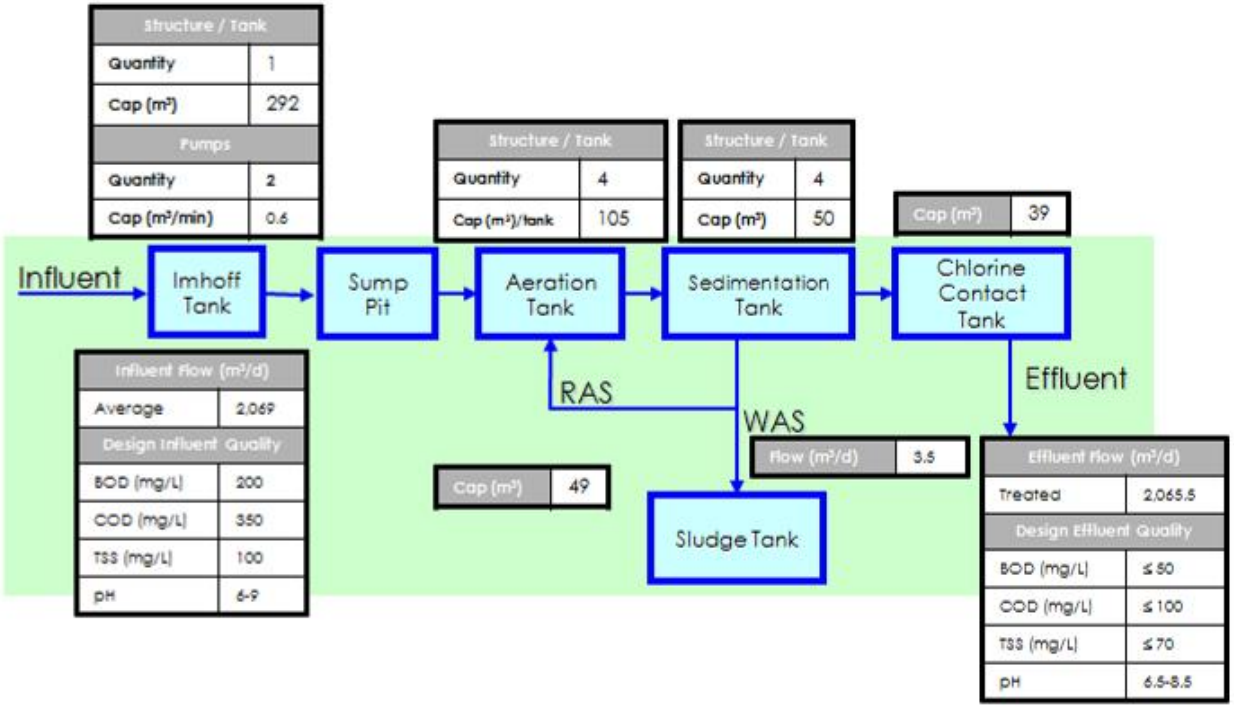

Figure 22 - Parameters used to design PhilAm WWTP (3).

The flow through the PhilAm WWTP is as follows: influent enters into an Imhoff tank, which removes solids by settling (and partially digests the solids anaerobically). Wastewater then flows to a sump pit from which it flows to the aeration tank and then to the sedimentation tank. From there, the liquid stream flows to a chlorine contact tank from which the flow is released to surface water. Solids are collected from the sedimentation tank, and those that are not recycled as RAS to the aeration tank are sent to a sludge tank. Though information about sludge processing is not clear from information provided by MWCI, it is suspected that the sludge tank is a holding tank and that the sludge is taken to a septage treatment facility for dewatering and land application.

MWCI could not provide a more detailed schematic for the PhilAm WWTP. Figure 22 illustrates the parameters used to design the PhilAm WWTP. Table 3 below provides measured values from April to December, 2008. Note that these values are significantly lower than design values.

Table 3 - Design and Measured Values (April to December, 2008) for PhilAm WWTP (3)

Influent	**units**	**Design Value**	**Measured Value**
Flow Rate	m^3/d	2069	715
BOD	mg/L	200	146
COD	mg/L	350	197
TSS	mg/L	100	100
Effluent			
BOD	mg/L	≤ 50	11
COD	mg/L	≤ 100	24
TSS	mg/L	≤ 70	10

The PhilAm WWTP receives low BOD in the influent (approximately 145 mg/L). A low influent BOD requires the maintenance of low MLSS because of the reduced influent substrate, but in order to maintain a good F/M ratio, MLSS must be further reduced. To ensure a good F/M ratio, the plant operates with a lower than average MLSS (normally around 1800 mg/L, though sometimes as low as 500, and sometimes as high as a 2500 mg/L; WWTPs typically operate around 2500 mg/L).

If the total tank volume is 420 m^3 and 80% of that is utilized, then reactor volume is essentially 336 m^3.

HRT is calculated then as follows:

$$\text{HRT} = \text{Volume/Flow rate}$$
$$\text{HRT} = 336\ m^3 / 715\ (m^3/d)$$
$$\text{HRT} = 0.48 \text{ days (11.4 hours)}$$

F/M ratio then is calculated as follows:

$$\text{F/M} = \text{BOD}/(\text{HRT}*\text{MLSS})$$
$$\text{F/M} = 146 \text{ mg/L} / (0.48 \text{ d} * 1800 \text{ mg/L})$$
$$\text{F/M} = 0.17\ d^{-1}$$

MWCI reports an average F/M ratio of 0.3 for samples measured from April to December, 2008, with a goal range of 0.2-0.5 d^{-1}. This calculation suggests that actual F/M ratios may

be shy of this goal.

The aeration tank at PhilAm utilizes coarse bubble diffusers which pass through draft tubes. This circulates the mixed liquor through the draft tubes via airlift action, allowing for simultaneous mixing and aeration. In practice, however, oxygen transfer is low, and typical residual dissolved oxygen concentrations in the mixed liquor are around 0.35 mg/L. This low DO content also explains the dark color of the sludge, an indication that fermentation is taking place. Changing the aeration system to one that provides an adequate oxygen supply (2 mg/L dissolved oxygen) would change the microbial population present (and perhaps improve plant performance), but would also increase sludge production.

Table 4 summarizes available operational data for PhilAm WWTP.

Table 4 - Available operational data for PhilAm WWTP

		Reported (3)			**Calculated**
		low	*high*	*average*	
HRT	hr	not reported	not reported	-	11.4
SRT	d	5	12	-	-
MLSS	mg/L	500	2500	1800	-
F/M Ratio	d-1	0.06	0.7	0.3	0.09

Makati South Wastewater Treatment Plant

The Makati South WWTP has a 10.5 MGD capacity and utilizes 73 km of sewer networks to serve a total area of 600 ha (approximately the green areas within the red circle in Figure 23).

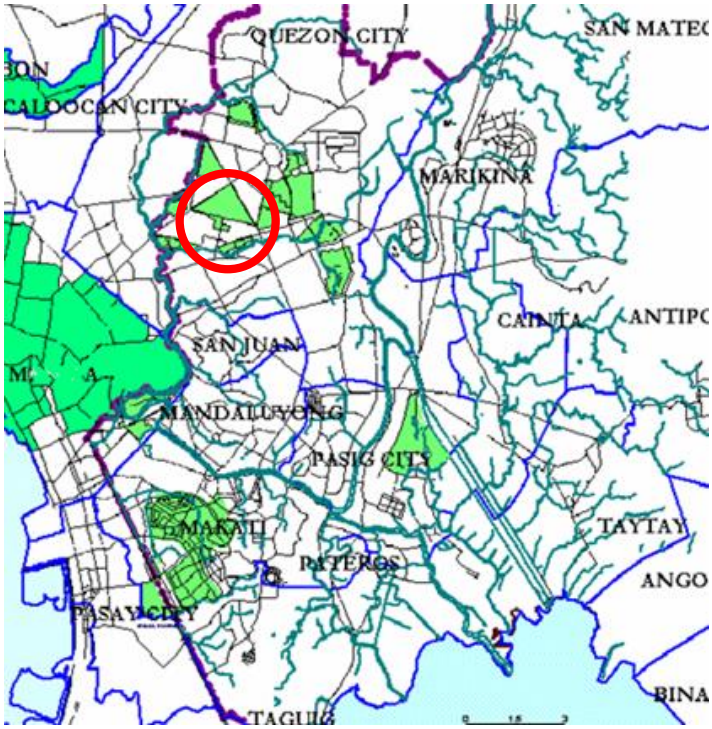

Figure 23 - Area served by Makati South WWTP (3).

Figure 24 and Figure 25 below show the progression of wastewater and solids through the Makati South WWTP.

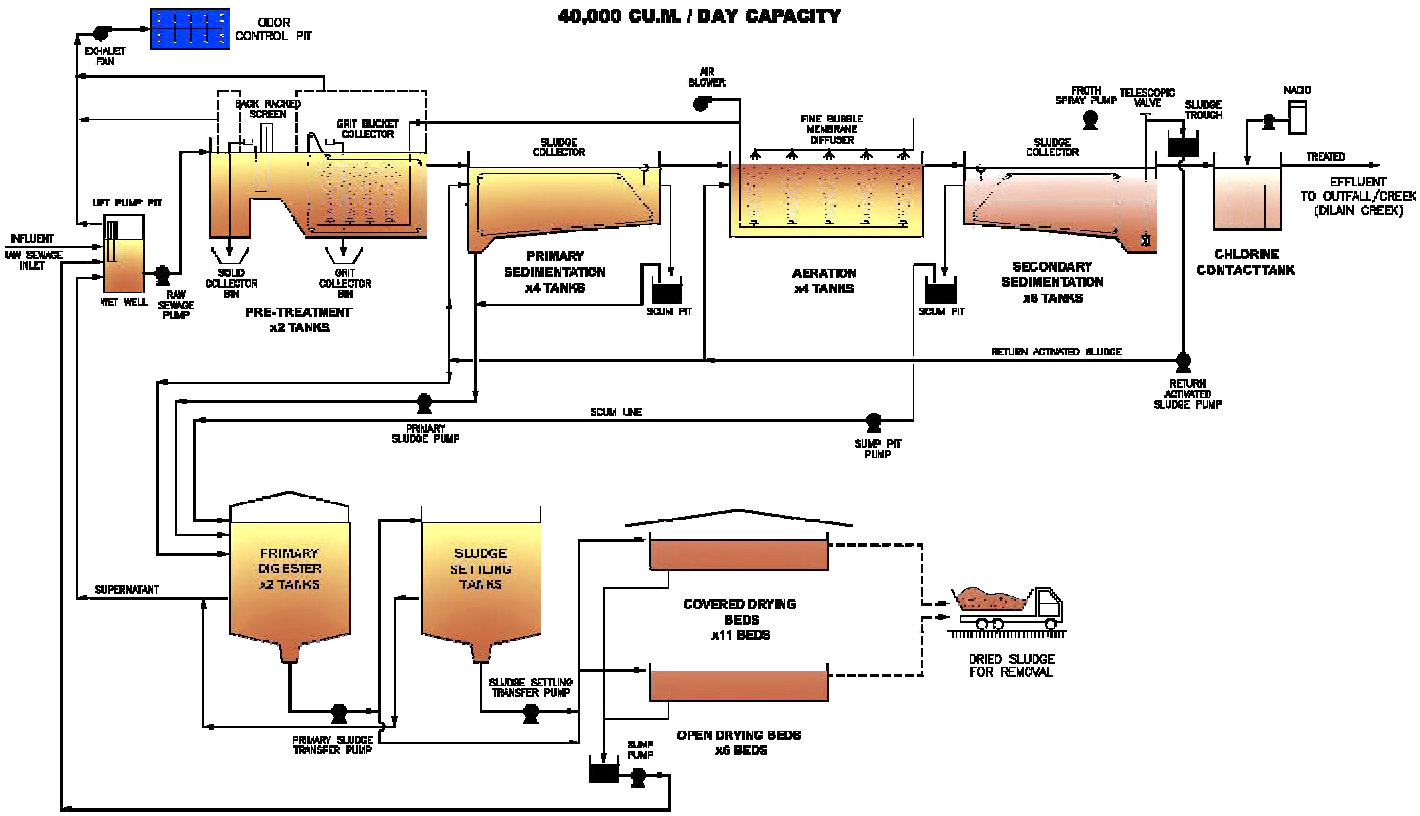

Figure 24 - Detailed schematic of MKS WWTP (3).

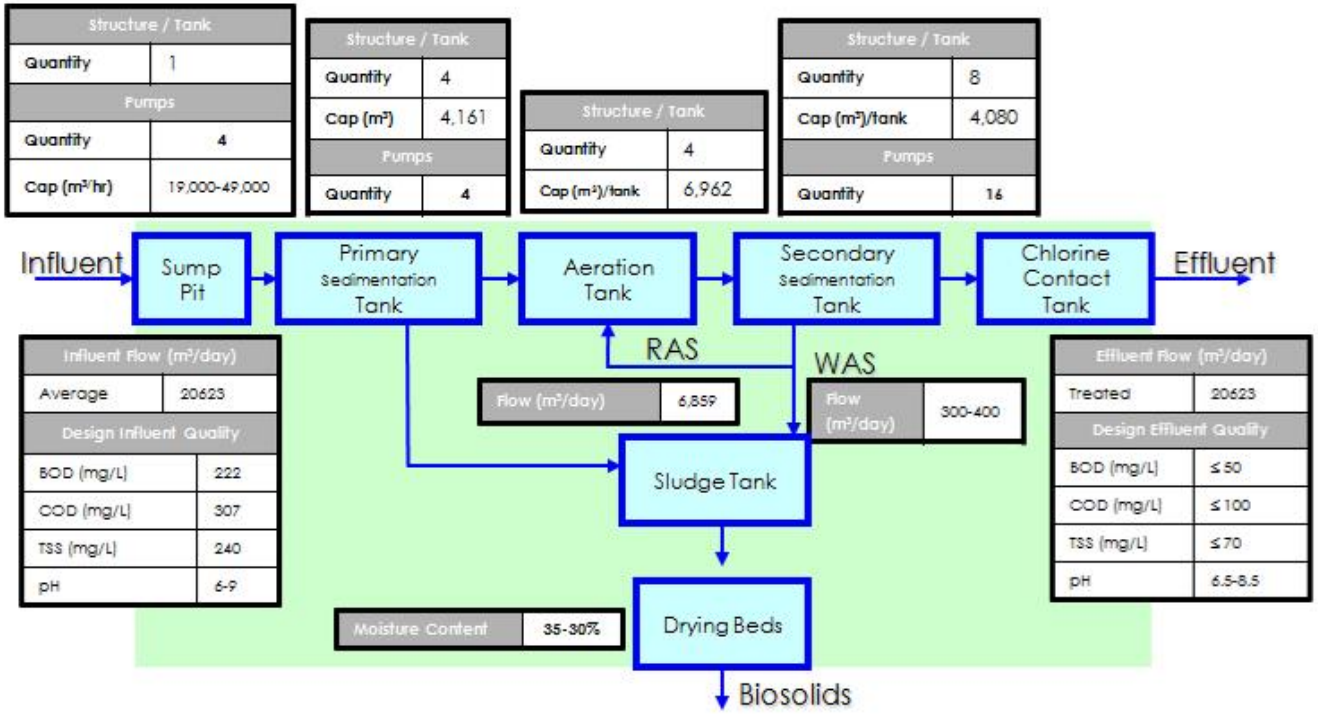

Figure 25 – Parameters Associated with Makati South WWTP (3)

Influent wastewater enters into a lift station, is sent to a bar screen, and then continues to a grit chamber. Flow continues to primary sedimentation, aeration, and clarification. At this point, the wastewater stream flows to chlorination and discharge. There is a solids recycle from the secondary clarifier back to the aeration tank. The solids stream collected from the primary sedimentation tank and the waste activated sludge from secondary sedimentation is sent to a digester, a sludge settling tank, and then to covered drying beds. Manila Water reports that a Waste-to-Energy methane recovery reactor has recently been installed on the solids digester, so the drying beds are now receiving only digested solids from this reactor (not included in diagram).

Figure 25 shows measured values for flow rate, BOD, COD and TSS in the MKS WWTP. Table 5 below summarizes the values provided in Figure 25. It should be noted that these are assumed to be measured values rather than design values; this could not be confirmed with MWCI.

Table 5 – Operational data from MKS WWTP (3)

Influent	**units**	**Measured Value**
Flow Rate	m^3/d	20623
BOD	mg/L	222
COD	mg/L	307
TSS	mg/L	240
Effluent		
BOD	mg/L	≤ 50
COD	mg/L	≤ 100
TSS	mg/L	≤ 70

The MKS WWTP receives average BOD in the influent (approximately 220 mg/L). MWCI reports that the MLSS in the plant is maintained around 2000-2500 mg/L.

If the total tank volume of 27848 m^3 and 80% of that is utilized, then reactor volume is essentially 22280 m^3.

HRT is calculated then as follows:

$$HRT = Volume/Flow\ rate$$
$$HRT = 22280\ m^3 / 20623\ (m^3/d)$$
$$HRT = 1.08\ days\ (25.9\ hours)$$

F/M ratio then is calculated as follows:

$$F/M = BOD/(HRT*MLSS)$$
$$F/M = 222\ mg/L / (1.08\ d * 2250\ mg/L)$$
$$F/M = 0.09\ d^{-1}$$

MWCI reports an average HRT of 8 hours for this plant (4 hours during peak times), and did not report an average F/M ratio. An F/M ratio of 0.09 d-1, however, is too low, and an HRT of nearly 26 hours is too long. Operating with an HRT of 8 hours, however, changes the F/M ratio to 0.36, which would be appropriate.

The aeration tank at Makati South utilizes fine bubble diffusers, and typical dissolved oxygen concentrations in this plant are around 1mg/L. Oxygen concentrations are lower in the Makati South WWTP than in the UP WWTP because the influent BOD is higher, which translates to a higher MLSS concentration and thus higher oxygen demands (and less surplus oxygen).

According to data from Manila Water, Makati South WWTP was the most reliable among the three plants at passing effluent standards for levels of coliforms, BOD and COD (3).

Table 6 below summarizes available operational data for MKS WWTP.

Table 6 - Available operational data for MKS WWTP

		Reported (3)		**Calculated**
		low	*high*	
HRT	hr	4	8	25.9
SRT	d	3	15	-
MLSS	mg/L	2000	2500	-
F/M Ratio	d-1	not reported	not reported	0.09

Materials and Methods

Sampling

Samples were collected from three WWTPs in Metro Manila, Philippines from January to June, 2008. Samples (approximately 500mL) were collected from the surface of aerated activated sludge basins at each of the WWTPs and were immediately transported to the University of the Philippines Institute of Biotechnology Laboratory. Samples were centrifuged in 50 mL tubes, and the supernatant was decanted. Pellets were stored at -20°C for several months before they were freeze-dried and transported back to North Carolina State University for DNA extraction. None of the treatment plants were experiencing foaming during collection.

Extraction of Bacterial DNA

Samples were reconstituted using RNAse free water, and DNA was extracted in triplicate using a lab method developed at North Carolina State University. This process first removes humic acids from the sample (which may interfere with extraction or future PCR related processes), and then lyses the cells in the samples to release the contained DNA. Remaining cellular debris and salts are removed and the precipitated DNA is eluted with TE buffer. The process is described in more detail below.

Approximately 0.25 g of each reconstituted sample was used for each extraction. Humic substances were precipitated prior to cell lysis using a low pH aluminum sulfate solution (100mM NaH_2PO_4, 100 mM $Al_2(SO_4)_3$; pH 6.0). To ensure humic acids were precipitated, the pH of the soil/aluminum sulfate solution was lowered to 6.0 using HCl. A lysis solution (100mM NaCl, 500 mM Tris, 10% SDS 1% Sodium Pyrophosphate; pH 9) was added and the pH was raised to 9.0 using NaOH (while ensuring the pH did not exceed 9.8). Glass

beads were added (100um diameter), and the samples were beat at max speed for one minute. The tubes were centrifuged (5 minutes, 13,200 rpm) and the supernatant (containing the DNA) was transferred to a clean tube. Ammonium acetate (7.5 M) was added (volume added = half of supernatant volume), the mixture was inverted to mix, and the tubes were centrifuged for 5-10 min at 13,200 rpm. At this point, the precipitate contained detergents, proteins and other cellular debris, so the supernatant (containing the DNA) is transferred to a new tube, isopropanol (100%; volume added equal to volume of supernatant) was added to the supernatant and incubated at room temperature for 15 minutes. The tubes were centrifuged (10 minutes, 13,200 rpm) to collect the DNA in the bottom of the tube. The supernatant was decanted, and the pellet was washed with 70% ethanol to remove remaining salts present in the pellet. The tubes were centrifuged again (3 min at 13,200 rpm) to reform the pellet. The ethanol was decanted and the pellets were dried for 5 to10 minutes. TE buffer (RNAse and DNAse-free; pH 8.0) was added and the tubes vortexed to solubilize the pellet. all DNA concentrations for the samples were quantified by measuring absorbance at 260 nm (Nanodrop, Thermo Fisher Scientific, MA, USA) and adjusted with TE buffer to concentrations of 30-60 ng/uL.

The Terminal Restriction Fragment Length Polymorphism Process (T-RFLP)

Species richness is defined as the number of species within a community, while species evenness is defined as the abundance of species populations within the community (23). These two parameters, central for characterizing microbial communities, are unable to be accurately quantified using culture-based methods because of the inability to culture some organisms, and because others are easily cultured, providing an inaccurate picture of the microbial community (23). Clone libraries (described in detail below) are one option to overcome culture-based methods, but creating clone libraries of a reasonable size will still yield results which omit some species.

Terminal Restriction Fragment Length Polymorphism (T-RFLP) was developed in response to these problems. T-RFLP works by applying a fluorescently labeled forward primer to the 5' end of a DNA sequence and then amplifying the DNA using PCR. A restriction enzyme is combined with the PCR product, which cuts the DNA sequences at a specific sequence from the 5' (fluorescently labeled) end. The fragments are separated such that only the fragments from the 5' end of the PCR product to the restriction site are considered "Terminal Restriction Fragments" (T-RFs); these fragments are used to characterize the microbial community. The results are presented in tabular or graphical form with the x-axis representing various fragment lengths and the y-axis representing the relative abundance of each species. This method can be used with DNA from complex microbial samples to reliably provide a means to understand community diversity (23). Figure 26 – Principle of Terminal fragment length polymorphism (T-RFLP) illustrates this process.

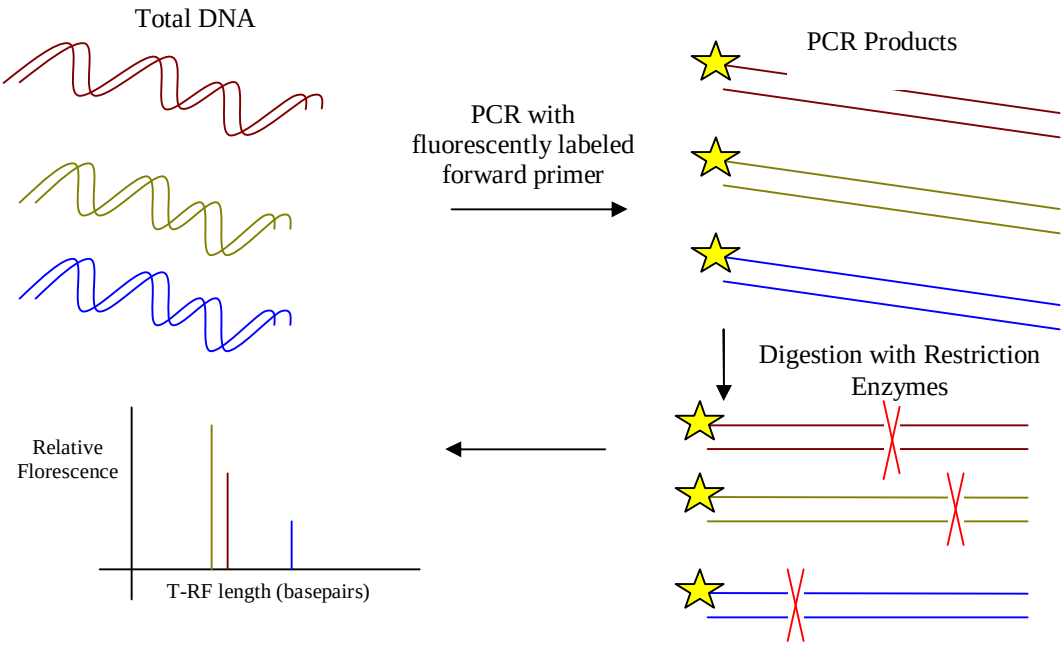

Figure 26 – Principle of Terminal fragment length polymorphism (T-RFLP)

PCR Amplification and T-RFLP

PCR was performed using primers specific for conserved 16S RNA gene sequences targeting the bacterial domain. Bacterial primers used were fluorescently labeled forward primer 8R (5'-AGAGTTTGATCCTGGCTCAG) and 1492R (5'-GGTTACCTTGTTACGACTT) (24). Reactions were conducted using 25 uL FailSafe PCR System Reaction Mix F (Epicentre; Madison, WI), 1 uL of each the forward and reverse primers (25 uM primer concentration), 0.5 uL FailSafe enzyme mix (Epicentre) per reaction, 3-15 ng per reaction of extracted DNA, and the remainder RNAse free water to bring the total reaction volume to 50uL. The PCR products were tested using agarose gel electrophoresis for similar band intensity, and samples that did not form strong bands were optimized by varying the DNA concentrations and

adding bovine serum albumin (1 uL/reaction).

Amplification was performed using the iCycler (Bio-Rad Laboratories, Hercules, CA) with the following program: an initial denaturing step at 95^{o}C for 3 min, followed by 30 cycles of denaturation at 95^{o}C for 1 min, annealing at 56^{o}C for 1 min, extension at 72^{o}C for 90 s, and final extension at 72^{o}C for 10 min.

The PCR amplicons, which were of equal size, were then purified using the Wizard PCR Preps DNA purification system (Promega, Madison, WI.) as directed by the supplier, and eluted with 25 µl sterile water. Purified PCR products (approximately 360 ng) were digested separately with roughly 5 U of tetrameric restriction endonucleases, *Hha*I, *Msp*I and *Rsa*I (Promega, Madison, WI), enzymes commonly used to target bacteria, in a 20µL reaction volume. The samples were digested for 4 h at 37 °C and inactivated for 20 min at 80 °C.

The digested fragments were then separated by capillary gel electrophoresis and visualized by automated DNA sequencer, which can only detect the fluorescently labeled fragments or terminal restriction fragments (T-RFs). T-RFs were analyzed by capillary electrophoresis at the North Carolina State University Genomic Sciences Laboratory. T-RFLP profiles were analyzed using Genescan^{TM} (Applied Biosystems). GeneScan numerical output from each electropherogram were extracted and transferred to Microsoft Excel.

Fragments that differ by less than 1 bp in size were considered identical. TRF peaks representing less than 1.0% of the total DNA presenting the sample were considered not significant and excluded from analysis. Samples were renormalized after these peaks were removed. Normalized T-RFLP profiles were then subjected to phylogenetic analysis. The

identity of the dominant microorganisms contributing to the T-RFs was obtained using the web-based phylogenetic assignment tool (PAT - http://trflp.limnology.wisc.edu/index.jsp). The T-RFLP PAT allows researchers to determine possible phylogenetic assignments based on a database of predicted T-RFs. Predicted T-RFs are obtained from *in silico* restriction enzyme digests (e.g. *Hha*I, *Msp*I, and *Rsa*I) of known 16S rDNA sequences amplified using the bacterial 16S rDNA forward primer 8F (AGAGTTTGATCCTGGCTCAG). The output from PAT is a list of species matches with their corresponding T-RFs (*Hha*I, *Msp*I, or *Rsa*I).

Output from PAT was pared down as follows: species matching the same TRF were looked up in the Uniprot database (http://www.uniprot.org/taxonomy/)
Species matching Eukaryota were removed from the output because the TRFLP performed targeted Bacteria. Species matching extremophiles were also removed from data before analysis. The taxonomies for the remaining species listed for each T-RF were compared and the highest common taxonomic level for each T-RF was assigned to an Operational Taxonomic Unit (OTU). Nearly all OTUs included an "Uncultured bacterium;" these bacteria are included in the tables of OTUs but were not considered when classifying the OTUs. Where PAT matched T-RFs of the same length with different organisms, the T-RFs were not combined; this explains why some fragment lengths of the same size match with different OTUs.

The final results are available in Appendix 4 as column charts showing the breakdown of the microbial communities into species, genera, or more specific groups where possible. Where T-RFs could not be matched with known organisms, the T-RFs remain unmatched.

The Cloning Process

In order to better understand the cloning results, it is necessary to first understand the cloning process.

During PCR amplification, Taq polymerase adds a deoxyadenosine (A) to the 3' end of the PCR product during PCR amplification. When topoisomerase I from the *Vaccinia* virus is introduced into the cloning reaction, it binds with the vector DNA at specific sites, cleaving the phosphodiester backbone after the 5'-CCCTT in one strand (12). This leaves a T overhang which is then available for the A overhang on the 3' end of the PCR product, allowing the PCR product to efficiently ligate with the vector (12).

The energy released in the cleaving of the phosphodiester backbone is then available to form a covalent bond between the 3' phosphate of the cleaved strand to a tyrosyl residue (Tyr-275) of toposiomerase I. This bond between the DNA and enzyme is then attacked by the 5' hydroxyl of the original cleaved strand, which reverses the reaction and releases topoisomerase (12). The TOPO TA cloning kit utilizes this reaction to clone PCR products into *E. coli* cells. Figure 27, below, is a graphical representation of this process.

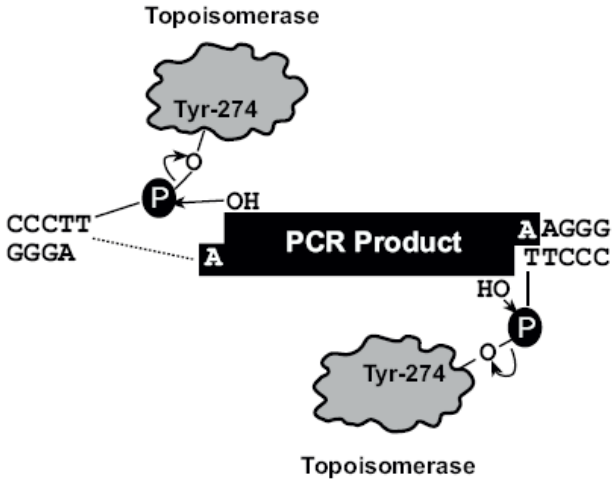

Figure 27 - TOPO Cloning reaction using topoisomerase I (12).

The TOPO cloning kit only allows for growth of cells that successfully take up the insert (PCR product) into the vector. This is possible because the lethal *E. coli* gene, *ccdB*, is located on the vector, fused to the C-terminus of the lacZα fragment. If the PCR product is successfully inserted into the vector, it disrupts expression of the [lethal] lacZα - *ccdB* gene. If this gene is disrupted, its lethal properties are disrupted as well, and the *E. coli* cells are allowed to grow. If the insert is not taken into the vector, however, the lacZα - *ccdB* gene is not disrupted, so its lethal properties are realized, and these cells are killed by the gene. In short, if the PCR product is successfully inserted into the vector, the cells will grow. If the PCR product fails to insert into the vector, no colonies should grow. Figure 28 below shows a map of the sequence surrounding the TOPO cloning site.

The vector also includes an ampicillin resistant gene. The presence of this gene allows for plating in agar that has been mixed with ampicillin, which allows for the growth only of organisms with an ampicillin resistant gene and preventing the growth of rogue bacteria present in the air or surroundings.

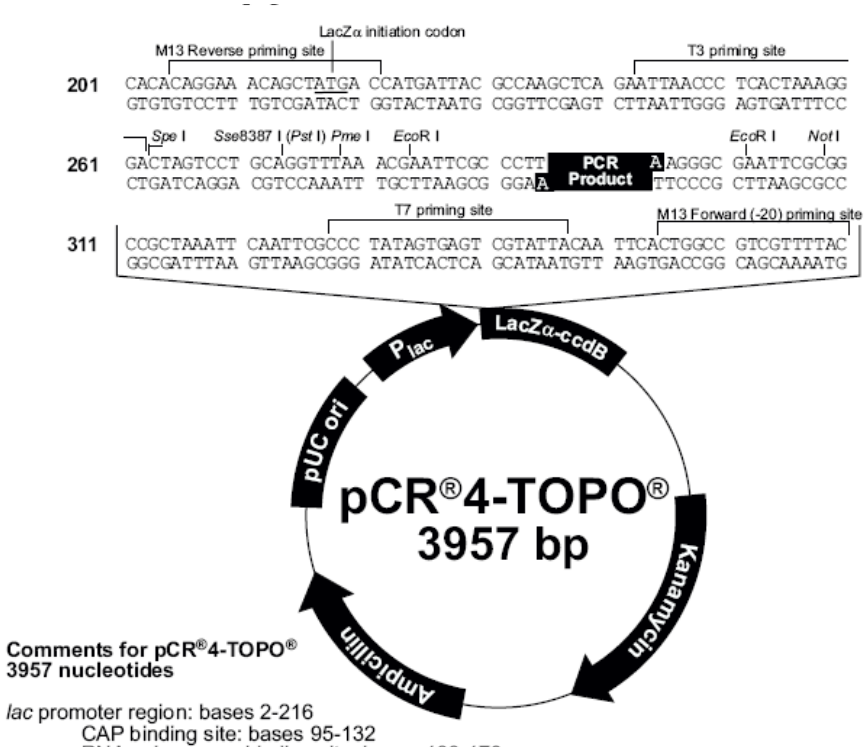

Figure 28 - pCR4 - TOPO vector map surrounding TOPO Cloning Site (12).

PCR Amplification and Cloning

PCR was performed with bacterial primers as described above.

After purification, the PCR product was combined with a salt solution, water and the TOPO vector according to the protocol provided by the supplier (Invitrogen; Carlsbad, CA). After the cloning reaction was complete, the vector was inserted into competent *E. coli* cells and the cells were plated on ampicillin plates and incubated.

Colonies were picked from plates and were grown up in liquid media at the Genomic Sciences Laboratory at North Carolina State University. GSL also performed plasmid

extraction on the colonies. The extracted plasmid concentrations were quantified (Nanodrop, Thermo Fisher Scientific, MA, USA). Samples were prepared for sequencing by adding 600 ng of plasmid, 6.4 pmol of forward primer and DI water for a final volume of 12 uL. Reverse reactions were also performed for each of the clones. The same primers used for the PCR reaction were used for sequencing (8F and 1492R).

Phylogenetic Analysis

Phylogenetic and molecular evolutionary analysis for 16S rRNA gene sequences were conducted using MEGA version 4 (Center for Evolutionary Functional Genomics, Tempe, AZ). Sequences were aligned using ClustalW algorithm and phylogenetic trees were constructed using the neighbor-joining method (Jukes-Cantor algorithm). Bootstrapping was performed to evaluate tree reliability (replications = 100). Sequences from related species and similar environmental clones were obtained from the National Center for Biotechnology Information (www.ncbi.nlm.nih.gov) and included in alignments.

Results and Discussion

T-RFLP results

T-RFLP is particularly helpful to view changes in microbial communities over time. By performing restriction digests on samples taken at different points in time, it is possible to see the changes in abundance of organisms during the sampling time period. T-RFLP can also be performed using different restriction enzymes on the same sample to determine which organisms are present in the wastewater with greater certainty. Both the analysis of populations using multiple enzymes and changes in microbial populations within the sampled plants will be discussed below, as well as a comparison among the microbial communities present in all three plants.

Results of the restriction analyses are presented here in the form of column charts showing the breakdown of the microbial communities into T-RFs. Where PAT provided matches with T-RFs, those T-RFs are labeled with the OTU names next to the fragment lengths in the chart legends. Tables with the possible organisms in each OTU are listed in Appendix 5. Where PAT was unable to provide organism matches, the T-RFs were left unaltered in the legends. In some cases, several samples had dominant organisms matching the same T-RFs, but PAT returned different possible organisms matching those T-RFs. For this reason, fragment lengths remain in the legends of these charts, and by viewing Appendix 5 it is possible to correlate organisms with appropriate OTUs.

It should be noted that only dominant microorganisms, that is, those with DNA contributing greater than 2% of the total DNA in the sample (i.e., bacterial populations are assumed to be > 2%), are included in these charts. This is because PCR-based fingerprinting techniques

such as T-RFLP can only detect the predominant species in the community. Non-dominant species are lumped together in each of the charts.

It should also be noted that samples were restricted in triplicate and the column charts show average values. In cases where single replicates were extremely different from the average, those samples were removed from the average and the averages recalculated. Using this criterion, the following sample replicates were removed from HhaI data processing: MKS 02/08a; from MspI processing: PHM 02/08c; and from RsaI processing: MKS 02/08a, PHM 02/08b, PHM 02/08c.

An example column chart for one of the samples, Makati South WWTP using restriction enzyme HhaI, is shown in Figure 29. One can see from this figure that the relative abundance of the organism with T-RF 568.90 (matching OTU8 corresponding to Burkholderiales in the class Betaproteobacteria) is 17%.

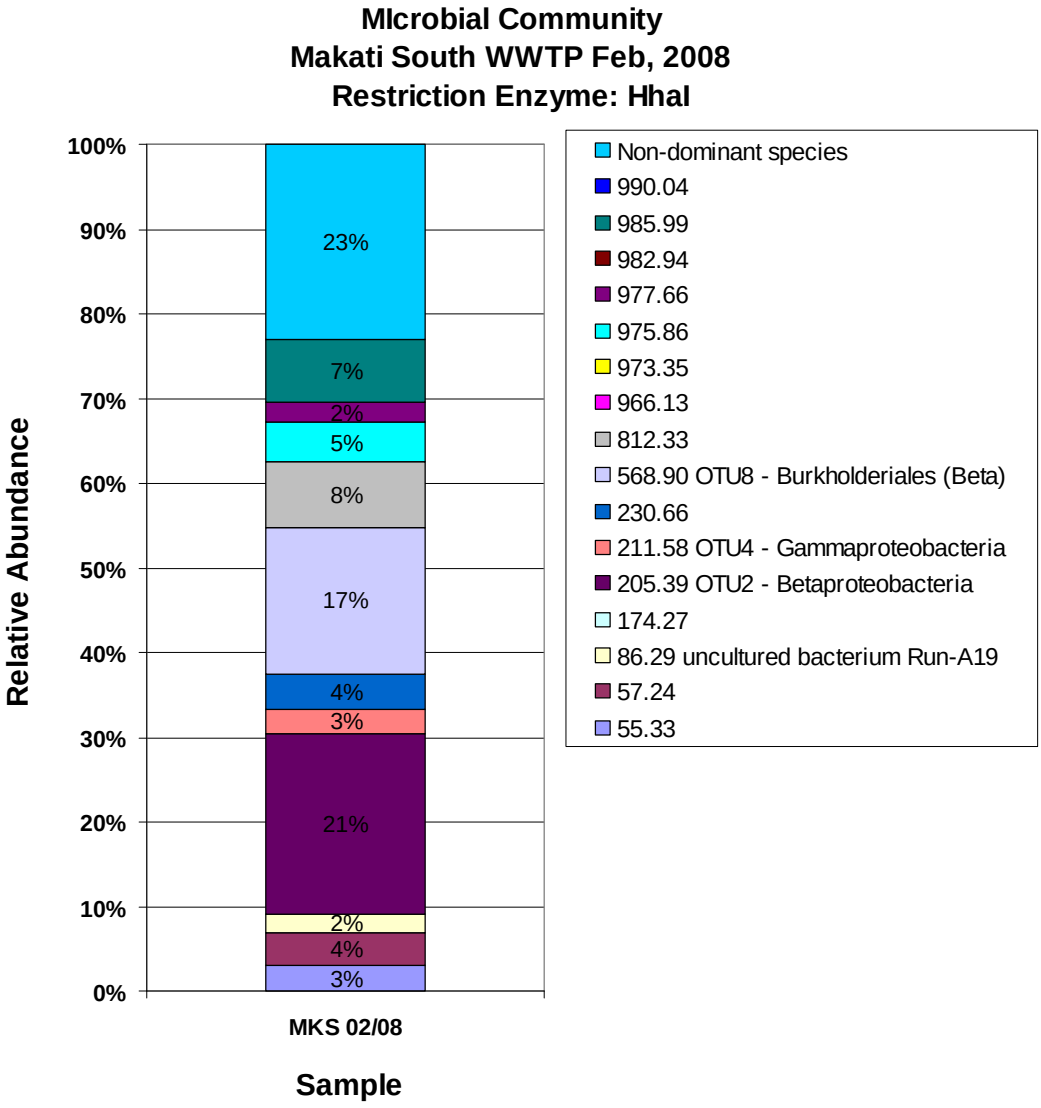

Figure 29 - Microbial Community at Makati South WWTP from February, 2008; restriction enzyme HhaI

Analysis of the same sample using multiple restriction enzymes

By performing T-RFLP using multiple restriction enzymes on the same sample, it is possible

to compare relative abundances among the charts and pare down the list of possible organisms returned from PAT when assigning OTUs to the T-RFs.

For these experiments, enzymes HhaI, MspI, and RsaI were used for the restriction and the resulting graphs should allow for some correlation between the samples to identify abundant organisms. That is, comparing sample MKS 02/08 among the three enzymes should yield similar relative abundances for each abundant organism.

Figure 30 below illustrates the concept of confirming the abundance of similar organisms using different restriction enzymes. While it is not possible to confirm the true identity of the organism, it seems likely that the organism matching 17% abundance restricted with HhaI (T-RF 568.80 – OTU8 – Burkholderiales (Betaproteobacteria)) correlates with the organism matching 15% abundance restricted with MspI (T-RF 140.71 OTU6 – Burkholderiales (Betaproteobacteria)).

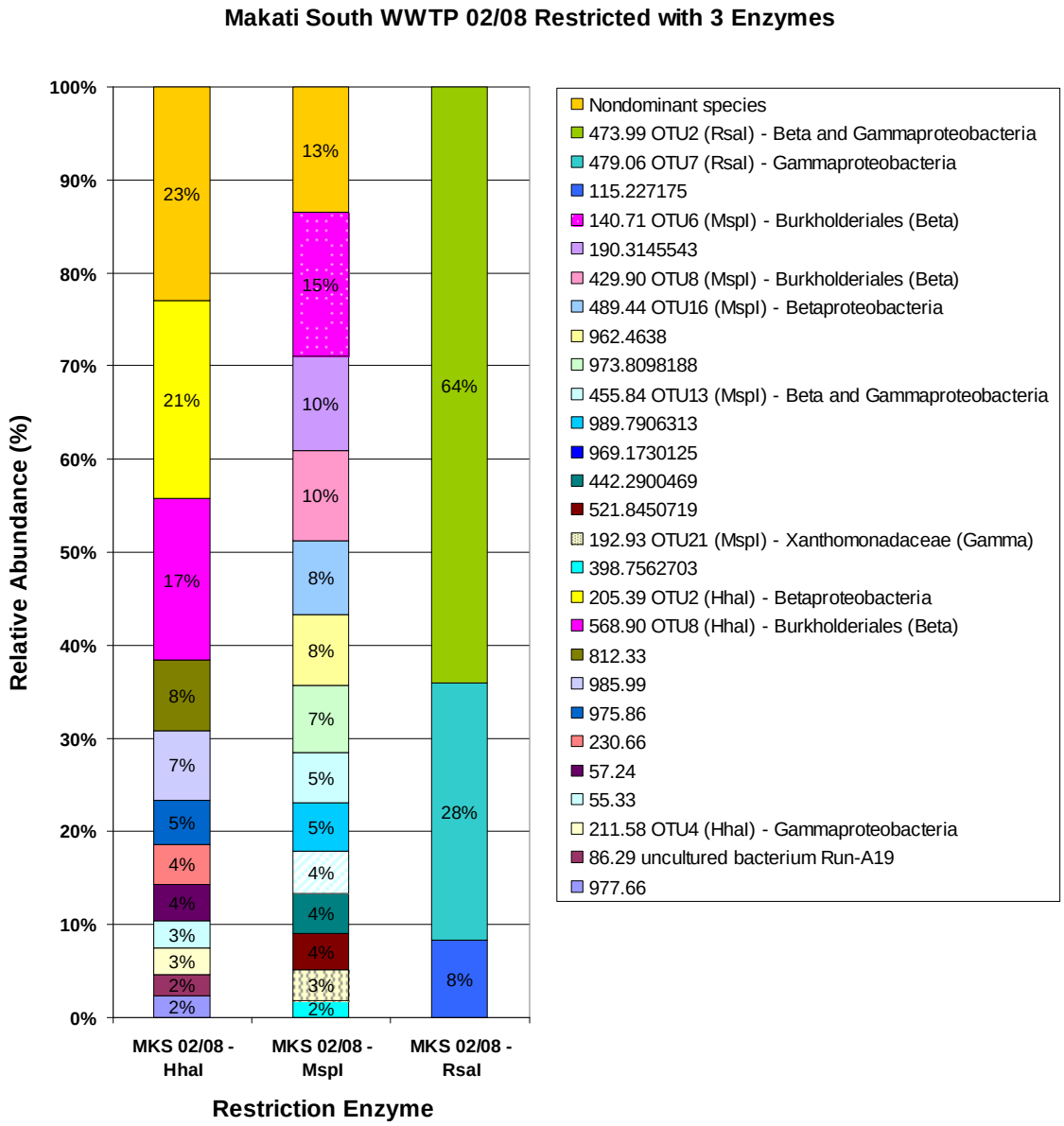

Figure 30 - Sample taken from Makati South WWTP in February, 2008 restricted with three enzymes

This same correlation can be seen between samples taken at the same WWTP in June, 2008, where organism matching 25% abundance restricted with HhaI (T-RF 568.80 – OUT8 – Burkholderiales (Betaproteobacteria)) correlates with the organism matching 21% abundance restricted with MspI (T-RF 140.71 OTU6 – Burkholderiales (Betaproteobacteria)). This is illustrated in Figure 31 below.

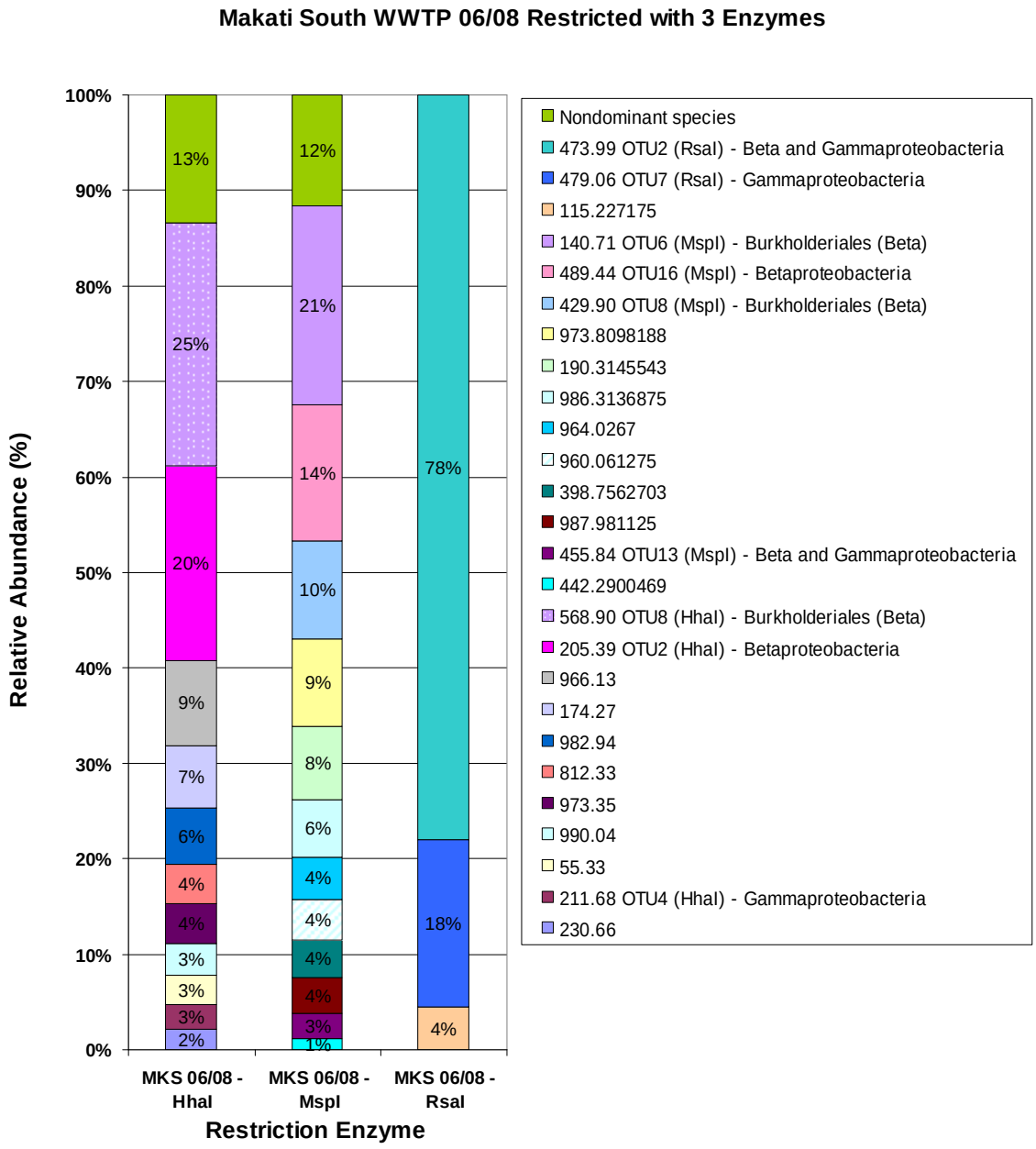

Figure 31 - Sample taken from Makati South WWTP in June, 2008 restricted with three enzymes

Since the columns in this chart represent DNA restricted from the same sample, one may conclude that the images should be highly similar. While some correlations can be observed in these charts, it is clear that the charts are not superimposable. This is because given the same organism, the enzymes cut in different regions of the 16S rRNA gene, and the resulting fragments may correlate with many organisms for one enzyme but with only one organism for another enzyme, i.e., the identification for enzyme-specific T-RFs are more definitive for some species than others. It is also clear that the enzyme RsaI was fairly unsuccessful as a T-RFLP restriction enzyme for these samples, since very few T-RFs were generated.

It is also difficult to draw correlations between T-RFs in these charts in particular because many of the T-RFs did not match with organisms in PAT's database. Nearly all T-RFs matched by PAT were in the β - or γ - Proteobacteria class, indicating that either the samples from these treatment plants are more heavily populated by β - and γ - Proteobacteria than plants surveyed in the US and Europe (1,2), or that the PAT database is more thorough in its cataloguing of β - and γ – Proteobacteria than other bacteria.

Samples from UP and PhilAm are provided below in Figure 32 and Figure 35 below. It was not possible to find strong, obvious correlations between the results from restricting with different enzymes for these samples.

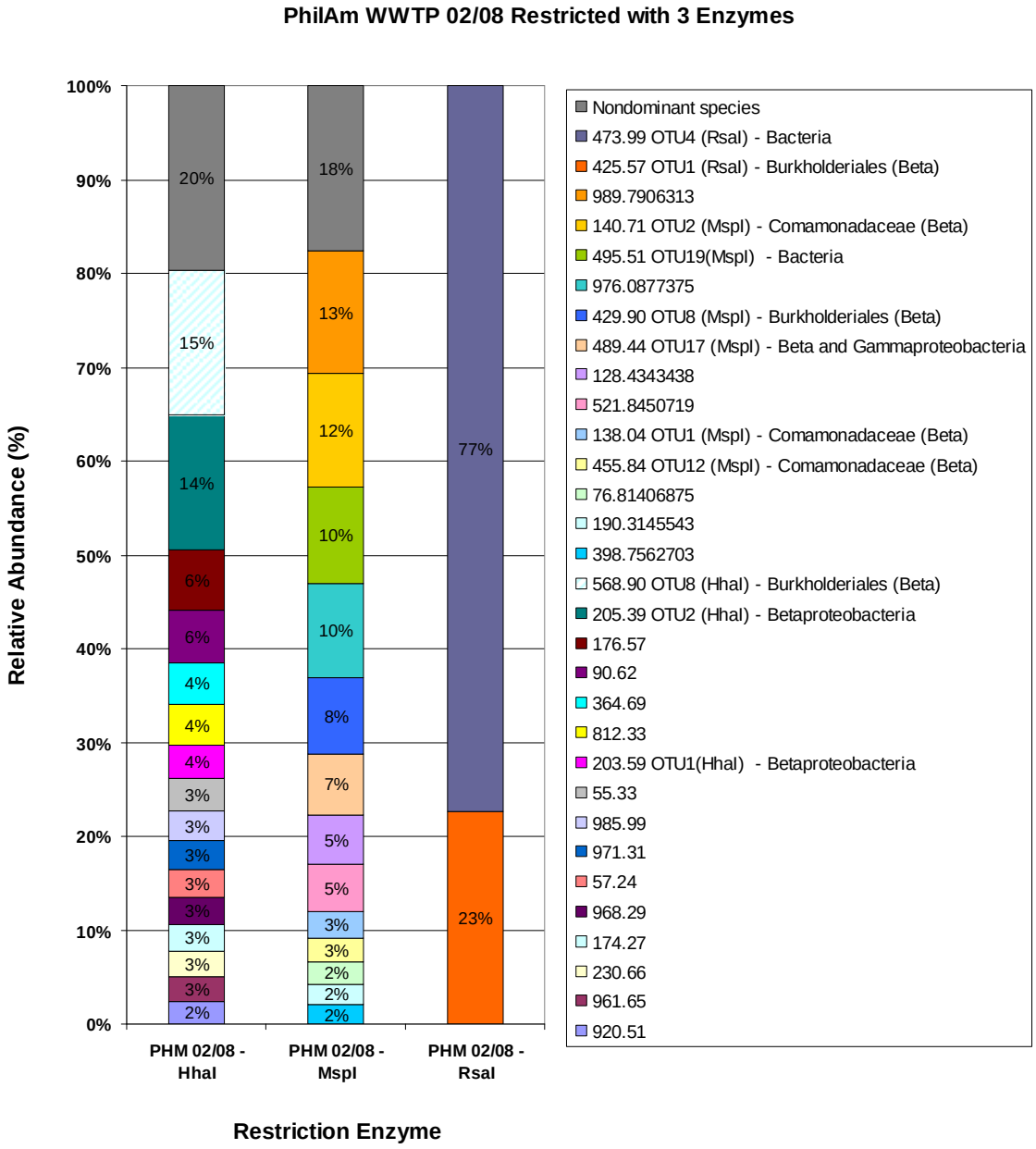

Figure 32 - Sample taken from PhilAm WWTP in February, 2008 restricted with three enzymes

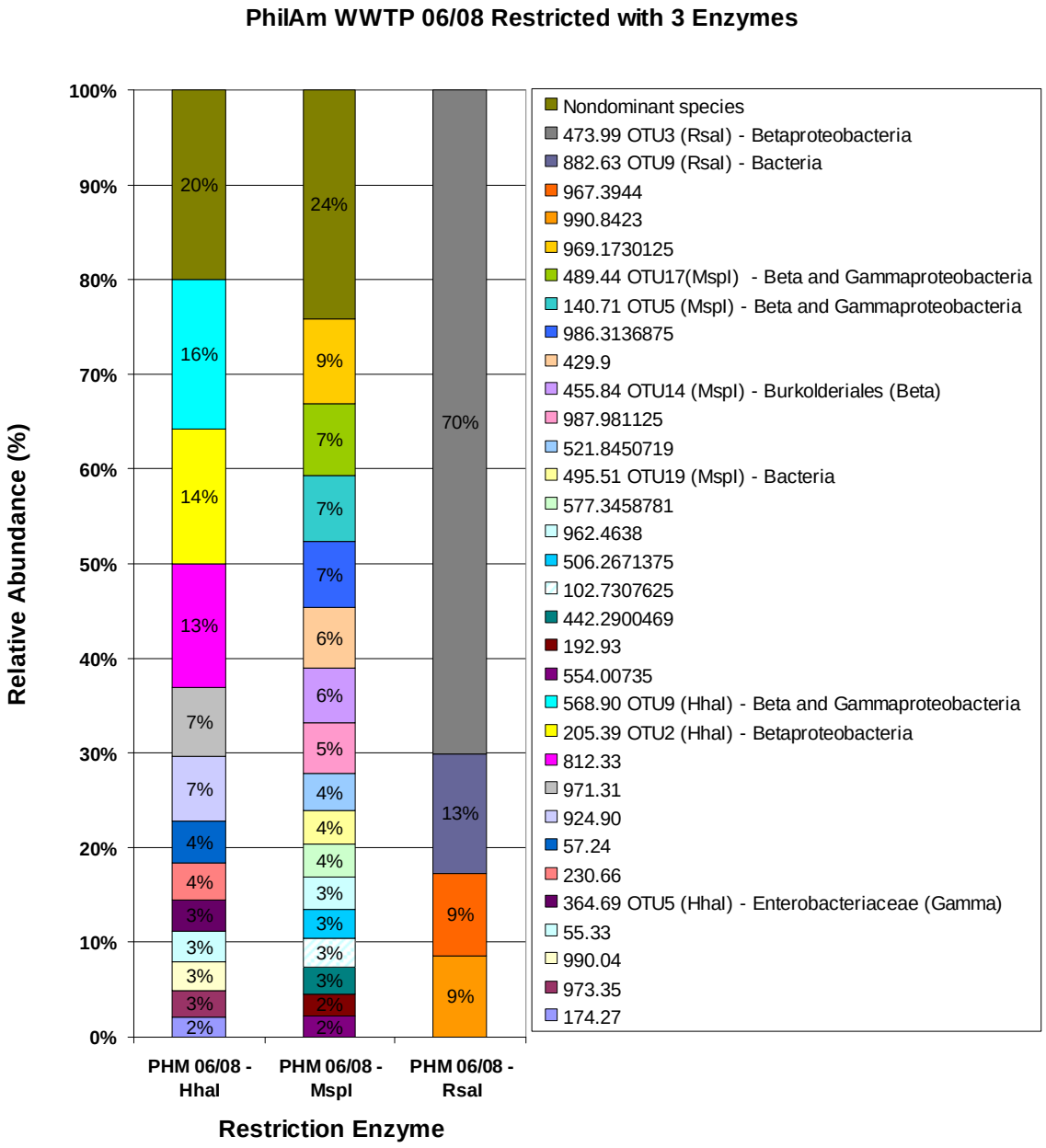

Figure 33 - Sample taken from PhilAm WWTP in June, 2008 restricted with three enzymes

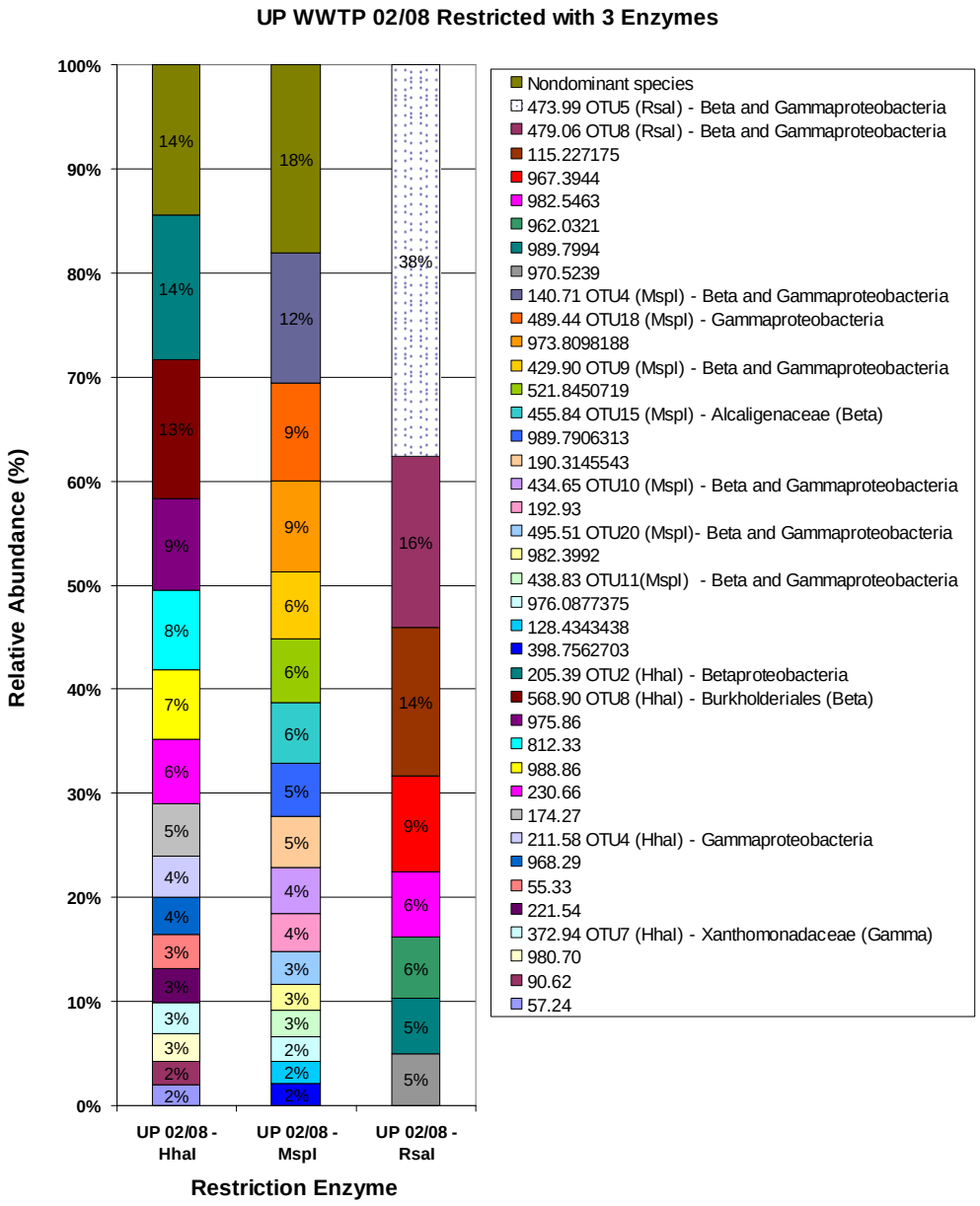

Figure 34 - Sample taken from UP WWTP in February, 2008 restricted with three enzymes

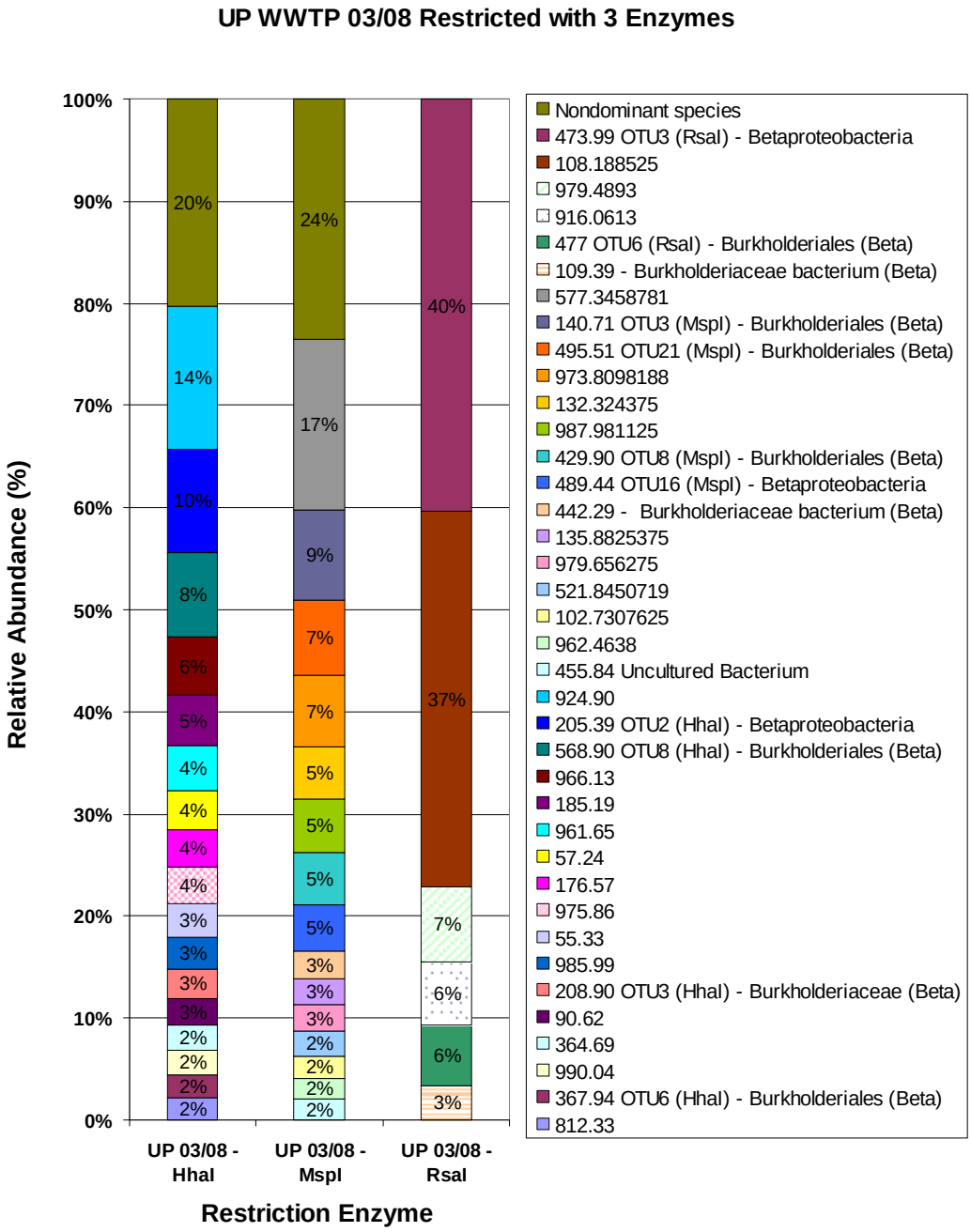

Figure 35 - Sample taken from UP WWTP in March, 2008 restricted with three enzymes

Changes over time and comparisons among WWTPs

By performing TRFLP with the same enzyme on samples from the same treatment plant at different times, it is possible to observe how the relative abundances of different organisms change during the sampling time period. In the following figures, sections of the columns that are the same color represent T-RFs of the same length. It should be noted, however, that in some instances PAT matched these T-RFs with different OTUs or matched one fragment but did not match the other; notes indicating this are in the legends of the graphs where applicable. Because the results from the three restriction enzymes do not correlate well with each other, the following charts were made with the samples restricted with the same enzyme over time. Figure 36, for example, illustrates that the relative abundance of the organism with T-RF 568.90 (matching OTU8 corresponding to Burkholderiales in the Betaproteobacteria class) is 17% in February and 25% in June for samples taken from Makati South WWTP.

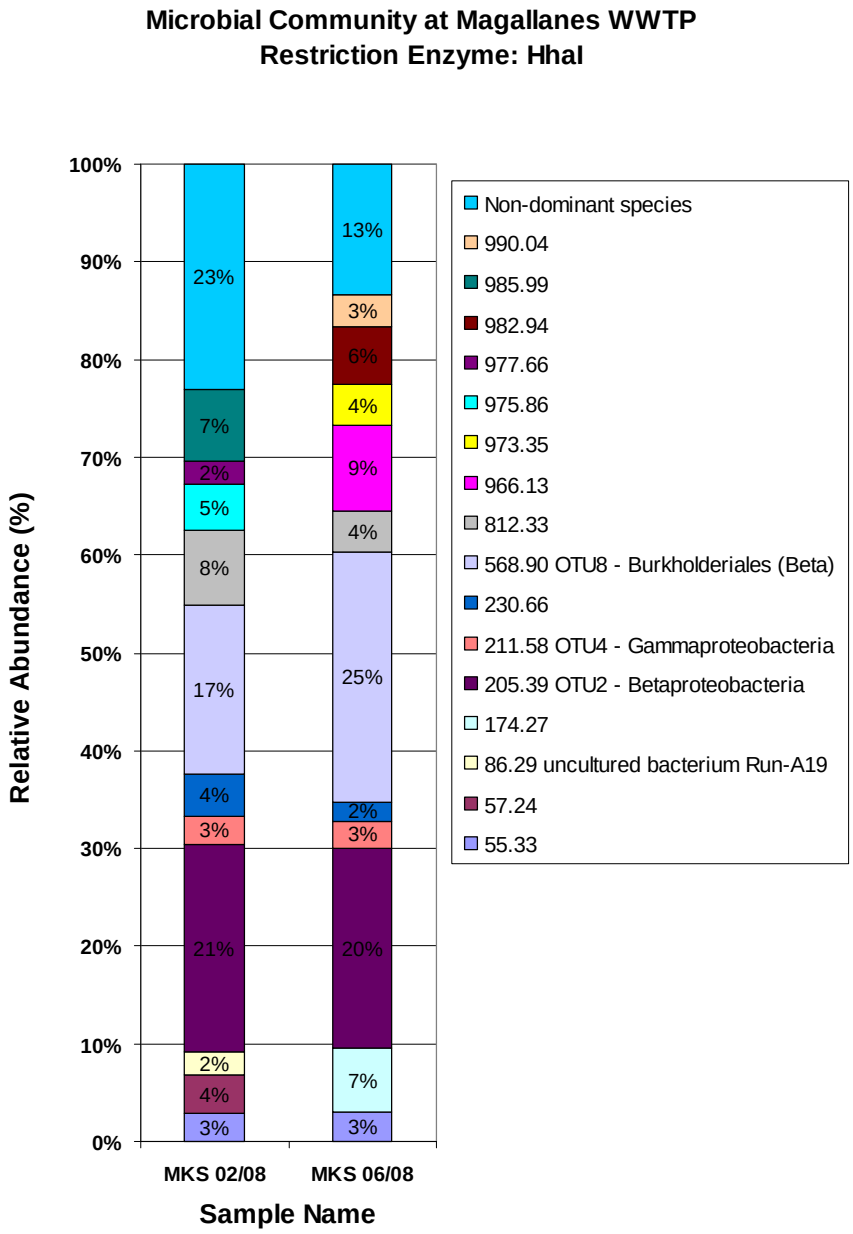

Figure 36 - Microbial community for samples taken at Makati South WWTP; restricted with enzyme HhaI.

Figure 37 shows microbial populations in each of the sampled WWTPs for samples taken at two different time points using the restriction enzyme HhaI. It is thus possible to compare changes within the same treatment plant over time, but it is also possible to observe correlations between the plants using these charts. Figure 37 below therefore includes a duplicate of information in the above chart (Figure 36) along with information from the PhilAm and UP WWTPs.

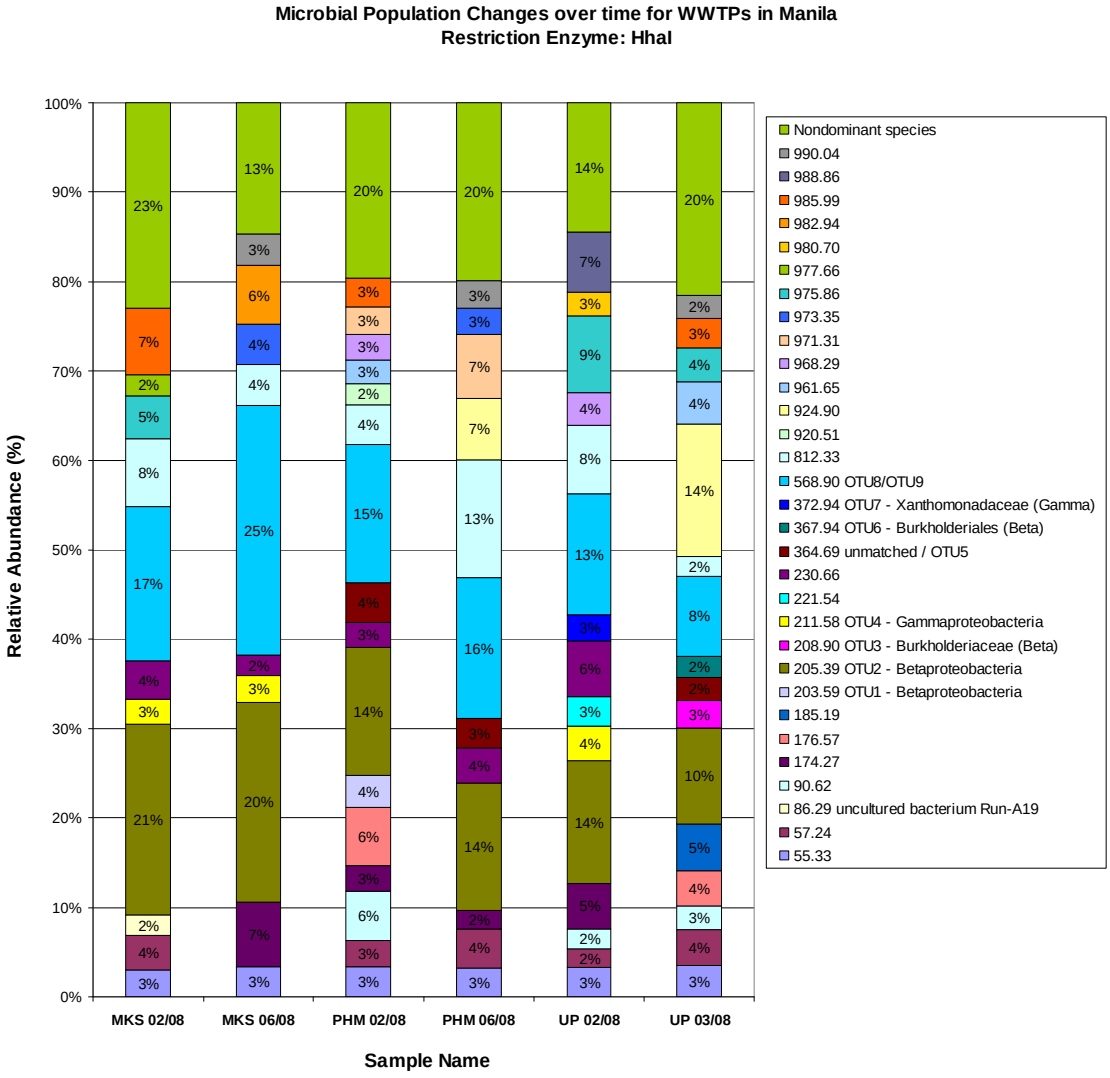

Figure 37 - Microbial Population Changes over time for WWTPs in Manila. Restriction enzyme: HhaI

Interestingly, several T-RFs are present in similar abundance in all samples; OTU 2 – Betaproteobacteria matching T-RF 203.59, for example, is present in similar abundance in all samples at all WWTPs. Fragment matching 568.90 is also present in all samples, but notice

that PAT matched this fragment with two different OTUs. See Appendix 5for more information on which samples matched with which OTUs and which organisms are in each OTU.

The microbial communities within the WWTPs changed over time. Fragment 966.13, for instance, is not present in any samples taken in February, but is present at 6% in the sample from the UP WWTP in March, and at 9% in the sample from Makati South in June. Similarly, T-RF 990.04 is not present in any of the February samples but is present in all of the later samples. This could be because of the increase in temperature that occurs after February, which is the coldest month in the Philippines, because of a change in rainfall, or because of social reasons, such as diet changes due attributable to seasonal, economical (the price of rice began to increase after February in response to global economic changes), or cultural changes.

Charts illustrating these same samples restricted using enzymes MspI (Figure 38) and RsaI (Figure 39) follow. Charts with higher resolution are available in Appendix 4; these charts are broken down into pairs of samples taken from the same plant for each restriction enzyme.

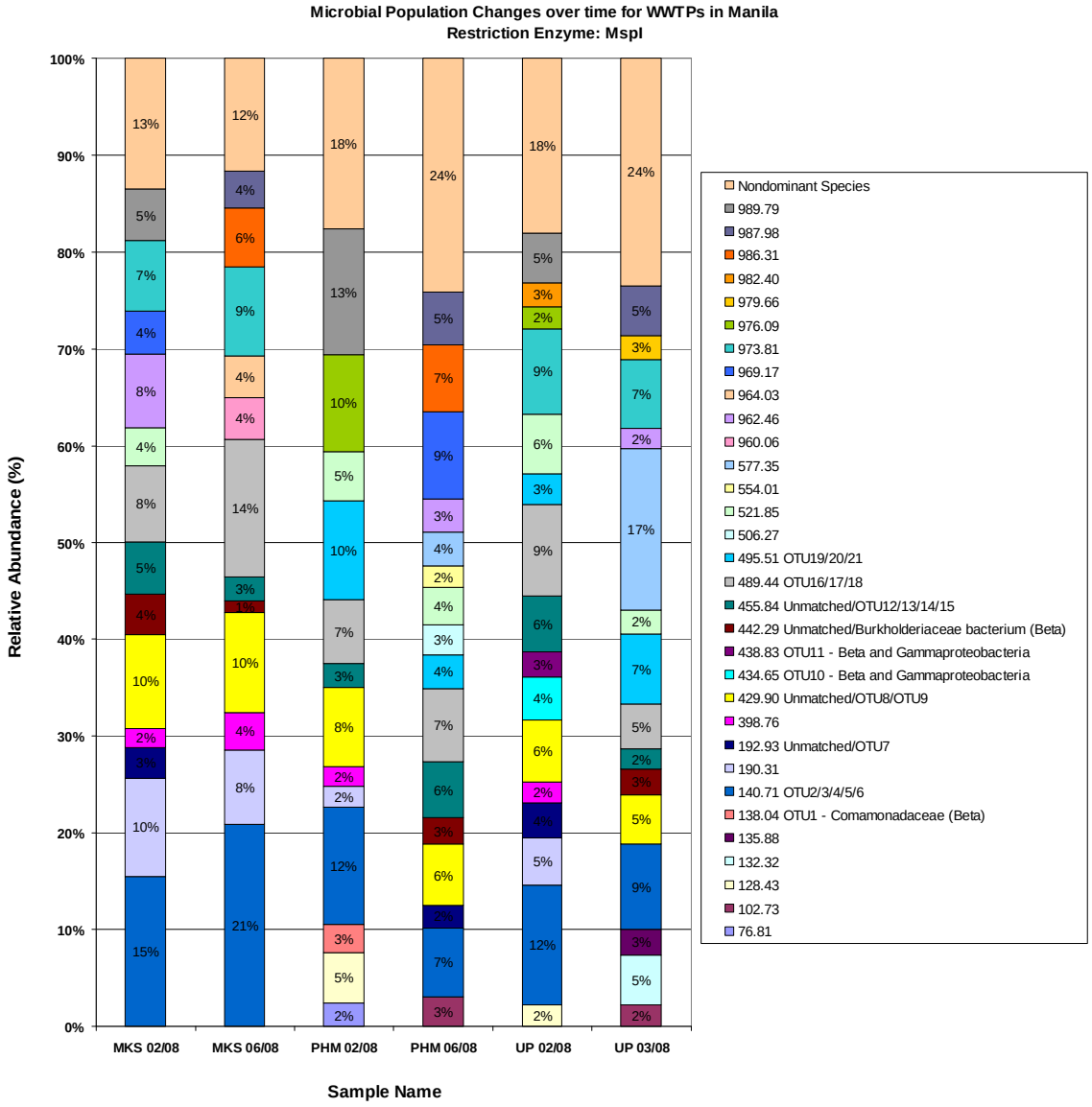

Figure 38 - Microbial Population Changes over time for WWTPs in Manila; restriction enzyme: MspI

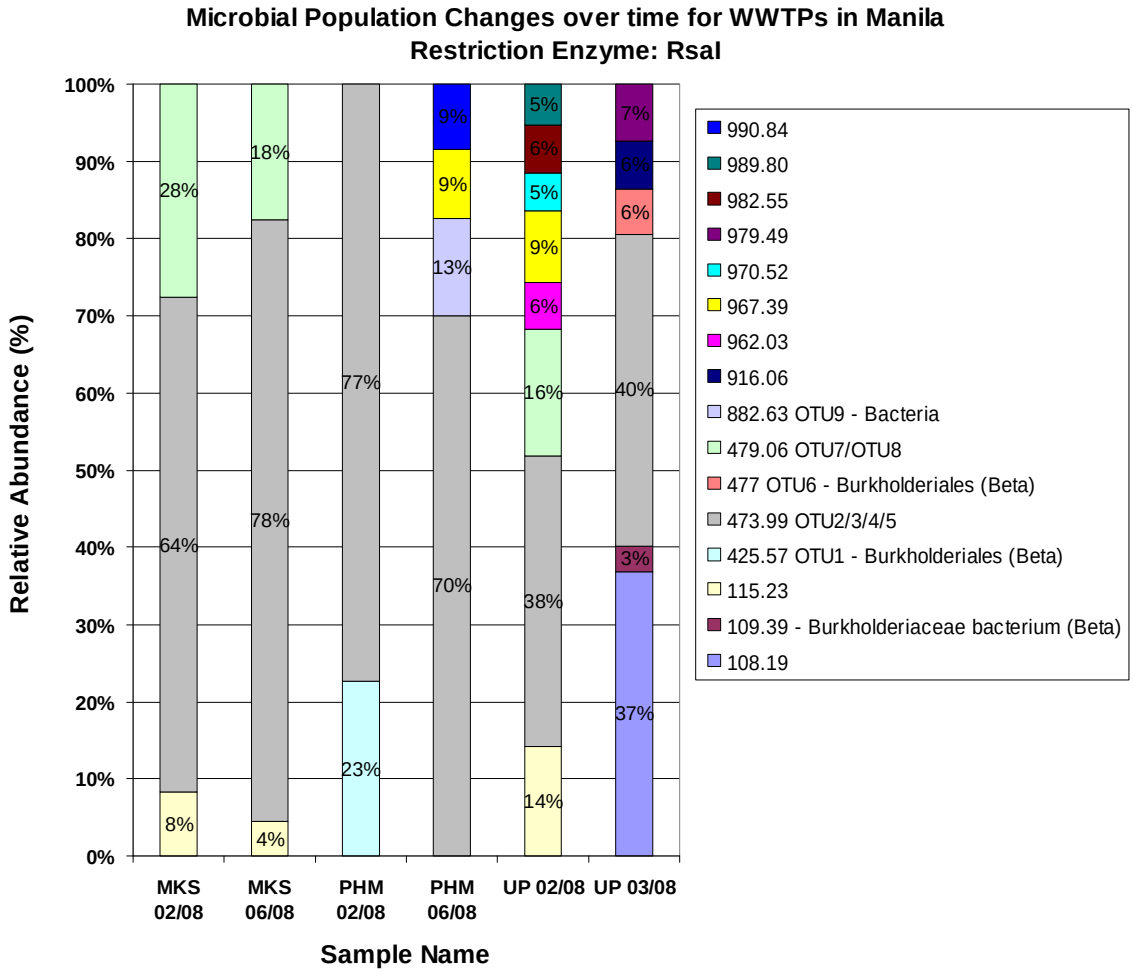

Figure 39 - Microbial Population Changes over time for WWTPs in Manila; restriction enzyme: RsaI

Another way of displaying TRFLP data is by creating multidimensional scaling (MDS) plots. Community Analysis Package 4 (CAP; Pisces Conservation Ltd, Lymington, United Kingdom) was used to create MDS plots for the samples using the same data that was used to create the column charts. These plots are nondimensional, so the axes have no physical meaning and are thus unlabeled. The plots only indicate how similar or dissimilar data points

are based on statistical analysis. Samples in these figures are labeled as follows: Sample location (UP, PHM, MKS) + date (0208 indicates February 2008) + sample replicate (a,b,c)). From Figure 40 below, it is clear that the samples taken from UP WWTP in February are similar to each other but are dissimilar to samples taken at UP in March. Conversely it is clear that samples taken at PhilAm in February are similar to those taken at PhilAm in June. This analysis is in agreement with the community structure presented in the column charts.

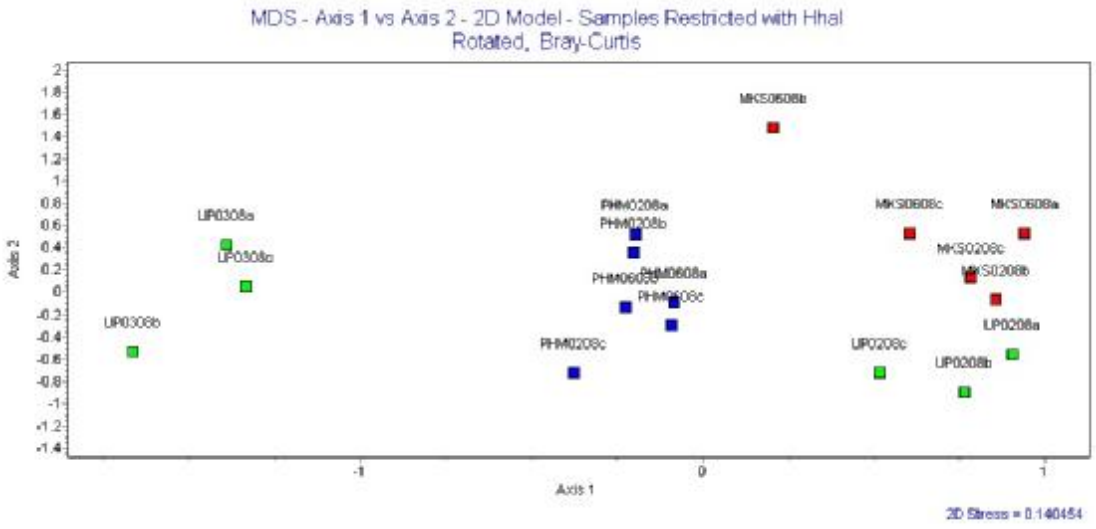

Figure 40 – Multidimensional scaling of all samples restricted with HhaI

Figure 41 below is of the same samples restricted with enzyme MspI. Similar patterns are observable here, where samples taken at UP in February differ significantly from those taken in March, but samples from PhilAm and Makati South are similar from February and June.

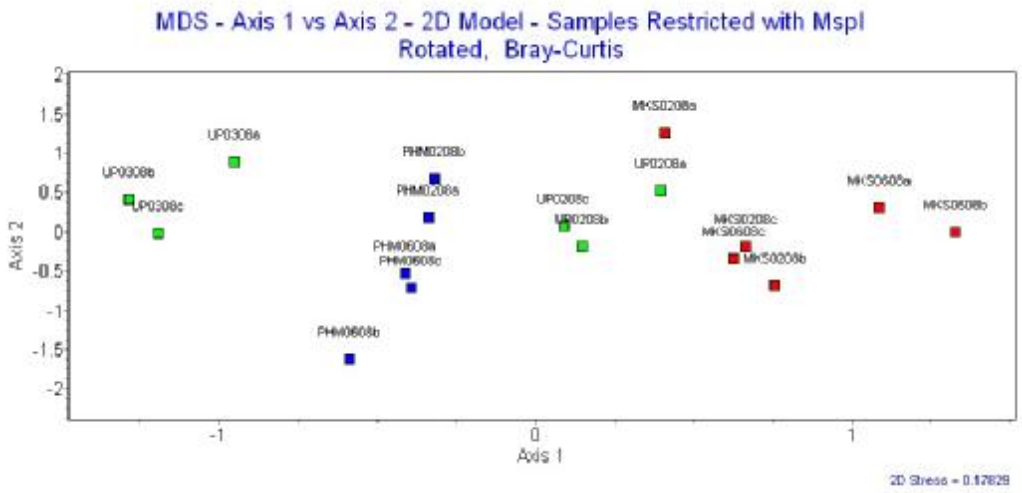

Figure 41 - Multidimensional scaling of all samples restricted with MspI.

The third restriction enzyme, RsaI, confirms the similarities further where UP samples from February and June cluster together but do not cluster with each other (see Figure 42). Notice here that only one sample from PhilAm in June is provided on the graph. For this enzyme, one of the other samples did not return any results and the third replicate was an outlier. With only one data point from June, it is not possible to know if the spacing on the graph represents true dissimilarity or if it is because of poor results.

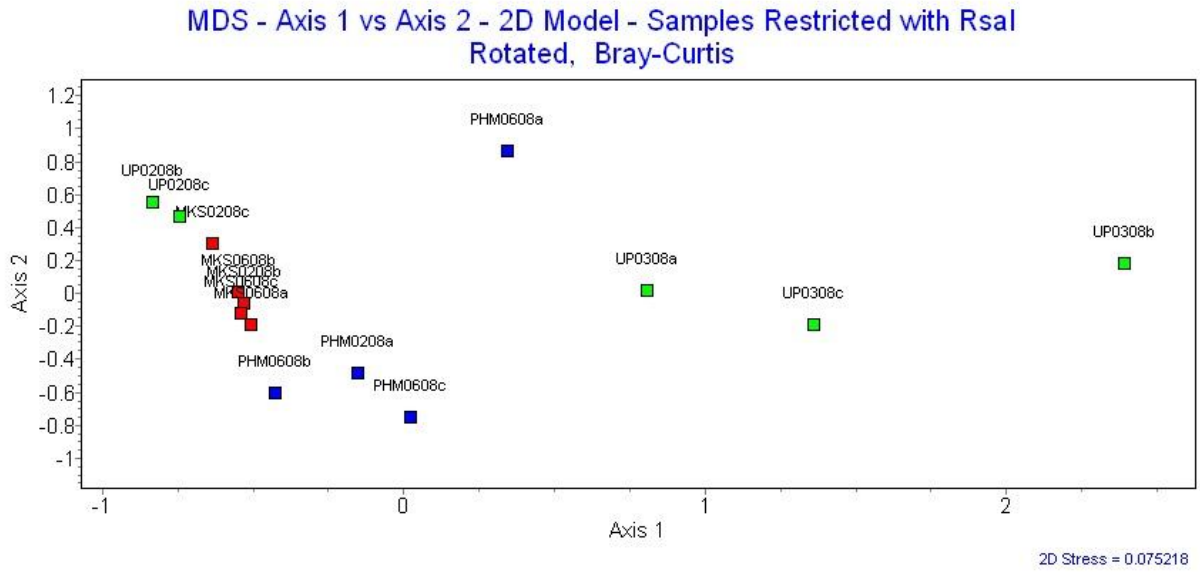

Figure 42 - Multidimensional scaling of all samples restricted with RsaI.

All three WWTPs are located in Manila, within a 30 mile radius of each other. These graphs indicate that for the UP WWTP, temporal changes are potentially more significant than spatial arrangement of the plants, since all samples taken at PHM and MKS group more closely together than the two samples taken at UP. This may be because of the Easter holiday that occurred just a few days before the second UP sampling event. Easter fell on March 23, 2008, and UP0308 was sampled on March 25. A high percentage of those living in Metro Manila return to their home provinces for the week preceding Easter, so the population supplying wastewater to the treatment plant would certainly have decreased during this period. These effects could have altered the influent characteristics in the WWTP significantly enough to change the microbial population.

The charts indicate the changes over time for samples collected at PHM and MKS appear to be less significant than at UP. Minor differences in the microbial populations in these plants

can perhaps be explained as spatial differences or differences in influent characteristics. MKS is receiving influent from one of the richest areas in Metro Manila, so perhaps the difference in diet allows for a different microbial community. More temporal and operational data would be needed to better determine reasons for the differing microbial communities. Two temporal data points are insufficient for this type of analysis.

Comparison of Diversity

Several indices are available for comparing diversity among different microbial community samples.

Richness (S) is a measure of the number of species present in a sample. With respect to T-RFLP results, richness will correlate with the number of T-RFs that are present in a sample, which higher richness indicating more species are present in the sample.

The Shannon Wiener Diversity Index (H) is another index used to characterize species diversity (25). It accounts for both abundance and evenness of a species present. The proportion of species *i* relative to the number of total species p_i is calculated, and multiplied by the natural log of this proportion ($\ln p_i$) as follows:

$$H = -\sum_{i=1}^{s} p_i \ln p_i$$

The Shannon Index ranges from 0 to ~4.6, with higher values indicating greater diversity (26). Evenness can then be calculated as the Shannon Index (H) divided by the natural log of the Richness (S), as follows:

$$E_H = H / H_{max} = H / \ln S$$

Evenness ranges from 0-1, with 1 indicating a perfectly even sample, where all species are present in the same abundance (26).

Table 7 below compares diversity among the six samples analyzed with T-RFLP (using restriction enzyme HhaI). The samples collected at UP in February and March, 2008, and the sample collected from PhilAm in February all have a species richness of 34 species. This is how many species were present in each of those samples collected. The Shannon Diversity Index indicates that the sample collected from UP in March, 2008 has the greatest diversity and evenness.

Table 7- Comparison of diversity among samples

	MKS 0208	**UP 0208**	**PHM 0208**	**UP 0308**	**MKS 0608**	**PHM 0608**
Species Richness	22	34	34	34	23	32
Shannon Wiener Diversity Index	2.56	2.99	3.10	3.19	2.47	2.89
Evenness	0.83	0.85	0.88	0.90	0.79	0.83

As discussed earlier in this chapter, high diversity in activated sludge reactors in WWTPs has been shown to correlate with good performance, while high diversity in other reactors (particularly anaerobic reactors) has been shown to be a detriment to the WWTP performance.

The Makati South WWTP was chosen for inclusion in this survey because it is the largest treatment plant in the country. Interestingly, according to these indices, MKS has the lowest

levels of species richness, diversity and evenness, but MWCI reports that MKS was the most reliable among the three plants at passing effluent standards for levels of coliforms, BOD and COD (3), indicating that lower diversity in the activated sludge process correlates with good system performance. This contradicts with studies referenced earlier in this chapter which indicate activated sludge reactors typically perform better with increased diversity. Perhaps this discrepancy can be attributed to the specific species found in these plants. The studies discussed previously focused on WWTPs in non-tropical countries, and thus are likely populated by different microorganisms than those in WWTPs in the Philippines. Perhaps the microorganisms found at the MKS plant are more efficient at treating the waste than the species found at UP and PhilAm, such that fewer species are acting more efficiently. Another possible explanation is that MKS maintains a lower richness, meaning functional redundancy is reduced. In this case, if some influent characteristic changes, perhaps the microbial population is less likely to be completely overturned (and the microbial population inside the plant more consistent) thus making the effluent properties more reliable.

The Phil-Am WWTP was chosen because it utilizes a unique air lift coarse bubble diffusion aeration system. Dissolved oxygen levels in this plant are too low, but the plant has high species richness and levels of diversity greater than those at MKS. This may be an indication that though there are many species present in the WWTP, they may be utilizing the substrates at low levels, or at inconsistent levels, and not producing effluent of reliable quality.

The UP WWTP was chosen because it serves the University of the Philippines campus and thus has different influent characteristics than the other two plants, which serve residential areas. The UP WWTP has the highest levels of richness, diversity and evenness, and also the highest levels of DO in any of the plants sampled. Higher levels of DO may allow for a larger range of microorganisms to grow in the plant, though this increased diversity does not

seem to correlate with improved system performance.

Cloning Results and Discussion

The samples collected in February 2008 (the same samples as reported in the T-RFLP section) were cloned to verify the T-RFLP results and to aid in realizing with more specificity the organisms represented by the T-RFs. The cloning reaction was assumed to be successful when colonies grew on the ampicillin plates, since this growth should have indicated the presence of the ampicillin resistant gene and the disruption of the *lac*Zα – *ccd*B gene (indicating successful insertion of the PCR insert into the vector). The inoculated agar plates grew colonies at a reasonably high density such that picking individual colonies for sequencing was possible (~25 individual colonies/plate were chosen for sequencing). Ninety six clones from each sample were picked from the agar plates, grown up in liquid media. Plasmids were extracted and sequenced in the forward and reverse direction.

Despite having grown (indicating successful disruption of the *lac*Zα – *ccd*B gene) in ampicillin media (indicating colonies were *E. coli*), results from the cloning reactions indicate that 211/288 sequences matched > 98% identity with the cloning vector in the NCBI database, indicating unsuccessful cloning reactions. This could be because the primers used for sequencing the clones matched with other locations on the vector, because the cloning kit was bad, or for another, unknown reason.

Primers M13 Forward and Reverse should have been used for sequencing the clones. Instead, due to a miscommunication, primers 8 Forward and 1492 Reverse were used for sequencing. When this yielded poor cloning efficiency, however, 3 samples with sequences matching the cloning vector were re-sequenced using the correct primers, and again the sequences matched with the cloning vector. This indicates the problem was not due to the

primers used for sequencing.

According to Invitrogen, the TOPO TA cloning kit should yield >90% efficiency with respect to the ability of the *lac*Zα – *ccd*B gene to successfully disrupt the growth of colonies whose plasmids did not take up the PCR inserts into their vectors. The highest efficiency with any of the samples cloned was 33% (UP WWTP). Though the cloning kit was less than 3 months old and was stored properly, the vector may have degraded and caused the reduced cloning efficiency.

Another potential problem suggested was that the PCR product was inserted in the wrong orientation in the vector. Because of the A overhang on the PCR product, it may be possible that the PCR product that was inserted was flipped 180°. In this case, sequencing using the same primers used for PCR may have caused the sequencing reaction to start at the PCR insert but move toward the vector. While this can neither be confirmed nor rejected, but it should be noted that when the samples were sequenced using the correct primers (M13F, M13R), the resulting sequence still matched with the vector.

Of the three hundred clones submitted for sequencing, only 77 returned sequences that did not match with the vector. Table 8 shows what percentage of positive clones matched with which percentage identity in the NCBI database. Only 36.4% of the positive clones matched any sequence in the database (including Uncultured Bacterium) with greater than 98% identity. This suggests at least 64% of the species cloned (49 clones) are new species.

Table 8 - Clones that matched with known organisms in the NCBI database

Successful Sequencing Reactions	77
≥ 95% identity with organism in NCBI database	75.3%
≥ 97% identity with organism in NCBI database	53.2%
≥ 98% identity with organism in NCBI database	36.4%

The clones which did successfully match with organisms in the NCBI database are presented in this section.

Figure 43 –Figure 45 below illustrate phylogenetic trees created using Mega (reference) for each of the samples individually, and Figure 46 is a representation of all samples combined. Each tree has the same anchoring organisms from phyla α - Proteobacteria, β - Proteobacteria, γ - Proteobacteria, δ - Proteobacteria, Bacteroides, Planctomyces, Actinobacteria, and Firmicutes. Some samples do not have clones that fit into each of the phyla, but they are included in all trees for consistency and purposes of cross-comparison.

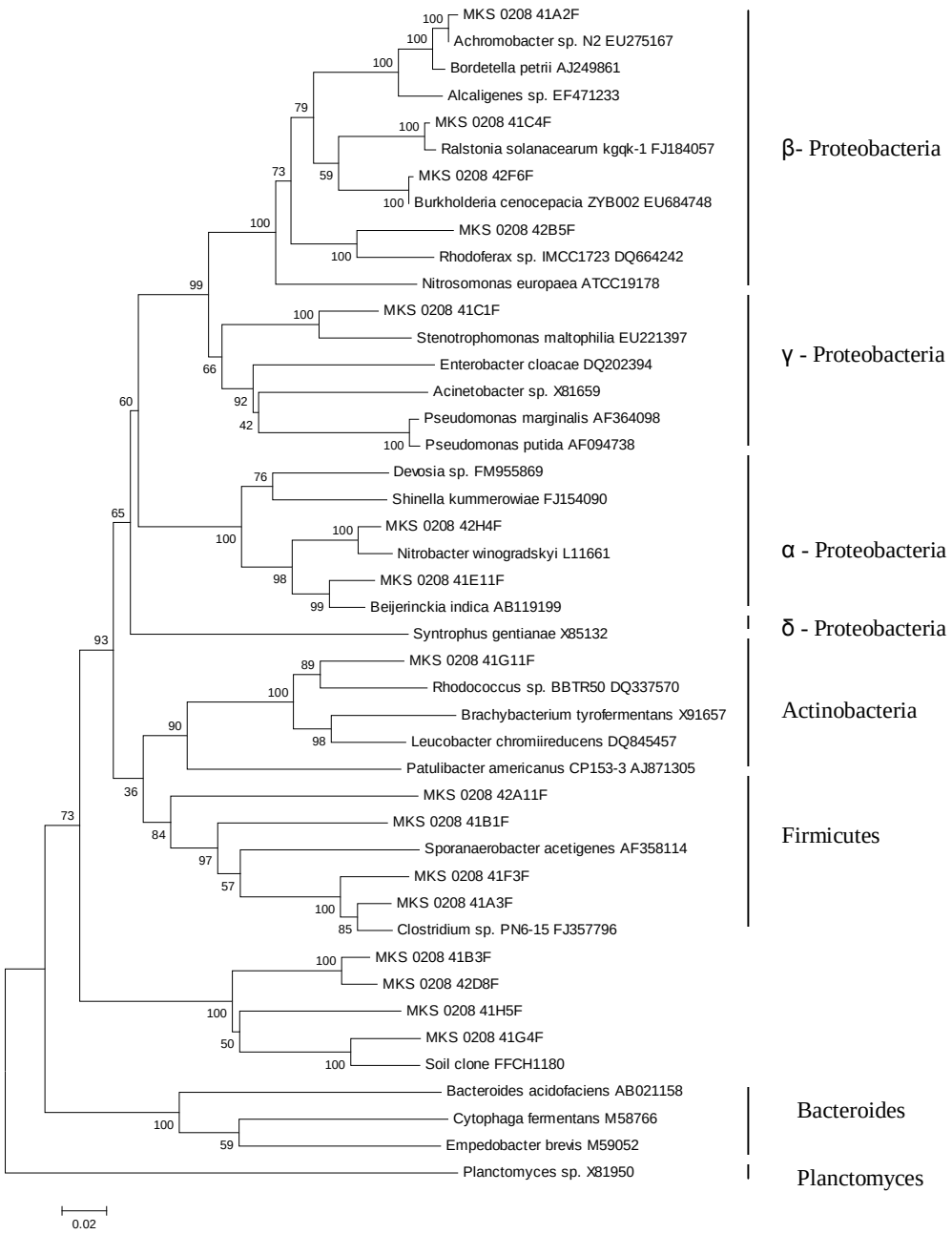

Figure 43 - Bootstrapped phylogenetic tree created from sample collected at Makati South WWTP in February, 2008.

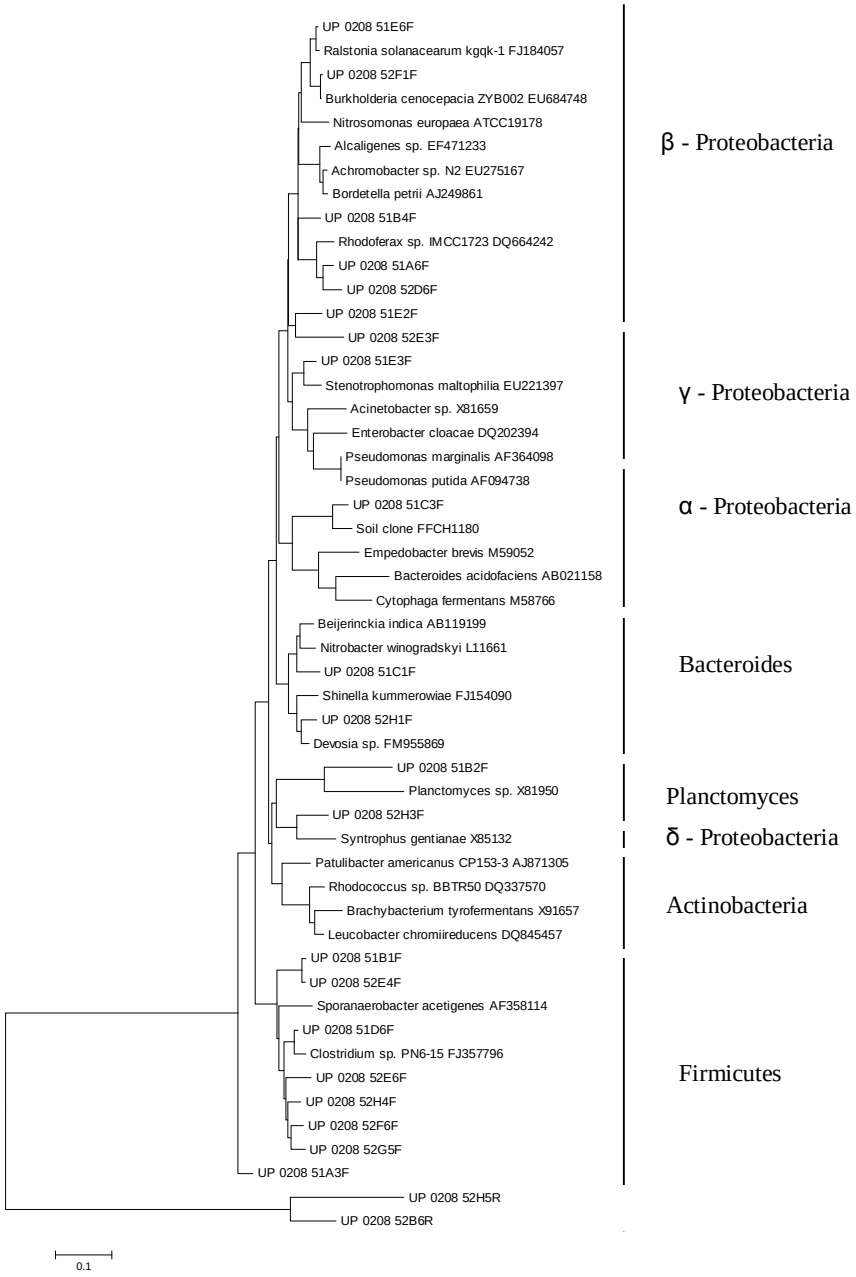

Figure 44 - Phylogenetic tree of representative Bacteria sequences recovered from Samples taken from UP WWTPs in February, 2008.

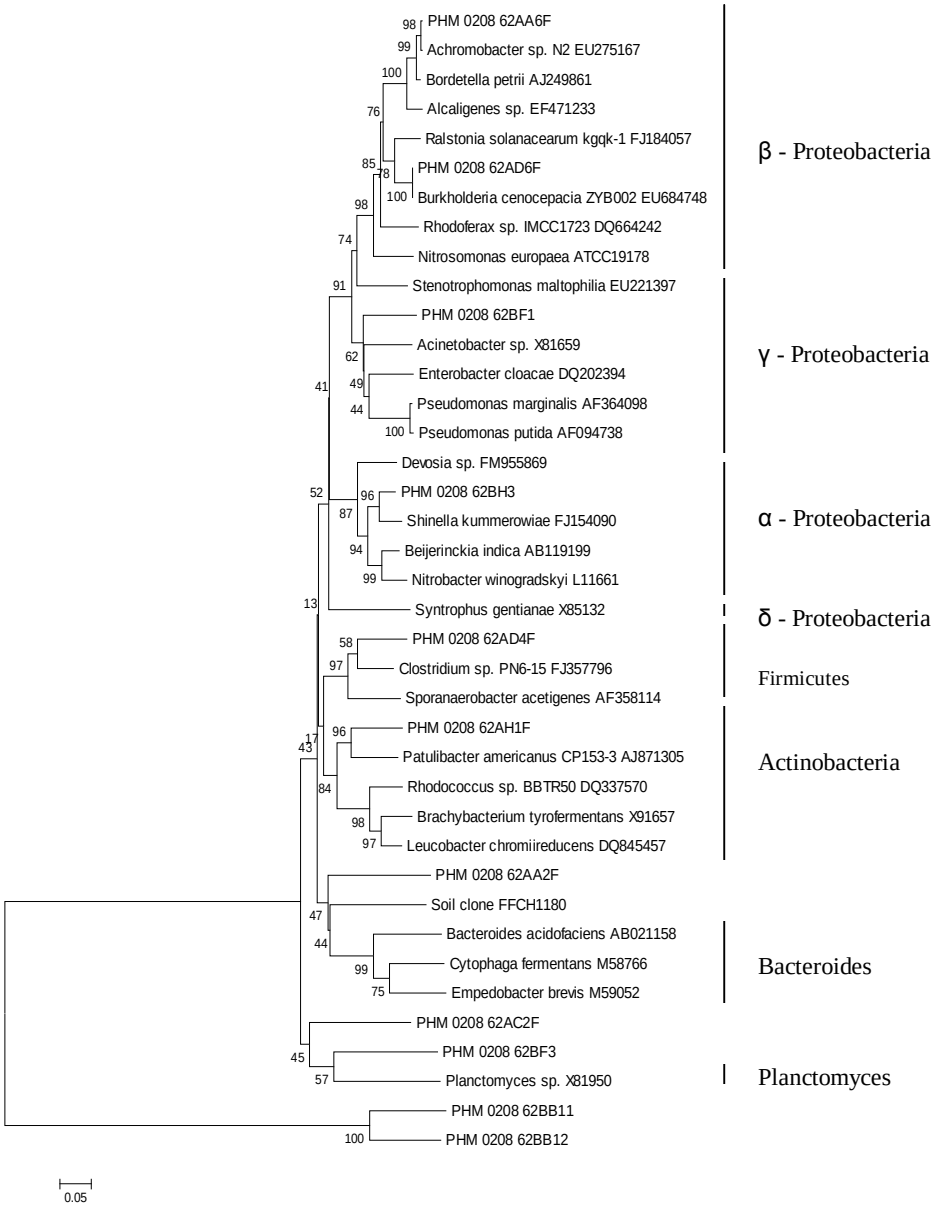

Figure 45 - Phylogenetic tree of representative Bacteria sequences recovered from Samples taken from PhilAm WWTPs in February, 2008.

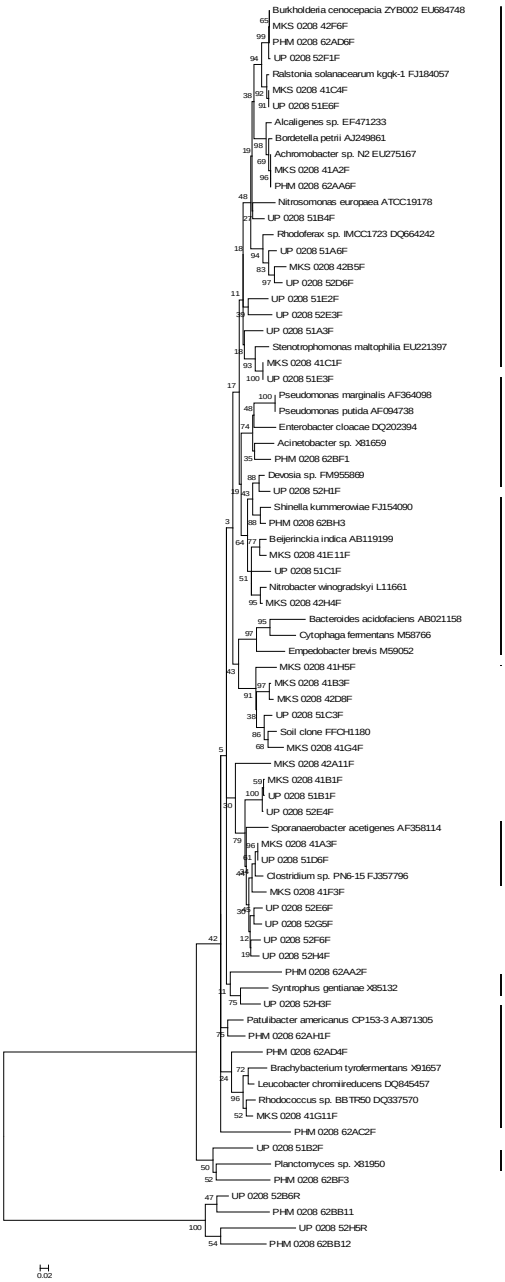

Figure 46 – Phylogenetic tree of representative Bacteria sequences recovered from Samples taken from Makati South, PhilAm and UP WWTPs in February, 2008. See Table 9 for distribution of Bacteria among the different plants.

The phylogenetic trees presented above are summarized in Table 9, with the clone libraries separated by phyla. The numbers in Table 9 represent the number of clones representing each phyla for each sample, with the total number of successful clones shown at the bottom of each column. Note that these totals represent the total number of successful clones out of the 96 sequenced for each sample.

Table 9 - Distribution (numerical) of *Bacteria* DNA sequences in WWTPs

Phylum/Order	**MKS**	**UP**	**PHM**
α - Proteobacteria	2	2	1
β - Proteobacteria	8	13	2
γ - Proteobacteria	4	2	1
δ - Proteobacteria	0	2	0
Actinobacteria	1	0	1
Firmicutes	8	9	1
Bacteroides	0	1	1
Planctomyces	0	1	2
unknown	4	2	2
Total	27	32	11

Figure 47 - Graphical representation of clone library statistics from samples taken in February, 2008.below represents the clone data in terms of percentages of each phyla present in successfully cloned samples. For instance, α – Proteobacteria comprise approximately 7% of organisms cloned from Makati South WWTP (February, 2008).

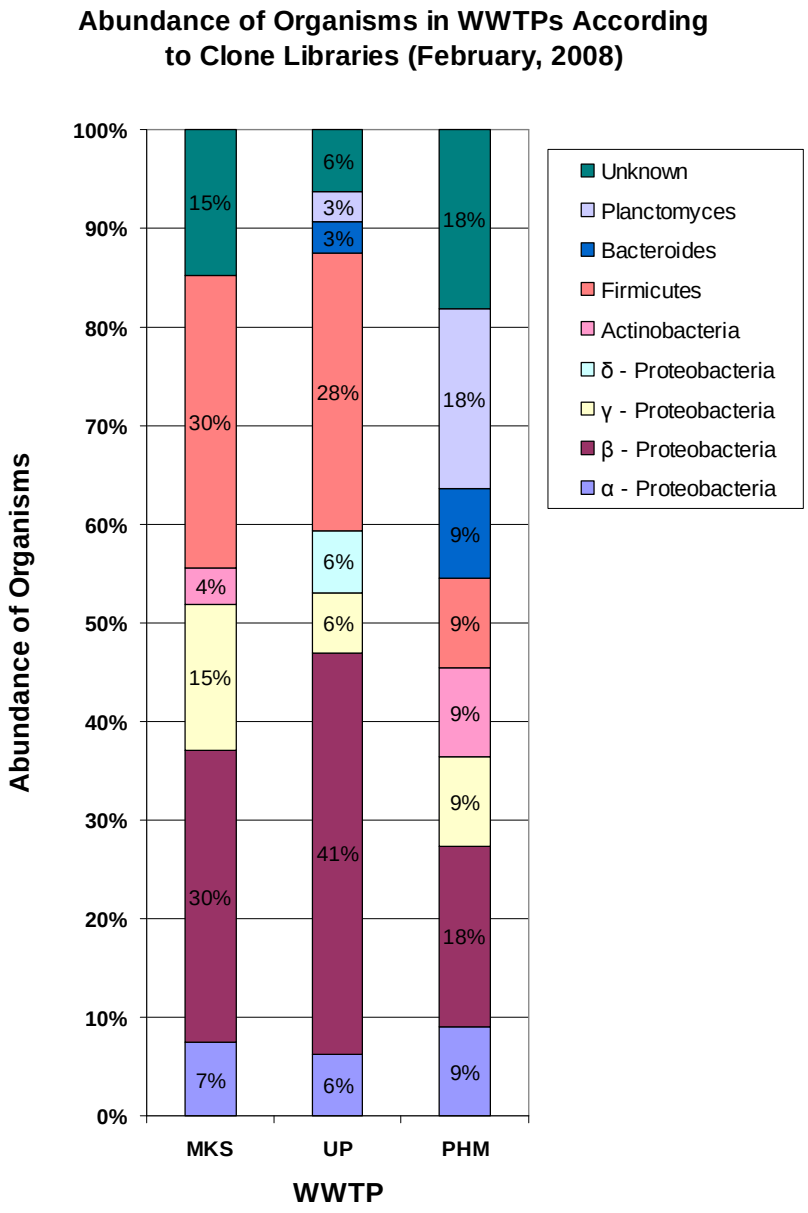

Figure 47 - Graphical representation of clone library statistics from samples taken in February, 2008.

Comparing this chart with Figure 15 - Bacterial Communities in WWTPs (1, 2) shows that α – Proteobacteria has lower abundance in the cloned samples of these WWTPs than in those sampled in studies presented in Figure 15. β- and γ- Proteobacteria match more closely with those samples taken in the US and Europe from Figure 15. Firmicutes are generally more abundant in the clone libraries from the samples in the Philippines. It should be noted, however, that because the clone libraries are very small, they cannot be considered necessarily representative of the actual microbial population.

Figure 48 below illustrates the results of restricting the February samples from the three plants with the enzyme HhaI. Betaproteobacteria comprises a known 14-21% of the samples, which correlates somewhat with the fraction of bacteria cloned and graphed in Figure 47. Unfortunately, most of the T-RFs returned with the T-RFLP data were unable to be matched with organisms, and as such it is impossible to say whether or not more of the species in the sample are. β - Proteobacteria, γ- Proteobacteria, etc.

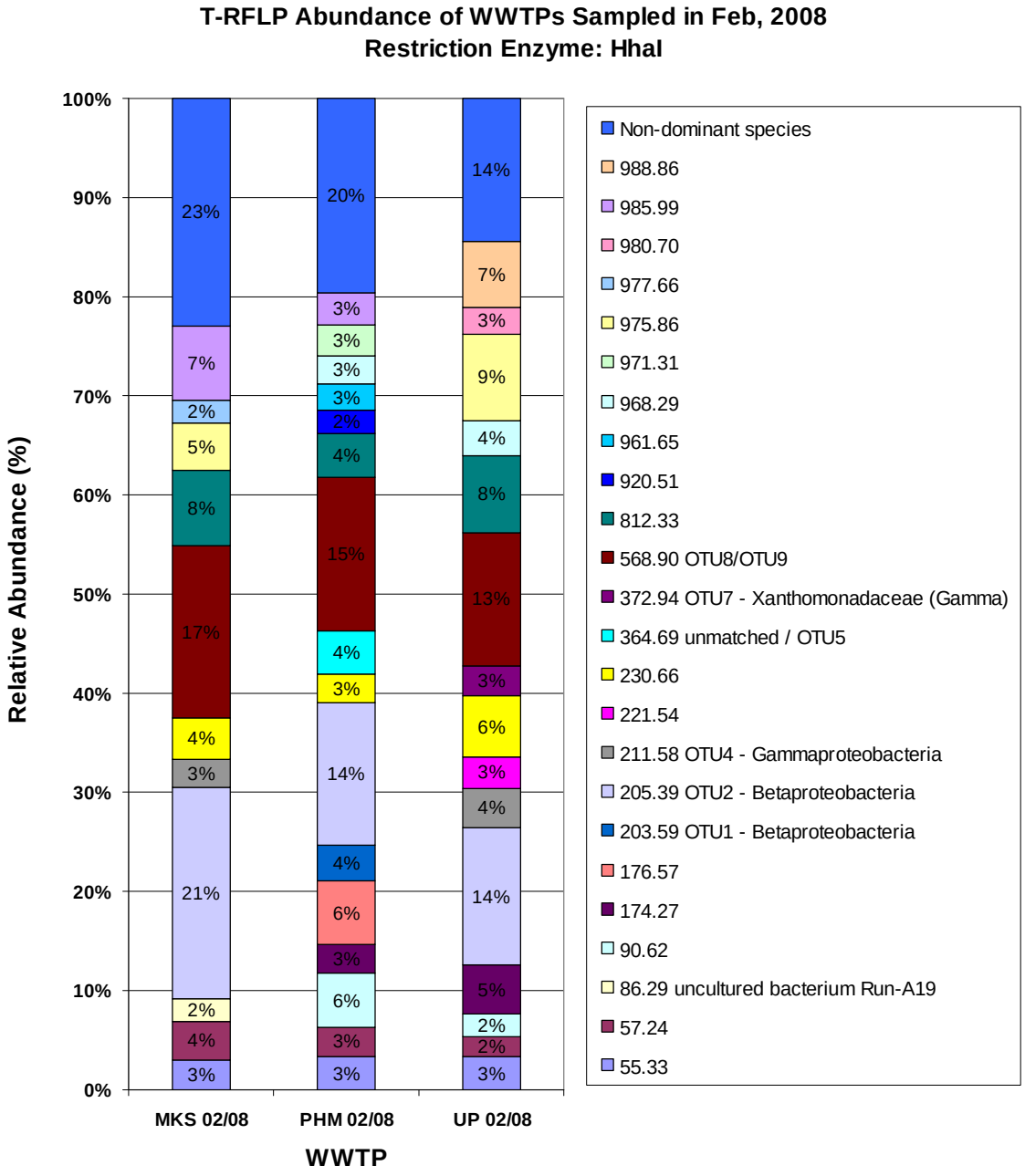

Figure 48 - T-RFLP Abundance of WWTPs Sampled in Feb, 2008 (Restriction Enzyme HhaI)

Implications and Suggestions for further research

Additional research is particularly needed in this area to better identify organisms present in the samples and determine their role in the wastewater treatment process. Forty nine clones did not match with known sequences in the NCBI database (< 98% identity match), indicating previously undescribed species.

Additional samples should be taken and analyzed with cloning. The cloning kit used in this experiment is likely to be the source of inefficiency, but as this cannot be confirmed, additional testing may be required.

If it is possible to learn more about the organisms present in the activated sludge basins in these WWTPs, attempts should be made to correlate these organisms with BOD and nutrient (N and P) removal.

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Appendices

Appendix 1 – Water Quality Results

Site Name		Philippine Std	Villarica
Location			Babak, Samal Island, Davao
parameter	units		
Acidity (as CaCO3)*	mg/L		106.4
Alkalinity (as CaCO3)*	mg/L		56
Arsenic	ppb	50	23
Carbon Dioxide*	mg/L		117.2
Chlorine Dioxide	mg/L		2.7; 1.7
Chlorine, Free	mg/L		0
Chlorine, Total	mg/L		0
Color	units	10	28; 26
Conductivity/TDS	uS/cm	500	647; 663
Copper, LR	mg/L	1	1.37
Hardness (as CaCO3)*	mg/L	300	386
Iron, Total	mg/L	1	0
Manganese, HR	mg/L	0.4	0.2
Nitrate (as N)	mg/L	11.3	10.3
Nitrite (as N)	mg/L	0.91	0.002
Ammonia, LR (as N)	mg/L		0.03
Phosphate - 2.75 L	mg/L		0.28
Silica, LR	mg/L		2.2 limit
Sulfate	mg/L	250	18
Sulfide	mg/L		0
Suspended Solids	mg/L		1
Temperature	C		31.6; 32.9
Turbidity	FAU	5	0; 3
Zinc	mg/L	5	0.12
pathogens (1)			+
pathogens (2)			+
pathogens (3)			+
pathogens (4)			+
pathogens (5)			+
E. coli x1	CFU/mL		0
Total Coliforms x1	CFU/mL		0
E. coli x1	CFU/mL		0
Total Coliforms x1	CFU/mL		0

Site Name		Philippine Std	Nazareno
Location			Babak, Samal Island, Davao
parameter	units		
Acidity (as CaCO3)*	mg/L		135
Alkalinity (as CaCO3)*	mg/L		320
Arsenic	ppb	50	8
Carbon Dioxide*	mg/L		83.8
Chloride	mg/L	250	16.4
Chlorine Dioxide	mg/L		0; 3.0
Chlorine, Free	mg/L		0.04
Chlorine, Total	mg/L		0.1
Color	units	10	0; 30
Conductivity/TDS	uS/cm	500	607
Copper, LR	mg/L	1	1.36
Hardness (as CaCO3)*	mg/L	300	320
Iron, Total	mg/L	1	0.01
Manganese, HR	mg/L	0.4	0.2
Nitrate (as N)	mg/L	11.3	35
Nitrite (as N)	mg/L	0.91	0.015
Ammonia, LR (as N)	mg/L		0.51
Phosphate - 2.75 L	mg/L		0.46
Silica, LR	mg/L		2.2 limit
Sulfate	mg/L	250	12
Sulfide	mg/L		0.19
Suspended Solids	mg/L		2
Temperature	C		29.5
Turbidity	FAU	5	1; 5
Zinc	mg/L	5	0.05
pathogens (1)			+
pathogens (2)			+
pathogens (3)			+
pathogens (4)			+
pathogens (5)			+
E. coli x1	CFU/mL		0
Total Coliforms x1	CFU/mL		0
E. coli x1	CFU/mL		0
Total Coliforms x1	CFU/mL		0

Site Name		Philippine Std	DMC Average
Location			Mahayga, Davao
parameter	units		
Acidity (as CaCO3)*	mg/L		247
Alkalinity (as CaCO3)*	mg/L		597
Arsenic	ppb	50	0
Carbon Dioxide*	mg/L		258
Chloride	mg/L	250	20
Chlorine Dioxide	mg/L		1.9
Chlorine, Free	mg/L		0.04
Chlorine, Total	mg/L		0.02
Color	units	10	17
Conductivity/TDS	uS/cm	500	1157
Copper, LR	mg/L	1	0.03
Hardness (as CaCO3)*	mg/L	300	
Iron, Total	mg/L	1	0.01
Manganese, HR	mg/L	0.4	0.0
Nitrate (as N)	mg/L	11.3	11.7
Nitrite (as N)	mg/L	0.91	0.069
Ammonia, LR (as N)	mg/L		0.49
pH	pH units	6.5-8.5	
Dissolved Oxygen	mg/L		
Phosphate - 2.75 L	mg/L		0.74
Silica, LR	mg/L		
Sulfate	mg/L	250	75
Sulfide	mg/L		0.00
Suspended Solids	mg/L		1
Temperature	C		
Turbidity	FAU	5	3
Zinc	mg/L	5	
pathogens (1)			+
pathogens (2)			+
pathogens (3)			+
pathogens (4)			+
pathogens (5)			+
E. coli x1	CFU/mL		0
Total Coliforms x1	CFU/mL		0
E. coli x1	CFU/mL		0
Total Coliforms x1	CFU/mL		0

Site Name		Philippine Std	San Nicolas
Location			Punta Dumalag, Davao
parameter	units		
Acidity (as CaCO3)*	mg/L		50.4
Alkalinity (as CaCO3)*	mg/L		152
Arsenic	ppb	50	0
Carbon Dioxide*	mg/L		unable to measure
Chloride	mg/L	250	14.4
Chlorine Dioxide	mg/L		0
Chlorine, Free	mg/L		0.06
Chlorine, Total	mg/L		0.19
Color	units	10	0
Conductivity/TDS	uS/cm	500	310
Copper, LR	mg/L	1	0
Hardness (as CaCO3)*	mg/L	300	134
Iron, Total	mg/L	1	0.04
Manganese, HR	mg/L	0.4	0.2
Nitrate (as N)	mg/L	11.3	35
Nitrite (as N)	mg/L	0.91	0.018
Ammonia, LR (as N)	mg/L		0
pH	pH units	6.5-8.5	
Dissolved Oxygen	mg/L		
Phosphate - 2.75 L	mg/L		0.72
Sulfate	mg/L	250	64
Sulfide	mg/L		0.02
Suspended Solids	mg/L		0
Temperature	C		
Turbidity	FAU	5	3
Zinc	mg/L	5	0.19
pathogens (1)			+
pathogens (2)			+
pathogens (3)			
pathogens (4)			
pathogens (5)			

Site Name		Philippine Std	South California
Location			Gravahan, Davao
parameter	units		
Acidity (as CaCO3)*	mg/L		78.8
Alkalinity (as CaCO3)*	mg/L		144
Arsenic	ppb	50	0
Carbon Dioxide*	mg/L		102
Chloride	mg/L	250	14.4
Chlorine Dioxide	mg/L		3
Chlorine, Free	mg/L		0.13
Chlorine, Total	mg/L		0.23
Color	units	10	30
Conductivity/TDS	uS/cm	500	308
Copper, LR	mg/L	1	0.01
Hardness (as CaCO3)*	mg/L	300	116
Iron, Total	mg/L	1	0.03
Manganese, HR	mg/L	0.4	0.3
Nitrate (as N)	mg/L	11.3	35
Nitrite (as N)	mg/L	0.91	0.005
Ammonia, LR (as N)	mg/L		0
pH	pH units	6.5-8.5	
Dissolved Oxygen	mg/L		
Phosphate - 2.75 L	mg/L		0.66
Sulfate	mg/L	250	39
Sulfide	mg/L		0.02
Suspended Solids	mg/L		2
Temperature	C		27.7
Turbidity	FAU	5	5
Zinc	mg/L	5	0
pathogens (1)			+
pathogens (2)			+

Site Name		Philippine Std	Sto. Rosario
Location			Calinan, Davao
parameter	units		
Acidity (as CaCO3)*	mg/L		118.8
Alkalinity (as CaCO3)*	mg/L		191
Arsenic	ppb	50	10
Carbon Dioxide*	mg/L		130
Chloride	mg/L	250	6.75
Chlorine Dioxide	mg/L		0
Chlorine, Free	mg/L		0.01
Chlorine, Total	mg/L		0
Color	units	10	0
Conductivity/TDS	uS/cm	500	374
Copper, LR	mg/L	1	0
Hardness (as CaCO3)*	mg/L	300	166
Iron, Total	mg/L	1	0
Manganese, HR	mg/L	0.4	0
Nitrate (as N)	mg/L	11.3	35
Nitrite (as N)	mg/L	0.91	0.004
Ammonia, LR (as N)	mg/L		0
pH	pH units	6.5-8.5	
Dissolved Oxygen	mg/L		3.1
Phosphate - 2.75 L	mg/L		0.96
Sulfate	mg/L	250	6
Sulfide	mg/L		0
Suspended Solids	mg/L		0
Temperature	C		27
Turbidity	FAU	5	0
Zinc	mg/L	5	0
pathogens (1)			-
pathogens (2)			-
E. coli x1	CFU/mL		0
Total Coliforms x1	CFU/mL		0

Site Name		Philippine Std	Violet Denison - Spring 1 (down hill)
Location			Kibalang, Marilog, Davao
parameter	units		
Acidity (as CaCO3)*	mg/L		152.4
Alkalinity (as CaCO3)*	mg/L		333
Arsenic	ppb	50	40
Carbon Dioxide*	mg/L		214
Chloride	mg/L	250	11.8
Chlorine Dioxide	mg/L		2.9
Chlorine, Free	mg/L		0.06
Chlorine, Total	mg/L		0.01
Color	units	10	40
Conductivity/TDS	uS/cm	500	674
Copper, LR	mg/L	1	0.91
Hardness (as CaCO3)*	mg/L	300	265
Iron, Total	mg/L	1	0.01
Manganese, HR	mg/L	0.4	0.1
Nitrate (as N)	mg/L	11.3	18.4
Nitrite (as N)	mg/L	0.91	0
Ammonia, LR (as N)	mg/L		0.02
pH	pH units	6.5-8.5	
Dissolved Oxygen	mg/L		
Phosphate - 2.75 L	mg/L		2.75
Sulfate	mg/L	250	25
Sulfide	mg/L		0
Suspended Solids	mg/L		0
Temperature	C		24.6
Turbidity	FAU	5	8
Zinc	mg/L	5	0.09
pathogens (1)			+
pathogens (2)			+
E. coli x1	CFU/mL		0
Total Coliforms x1	CFU/mL		0
E. coli x1	CFU/mL		0
Total Coliforms x1	CFU/mL		0

Site Name		Philippine Std	Violet Denison - Spring 2 (by chapel)
Location			Kibalang, Marilog, Davao
parameter	units		
Acidity (as CaCO3)*	mg/L		71.2
Alkalinity (as CaCO3)*	mg/L		145
Arsenic	ppb	50	0
Carbon Dioxide*	mg/L		94
Chloride	mg/L	250	7.6
Chlorine Dioxide	mg/L		1.3
Chlorine, Free	mg/L		0.02
Chlorine, Total	mg/L		0.03
Color	units	10	11
Conductivity/TDS	uS/cm	500	289
Copper, LR	mg/L	1	0.02
Hardness (as CaCO3)*	mg/L	300	145
Iron, Total	mg/L	1	0.01
Manganese, HR	mg/L	0.4	0
Nitrate (as N)	mg/L	11.3	8.7
Nitrite (as N)	mg/L	0.91	0.002
Ammonia, LR (as N)	mg/L		0
pH	pH units	6.5-8.5	
Dissolved Oxygen	mg/L		
Phosphate - 2.75 L	mg/L		0.1
Sulfate	mg/L	250	34
Sulfide	mg/L		0.02
Suspended Solids	mg/L		0
Temperature	C		27.1
Turbidity	FAU	5	2
Zinc	mg/L	5	0.04
pathogens (1)			+
pathogens (2)			+
E. coli x1	CFU/mL		0
Total Coliforms x1	CFU/mL		0
E. coli x1	CFU/mL		0
Total Coliforms x1	CFU/mL		0

Site Name		Philippine Std	Robinhood - 12' Open Well
Location			Valencia, Bukidnon
parameter	units		
Acidity (as CaCO3)*	mg/L		175.2
Alkalinity (as CaCO3)*	mg/L		17.6
Arsenic	ppb	50	0
Carbon Dioxide*	mg/L		122
Chloride	mg/L	250	9.7
Chlorine Dioxide	mg/L		5.1
Chlorine, Free	mg/L		0.04
Chlorine, Total	mg/L		0.03
Color	units	10	63
Conductivity/TDS	uS/cm	500	33.5
Copper, LR	mg/L	1	0
Hardness (as CaCO3)*	mg/L	300	30
Iron, Total	mg/L	1	0.06
Manganese, HR	mg/L	0.4	0.4
Nitrate (as N)	mg/L	11.3	13
Nitrite (as N)	mg/L	0.91	0.007
Ammonia, LR (as N)	mg/L		0
pH	pH units	6.5-8.5	
Dissolved Oxygen	mg/L		
Phosphate - 2.75 L	mg/L		2.75
Sulfate	mg/L	250	51
Sulfide	mg/L		0.03
Suspended Solids	mg/L		9
Temperature	C		28.3
Turbidity	FAU	5	10
Zinc	mg/L	5	0.11
pathogens (1)			+
E. coli x1	CFU/mL		0
Total Coliforms x1	CFU/mL		4
E. coli x1	CFU/mL		0
Total Coliforms x1	CFU/mL		6

Site Name		Philippine Std	Robinhood - Valencia City Water
Location			Valencia, Bukidnon
parameter	units		
Acidity (as CaCO3)*	mg/L		93
Alkalinity (as CaCO3)*	mg/L		128
Arsenic	ppb	50	0
Carbon Dioxide*	mg/L		104
Chloride	mg/L	250	21
Chlorine Dioxide	mg/L		0
Chlorine, Free	mg/L		0.11
Chlorine, Total	mg/L		0.11
Color	units	10	0
Conductivity/TDS	uS/cm	500	154.1
Copper, LR	mg/L	1	0
Hardness (as CaCO3)*	mg/L	300	65
Iron, Total	mg/L	1	0
Manganese, HR	mg/L	0.4	0.1
Nitrate (as N)	mg/L	11.3	0.2
Nitrite (as N)	mg/L	0.91	0.012
Ammonia, LR (as N)	mg/L		0
pH	pH units	6.5-8.5	
Dissolved Oxygen	mg/L		
Phosphate - 2.75 L	mg/L		0.68
Sulfate	mg/L	250	80 L
Sulfide	mg/L		0
Suspended Solids	mg/L		0
Temperature	C		31.2
Turbidity	FAU	5	0
Zinc	mg/L	5	0.1
pathogens (1)			-
E. coli x1	CFU/mL		0
Total Coliforms x1	CFU/mL		0
E. coli x1	CFU/mL		0
Total Coliforms x1	CFU/mL		0

Site Name		Philippine Std	Robinhood 30' closed Well
Location			Valencia, Bukidnon
parameter	units		
Acidity (as CaCO3)*	mg/L		105
Alkalinity (as CaCO3)*	mg/L		28
Arsenic	ppb	50	0
Carbon Dioxide*	mg/L		120
Chloride	mg/L	250	16.5
Chlorine Dioxide	mg/L		0
Chlorine, Free	mg/L		0.02
Chlorine, Total	mg/L		0.02
Color	units	10	0
Conductivity/TDS	uS/cm	500	29.6
Copper, LR	mg/L	1	0
Hardness (as CaCO3)*	mg/L	300	16
Iron, Total	mg/L	1	0.06
Manganese, HR	mg/L	0.4	0.3
Nitrate (as N)	mg/L	11.3	35
Nitrite (as N)	mg/L	0.91	0.003
Ammonia, LR (as N)	mg/L		0
pH	pH units	6.5-8.5	
Dissolved Oxygen	mg/L		4.1
Phosphate - 2.75 L	mg/L		2.75
Sulfate	mg/L	250	24
Sulfide	mg/L		0.02
Suspended Solids	mg/L		0
Temperature	C		28.9
Turbidity	FAU	5	0
Zinc	mg/L	5	0.12
pathogens (1)			+
E. coli x1	CFU/mL		0
Total Coliforms x1	CFU/mL		0
E. coli x1	CFU/mL		0
Total Coliforms x1	CFU/mL		0

Site Name		Philippine Std	Robinhood 35' Closed Well
Location			Valencia, Bukidnon
parameter	units		
Acidity (as CaCO3)*	mg/L		69
Alkalinity (as CaCO3)*	mg/L		5.9
Arsenic	ppb	50	0
Carbon Dioxide*	mg/L		112
Chloride	mg/L	250	26.7
Chlorine Dioxide	mg/L		8
Chlorine, Free	mg/L		0.04
Chlorine, Total	mg/L		0.03
Color	units	10	99
Conductivity/TDS	uS/cm	500	20.9
Copper, LR	mg/L	1	0.02
Hardness (as CaCO3)*	mg/L	300	6
Iron, Total	mg/L	1	0.09
Manganese, HR	mg/L	0.4	0.2
Nitrate (as N)	mg/L	11.3	35
Nitrite (as N)	mg/L	0.91	0
Ammonia, LR (as N)	mg/L		0.03
pH	pH units	6.5-8.5	
Dissolved Oxygen	mg/L		5.8
Phosphate - 2.75 L	mg/L		2.75
Sulfate	mg/L	250	38
Sulfide	mg/L		0.04
Suspended Solids	mg/L		8
Temperature	C		26.8
Turbidity	FAU	5	12
Zinc	mg/L	5	0.08
pathogens (1)			+
E. coli x1	CFU/mL		0
Total Coliforms x1	CFU/mL		2
E. coli x1	CFU/mL		0
Total Coliforms x1	CFU/mL		0

Site Name		Philippine Std	Paradise
Location			Valencia, Bukidnon
parameter	units		
Acidity (as CaCO3)*	mg/L		170.4
Alkalinity (as CaCO3)*	mg/L		28.8
Arsenic	ppb	50	0
Carbon Dioxide*	mg/L		180
Chloride	mg/L	250	13.4
Chlorine Dioxide	mg/L		0
Chlorine, Free	mg/L		0.04
Chlorine, Total	mg/L		0.02
Color	units	10	0
Conductivity/TDS	uS/cm	500	90.8
Copper, LR	mg/L	1	0.02
Hardness (as CaCO3)*	mg/L	300	32.2
Iron, Total	mg/L	1	0.05
Manganese, HR	mg/L	0.4	0.2
Nitrate (as N)	mg/L	11.3	18.4
Nitrite (as N)	mg/L	0.91	0.069
Ammonia, LR (as N)	mg/L		0.01
pH	pH units	6.5-8.5	
Dissolved Oxygen	mg/L		
Phosphate - 2.75 L	mg/L		2.75
Sulfate	mg/L	250	15
Sulfide	mg/L		0.01
Suspended Solids	mg/L		0
Temperature	C		29.5
Turbidity	FAU	5	0
Zinc	mg/L	5	0.13
pathogens (1)			+
E. coli x1	CFU/mL		0
Total Coliforms x1	CFU/mL		0
E. coli x1	CFU/mL		0
Total Coliforms x1	CFU/mL		0

Site Name		Philippine Std	Ray Of Hope City Jail
Location			Maa, Davao City
parameter	units		
Acidity (as CaCO3)*	mg/L		128.8
Alkalinity (as CaCO3)*	mg/L		234 (yellow)
Arsenic	ppb	50	0
Carbon Dioxide*	mg/L		97.8
Chloride	mg/L	250	8.3
Chlorine Dioxide	mg/L		2.1
Chlorine, Free	mg/L		0.37
Chlorine, Total	mg/L		0.83
Color	units	10	24
Conductivity/TDS	uS/cm	500	263
Copper, LR	mg/L	1	0.02
Hardness (as CaCO3)*	mg/L	300	119
Iron, Total	mg/L	1	0.03
Manganese, HR	mg/L	0.4	0.2
Nitrate (as N)	mg/L	11.3	0.5
Nitrite (as N)	mg/L	0.91	0.012
Ammonia, LR (as N)	mg/L		0
pH	pH units	6.5-8.5	--
Dissolved Oxygen	mg/L		--
Phosphate - 2.75 L	mg/L		2.75
Sulfate	mg/L	250	73
Sulfide	mg/L		0.1
Suspended Solids	mg/L		2
Temperature	C		29.1
Turbidity	FAU	5	3
Zinc	mg/L	5	0.46
pathogens (1)			+
pathogens (2)			+
pathogens (3)			+
pathogens (4)			+
pathogens (5)			+
E. coli x1	CFU/mL		0
Total Coliforms x1	CFU/mL		0
E. coli x1	CFU/mL		0
Total Coliforms x1	CFU/mL		0

Site Name		Philippine Std	Riverdrive Spring
Location			Davao
parameter	units		
Acidity (as CaCO3)*	mg/L		149
Alkalinity (as CaCO3)*	mg/L		222
Arsenic	ppb	50	0
Carbon Dioxide*	mg/L		116.8
Chloride	mg/L	250	40
Chlorine Dioxide	mg/L		2
Chlorine, Free	mg/L		0.02
Chlorine, Total	mg/L		0.02
Color	units	10	23
Conductivity/TDS	uS/cm	500	563
Copper, LR	mg/L	1	0.01
Hardness (as CaCO3)*	mg/L	300	271
Iron, Total	mg/L	1	0.01
Manganese, HR	mg/L	0.4	0.1
Nitrate (as N)	mg/L	11.3	9.6
Nitrite (as N)	mg/L	0.91	0.068
Ammonia, LR (as N)	mg/L		0
pH	pH units	6.5-8.5	--
Dissolved Oxygen	mg/L		--
Phosphate - 2.75 L	mg/L		2.75
Sulfate	mg/L	250	51
Sulfide	mg/L		0.01
Suspended Solids	mg/L		7
Temperature	C		29.5
Turbidity	FAU	5	2
Zinc	mg/L	5	--
pathogens (1)			+
pathogens (2)			+
pathogens (3)			+
E. coli x1	CFU/mL		0
Total Coliforms x1	CFU/mL		7

Site Name		Philippine Std	Riverdrive DCWD
Location			Davao
parameter	units		
Acidity (as CaCO3)*	mg/L		102
Alkalinity (as CaCO3)*	mg/L		140
Arsenic	ppb	50	0
Carbon Dioxide*	mg/L		77.8
Chloride	mg/L	250	--
Chlorine Dioxide	mg/L		2.3
Chlorine, Free	mg/L		1.09
Chlorine, Total	mg/L		0.99
Color	units	10	35
Conductivity/TDS	uS/cm	500	238
Copper, LR	mg/L	1	0.02
Hardness (as CaCO3)*	mg/L	300	106
Iron, Total	mg/L	1	0
Manganese, HR	mg/L	0.4	0
Nitrate (as N)	mg/L	11.3	1.1
Nitrite (as N)	mg/L	0.91	0.017
Ammonia, LR (as N)	mg/L		0.01
pH	pH units	6.5-8.5	--
Dissolved Oxygen	mg/L		--
Phosphate - 2.75 L	mg/L		2.75
Sulfate	mg/L	250	80 L
Sulfide	mg/L		0
Suspended Solids	mg/L		2
Temperature	C		30.3
Turbidity	FAU	5	4
Zinc	mg/L	5	--
pathogens (1)			+
pathogens (2)			+
pathogens (3)			+
pathogens (4)			+
pathogens (5)			+
E. coli x1	CFU/mL		0
Total Coliforms x1	CFU/mL		0
E. coli x1	CFU/mL		0
Total Coliforms x1	CFU/mL		0

Site Name		Philippine Std	Pueblo Antonio Average
Location			Davao
parameter	units		
Acidity (as CaCO3)*	mg/L		30
Alkalinity (as CaCO3)*	mg/L		19
Arsenic	ppb	50	70
Carbon Dioxide*	mg/L		22
Chloride	mg/L	250	13
Chlorine Dioxide	mg/L		3.4
Chlorine, Free	mg/L		0.04
Chlorine, Total	mg/L		0.06
Color	units	10	37
Conductivity/TDS	uS/cm	500	72
Copper, LR	mg/L	1	0.00
Hardness (as CaCO3)*	mg/L	300	
Iron, Total	mg/L	1	0.01
Manganese, HR	mg/L	0.4	0.1
Nitrate (as N)	mg/L	11.3	11.3
Nitrite (as N)	mg/L	0.91	0.016
Ammonia, LR (as N)	mg/L		0.00
pH	pH units	6.5-8.5	
Dissolved Oxygen	mg/L		
Phosphate - 2.75 L	mg/L		
Sulfate	mg/L	250	10
Sulfide	mg/L		0.01
Suspended Solids	mg/L		3
Temperature	C		
Turbidity	FAU	5	5
Zinc	mg/L	5	
pathogens (1)			+
pathogens (2)			+
pathogens (3)			+
pathogens (4)			+
pathogens (5)			+
E. coli x1	CFU/mL		0.7
Total Coliforms x1	CFU/mL		12.3

Site Name		Philippine Std	Sta Martha Average
Location			Davao
parameter	units		
Acidity (as CaCO3)*	mg/L		104
Alkalinity (as CaCO3)*	mg/L		113
Arsenic	ppb	50	0
Carbon Dioxide*	mg/L		113
Chloride	mg/L	250	15
Chlorine Dioxide	mg/L		0.0
Chlorine, Free	mg/L		1.13
Chlorine, Total	mg/L		1.06
Color	units	10	31
Conductivity/TDS	uS/cm	500	237
Copper, LR	mg/L	1	0.01
Hardness (as CaCO3)*	mg/L	300	
Iron, Total	mg/L	1	0.10
Manganese, HR	mg/L	0.4	0.1
Nitrate (as N)	mg/L	11.3	7.7
Nitrite (as N)	mg/L	0.91	0.013
Ammonia, LR (as N)	mg/L		0.01
pH	pH units	6.5-8.5	
Dissolved Oxygen	mg/L		
Phosphate - 2.75 L	mg/L		6.23
Sulfate	mg/L	250	20
Sulfide	mg/L		0.00
Suspended Solids	mg/L		0
Temperature	C		
Turbidity	FAU	5	4
Zinc	mg/L	5	
pathogens (1)			+
pathogens (2)			+
pathogens (3)			+
pathogens (4)			+
pathogens (5)			-
E. coli x1	CFU/mL		0
Total Coliforms x1	CFU/mL		0.3

Site Name		Philippine Std	Amparo Spring Average
Location			Manila
parameter	units		
Acidity (as CaCO3)*	mg/L		216
Alkalinity (as CaCO3)*	mg/L		173
Arsenic	ppb	50	0
Carbon Dioxide*	mg/L		198
Chloride	mg/L	250	31
Chlorine Dioxide	mg/L		0.0
Chlorine, Free	mg/L		0.00
Chlorine, Total	mg/L		0.00
Color	units	10	0
Conductivity/TDS	uS/cm	500	403
Copper, LR	mg/L	1	0.00
Hardness (as CaCO3)*	mg/L	300	
Iron, Total	mg/L	1	0.00
Manganese, HR	mg/L	0.4	0.1
Nitrate (as N)	mg/L	11.3	0.5
Nitrite (as N)	mg/L	0.91	0.010
Ammonia, LR (as N)	mg/L		0.10
pH	pH units	6.5-8.5	
Dissolved Oxygen	mg/L		
Phosphate - 2.75 L	mg/L		2.75
Sulfate	mg/L	250	47
Sulfide	mg/L		0.00
Suspended Solids	mg/L		0
Temperature	C		
Turbidity	FAU	5	0
Zinc	mg/L	5	
pathogens (1)			+
pathogens (2)			+
pathogens (3)			+
pathogens (4)			+
pathogens (5)			+
E. coli x1	CFU/mL		0
Total Coliforms x1	CFU/mL		3
E. coli x10	CFU/mL		0
Total Coliforms x10	CFU/mL		2

Site Name		Philippine Std	Amparo Maynilad Average
Location			Manila
parameter	units		
Acidity (as CaCO3)*	mg/L		63
Alkalinity (as CaCO3)*	mg/L		72
Arsenic	ppb	50	0
Carbon Dioxide*	mg/L		56
Chloride	mg/L	250	12
Chlorine Dioxide	mg/L		0.0
Chlorine, Free	mg/L		0.92
Chlorine, Total	mg/L		1.05
Color	units	10	32
Conductivity/TDS	uS/cm	500	142
Copper, LR	mg/L	1	0.02
Hardness (as CaCO3)*	mg/L	300	
Iron, Total	mg/L	1	0.00
Manganese, HR	mg/L	0.4	0.3
Nitrate (as N)	mg/L	11.3	12.9
Nitrite (as N)	mg/L	0.91	0.004
Ammonia, LR (as N)	mg/L		0.00
pH	pH units	6.5-8.5	
Dissolved Oxygen	mg/L		
Phosphate - 2.75 L	mg/L		0.79
Sulfate	mg/L	250	18
Sulfide	mg/L		0.00
Suspended Solids	mg/L		6
Temperature	C		
Turbidity	FAU	5	4
Zinc	mg/L	5	
pathogens (1)			+
pathogens (2)			+
pathogens (3)			+
pathogens (4)			+
pathogens (5)			+
E. coli x1	CFU/mL		0
Total Coliforms x1	CFU/mL		0
E. coli x10	CFU/mL		0
Total Coliforms x10	CFU/mL		0

Site Name		Philippine Std	Reunion Onsite Well
Location			Anus, San Jose, Batangas
parameter	units		
Acidity (as CaCO3)*	mg/L		179.5
Alkalinity (as CaCO3)*	mg/L		121
Arsenic	ppb	50	0
Carbon Dioxide*	mg/L		142
Chloride	mg/L	250	28.1
Chlorine Dioxide	mg/L		1.1
Chlorine, Free	mg/L		0.005
Chlorine, Total	mg/L		0.03
Color	units	10	0
Conductivity/TDS	uS/cm	500	369
Copper, LR	mg/L	1	0.03
Hardness (as CaCO3)*	mg/L	300	135.6
Iron, Total	mg/L	1	0.02
Manganese, HR	mg/L	0.4	0
Nitrate (as N)	mg/L	11.3	2.775
Nitrite (as N)	mg/L	0.91	0.0225
Ammonia, LR (as N)	mg/L		0.015
pH	pH units	6.5-8.5	
Dissolved Oxygen	mg/L		
Phosphate - 2.75 L	mg/L		2.225
Sulfate	mg/L	250	32
Sulfide	mg/L		0.005
Suspended Solids	mg/L		0
Temperature	C		28.15
Turbidity	FAU	5	0
Zinc	mg/L	5	
pathogens (1)			+
pathogens (2)			+
E. coli x1	CFU/mL		0
Total Coliforms x1	CFU/mL		7.5
E. coli x10	CFU/mL		0
Total Coliforms x10	CFU/mL		1.5

Site Name		Philippine Std	GK Reunion Barangay Water
Location			Anus, San Jose, Batangas
parameter	units		
Acidity (as CaCO3)*	mg/L		177
Alkalinity (as CaCO3)*	mg/L		125
Arsenic	ppb	50	17
Carbon Dioxide*	mg/L		140
Chloride	mg/L	250	36.25
Chlorine Dioxide	mg/L		2.6
Chlorine, Free	mg/L		0.03
Chlorine, Total	mg/L		0.01
Color	units	10	33
Conductivity/TDS	uS/cm	500	326
Copper, LR	mg/L	1	0
Hardness (as CaCO3)*	mg/L	300	128
Iron, Total	mg/L	1	0
Manganese, HR	mg/L	0.4	0.2
Nitrate (as N)	mg/L	11.3	0.1
Nitrite (as N)	mg/L	0.91	0.004
Ammonia, LR (as N)	mg/L		0.01
pH	pH units	6.5-8.5	
Dissolved Oxygen	mg/L		
Phosphate - 2.75 L	mg/L		2.75
Sulfate	mg/L	250	21
Sulfide	mg/L		0.01
Suspended Solids	mg/L		1
Temperature	C		27.5
Turbidity	FAU	5	2
Zinc	mg/L	5	
pathogens (1)			+
pathogens (2)			+
E. coli x1	CFU/mL		0
Total Coliforms x1	CFU/mL		0
E. coli x10	CFU/mL		0
Total Coliforms x10	CFU/mL		0

Site Name		Philippine Std	Shell Libjo Well #1
Location			Libjo, Batangas
parameter	units		
Acidity (as CaCO3)*	mg/L		154
Alkalinity (as CaCO3)*	mg/L		295
Arsenic	ppb	50	0
Carbon Dioxide*	mg/L		208
Chloride	mg/L	250	34.75
Chlorine Dioxide	mg/L		0
Chlorine, Free	mg/L		--
Chlorine, Total	mg/L		--
Color	units	10	0
Conductivity/TDS	uS/cm	500	588
Copper, LR	mg/L	1	0.02
Hardness (as CaCO3)*	mg/L	300	224
Iron, Total	mg/L	1	0.03
Manganese, HR	mg/L	0.4	0.3
Nitrate (as N)	mg/L	11.3	12
Nitrite (as N)	mg/L	0.91	0.008
Ammonia, LR (as N)	mg/L		0.02
pH	pH units	6.5-8.5	
Dissolved Oxygen	mg/L		3.9
Phosphate - 2.75 L	mg/L		2.5
Sulfate	mg/L	250	41
Sulfide	mg/L		0
Suspended Solids	mg/L		0
Temperature	C		
Turbidity	FAU	5	0
Zinc	mg/L	5	
pathogens (1)			+
pathogens (2)			+
E. coli x1	CFU/mL		0
Total Coliforms x1	CFU/mL		0
E. coli x10	CFU/mL		0
Total Coliforms x10	CFU/mL		0

Site Name		Philippine Std	Shell Libjo Well #2
Location			Libjo, Batangas
parameter	units		
Acidity (as CaCO3)*	mg/L		151
Alkalinity (as CaCO3)*	mg/L		264
Arsenic	ppb	50	10
Carbon Dioxide*	mg/L		134
Chloride	mg/L	250	40
Chlorine Dioxide	mg/L		1.7
Chlorine, Free	mg/L		
Chlorine, Total	mg/L		
Color	units	10	25
Conductivity/TDS	uS/cm	500	593
Copper, LR	mg/L	1	0
Hardness (as CaCO3)*	mg/L	300	222
Iron, Total	mg/L	1	0.13
Manganese, HR	mg/L	0.4	0.3
Nitrate (as N)	mg/L	11.3	0.4
Nitrite (as N)	mg/L	0.91	0.009
Ammonia, LR (as N)	mg/L		0
pH	pH units	6.5-8.5	
Dissolved Oxygen	mg/L		2.9
Phosphate - 2.75 L	mg/L		1.23
Sulfate	mg/L	250	9
Sulfide	mg/L		0
Suspended Solids	mg/L		0
Temperature	C		
Turbidity	FAU	5	3
Zinc	mg/L	5	
pathogens (1)			+
pathogens (2)			+
E. coli x1	CFU/mL		0
Total Coliforms x1	CFU/mL		0
E. coli x10	CFU/mL		0
Total Coliforms x10	CFU/mL		0

Site Name		Philippine Std	Sico 1 EBD Well #1
Location			Batangas
parameter	units		
Acidity (as CaCO3)*	mg/L		300
Alkalinity (as CaCO3)*	mg/L		335
Arsenic	ppb	50	0
Carbon Dioxide*	mg/L		340
Chloride	mg/L	250	124.5
Chlorine Dioxide	mg/L		1
Chlorine, Free	mg/L		
Chlorine, Total	mg/L		
Color	units	10	2
Conductivity/TDS	uS/cm	500	1088
Copper, LR	mg/L	1	1.7
Hardness (as CaCO3)*	mg/L	300	459
Iron, Total	mg/L	1	0.19
Manganese, HR	mg/L	0.4	0.1
Nitrate (as N)	mg/L	11.3	17.5
Nitrite (as N)	mg/L	0.91	0.089
Ammonia, LR (as N)	mg/L		0
pH	pH units	6.5-8.5	
Dissolved Oxygen	mg/L		2.9
Phosphate - 2.75 L	mg/L		2.75
Sulfate	mg/L	250	19
Sulfide	mg/L		0
Suspended Solids	mg/L		1
Temperature	C		
Turbidity	FAU	5	0
Zinc	mg/L	5	
pathogens (1)			+
pathogens (2)			+
E. coli x1	CFU/mL		0
Total Coliforms x1	CFU/mL		0
E. coli x10	CFU/mL		0
Total Coliforms x10	CFU/mL		0

Site Name		Philippine Std	Sico 1 EBD Well #2
Location			Batangas
parameter	units		
Acidity (as CaCO3)*	mg/L		280
Alkalinity (as CaCO3)*	mg/L		306
Arsenic	ppb	50	0
Carbon Dioxide*	mg/L		240
Chloride	mg/L	250	62
Chlorine Dioxide	mg/L		0.5
Chlorine, Free	mg/L		
Chlorine, Total	mg/L		
Color	units	10	2
Conductivity/TDS	uS/cm	500	762
Copper, LR	mg/L	1	0.37
Hardness (as CaCO3)*	mg/L	300	258
Iron, Total	mg/L	1	0.05
Manganese, HR	mg/L	0.4	0.2
Nitrate (as N)	mg/L	11.3	1
Nitrite (as N)	mg/L	0.91	0.024
Ammonia, LR (as N)	mg/L		0
pH	pH units	6.5-8.5	
Dissolved Oxygen	mg/L		3.9
Phosphate - 2.75 L	mg/L		0.087
Sulfate	mg/L	250	68
Sulfide	mg/L		0.01
Suspended Solids	mg/L		1
Temperature	C		
Turbidity	FAU	5	0
Zinc	mg/L	5	
pathogens (1)			+
pathogens (2)			+
E. coli x1	CFU/mL		8
Total Coliforms x1	CFU/mL		~100
E. coli x10	CFU/mL		2
Total Coliforms x10	CFU/mL		~80

Site Name		Philippine Std	GK OKS Tank
Location			Batangas
parameter	units		
Acidity (as CaCO3)*	mg/L		118
Alkalinity (as CaCO3)*	mg/L		118
Arsenic	ppb	50	0
Carbon Dioxide*	mg/L		122
Chloride	mg/L	250	56.75
Chlorine Dioxide	mg/L		0
Chlorine, Free	mg/L		
Chlorine, Total	mg/L		
Color	units	10	0
Conductivity/TDS	uS/cm	500	403
Copper, LR	mg/L	1	0.05
Hardness (as CaCO3)*	mg/L	300	143
Iron, Total	mg/L	1	0.06
Manganese, HR	mg/L	0.4	0
Nitrate (as N)	mg/L	11.3	0.4
Nitrite (as N)	mg/L	0.91	0.004
Ammonia, LR (as N)	mg/L		0.04
pH	pH units	6.5-8.5	
Dissolved Oxygen	mg/L		
Phosphate - 2.75 L	mg/L		2.75
Sulfate	mg/L	250	10
Sulfide	mg/L		0
Suspended Solids	mg/L		0
Temperature	C		
Turbidity	FAU	5	0
Zinc	mg/L	5	
pathogens (1)			+
pathogens (2)			+
E. coli x1	CFU/mL		0
Total Coliforms x1	CFU/mL		0
E. coli x10	CFU/mL		0
Total Coliforms x10	CFU/mL		0

Site Name		Philippine Std	GK OKS 40' Well
Location			Batangas
parameter	units		
Acidity (as CaCO3)*	mg/L		114
Alkalinity (as CaCO3)*	mg/L		130
Arsenic	ppb	50	0
Carbon Dioxide*	mg/L		126
Chloride	mg/L	250	54.75
Chlorine Dioxide	mg/L		0
Chlorine, Free	mg/L		
Chlorine, Total	mg/L		
Color	units	10	0
Conductivity/TDS	uS/cm	500	391
Copper, LR	mg/L	1	0.03
Hardness (as CaCO3)*	mg/L	300	146
Iron, Total	mg/L	1	0.1
Manganese, HR	mg/L	0.4	0.5
Nitrate (as N)	mg/L	11.3	1.3
Nitrite (as N)	mg/L	0.91	0.021
Ammonia, LR (as N)	mg/L		0
pH	pH units	6.5-8.5	
Dissolved Oxygen	mg/L		
Phosphate - 2.75 L	mg/L		2.75
Sulfate	mg/L	250	4
Sulfide	mg/L		0
Suspended Solids	mg/L		0
Temperature	C		
Turbidity	FAU	5	0
Zinc	mg/L	5	
pathogens (1)			+
pathogens (2)			+
E. coli x1	CFU/mL		0
Total Coliforms x1	CFU/mL		2
E. coli x10	CFU/mL		0
Total Coliforms x10	CFU/mL		0

Site Name		Philippine Std	GK OKS 80' Well
Location			Batangas
parameter	units		
Acidity (as CaCO3)*	mg/L		140
Alkalinity (as CaCO3)*	mg/L		126
Arsenic	ppb	50	0
Carbon Dioxide*	mg/L		160
Chloride	mg/L	250	53.75
Chlorine Dioxide	mg/L		0
Chlorine, Free	mg/L		
Chlorine, Total	mg/L		
Color	units	10	0
Conductivity/TDS	uS/cm	500	366
Copper, LR	mg/L	1	0.06
Hardness (as CaCO3)*	mg/L	300	128
Iron, Total	mg/L	1	0.05
Manganese, HR	mg/L	0.4	0.4
Nitrate (as N)	mg/L	11.3	0.3
Nitrite (as N)	mg/L	0.91	0.012
Ammonia, LR (as N)	mg/L		0
pH	pH units	6.5-8.5	
Dissolved Oxygen	mg/L		
Phosphate - 2.75 L	mg/L		1.34
Sulfate	mg/L	250	11
Sulfide	mg/L		0
Suspended Solids	mg/L		0
Temperature	C		
Turbidity	FAU	5	0
Zinc	mg/L	5	
pathogens (1)			+
pathogens (2)			+
E. coli x1	CFU/mL		0
Total Coliforms x1	CFU/mL		0
E. coli x10	CFU/mL		0
Total Coliforms x10	CFU/mL		0

Site Name		Philippine Std	GK San Juan
Location			Batangas
parameter	units		
Acidity (as CaCO3)*	mg/L		226
Alkalinity (as CaCO3)*	mg/L		261
Arsenic	ppb	50	0
Carbon Dioxide*	mg/L		232
Chloride	mg/L	250	46.75
Chlorine Dioxide	mg/L		0
Chlorine, Free	mg/L		
Chlorine, Total	mg/L		
Color	units	10	0
Conductivity/TDS	uS/cm	500	620
Copper, LR	mg/L	1	0.06
Hardness (as CaCO3)*	mg/L	300	248
Iron, Total	mg/L	1	0.14
Manganese, HR	mg/L	0.4	0.2
Nitrate (as N)	mg/L	11.3	0.1
Nitrite (as N)	mg/L	0.91	0.009
Ammonia, LR (as N)	mg/L		0.04
pH	pH units	6.5-8.5	
Dissolved Oxygen	mg/L		
Phosphate - 2.75 L	mg/L		2.5
Sulfate	mg/L	250	3
Sulfide	mg/L		0.01
Suspended Solids	mg/L		0
Temperature	C		
Turbidity	FAU	5	0
Zinc	mg/L	5	
pathogens (1)			+
pathogens (2)			+
E. coli x1	CFU/mL		20
Total Coliforms x1	CFU/mL		115
E. coli x10	CFU/mL		22
Total Coliforms x10	CFU/mL		~80

Site Name		Philippine Std	GK Buhay Nasapa
Location			Batangas
parameter	units		
Acidity (as CaCO3)*	mg/L		232
Alkalinity (as CaCO3)*	mg/L		312
Arsenic	ppb	50	10
Carbon Dioxide*	mg/L		192
Chloride	mg/L	250	51.25
Chlorine Dioxide	mg/L		0*
Chlorine, Free	mg/L		
Chlorine, Total	mg/L		
Color	units	10	0
Conductivity/TDS	uS/cm	500	746
Copper, LR	mg/L	1	0.07
Hardness (as CaCO3)*	mg/L	300	273
Iron, Total	mg/L	1	0.04
Manganese, HR	mg/L	0.4	0.2
Nitrate (as N)	mg/L	11.3	0.4
Nitrite (as N)	mg/L	0.91	0.001
Ammonia, LR (as N)	mg/L		0.05
pH	pH units	6.5-8.5	
Dissolved Oxygen	mg/L		3.6
Phosphate - 2.75 L	mg/L		2.75
Sulfate	mg/L	250	10
Sulfide	mg/L		0.01
Suspended Solids	mg/L		0
Temperature	C		
Turbidity	FAU	5	0
Zinc	mg/L	5	
pathogens (1)			+
pathogens (2)			+
E. coli x1	CFU/mL		0
Total Coliforms x1	CFU/mL		1
E. coli x10	CFU/mL		0
Total Coliforms x10	CFU/mL		32 *unreliable

Site Name		Philippine Std	GK San Francisco
Location			Batangas
parameter	units		
Acidity (as CaCO3)*	mg/L		116
Alkalinity (as CaCO3)*	mg/L		170
Arsenic	ppb	50	30
Carbon Dioxide*	mg/L		120
Chloride	mg/L	250	219
Chlorine Dioxide	mg/L		0
Chlorine, Free	mg/L		
Chlorine, Total	mg/L		
Color	units	10	0
Conductivity/TDS	uS/cm	500	1078
Copper, LR	mg/L	1	0.02
Hardness (as CaCO3)*	mg/L	300	370
Iron, Total	mg/L	1	0.01
Manganese, HR	mg/L	0.4	0.4
Nitrate (as N)	mg/L	11.3	8.5
Nitrite (as N)	mg/L	0.91	0.046
Ammonia, LR (as N)	mg/L		0.22
pH	pH units	6.5-8.5	
Dissolved Oxygen	mg/L		
Phosphate - 2.75 L	mg/L		2.75
Sulfate	mg/L	250	30
Sulfide	mg/L		0
Suspended Solids	mg/L		0
Temperature	C		
Turbidity	FAU	5	0
Zinc	mg/L	5	
pathogens (1)			+
pathogens (2)			+
E. coli x1	CFU/mL		0
Total Coliforms x1	CFU/mL		2
E. coli x10	CFU/mL		0
Total Coliforms x10	CFU/mL		2

Site Name		Philippine Std	GK 1st Calacan Well #1
Location			Batangas
parameter	units		
Acidity (as CaCO3)*	mg/L		147
Alkalinity (as CaCO3)*	mg/L		163
Arsenic	ppb	50	0
Carbon Dioxide*	mg/L		122
Chloride	mg/L	250	34
Chlorine Dioxide	mg/L		0
Chlorine, Free	mg/L		
Chlorine, Total	mg/L		
Color	units	10	0
Conductivity/TDS	uS/cm	500	508
Copper, LR	mg/L	1	0.02
Hardness (as CaCO3)*	mg/L	300	196
Iron, Total	mg/L	1	0.06
Manganese, HR	mg/L	0.4	0.4
Nitrate (as N)	mg/L	11.3	0.4
Nitrite (as N)	mg/L	0.91	0.019
Ammonia, LR (as N)	mg/L		0.01
pH	pH units	6.5-8.5	
Dissolved Oxygen	mg/L		
Phosphate - 2.75 L	mg/L		0.87
Sulfate	mg/L	250	49
Sulfide	mg/L		0
Suspended Solids	mg/L		0
Temperature	C		
Turbidity	FAU	5	1
Zinc	mg/L	5	
pathogens (1)			+
pathogens (2)			+
E. coli x1	CFU/mL		0
Total Coliforms x1	CFU/mL		0
E. coli x10	CFU/mL		0
Total Coliforms x10	CFU/mL		0

Site Name		Philippine Std	GK 1st Calacan Well #2
Location			Batangas
parameter	units		
Acidity (as CaCO3)*	mg/L		157
Alkalinity (as CaCO3)*	mg/L		188
Arsenic	ppb	50	5
Carbon Dioxide*	mg/L		140
Chloride	mg/L	250	39
Chlorine Dioxide	mg/L		0*
Chlorine, Free	mg/L		
Chlorine, Total	mg/L		
Color	units	10	0
Conductivity/TDS	uS/cm	500	553
Copper, LR	mg/L	1	0
Hardness (as CaCO3)*	mg/L	300	216
Iron, Total	mg/L	1	0
Manganese, HR	mg/L	0.4	0.1
Nitrate (as N)	mg/L	11.3	9.95
Nitrite (as N)	mg/L	0.91	0.019
Ammonia, LR (as N)	mg/L		0
pH	pH units	6.5-8.5	
Dissolved Oxygen	mg/L		
Phosphate - 2.75 L	mg/L		2.75
Sulfate	mg/L	250	66
Sulfide	mg/L		0
Suspended Solids	mg/L		0
Temperature	C		
Turbidity	FAU	5	0
Zinc	mg/L	5	
pathogens (1)			+
pathogens (2)			+
E. coli x1	CFU/mL		0
Total Coliforms x1	CFU/mL		1
E. coli x10	CFU/mL		0
Total Coliforms x10	CFU/mL		0

Site Name		Philippine Std	Brookside Filtered Average
Location			Manila
parameter	units		
Acidity (as CaCO3)*	mg/L		98
Alkalinity (as CaCO3)*	mg/L		70
Arsenic	ppb	50	0
Carbon Dioxide*	mg/L		147
Chloride	mg/L	250	13
Chlorine Dioxide	mg/L		0.0
Chlorine, Free	mg/L		0.02
Chlorine, Total	mg/L		0.03
Color	units	10	6
Conductivity/TDS	uS/cm	500	148
Copper, LR	mg/L	1	0.00
Hardness (as CaCO3)*	mg/L	300	
Iron, Total	mg/L	1	0.03
Manganese, HR	mg/L	0.4	0.1
Nitrate (as N)	mg/L	11.3	0.6
Nitrite (as N)	mg/L	0.91	0.003
Ammonia, LR (as N)	mg/L		0.00
pH	pH units	6.5-8.5	6
Dissolved Oxygen	mg/L		
Phosphate - 2.75 L	mg/L		1.54
Sulfate	mg/L	250	5
Sulfide	mg/L		0.01
Suspended Solids	mg/L		0
Temperature	C		
Turbidity	FAU	5	1
Zinc	mg/L	5	
pathogens (1)			+
pathogens (2)			+
E. coli x1	CFU/mL		0
Total Coliforms x1	CFU/mL		0
E. coli x10	CFU/mL		0
Total Coliforms x10	CFU/mL		0

Site Name		Philippine Std	Brookside Maynilad Average
Location			Manila
parameter	units		
Acidity (as CaCO3)*	mg/L		141
Alkalinity (as CaCO3)*	mg/L		72
Arsenic	ppb	50	0
Carbon Dioxide*	mg/L		155
Chloride	mg/L	250	12
Chlorine Dioxide	mg/L		0.0
Chlorine, Free	mg/L		0.41
Chlorine, Total	mg/L		0.48
Color	units	10	6
Conductivity/TDS	uS/cm	500	145
Copper, LR	mg/L	1	0.02
Hardness (as CaCO3)*	mg/L	300	
Iron, Total	mg/L	1	0.00
Manganese, HR	mg/L	0.4	0.1
Nitrate (as N)	mg/L	11.3	0.4
Nitrite (as N)	mg/L	0.91	0.003
Ammonia, LR (as N)	mg/L		0.03
pH	pH units	6.5-8.5	6
Dissolved Oxygen	mg/L		
Phosphate - 2.75 L	mg/L		0.34
Sulfate	mg/L	250	6
Sulfide	mg/L		0.00
Suspended Solids	mg/L		0
Temperature	C		
Turbidity	FAU	5	1
Zinc	mg/L	5	
pathogens (1)			+
pathogens (2)			+
E. coli x1	CFU/mL		0
Total Coliforms x1	CFU/mL		0
E. coli x10	CFU/mL		0
Total Coliforms x10	CFU/mL		0

Site Name		Philippine Std	Police Well Average
Location			Taguig
parameter	units		
Acidity (as CaCO3)*	mg/L		243
Alkalinity (as CaCO3)*	mg/L		327
Arsenic	ppb	50	10
Carbon Dioxide*	mg/L		218
Chloride	mg/L	250	238
Chlorine Dioxide	mg/L		2.6
Chlorine, Free	mg/L		0.03
Chlorine, Total	mg/L		0.01
Color	units	10	33
Conductivity/TDS	uS/cm	500	1296
Copper, LR	mg/L	1	1.30
Hardness (as CaCO3)*	mg/L	300	
Iron, Total	mg/L	1	0.09
Manganese, HR	mg/L	0.4	0.2
Nitrate (as N)	mg/L	11.3	3.8
Nitrite (as N)	mg/L	0.91	0.009
Ammonia, LR (as N)	mg/L		0.02
pH	pH units	6.5-8.5	8
Dissolved Oxygen	mg/L		
Phosphate - 2.75 L	mg/L		2.01
Sulfate	mg/L	250	100
Sulfide	mg/L		0.00
Suspended Solids	mg/L		3
Temperature	C		
Turbidity	FAU	5	4
Zinc	mg/L	5	
pathogens (1)			+
pathogens (2)			+
E. coli x1	CFU/mL		0
Total Coliforms x1	CFU/mL		7
E. coli x10	CFU/mL		2
Total Coliforms x10	CFU/mL		10

Site Name		Philippine Std	Police Tank Average
Location			Taguig
parameter	units		
Acidity (as CaCO3)*	mg/L		303
Alkalinity (as CaCO3)*	mg/L		305
Arsenic	ppb	50	0
Carbon Dioxide*	mg/L		
Chloride	mg/L	250	293
Chlorine Dioxide	mg/L		
Chlorine, Free	mg/L		
Chlorine, Total	mg/L		
Color	units	10	0
Conductivity/TDS	uS/cm	500	1281
Copper, LR	mg/L	1	0.89
Hardness (as CaCO3)*	mg/L	300	
Iron, Total	mg/L	1	0.04
Manganese, HR	mg/L	0.4	0.2
Nitrate (as N)	mg/L	11.3	0.7
Nitrite (as N)	mg/L	0.91	0.009
Ammonia, LR (as N)	mg/L		0.00
pH	pH units	6.5-8.5	7
Dissolved Oxygen	mg/L		
Phosphate - 2.75 L	mg/L		1.92
Sulfate	mg/L	250	110
Sulfide	mg/L		
Suspended Solids	mg/L		0
Temperature	C		
Turbidity	FAU	5	0
Zinc	mg/L	5	
pathogens (1)			+
pathogens (2)			+
E. coli x1	CFU/mL		0
Total Coliforms x1	CFU/mL		2
E. coli x10	CFU/mL		0
Total Coliforms x10	CFU/mL		3

Site Name		Philippine Std	Banyuhay Spring Average
Location			Rizal
parameter	units		
Acidity (as CaCO3)*	mg/L		190
Alkalinity (as CaCO3)*	mg/L		164
Arsenic	ppb	50	0
Carbon Dioxide*	mg/L		
Chloride	mg/L	250	9
Chlorine Dioxide	mg/L		
Chlorine, Free	mg/L		
Chlorine, Total	mg/L		
Color	units	10	34
Conductivity/TDS	uS/cm	500	284
Copper, LR	mg/L	1	0.00
Hardness (as CaCO3)*	mg/L	300	
Iron, Total	mg/L	1	0.01
Manganese, HR	mg/L	0.4	0.1
Nitrate (as N)	mg/L	11.3	1.4
Nitrite (as N)	mg/L	0.91	0.008
Ammonia, LR (as N)	mg/L		0.00
pH	pH units	6.5-8.5	6
Dissolved Oxygen	mg/L		
Phosphate - 2.75 L	mg/L		1.97
Sulfate	mg/L	250	5
Sulfide	mg/L		
Suspended Solids	mg/L		2
Temperature	C		
Turbidity	FAU	5	4
Zinc	mg/L	5	
pathogens (1)			+
pathogens (2)			+
E. coli x1	CFU/mL		0
Total Coliforms x1	CFU/mL		6
E. coli x10	CFU/mL		0
Total Coliforms x10	CFU/mL		3

Site Name		Philippine Std	Maislap Average
Location			Rizal
parameter	units		
Acidity (as CaCO3)*	mg/L		170
Alkalinity (as CaCO3)*	mg/L		248
Arsenic	ppb	50	0
Carbon Dioxide*	mg/L		
Chloride	mg/L	250	37
Chlorine Dioxide	mg/L		
Chlorine, Free	mg/L		
Chlorine, Total	mg/L		
Color	units	10	42
Conductivity/TDS	uS/cm	500	505
Copper, LR	mg/L	1	0.01
Hardness (as CaCO3)*	mg/L	300	
Iron, Total	mg/L	1	0.25
Manganese, HR	mg/L	0.4	0.0
Nitrate (as N)	mg/L	11.3	0.6
Nitrite (as N)	mg/L	0.91	0.002
Ammonia, LR (as N)	mg/L		0.00
pH	pH units	6.5-8.5	8
Dissolved Oxygen	mg/L		
Phosphate - 2.75 L	mg/L		1.92
Sulfate	mg/L	250	27
Sulfide	mg/L		
Suspended Solids	mg/L		3
Temperature	C		
Turbidity	FAU	5	5
Zinc	mg/L	5	
pathogens (1)			+
pathogens (2)			+
E. coli x1	CFU/mL		0
Total Coliforms x1	CFU/mL		0
E. coli x10	CFU/mL		0
Total Coliforms x10	CFU/mL		0

Site Name		Philippine Std	Trece Martires Aquabest Treated Average
Location			Cavite
parameter	units		
Acidity (as CaCO3)*	mg/L		230
Alkalinity (as CaCO3)*	mg/L		33
Arsenic	ppb	50	0
Carbon Dioxide*	mg/L		155
Chloride	mg/L	250	20
Chlorine Dioxide	mg/L		2.5
Chlorine, Free	mg/L		0.05
Chlorine, Total	mg/L		0.03
Color	units	10	17
Conductivity/TDS	uS/cm	500	79
Copper, LR	mg/L	1	0.00
Hardness (as CaCO3)*	mg/L	300	
Iron, Total	mg/L	1	0.02
Manganese, HR	mg/L	0.4	0.1
Nitrate (as N)	mg/L	11.3	0.4
Nitrite (as N)	mg/L	0.91	0.005
Ammonia, LR (as N)	mg/L		0.07
pH	pH units	6.5-8.5	5
Dissolved Oxygen	mg/L		
Phosphate - 2.75 L	mg/L		1.59
Sulfate	mg/L	250	6
Sulfide	mg/L		0.00
Suspended Solids	mg/L		2
Temperature	C		
Turbidity	FAU	5	2
Zinc	mg/L	5	
pathogens (1)			+
pathogens (2)			+
E. coli x1	CFU/mL		0
Total Coliforms x1	CFU/mL		0
E. coli x10	CFU/mL		0
Total Coliforms x10	CFU/mL		0

Site Name		Philippine Std	Trece Martires Aquabest Untreated Average
Location			Cavite
parameter	units		
Acidity (as CaCO3)*	mg/L		325
Alkalinity (as CaCO3)*	mg/L		124
Arsenic	ppb	50	0
Carbon Dioxide*	mg/L		293
Chloride	mg/L	250	38
Chlorine Dioxide	mg/L		1.9
Chlorine, Free	mg/L		0.04
Chlorine, Total	mg/L		0.00
Color	units	10	31
Conductivity/TDS	uS/cm	500	308
Copper, LR	mg/L	1	0.02
Hardness (as CaCO3)*	mg/L	300	
Iron, Total	mg/L	1	0.08
Manganese, HR	mg/L	0.4	0.2
Nitrate (as N)	mg/L	11.3	1.0
Nitrite (as N)	mg/L	0.91	0.005
Ammonia, LR (as N)	mg/L		0.02
pH	pH units	6.5-8.5	6
Dissolved Oxygen	mg/L		
Phosphate - 2.75 L	mg/L		2.75
Sulfate	mg/L	250	10
Sulfide	mg/L		0.00
Suspended Solids	mg/L		3
Temperature	C		
Turbidity	FAU	5	3
Zinc	mg/L	5	
pathogens (1)			+
pathogens (2)			+
E. coli x1	CFU/mL		9
Total Coliforms x1	CFU/mL		29
E. coli x10	CFU/mL		5
Total Coliforms x10	CFU/mL		27

Site Name		Philippine Std	Trece Martires Manila Water Average
Location			Cavite
parameter	units		
Acidity (as CaCO3)*	mg/L		226
Alkalinity (as CaCO3)*	mg/L		194
Arsenic	ppb	50	0
Carbon Dioxide*	mg/L		198
Chloride	mg/L	250	34
Chlorine Dioxide	mg/L		1.9
Chlorine, Free	mg/L		0.04
Chlorine, Total	mg/L		0.00
Color	units	10	25
Conductivity/TDS	uS/cm	500	369
Copper, LR	mg/L	1	0.01
Hardness (as CaCO3)*	mg/L	300	
Iron, Total	mg/L	1	0.03
Manganese, HR	mg/L	0.4	0.1
Nitrate (as N)	mg/L	11.3	1.0
Nitrite (as N)	mg/L	0.91	0.001
Ammonia, LR (as N)	mg/L		0.06
pH	pH units	6.5-8.5	7
Dissolved Oxygen	mg/L		
Phosphate - 2.75 L	mg/L		2.18
Sulfate	mg/L	250	26
Sulfide	mg/L		0.00
Suspended Solids	mg/L		2
Temperature	C		
Turbidity	FAU	5	2
Zinc	mg/L	5	
pathogens (1)			+
pathogens (2)			+
E. coli x1	CFU/mL		0
Total Coliforms x1	CFU/mL		2
E. coli x10	CFU/mL		0
Total Coliforms x10	CFU/mL		0

Appendix 2 –Photographs of 3M Petrifrilm Plates

The following photographs were taken of 3M Petrifilm Plates after 24 hours of incubation. Bubbles on the plates are not indicative of the presence of coliforms unless they accompany colonies. Colonies not accompanied by gas bubbles are not true colonies. A brief description accompanies several of the plates to allow for easier understanding of colony counting. Appendix 1 contains tables with colony counts for each sample.

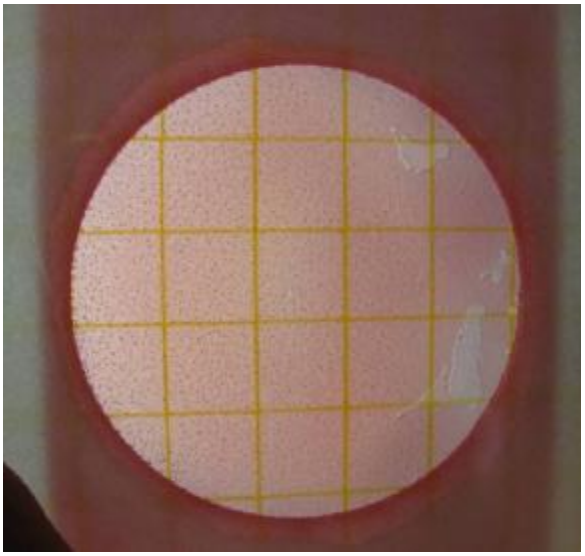

Figure 49 - Santo Rosario Well x1 (Davao)

This film has no coliforms. Smaller bubbles are only background bubbles; larger bubbles were trapped under the film during sample collection and are not associated with colonies.

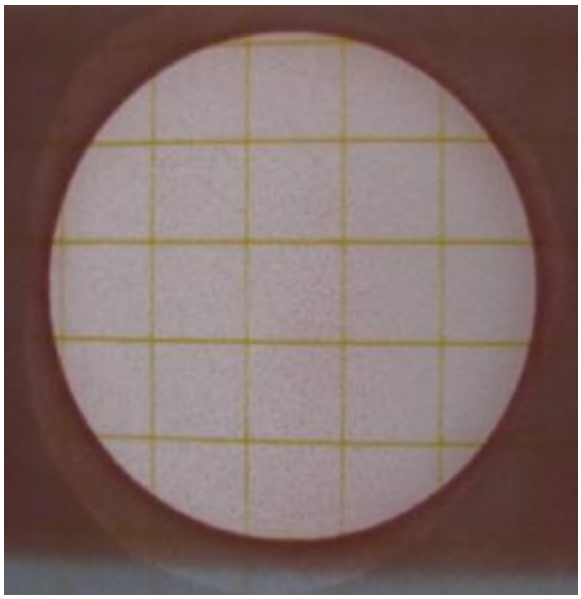

Figure 50 - Violet Denison Spring near Chapel x1 Sample 1 (Davao).

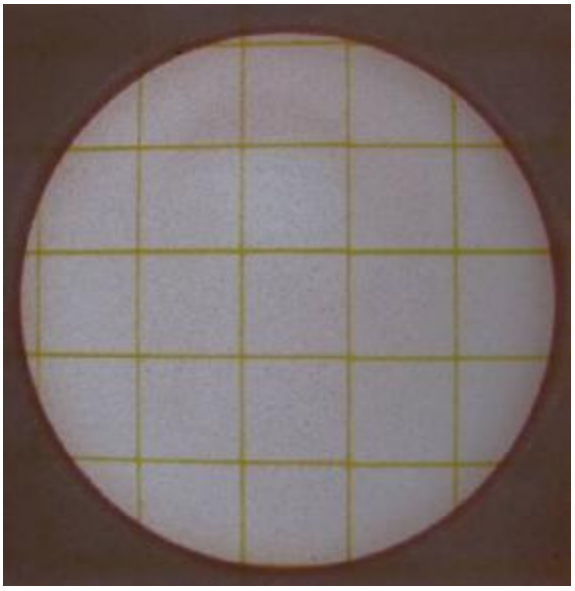

Figure 51 - Violet Denison Spring near Chapel x1 Sample 2 (Davao)

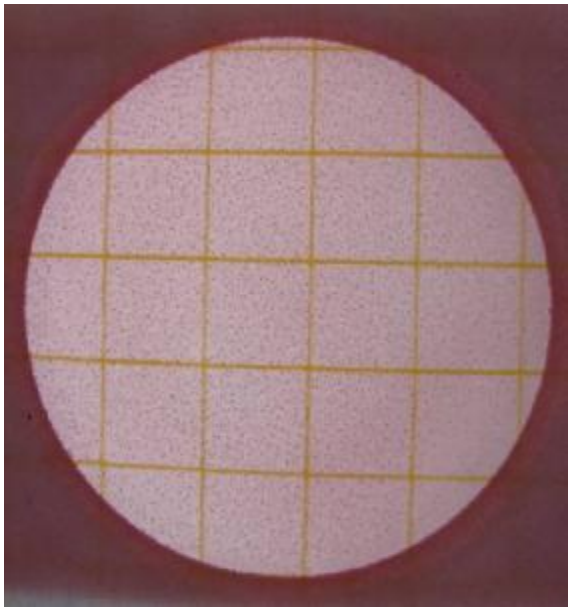

Figure 52 - Violet Denison Spring Down Hill x1 Sample 1 (Davao)

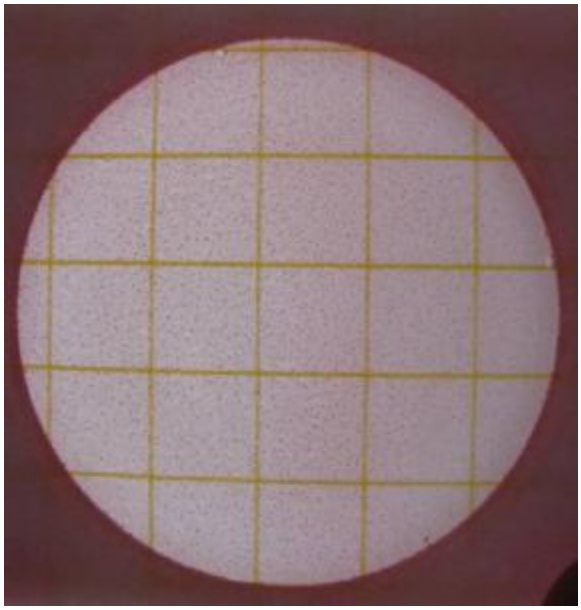

Figure 53 - Violet Denison Spring Down Hill x1 Sample 2 (Davao)

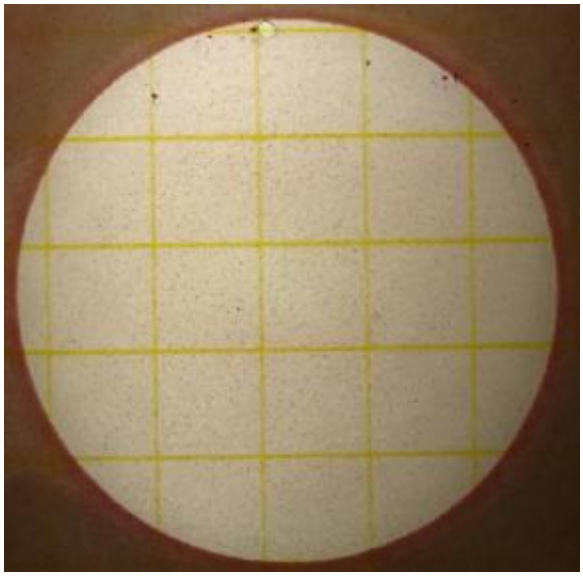

Figure 54 - Robinhood Shallow Open Well x1 Sample 1 (Bukidnon)

This film contains 4 coliforms. A coliform is apparent if a gas bubble accompanies a red colony. *E. coli* colonies are blue, but are not present on this film.

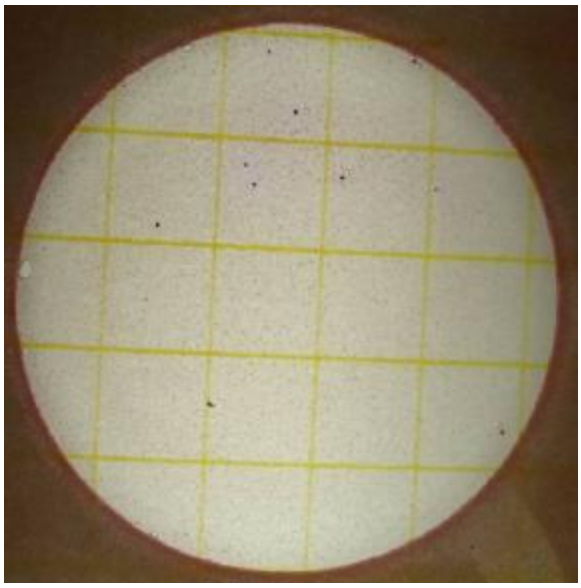

Figure 55 - Robinhood Shallow Open Well x1 Sample 2 (Bukidnon)

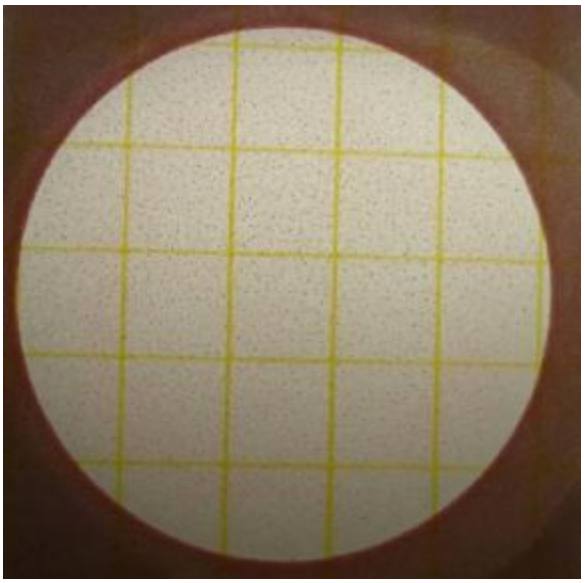

Figure 56 - Robinhood - Valencia Water District x1 Sample 1 (Bukidnon)

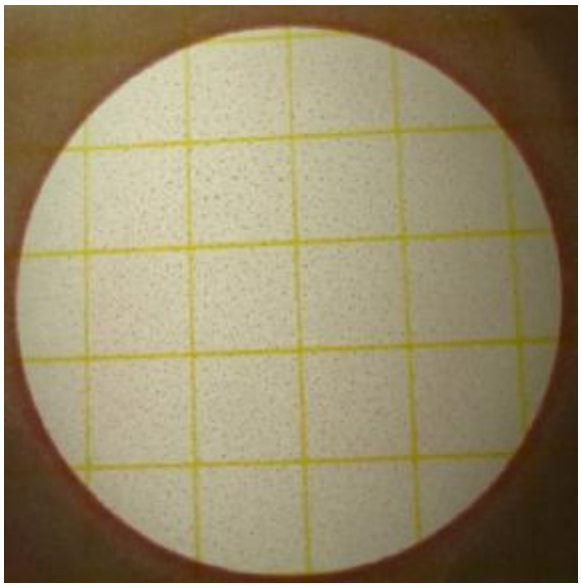

Figure 57 - Robinhood - Valencia Water District x1 Sample 2 (Bukidnon)

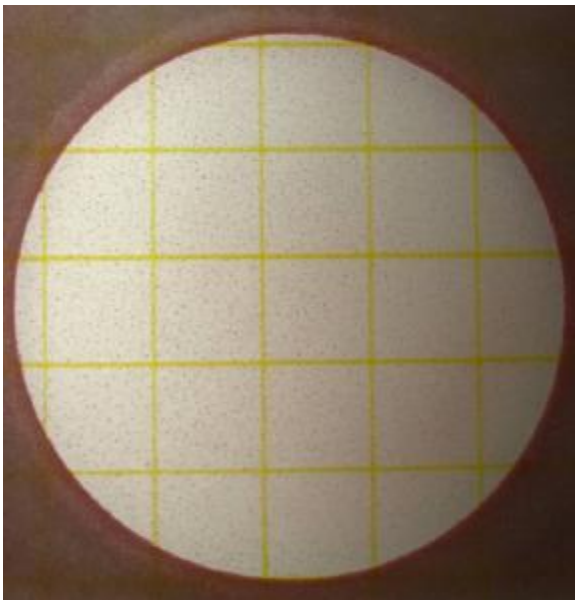

Figure 58 - Robinhood - Closed 30' Well x1 Sample 1 (Bukidnon)

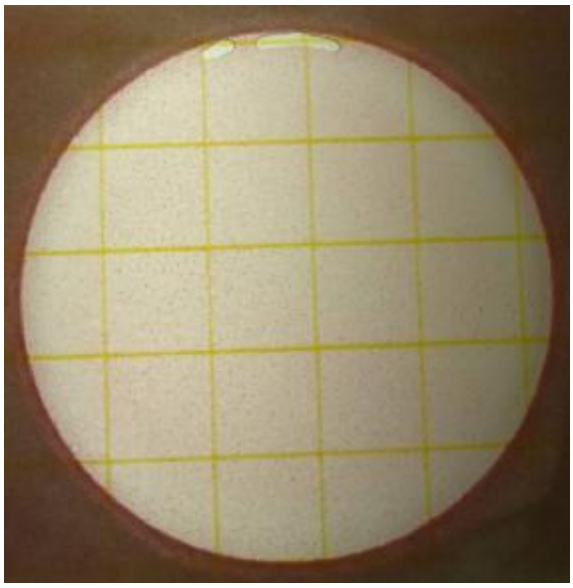

Figure 59 - Robinhood - Closed 30' Well x1 Sample 2 (Bukidnon)

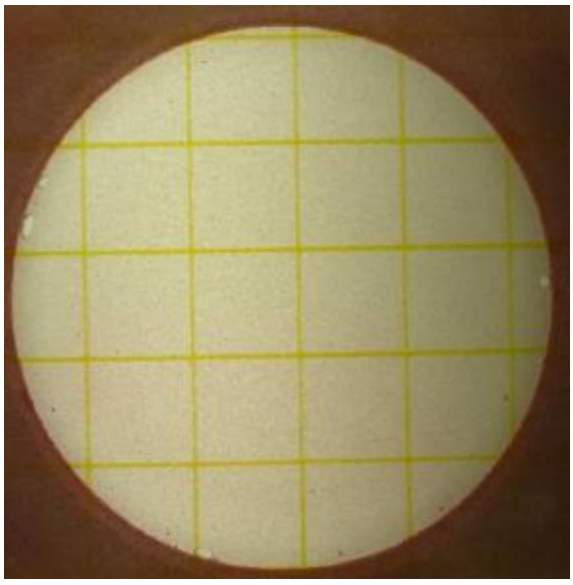

Figure 60 - Robinhood - Closed 35' Well x1 Sample 1 (Bukidnon)

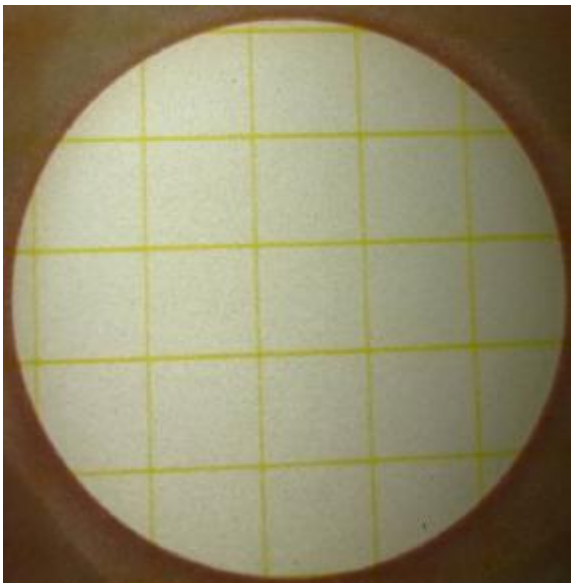

Figure 61 - Robinhood - Closed 35' Well x1 Sample 2 (Bukidnon)

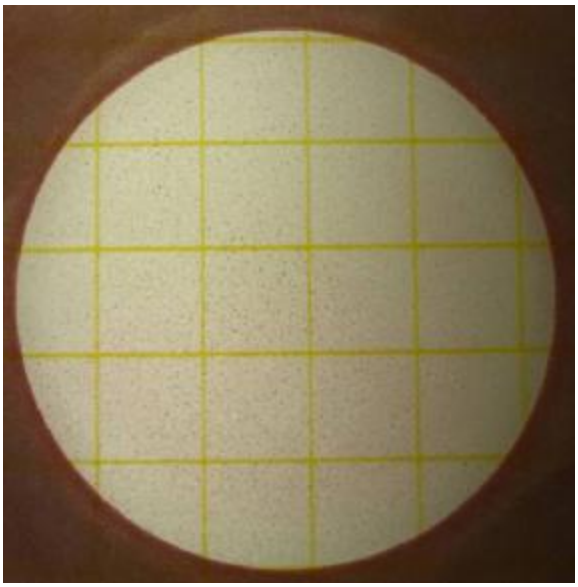

Figure 62 - Paradise 45' Well x1 Sample 1 (Bukidnon)

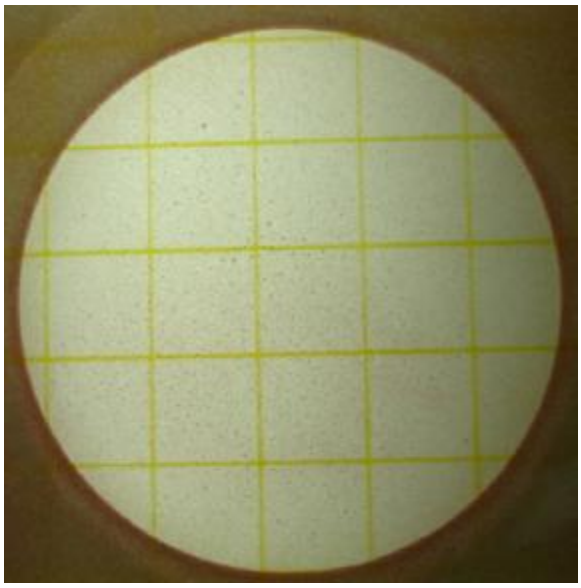

Figure 63 - Paradise 45' Well x1 Sample 2 (Bukidnon)

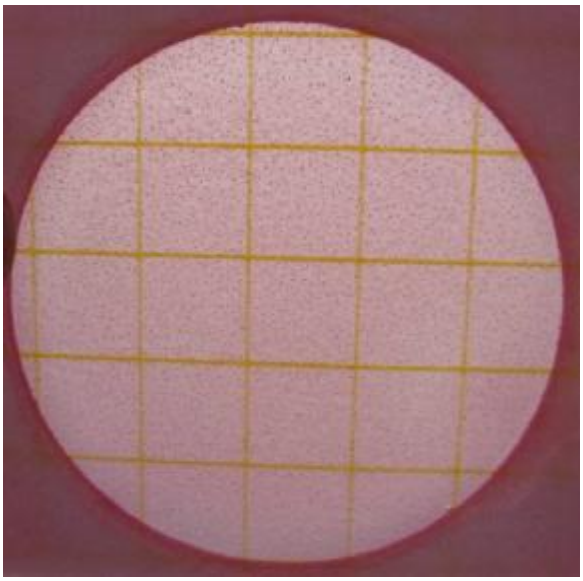

Figure 64 - Ray of Hope City Jail DCWD x1 Sample 1 (Davao)

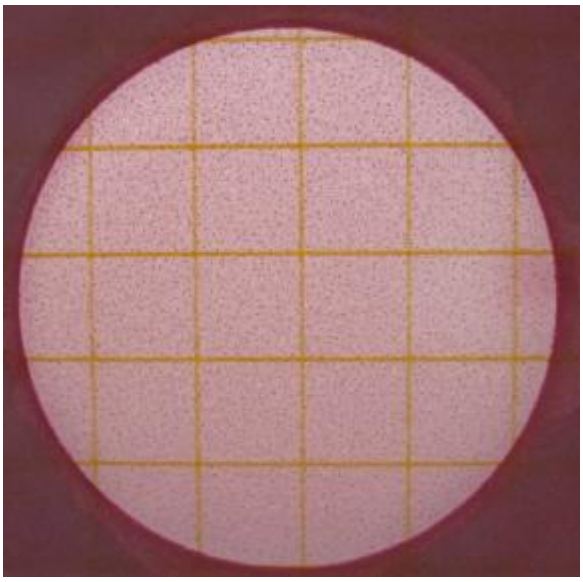

Figure 65 - Ray of Hope City Jail DCWD x1 Sample 2 (Davao)

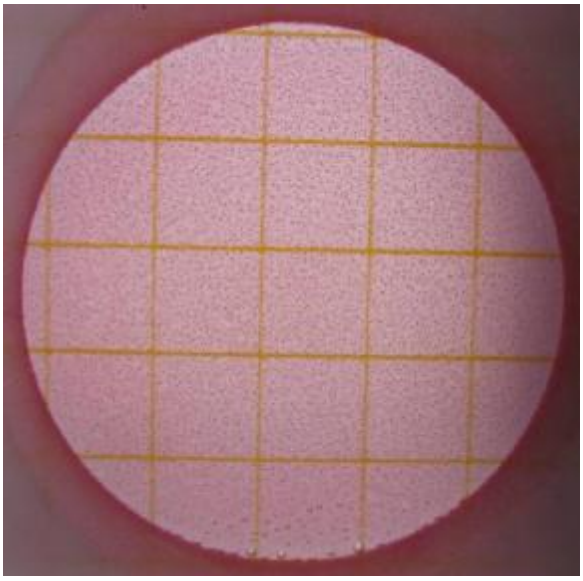

Figure 66 - Riverdrive DCWD x1 Sample 1 (Davao)

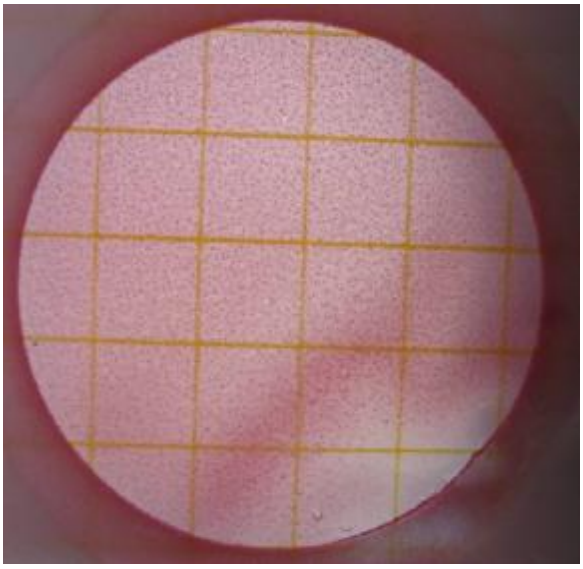

Figure 67 - Riverdrive DCWD x1 Sample 2 (Davao)

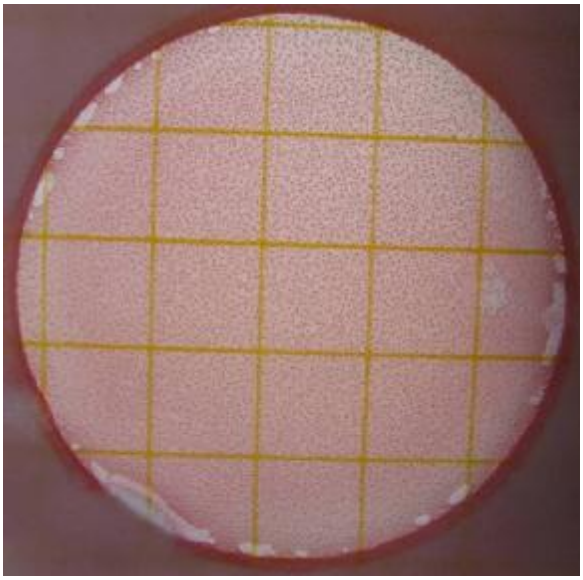

Figure 68 - DMC Sampling Event 1 x1 Sample 1 (Davao)

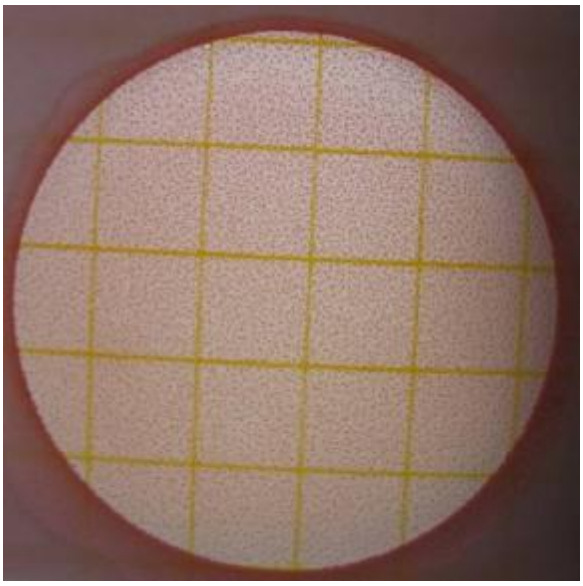

Figure 69 - DMC Sampling Event 1 x1 Sample 2 (Davao)

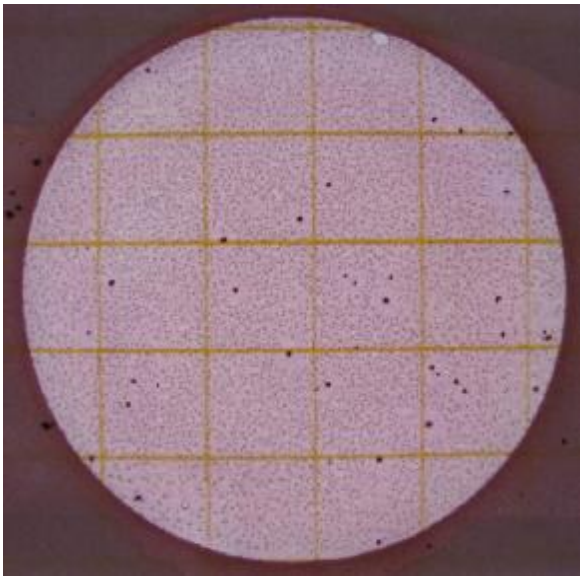

Figure 70 - Riverdrive Spring x1 (Davao)

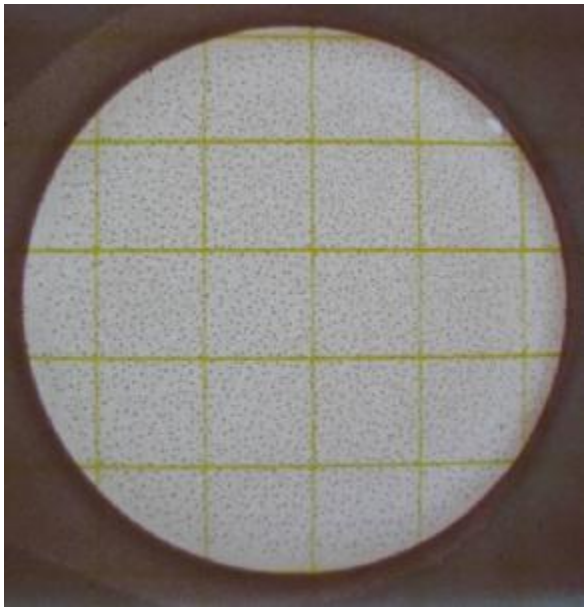

Figure 71 - Villarica Well x1 Sample 1 (Samal Island)

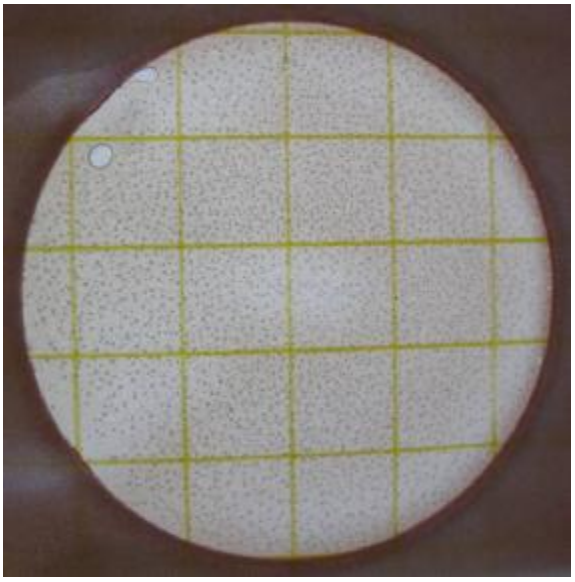

Figure 72 - Villarica Well x1 Sample 2 (Samal Island)

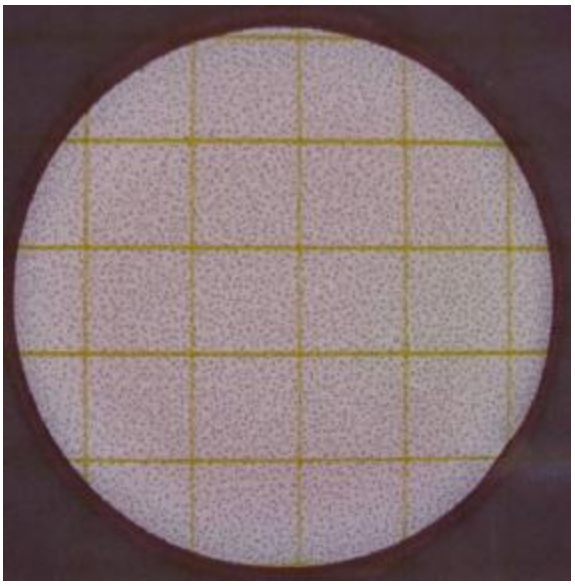

Figure 73 - Nazareno 30’ Well x1 Sample 1 (Samal Island)

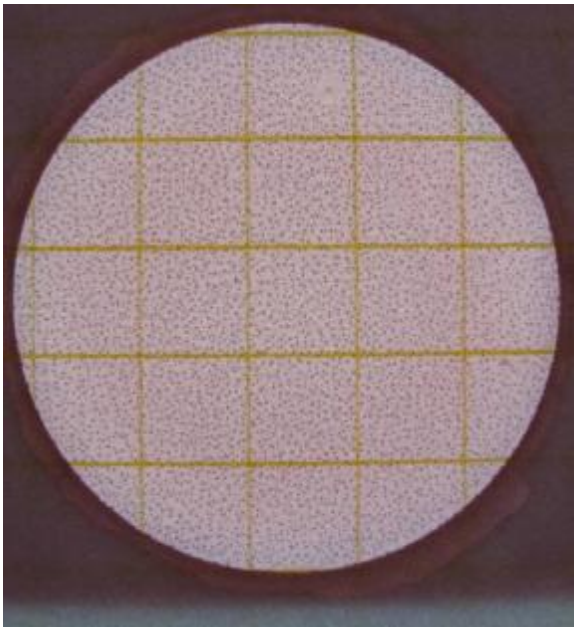

Figure 74 - Nazareno 30’ Well x1 Sample 2 (Samal Island)

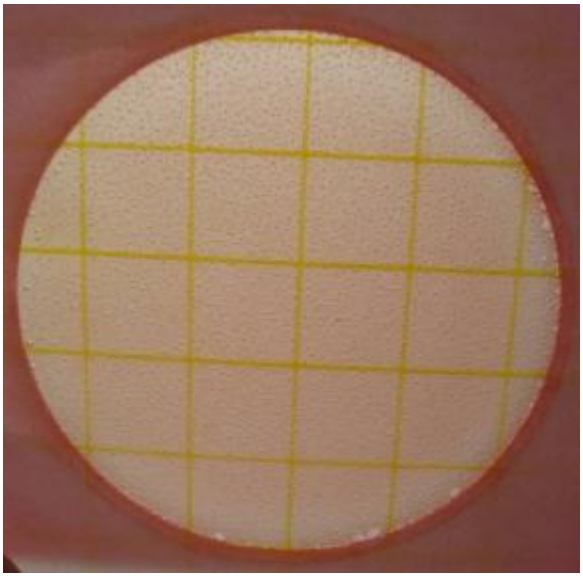

Figure 75 - DMC Sampling Envent 2 Sample 1 x1 (Davao)

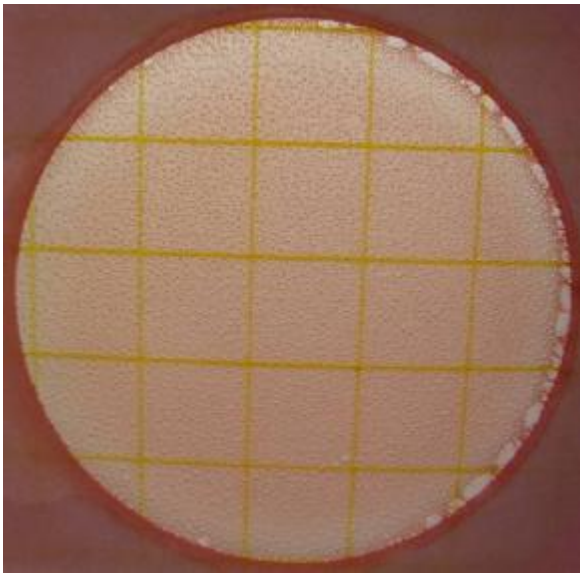

Figure 76 - DMC Sampling Envent 2 Sample 2 x1 (Davao)

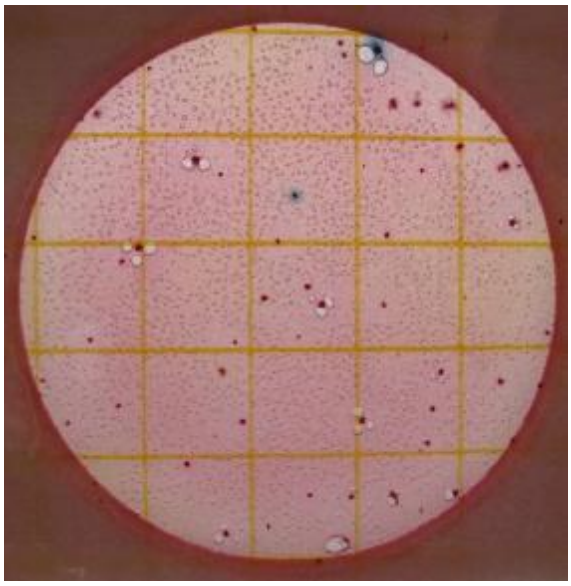

Figure 77 - Pueblo Antonio Sample 1 x1 (Davao)

This sample contains both coliforms and an *E. coli* colony, which is the blue colony with gas bubbles. The other blue colony does not have gas bubbles associated with it, and is not counted as an *E. coli* colony.

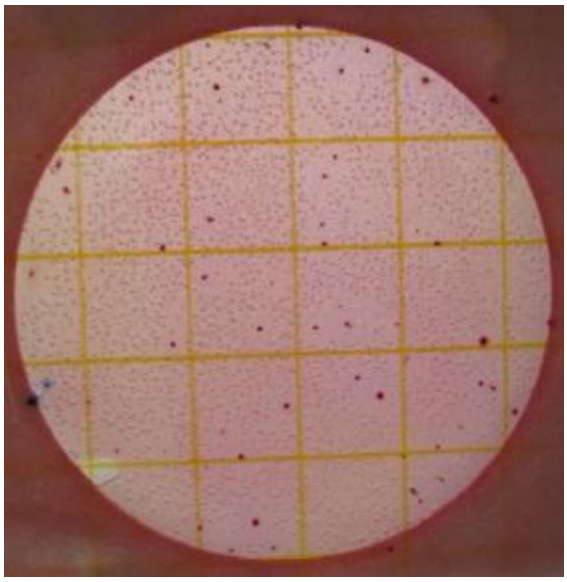

Figure 78 - Pueblo Antonio Sample 2 x1 (Davao)

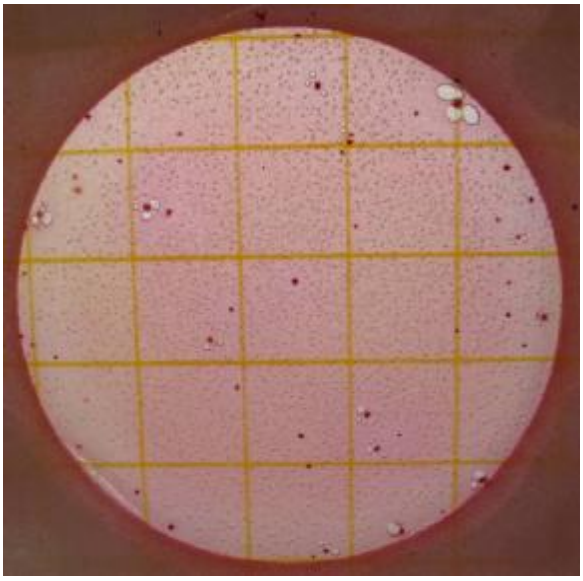

Figure 79 - Pueblo Antonio Sample 3 x1 (Davao)

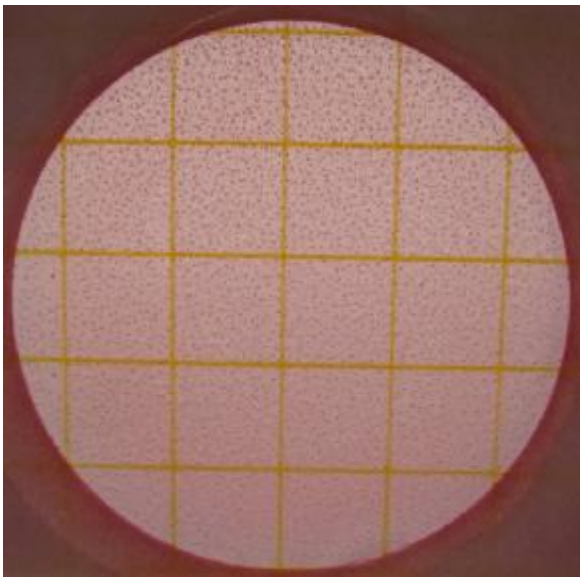

Figure 80 - Santa Martha Sample 1 x1 (Davao)

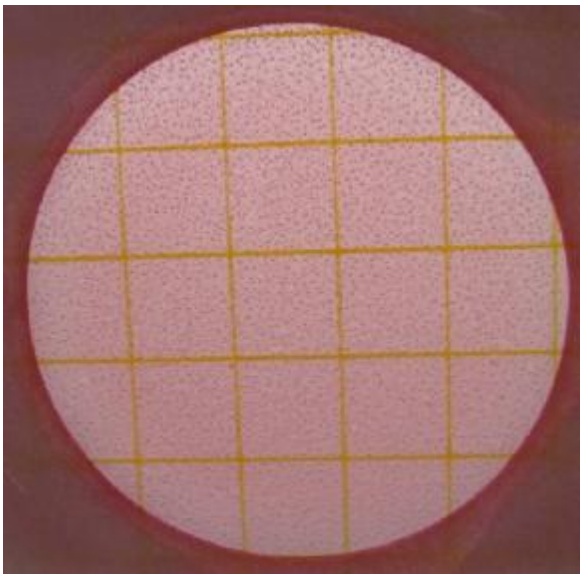

Figure 81 - Santa Martha Sample 2 x1 (Davao)

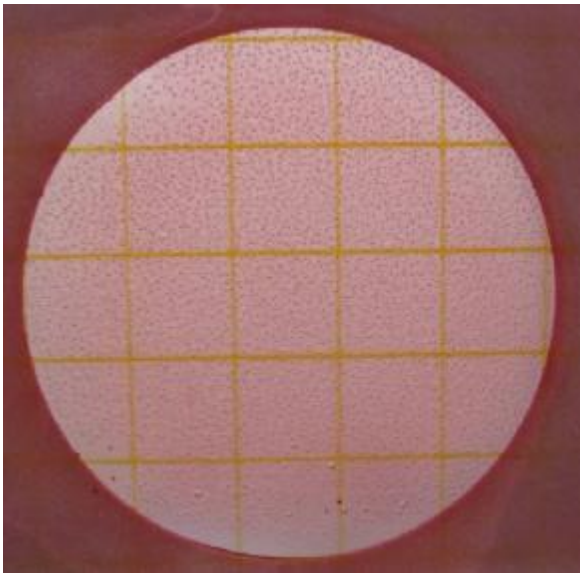

Figure 82 - Santa Martha Sample 3 x1 (Davao)

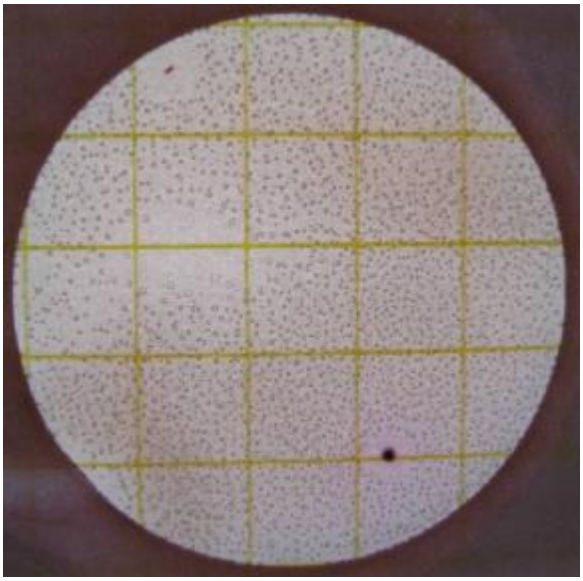

Figure 83 - DMC Sampling Event 3 Sample 1 x1 (Davao)

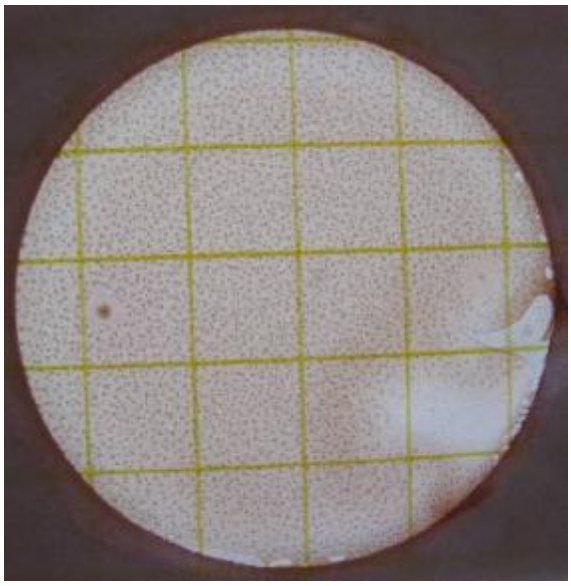

Figure 84 - DMC Sampling Event 3 Sample 2 x1 (Davao)

The gel on this film looks different to the right because the gel was compressed and moved (error in handling).

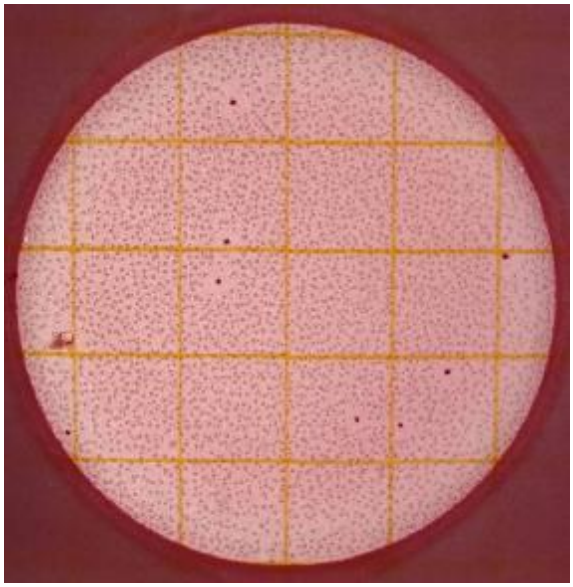

Figure 85 - Amparo Spring x10 (Manila)

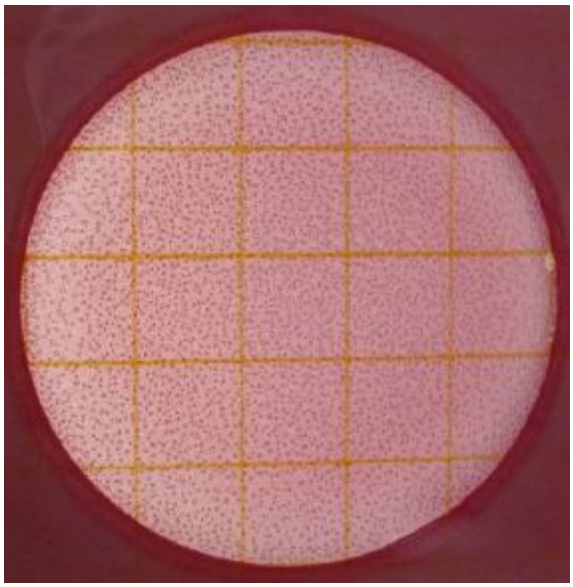

Figure 86 - Amparo Maynilad x1 (Manila)

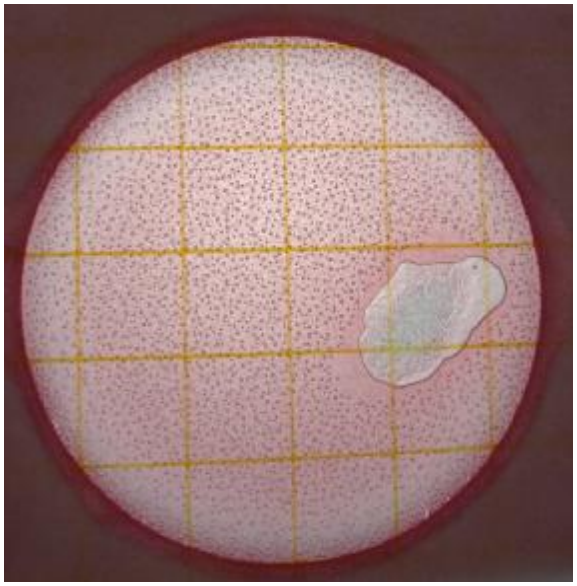

Figure 87 - Amparo Maynilad x10 (Manila)

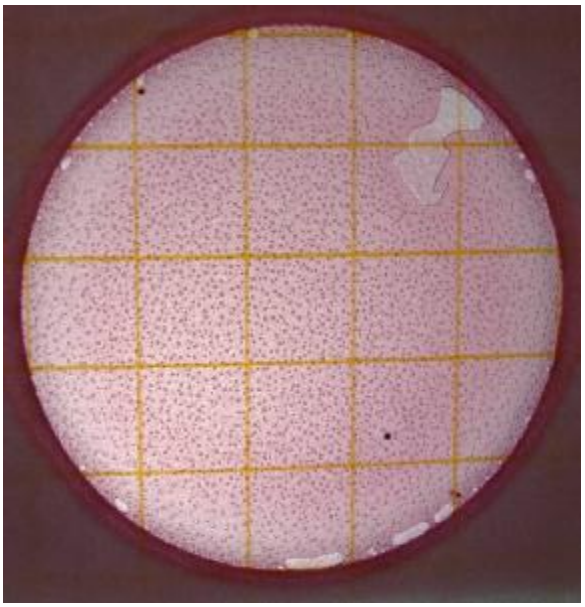

Figure 88 - Ampao Spring x1 (Manila)

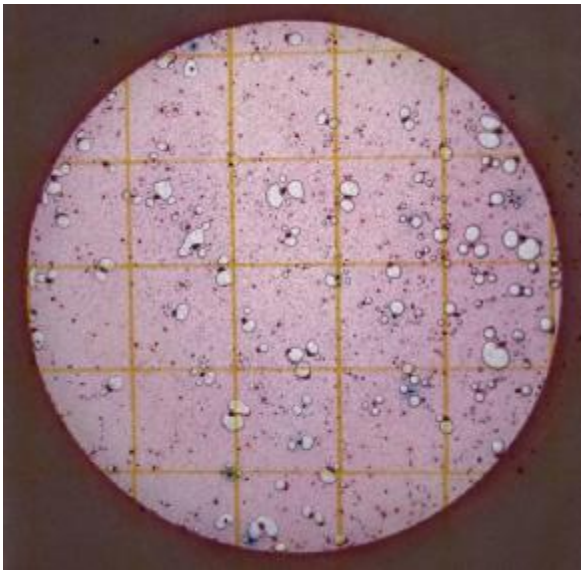

Figure 89 - Sico1 EBD Well #2 x1 (Batangas)

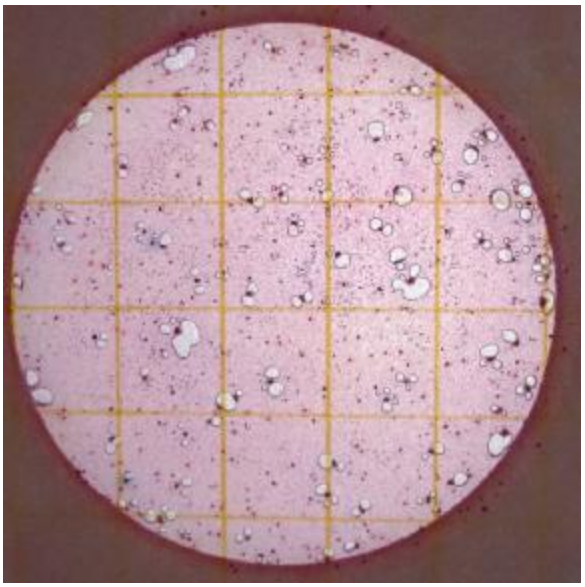

Figure 90 - Sico1 EBD Well #2 x10 (Batangas)

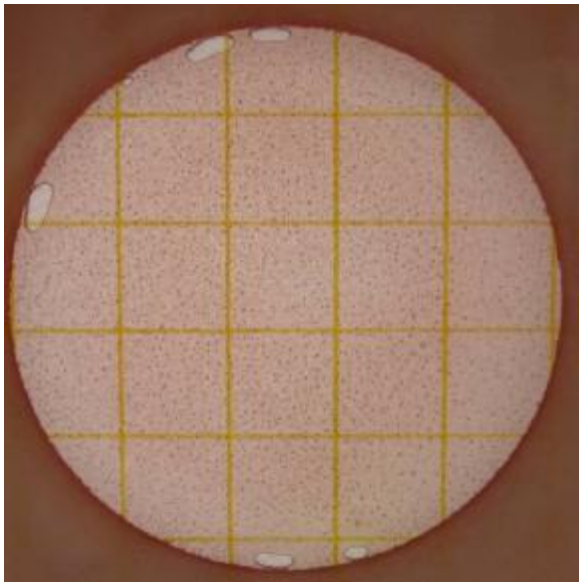

Figure 91 - Sico1 EBD Well #1 x1 (Batangas)

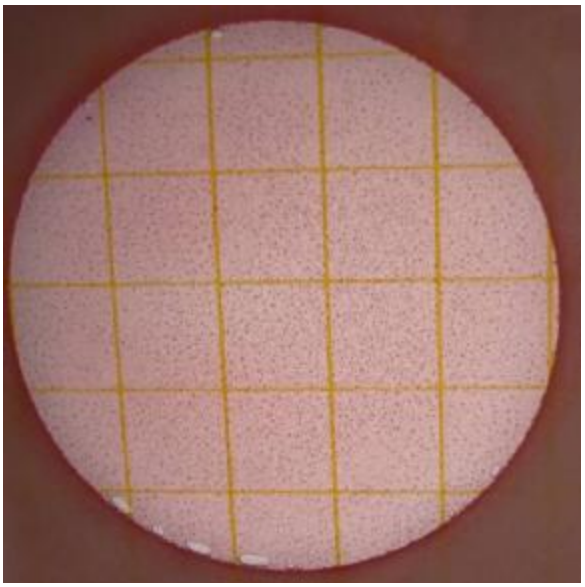

Figure 92 - Sico1 EBD Well #1 x10 (Batangas)

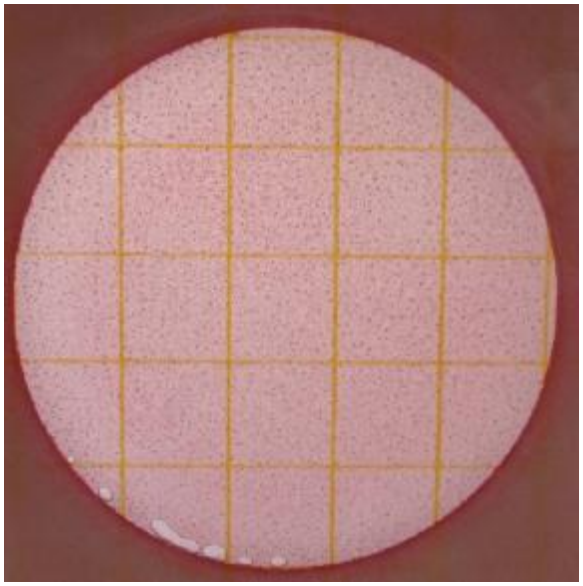

Figure 93 - Shell Livjo Well #1 x1 (Batangas)

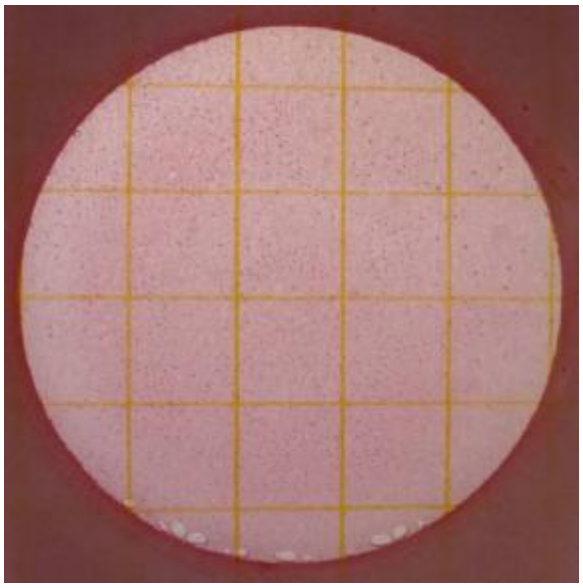

Figure 94 - Shell Livjo Well #1 x10 (Batangas)

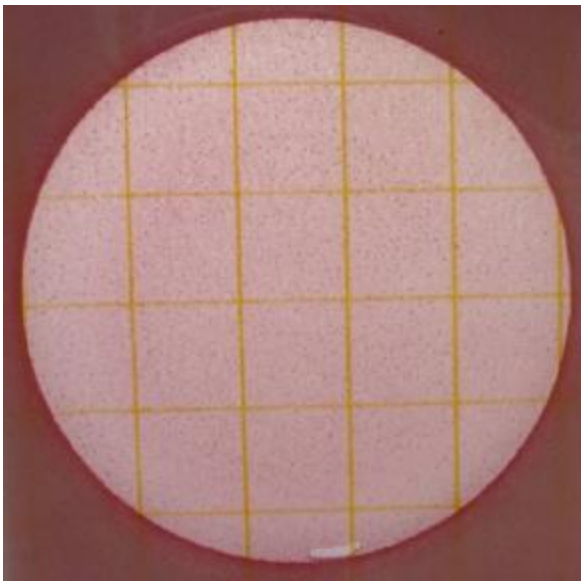

Figure 95 - Shell Livjo Well #2 x10 (Batangas)

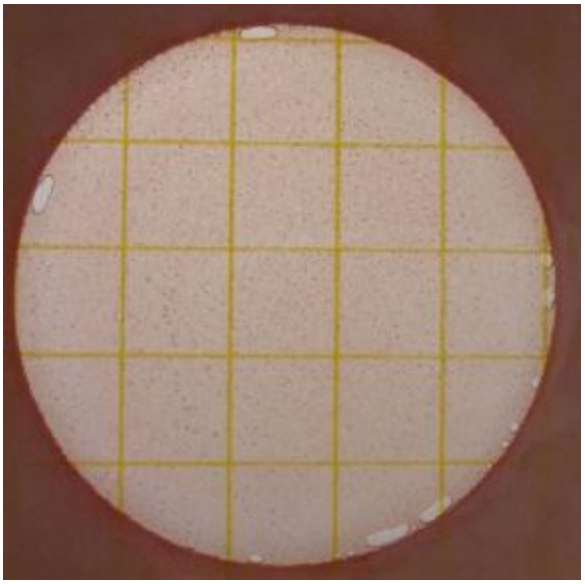

Figure 96 - Shell Livjo Well #2 x1 (Batangas)

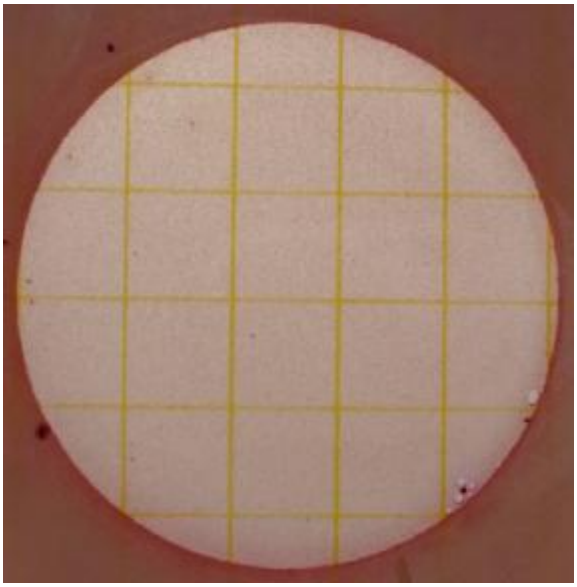

Figure 97 - Reunion Onsite Well x1 (Batangas)

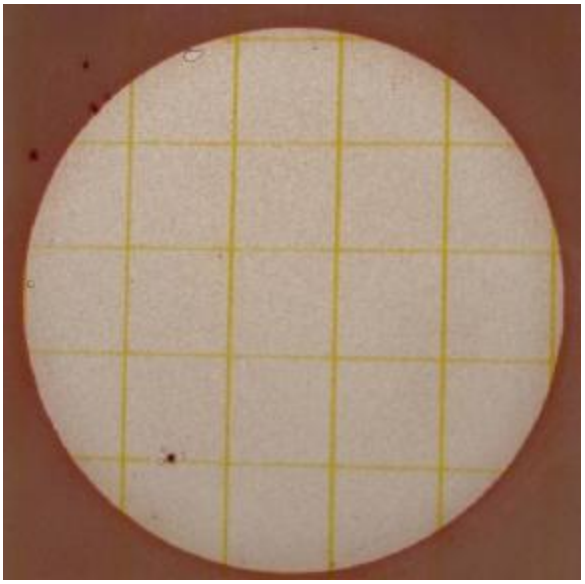

Figure 98 - Reunion Onsite Well x10 (Batangas)

Figure 99 - San Juan Well x1 (Batangas)

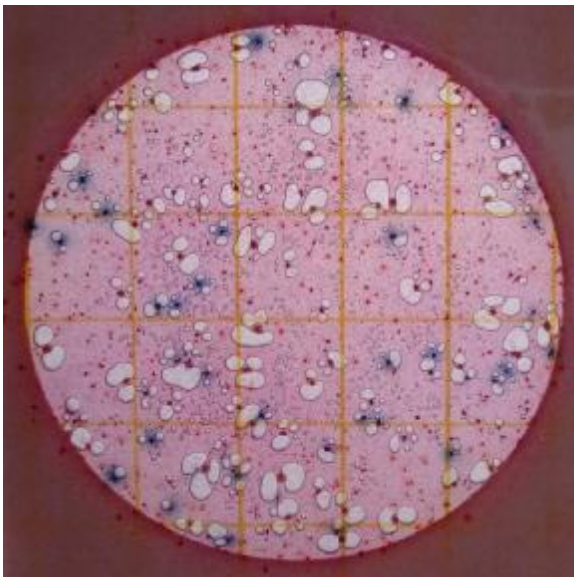

Figure 100 - San Juan Well x10 (Batangas)

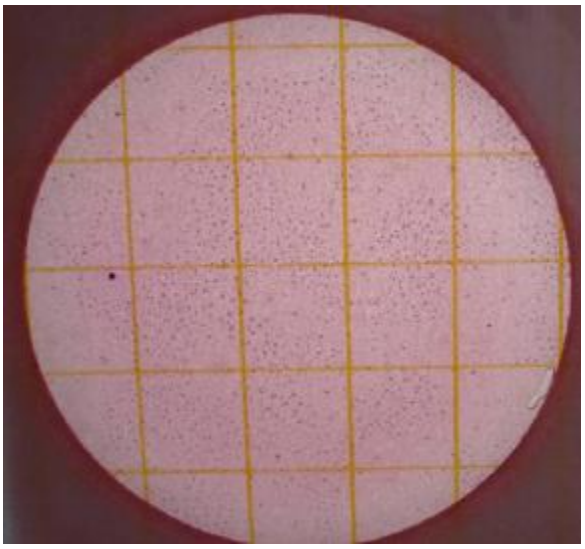

Figure 101 - OKS Tank x1 (Batangas)

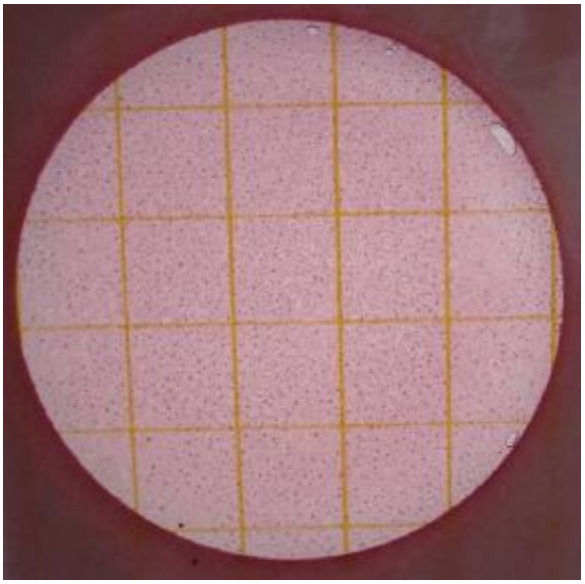

Figure 102 - OKS Tank x10 (Batangas)

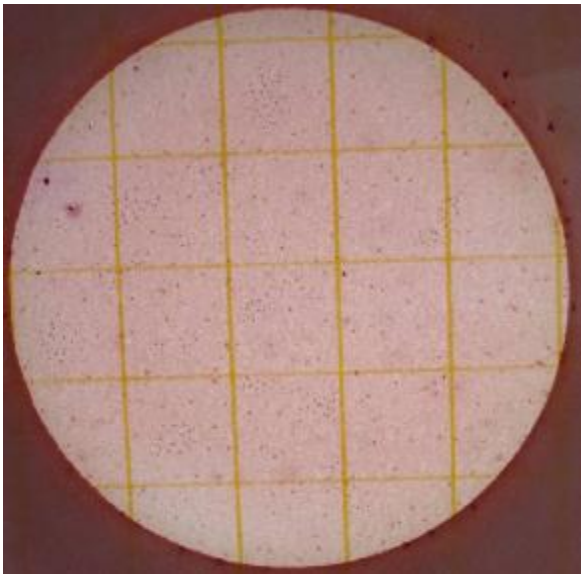

Figure 103 - OKS 40' Well x10 (Batangas)

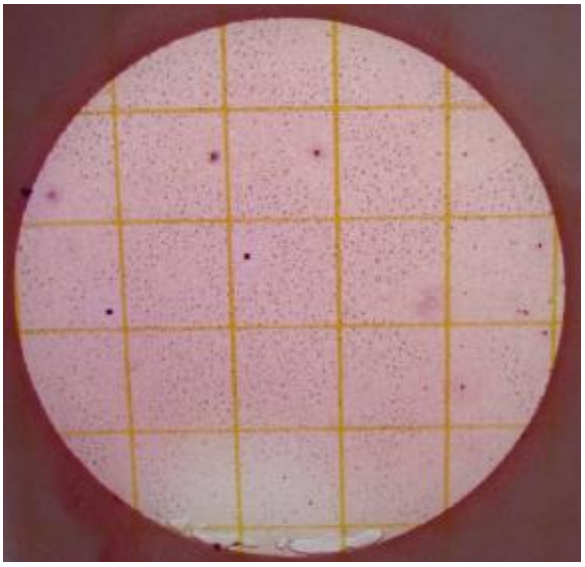

Figure 104 - OKS 40' Well x1 (Batangas)

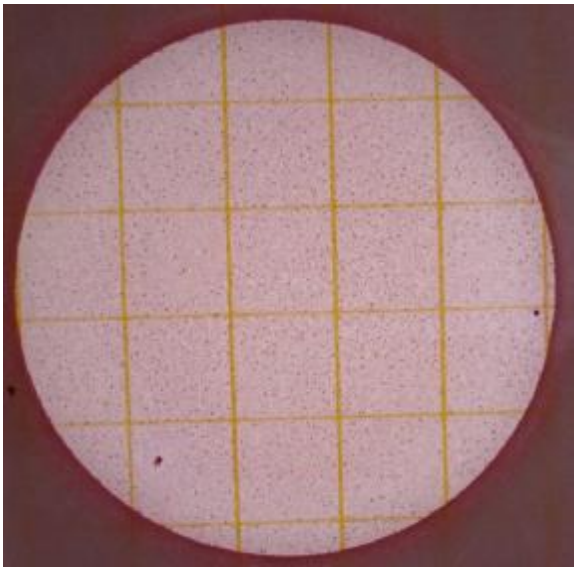

Figure 105 - OKS 80' Well x10 (Batangas)

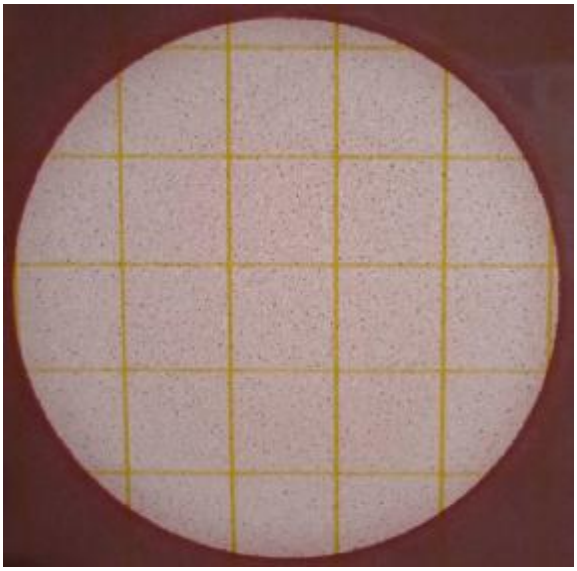

Figure 106 - OKS 80' Well x1 (Batangas)

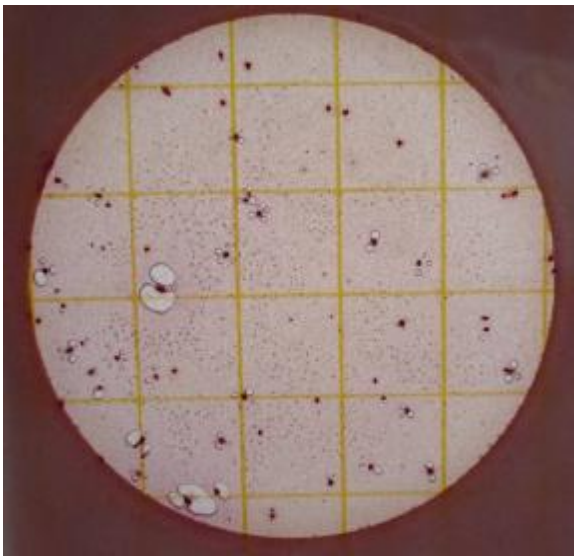

Figure 107 - Buhay Nasapa 50' Well x10 (Batangas)

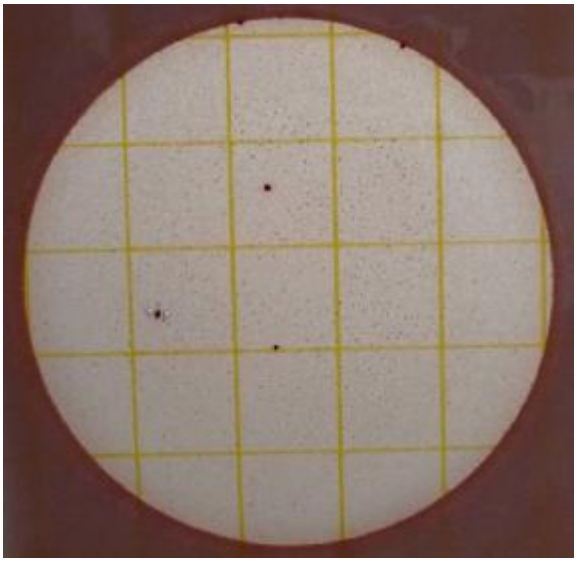

Figure 108 - Buhay Nasapa 50' Well x1 (Batangas)

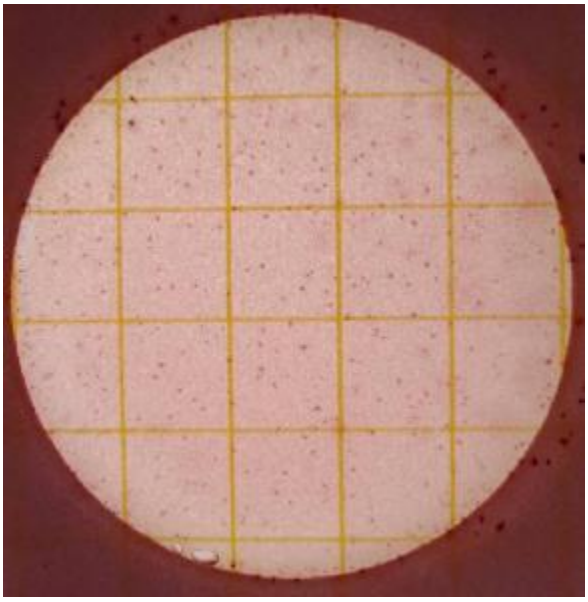

Figure 109 - First Calacan Well #2 x1 (Batangas)

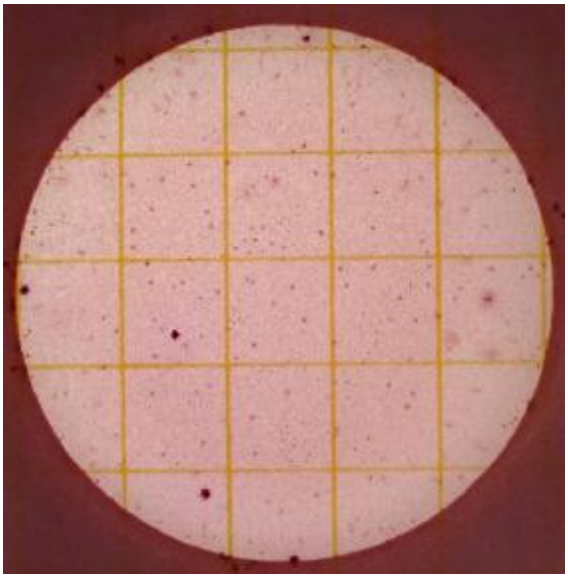

Figure 110 - First Calacan Well #2 x10 (Batangas)

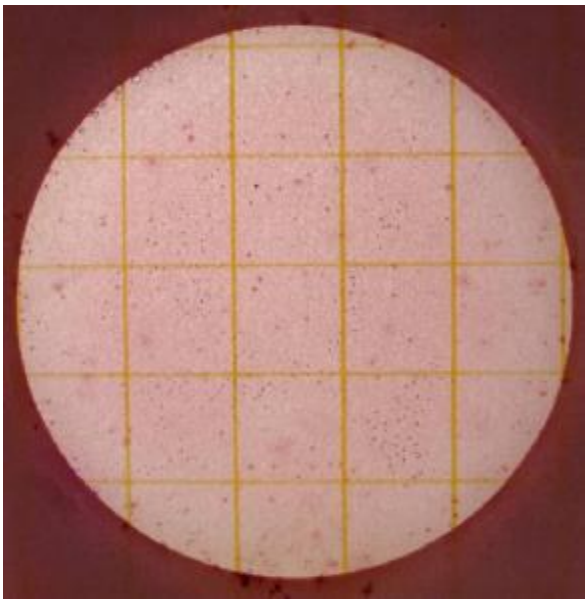

Figure 111 - First Calacan Well #1 x10 (Batangas)

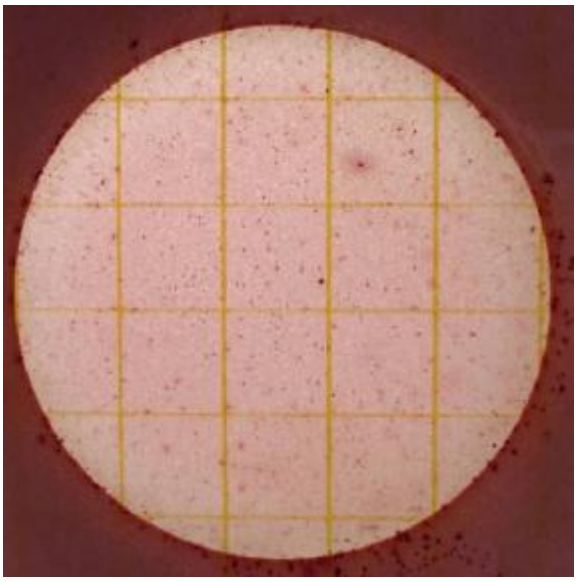

Figure 112 - First Calacan Well #1 x1 (Batangas)

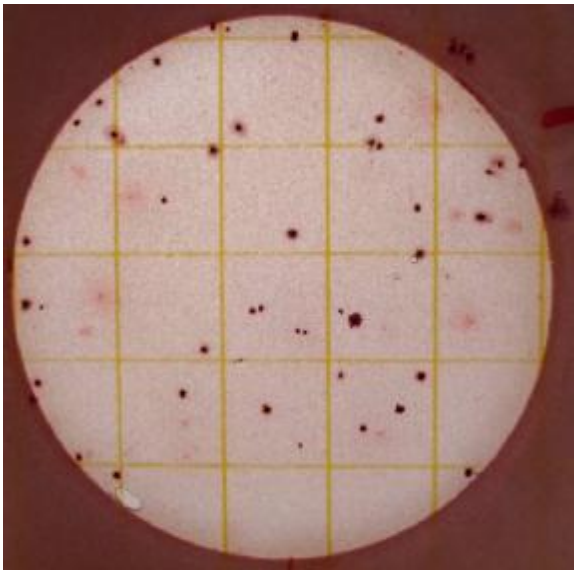

Figure 113 - Reunion Onsite Well x10 (Batangas)

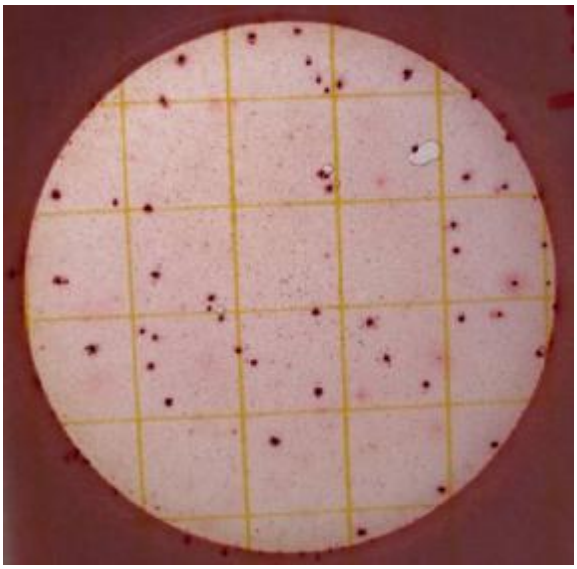

Figure 114 - Reunion Onsite Well x1 (Batangas)

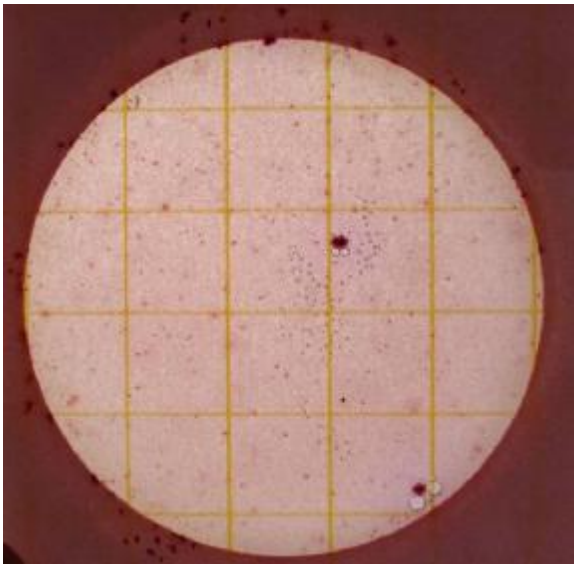

Figure 115 - San Francisco Javier Well x1 (Batangas)

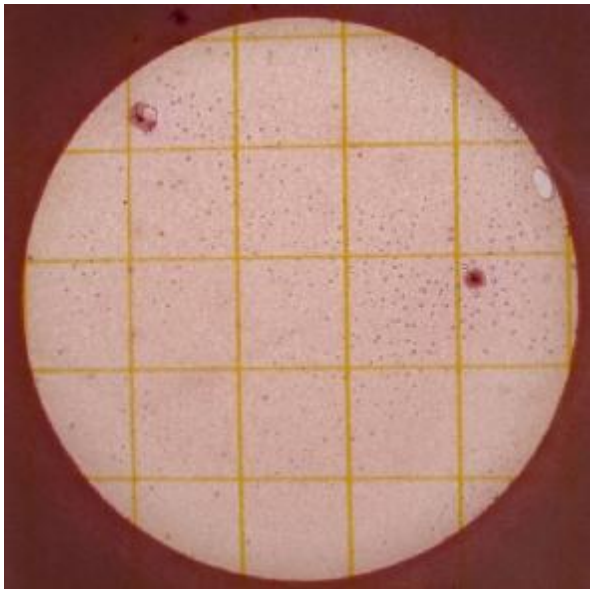

Figure 116 - San Francisco Javier Well x10 (Batangas)

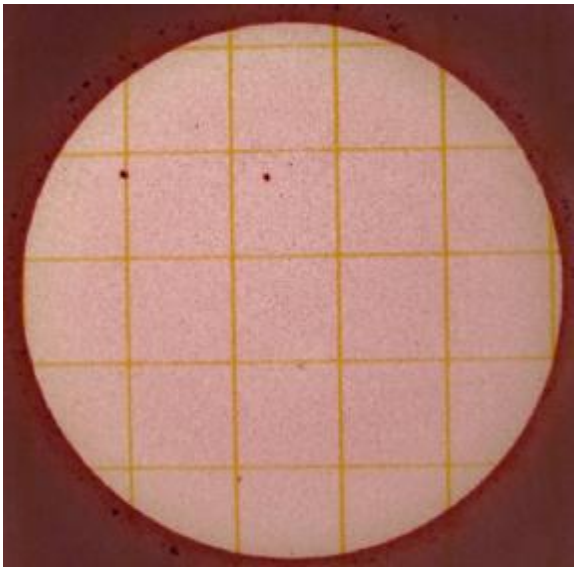

Figure 117 - Reunion Barangay Water x10 (Batangas)

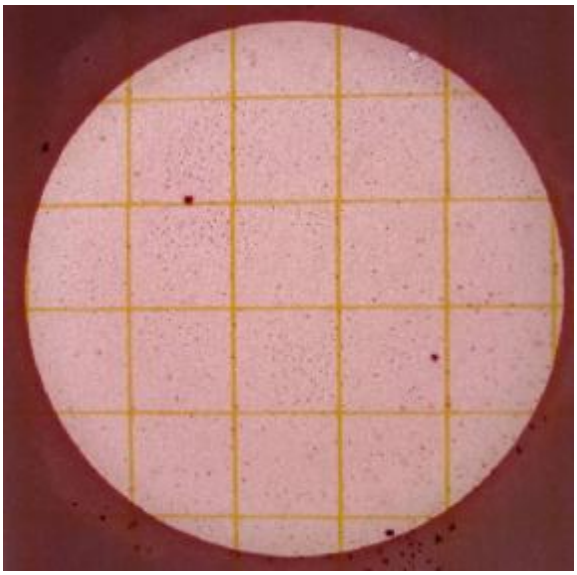

Figure 118 - Reunion Barangay Water x1 (Batangas)

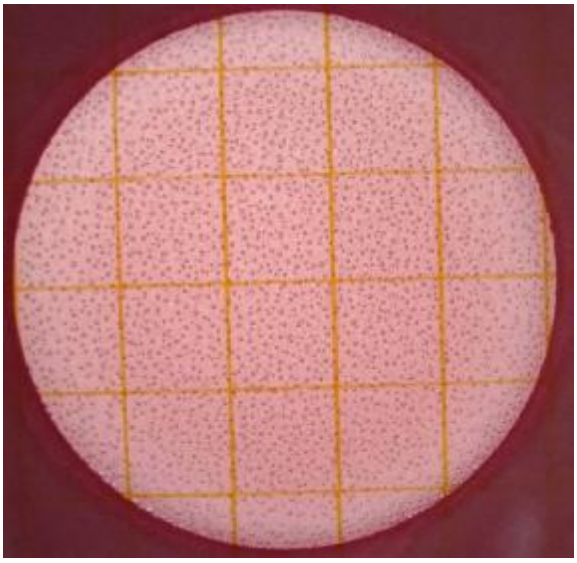

Figure 119 - Brookside Filtered Groundwater x1 (Manila)

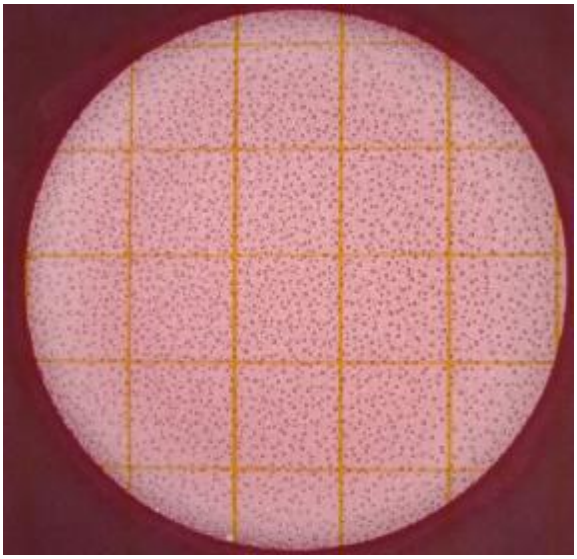

Figure 120 - Brookside Filtered Groundwater x10 (Manila)

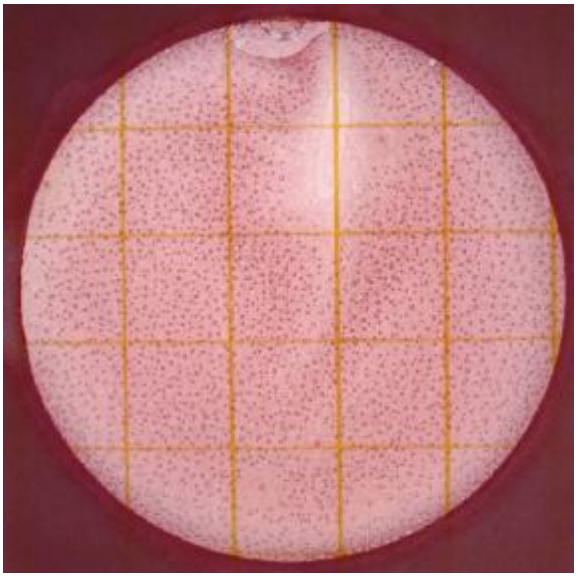

Figure 121 - Brookside Maynilad Water x10 (Manila)

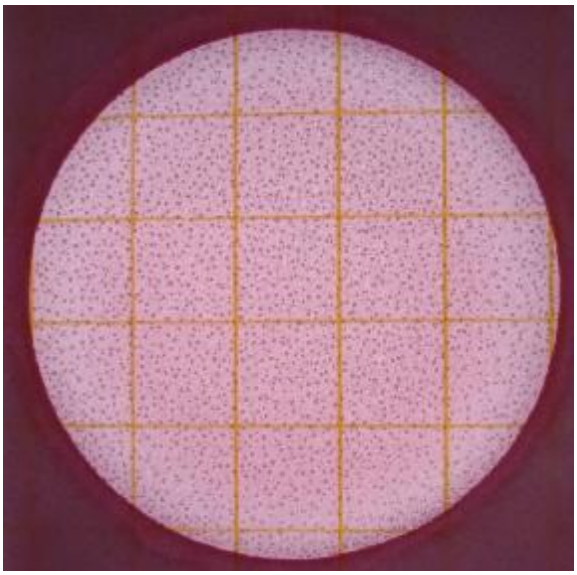

Figure 122 - Brookside Maynilad Water x1 (Manila)

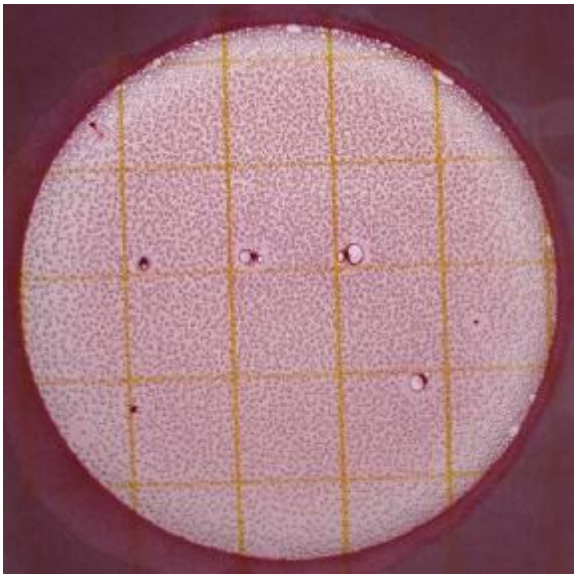

Figure 123 - Police Well x1 (Manila)

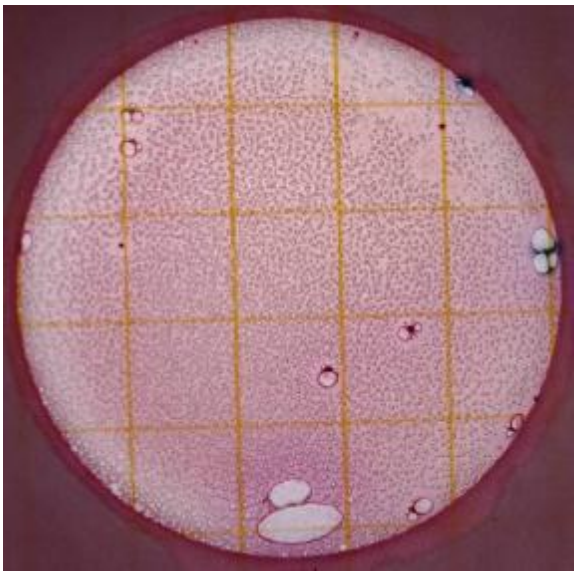

Figure 124 - Police Well x10 (Manila)

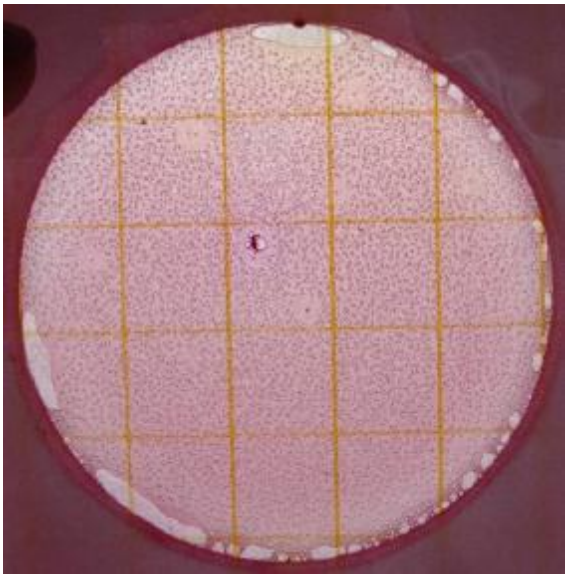

Figure 125 - Police Tank x1 (Manila)

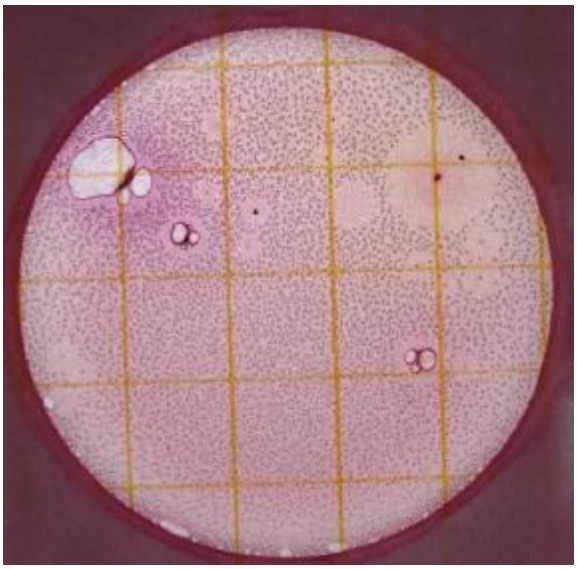

Figure 126 - Police Tank x10 (Manila)

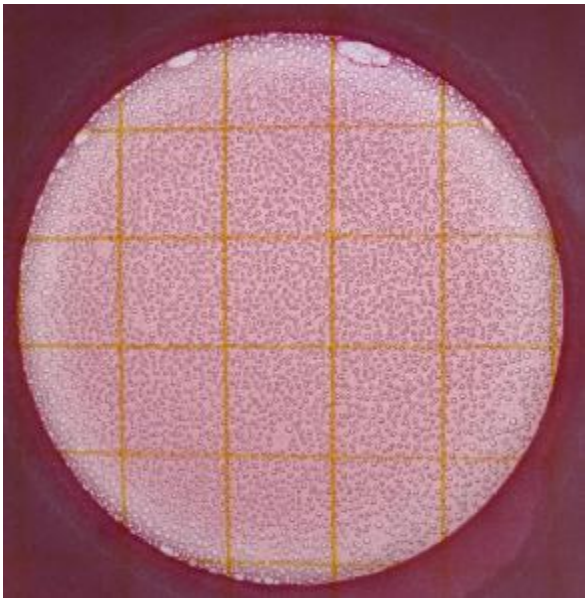

Figure 127- Maislap Well x1 (Manila)

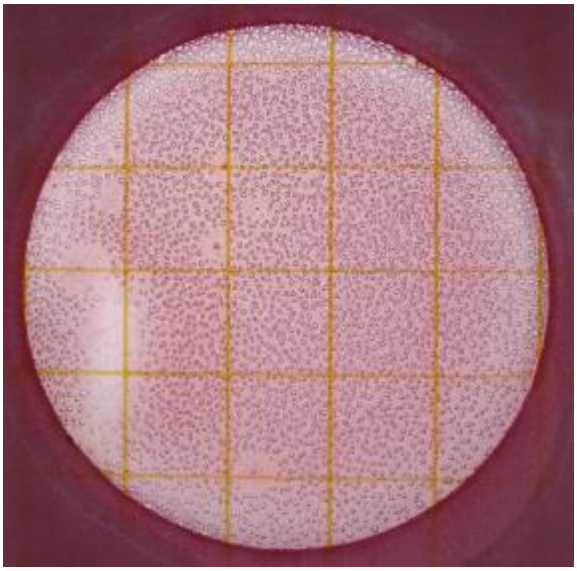

Figure 128- Maislap Well x10 (Manila)

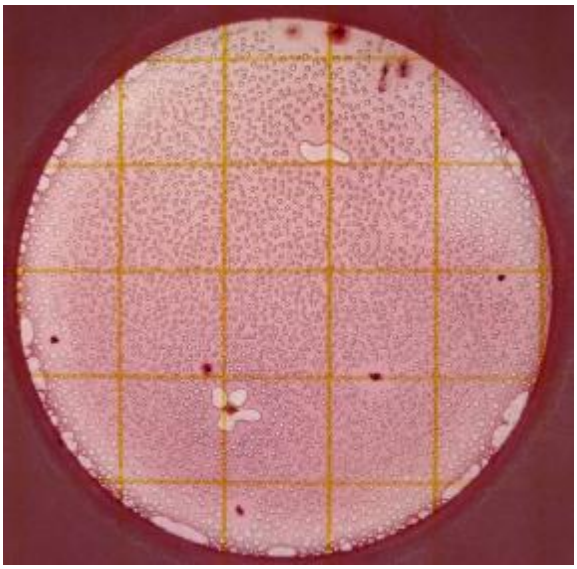

Figure 129 - Banyuhay Spring x10 (Manila)

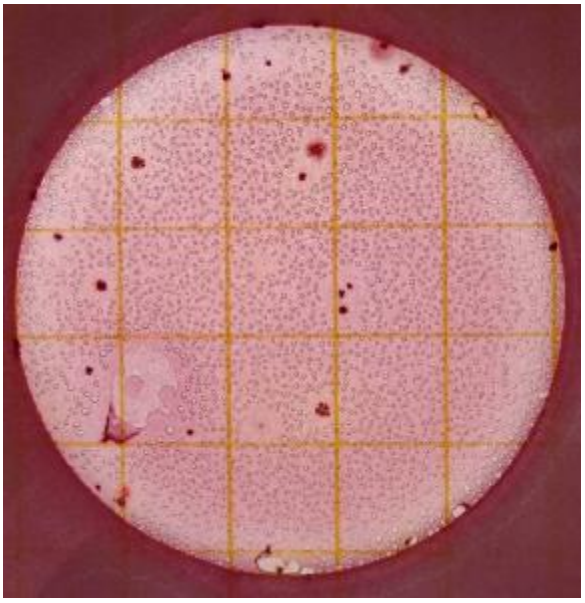

Figure 130 - Banyuhay Spring x1 (Manila)

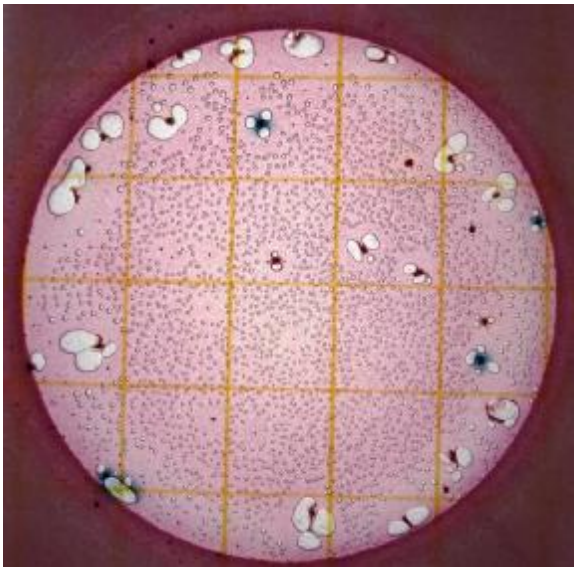

Figure 131 - Trece Martires Groundwater x10 (Cavite)

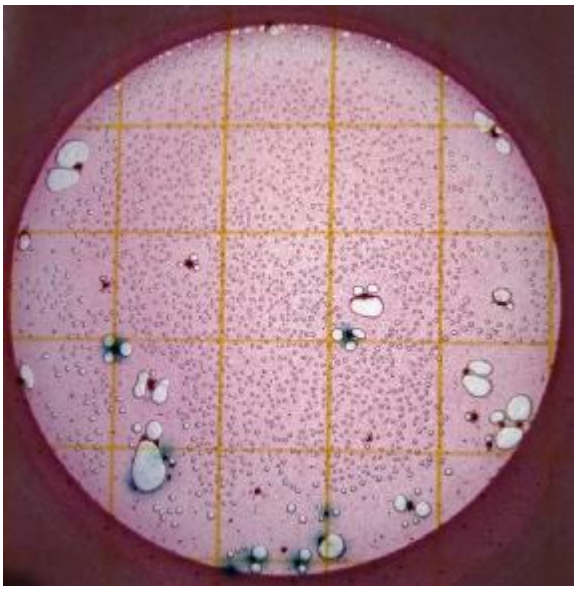

Figure 132 - Trece Martires Groundwater x1 (Cavite)

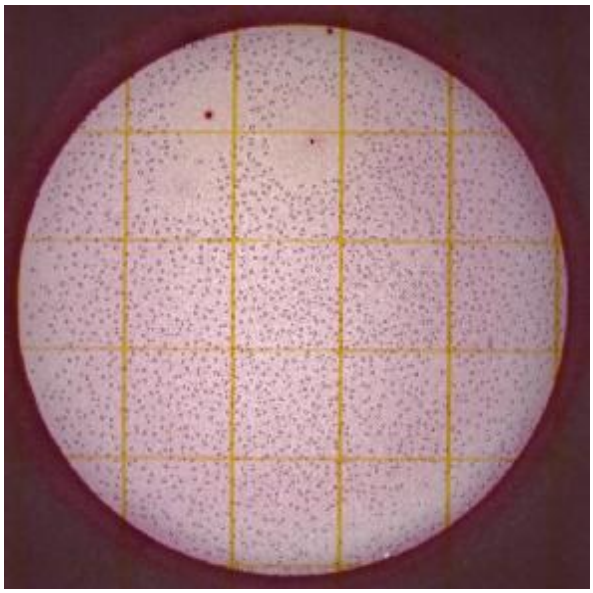

Figure 133 - Trece Martires Filtered Groundwater x1 (Cavite)

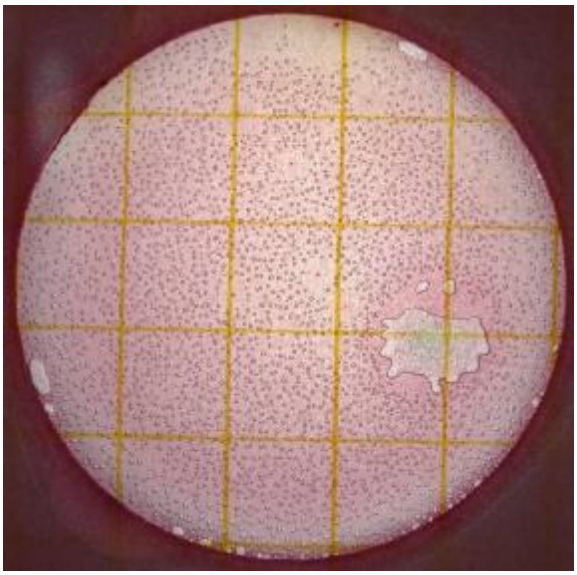

Figure 134 - Trece Martires Manila Water x10 (Cavite)

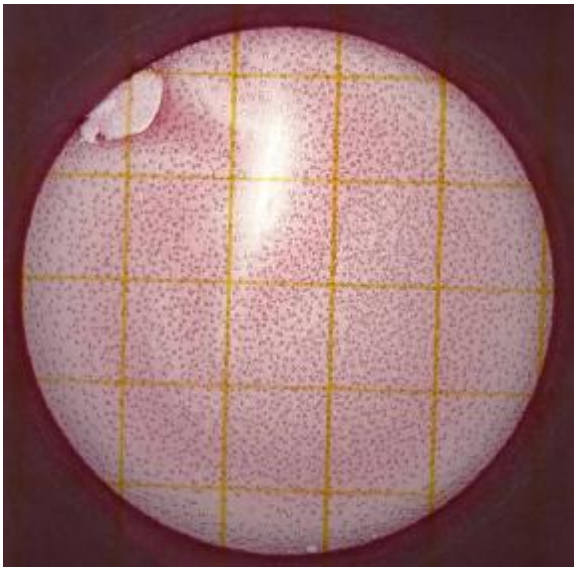

Figure 135 - Trece Martires Manila Water x1 (Cavite)

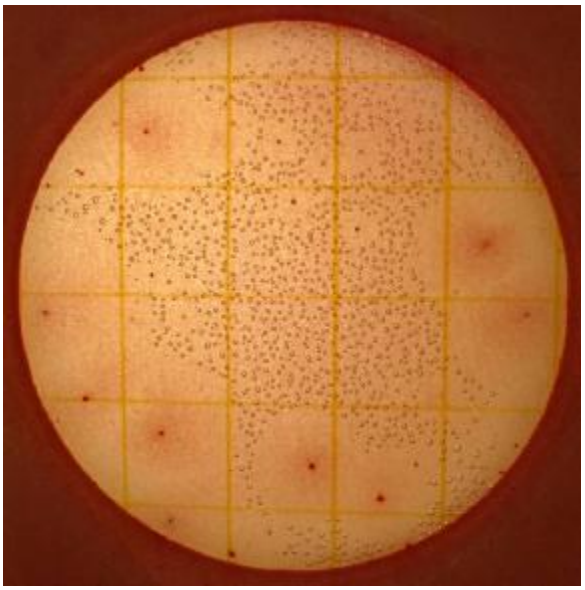

Figure 136 - Trece Martires Filtered Groundwater x10 (Cavite)

Appendix 3 – Interview Questionnaire

Table 10 - Interview Questions and Responses

Interview Questions			**Analysis of Responses (154 interviewed)**	
Site Name **Location** Beneficiary Name House Number Gender Age				
Family Size			5.4	average family size
How long have you lived in the village?			3.7	average years in village
Where did you live before you moved to the GK Village?			various	
What is the source of livelihood for your household?			various	
Health	How many incidents of diarrhea do you or anyone in	Before GK village	1.7	average incidences before (not including outlier)

Interview Questions			Analysis of Responses (154 interviewed)	
	your family experience in a year?	After GK village	1.2	average incidences now
			13.6	% report an increase in diarrhea since moving to GK
			28.6	% families report a decrease in diarrhea since moving to GK
			0.5	net improvement in cases of diarrhea per family per year
			14.9	% net decrease in incidences of diarrhea
Water	Potable water supply	What is the potable water source you use?	43.5	% ever drinking from on-site water source
			47.4	% drink from water district
			11.7	% ever buy purified water
			2.6	% of people change their practices depending on the season.
		Do you treat the water ?	varies	

Interview Questions			Analysis of Responses (154 interviewed)	
		Drinking water from on-site source with treatment *	20.9	% of people treat the water from their on-site source under any circumstance
		How many liters per day is your household drinking?	1.4	average L/p/d
		What is the nonpotable water source you use?	same as for potable	
		Are you satisfied with your water source?	85.1	% of people report that they are satisfied
	Comparison before and after	What was the water source you used at your previous residence?	varies	
		Has the amount of water available to you increased, decreaesed, or stayed the same since you moved to the GK village?	55.2	% reported water availability stayed the same
			36.4	% people report increased water supply since GK
			8.4	% people report decreased water supply since GK
			27.9	net % with increased water supply

Interview Questions			Analysis of Responses (154 interviewed)	
		Have your water uses changed since you moved to GK?	66.9	% report their water usage has stayed the same
			16.9	% report increased water use for hygiene purposes
Wastewater	Septic Tanks	Where is your septic tank located? (Yes) indicates the interviewee knew the location. (No) indicates they did nto.	99.4	% knew where their septic tanks were located
		What will you do when your septic tank becomes full?	24.7	%don't know what to do
			22.7	% plan to build new
			41.6	% will pump
			5.8	% are inaccessible for pumping
		Have you experienced any problems with your septic tank?	1.3	% of people report problems with their septic systems
		Are you satisfied with your septic tank?	95.4	% report they are satisfied with their septic tanks

Interview Questions			Analysis of Responses (154 interviewed)	
	Before GK village	What was your wastewater disposal system before you moved to the GK vilalge?	various	
		Is the current sanitation system an improvement over the system used before moving to GK village? *	28.5	% have improved sanitation now
Solid waste	Does the municipality collect any of your waste?		55.2	%utilize city collection
	Do you segregate your biodegradable and non-biodegradable waste? (yes) segregates (no) mixed waste		64.3	%segregate waste
	Do you recycle any of your waste?		85.7	%recycle
	Do you burn any of your waste?		50.0	% burn at least some of their waste
	Before GK village	What were your methods of solid waste disposal before you moved to the GK village?	various	

Interview Questions			Analysis of Responses (154 interviewed)	
		Have the practices improved? *	29.9	% net improvement in residents' practices
* indicates question was not asked directly but was inferred from interviewee's response				

Appendix 4 – TRFLP Charts depicting microbial community.

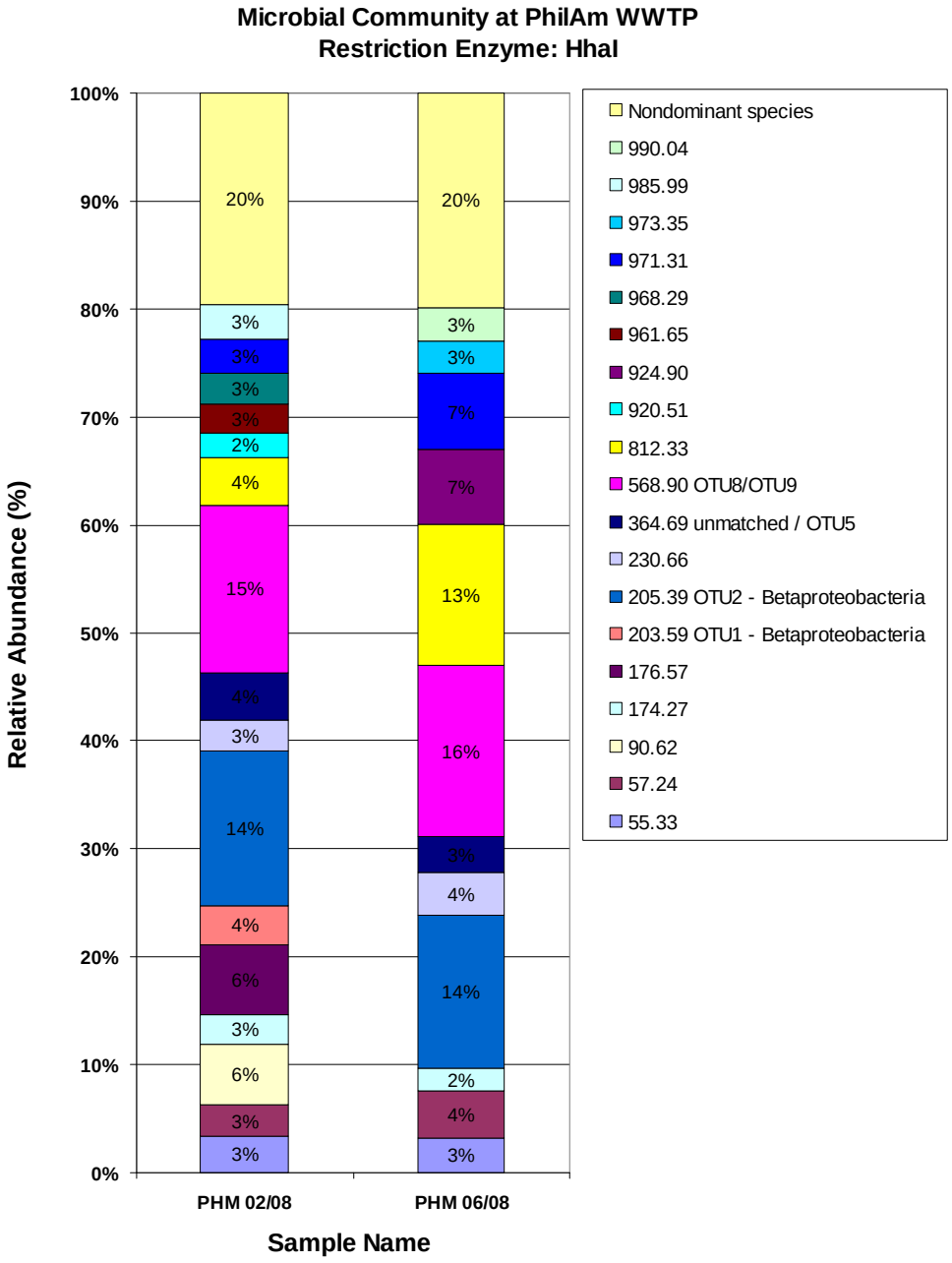

Figure 137 - Microbial Community at PhilAm WWTP; Restriction Enzyme HhaI

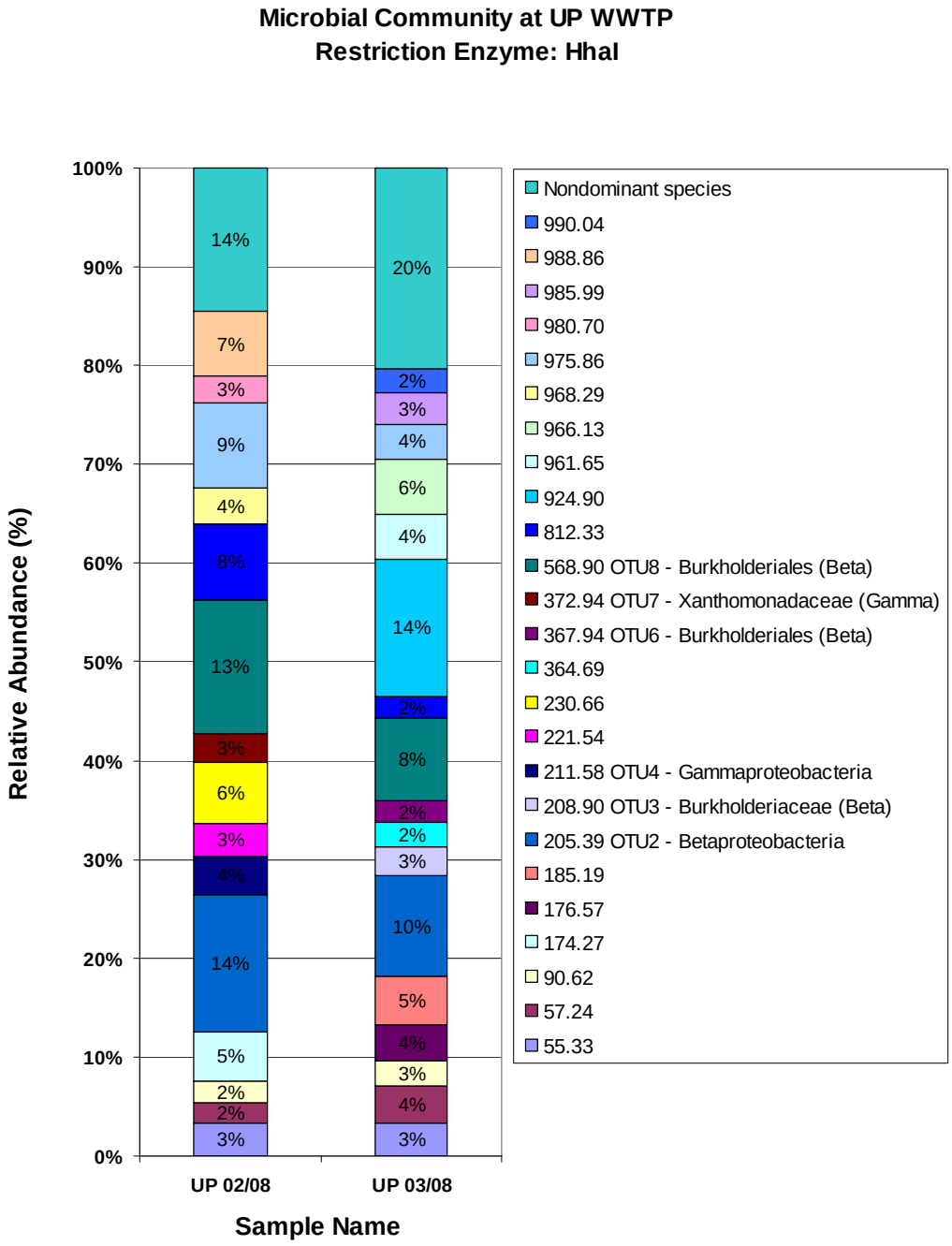

Figure 138 - Microbial Community at UP WWTP; Restriction Enzyme HhaI

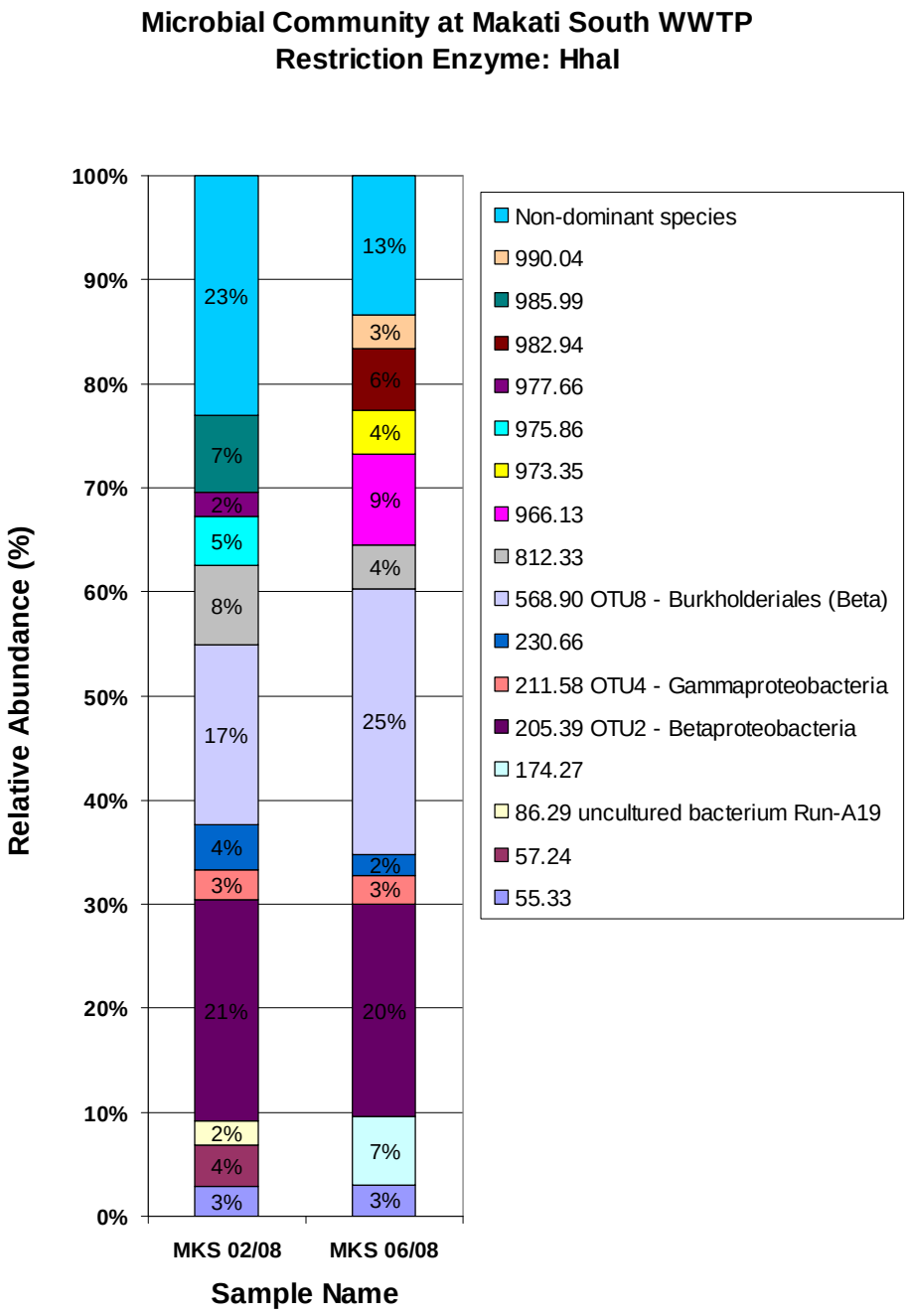

Figure 139 - Microbial Community at MKS WWTP; Restriction Enzyme HhaI

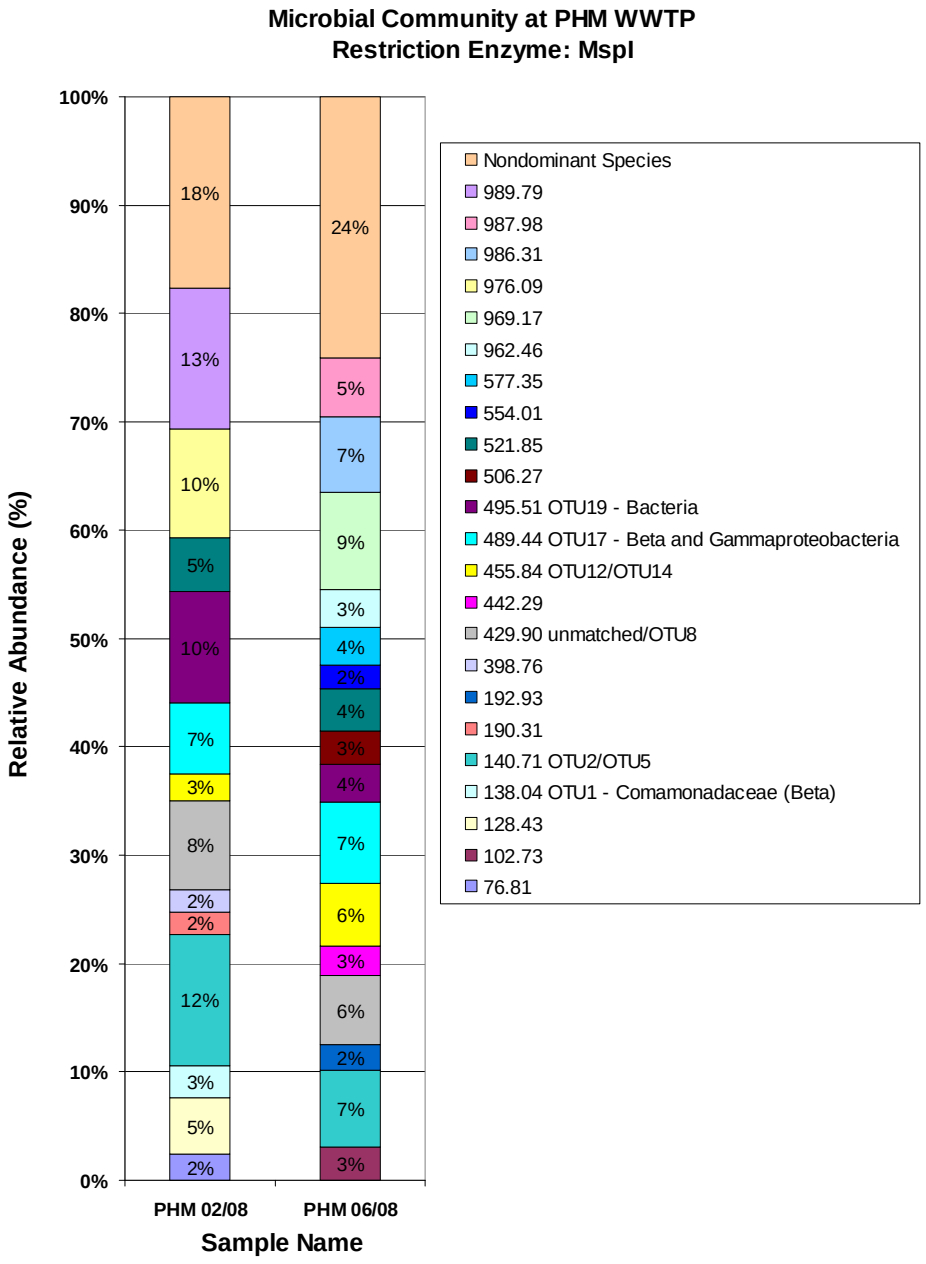

Figure 140 - Microbial Community at PHM WWTP; Restriction Enzyme: MspI

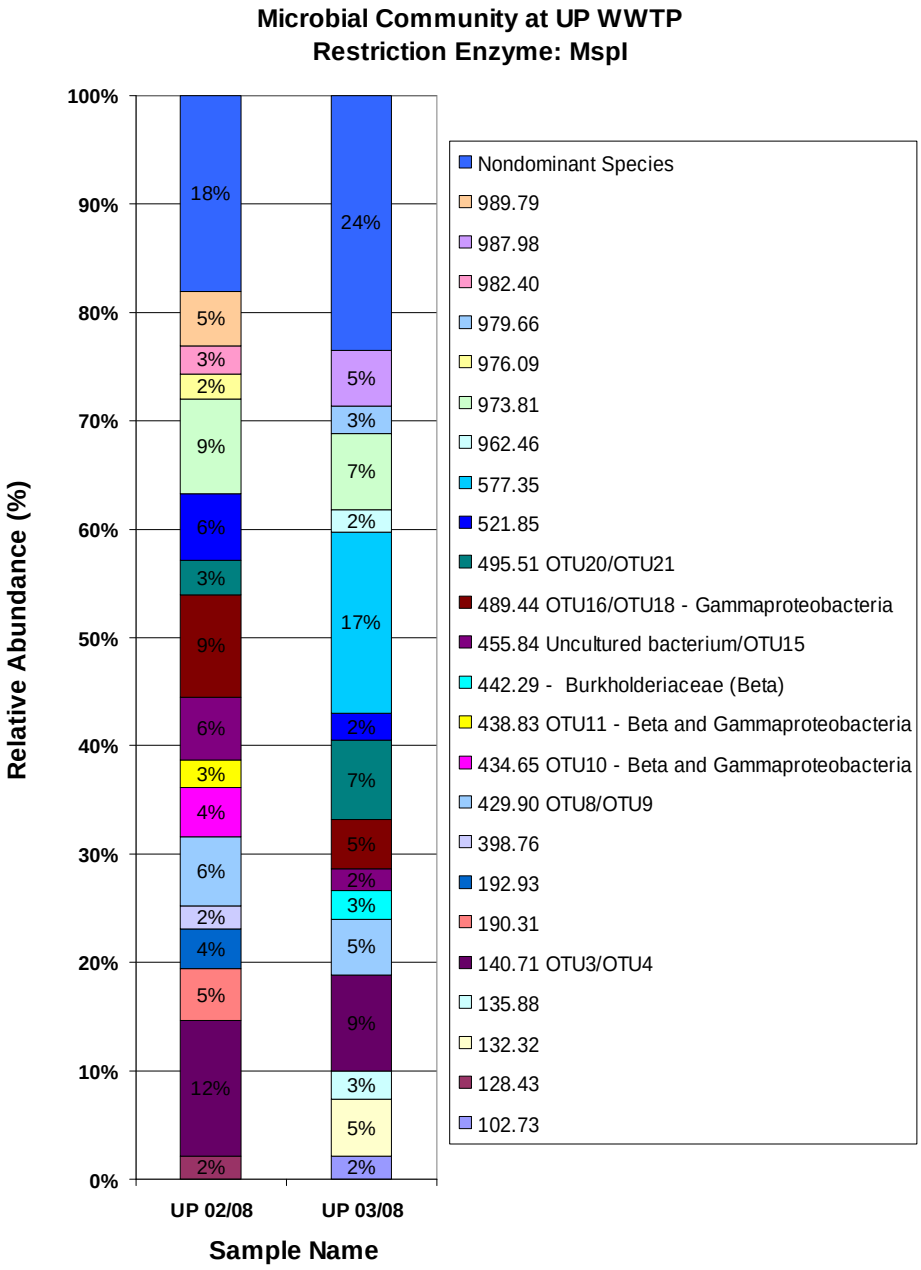

Figure 141 - Microbial Community at UP WWTP; Restriction Enzyme: MspI

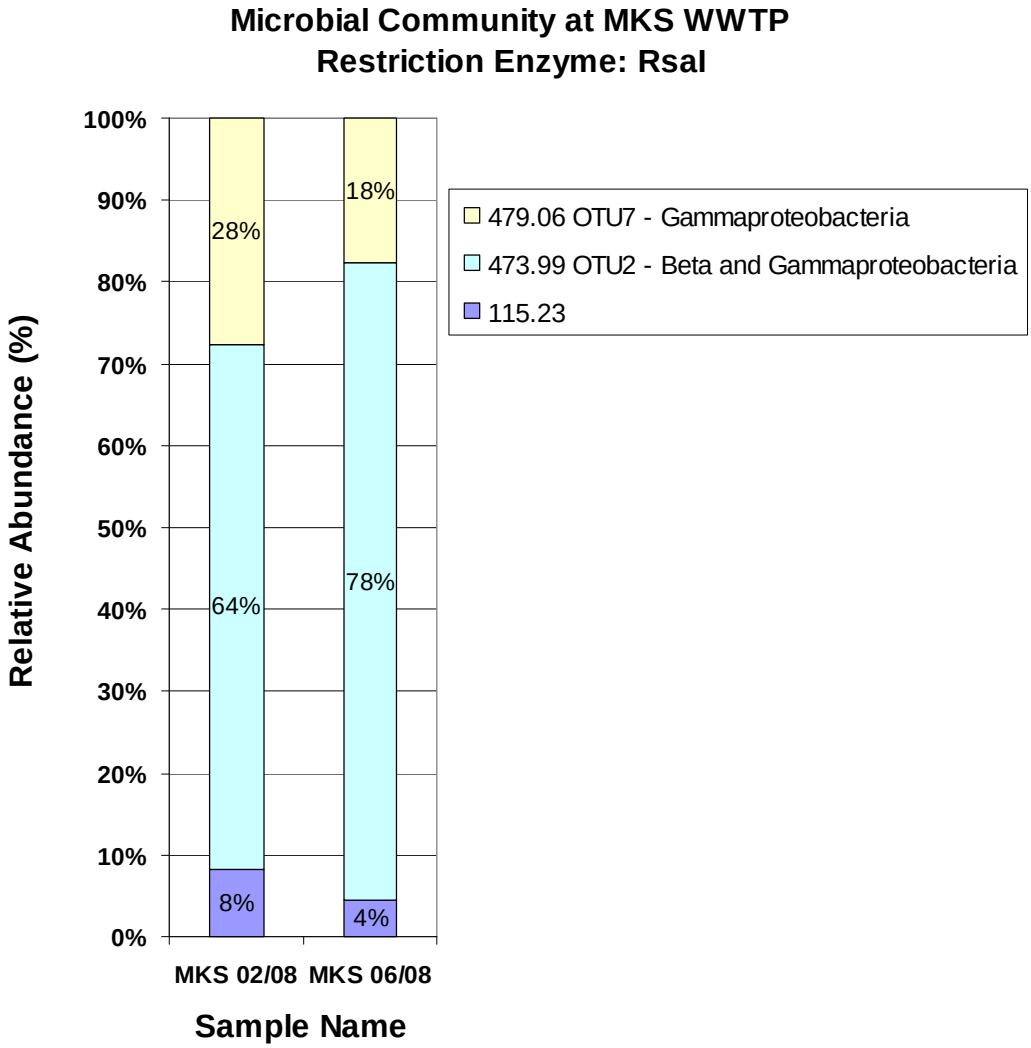

Figure 142 - Microbial Community at MKS WWTP; Restriction Enzyme: MspI

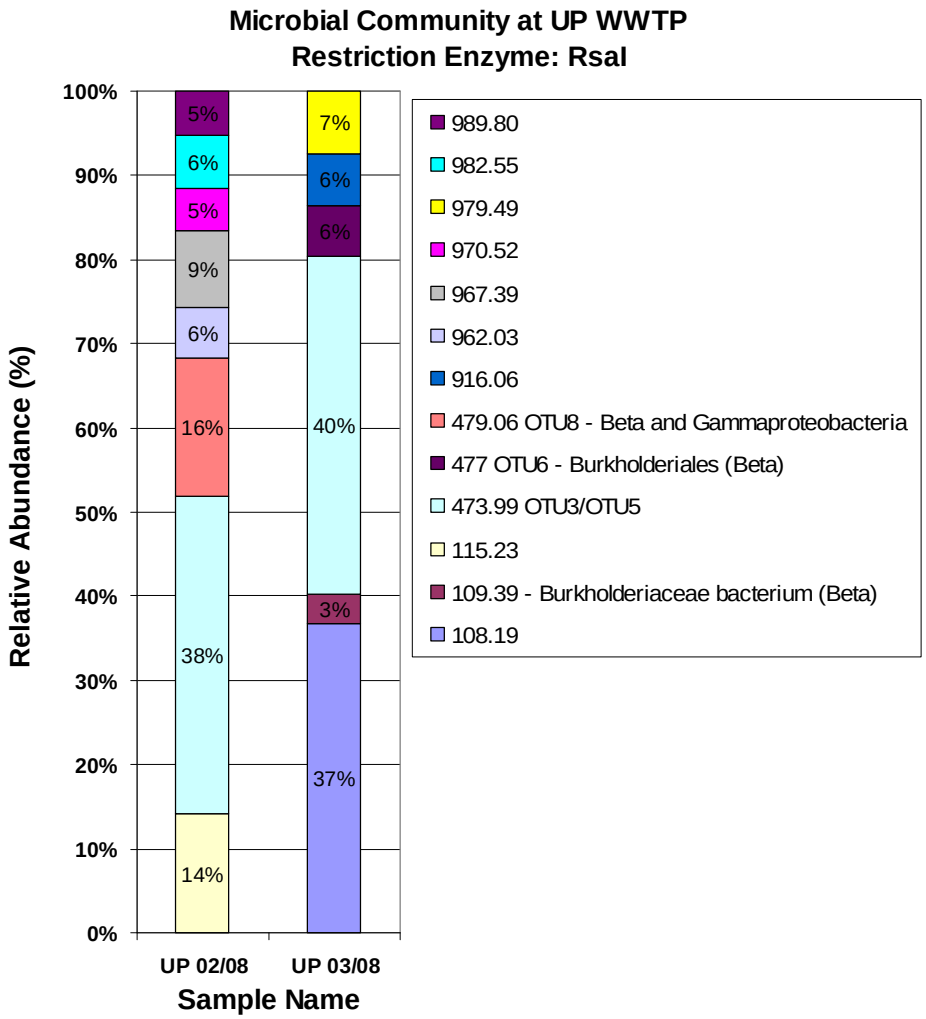

Figure 143 - Microbial Community at UP WWTP; Restriction Enzyme: RsaI

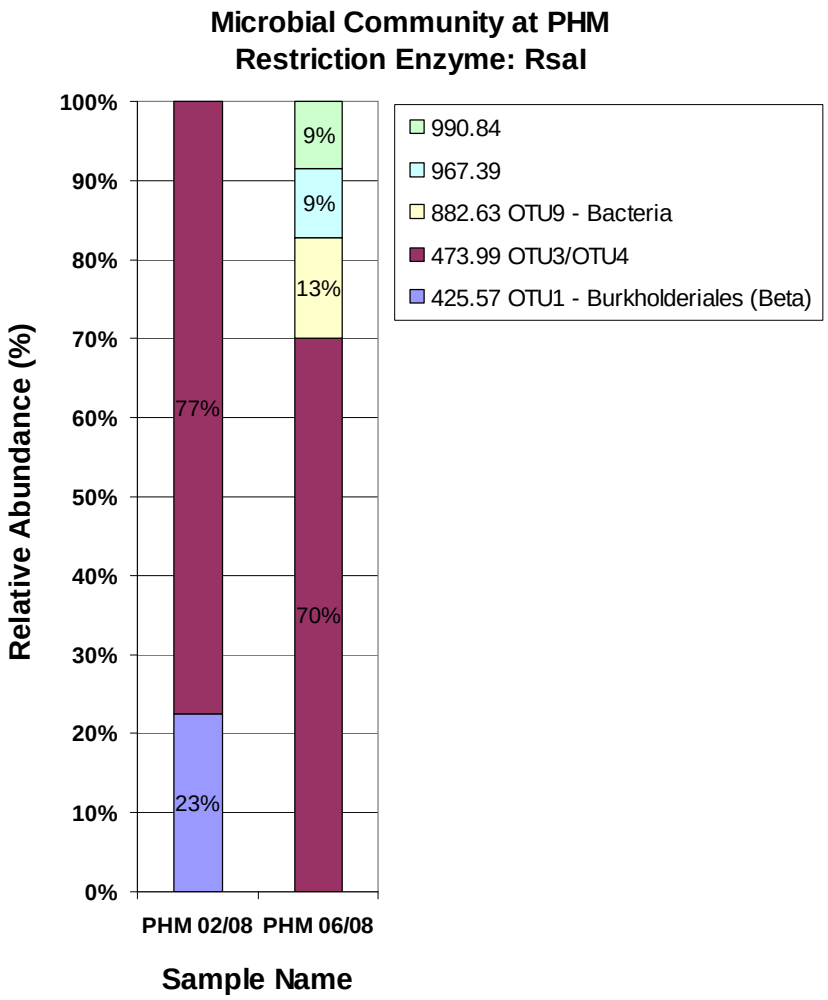

Figure 144 - Microbial Community at PHM WWTP; Restriction Enzyme: RsaI

Appendix 5 – OTU Tables – Results returned from PAT

Table 11 - OTUs from RE HhaI

HhaI OTU LABEL with Highest common taxonomic unit among species returned	Species	T-RF
uncultured bacterium Run-A19.	uncultured bacterium Run-A19.	86.29
OTU1 - Betaproteobacteria	Acidovorax	203.59
-	aniline-degrading bacterium HY99.	203.59
-	Comamonas	203.59
-	Delftia	203.59
-	Diaphorobacter	203.59
-	Leptothrix	203.59
-	Malikia granosa (T) type strain: P1.	203.59
-	Mitsuaria chitosanitabida 12.	203.59
-	uncultured bacterium	203.59
OTU2 - Betaproteobacteria	Acidovorax	205.39
-	Alcaligenes sp. 3013.	205.39
-	aniline-degrading bacterium HY99.	205.39
-	Burkholderia sp. AK-5.	205.39
-	Comamonas	205.39
-	Cupriavidus necator	205.39

HhaI OTU LABEL with Highest common taxonomic unit among species returned	Species	T-RF
-	Delftia	205.39
-	Diaphorobacter	205.39
-	Kinetoplastibacterium crithidii.	205.39
-	Leptothrix sp. S1.1.	205.39
-	Malikia granosa (T) type strain: P1.	205.39
-	Mitsuaria chitosanitabida 12.	205.39
-	Rubrivivax sp. 3016.	205.39
-	uncultured bacterium	205.39
-	Alcaligenes sp. 3013.	205.39
-	Burkholderia sp. AK-5.	205.39
-	Cupriavidus necator	205.39
-	Kinetoplastibacterium crithidii.	205.39
-	Mitsuaria chitosanitabida 12.	205.39
-	Rubrivivax sp. 3016.	205.39
-	uncultured bacterium	205.39
OTU3 - Burkholderiaceae (Beta)	Burkholderia	208.90
-	uncultured bacterium	208.90
OTU4 - Gammaproteobacteria	Dyella sp. CHNCT13.	211.58
-	Frateuria aurantia IFO3249.	211.58
-	Pseudomonas sp. An18.	211.58
-	Stenotrophomonas	211.58

HhaI OTU LABEL with Highest common taxonomic unit among species returned	Species	T-RF
-	Xanthomonas	211.58
-	uncultured bacterium	211.58
-	Frateuria aurantia IFO3249.	211.58
-	Pseudomonas sp. An18.	211.58
-	Stenotrophomonas	211.58
-	Xanthomonadaceae	211.58
-	uncultured bacterium	211.58
OTU5 - Enterobacteriaceae (Gamma)	Serratia sp. EP26.	364.69
-	uncultured bacterium	364.69
OTU6 - Burkholderiales (Beta)	Burkholderia	367.94
-	Herbaspirillum	367.94
-	uncultured bacterium	367.94
OTU7 - Xanthomonadaceae (Gamma)	Lysobacter sp. BBCT65.	372.94
-	Pseudoxanthomonas mexicana (T) AMX 26B.	372.94
-	Xanthomonas sp. Tibet-IB54.	372.94
-	Xylella fastidiosa	372.94
-	uncultured bacterium	372.94
OTU8 - Burkholderiales (Beta)	Achromobacter	568.90
-	Acidovorax sp. 98-63833.	568.90
-	Alcaligenaceae	568.90

HhaI OTU LABEL with Highest common taxonomic unit among species returned	Species	T-RF
-	Bordetella	568.90
-	Burkholderiaceae	568.90
-	Comamonas	568.90
-	Moritella	568.90
-	uncultured bacterium	568.90
-	Achromobacter	568.90
-	Alcaligenaceae	568.90
-	Bordetella	568.90
-	Burkholderiaceae	568.90
-	uncultured bacterium	568.90
OTU9 - Beta and Gammaproteobacteria	Achromobacter	568.90
-	Alcaligenaceae	568.90
-	Alkalilimnicola ehrlichei	568.90
-	Bordetella	568.90
-	Burkholderiaceae	568.90
-	dibenzofuran-degrading bacterium DBF-MAK.	568.90
-	Glaciecola sp. BSi20138.	568.90
-	Marinomonas	568.90
-	Piscirickettsia	568.90
-	Psychromonas	568.90

HhaI OTU LABEL with Highest common taxonomic unit among species returned	Species	T-RF
-	uncultured bacterium	568.90

Table 12 - OTUs from RE MspI

MspI OTU LABEL with Highest common taxonomic unit among species returned	Species	T-RF
OTU1 - Comamonadaceae (Beta)	Acidovorax	138.04
-	aniline-degrading bacterium HY99.	138.04
-	Diaphorobacter	138.04
-	uncultured bacterium	138.04
OTU2 - Comamonadaceae (Beta)	Comamonas sp. 141S.	140.71
-	Diaphorobacter	140.71
-	uncultured bacterium	140.71
OTU3 - Burkholderiales (Beta)	Achromobacter xylosoxidans subsp. xylosoxidans E.	140.71
-	Burkholderiaceae bacterium	140.71
-	uncultured bacterium	140.71
OTU4 - Beta and Gammaproteobacteria	Burkholderiaceae bacterium	140.71
-	Frateuria aurantia IFO3249.	140.71
-	Kinetoplastibacterium crithidii.	140.71
-	Mitsuaria chitosanitabida 12.	140.71
-	uncultured bacterium	140.71
OTU5 - Beta and Gammaproteobacteria	Alcaligenes sp. 3013.	140.71
-	Alkalilimnicola ehrlichei MLHE-1.	140.71

MspI OTU LABEL with Highest common taxonomic unit among species returned	Species	T-RF
-	Burkholderia	140.71
-	Rubrivivax sp. 3016.	140.71
-	uncultured bacterium	140.71
OTU6 - Burkholderiales (Beta)	Alcaligenes sp. 3013.	140.71
-	Burkholderia	140.71
-	Rubrivivax sp. 3016.	140.71
-	uncultured bacterium	140.71
OTU7 - Xanthomonadaceae (Gamma)	Stenotrophomonas sp. Toyama-1.	192.93
-	Xanthomonas sp. oral clone AY088.	192.93
OTU8 - Burkholderiales (Beta)	Acidovorax sp. JS42.	429.90
-	Comamonas	429.90
-	Diaphorobacter nitroreducens KSP3.	429.90
-	uncultured bacterium	429.90
-	Cupriavidus necator VKPM	429.90
-	uncultured bacterium	429.90
-	Achromobacter	429.90
-	Burkholderiaceae	429.90
-	uncultured bacterium	429.90
OTU9 - Beta and Gammaproteobacteria	Burkholderiaceae	429.90

MspI OTU LABEL with Highest common taxonomic unit among species returned	Species	T-RF
-	Xanthomonas sp. oral clone AY088.	429.90
-	uncultured bacterium	429.90
OTU10 - Beta and Gammaproteobacteria	Achromobacter sp. IC074.	434.65
-	Bordetella	434.65
-	Stenotrophomonas sp. Toyama-1.	434.65
-	uncultured bacterium	434.65
-	Xylella fastidiosa CaVIc2.	434.65
OTU11 - Beta and Gammaproteobacteria	Bordetella	438.83
-	Pseudoxanthomonas mexicana (T) AMX 26B.	438.83
-	uncultured bacterium	438.83
Burkholderiaceae bacterium	Burkholderiaceae bacterium	442.29
OTU12- Comamonadaceae (Beta)	Acidovorax sp. JS42.	455.84
-	aniline-degrading bacterium HY99.	455.84
-	Comamonas sp. 153S.	455.84
-	Diaphorobacter nitroreducens KSP4.	455.84
-	uncultured bacterium	455.84
OTU13 - Beta and Gammaproteobacteria	Frateuria aurantia IFO3249.	455.84

MspI OTU LABEL with Highest common taxonomic unit among species returned	Species	T-RF
-	Mitsuaria chitosanitabida 12.	455.84
-	Pseudomonas sp. An18.	455.84
-	Stenotrophomonas	455.84
-	Xanthomonas	455.84
-	uncultured bacterium	455.84
OTU14 - Burkolderiales (Beta)	Mitsuaria chitosanitabida 12.	455.84
-	uncultured bacterium P13-28.	455.84
OTU15 - Alcaligenaceae (Beta)	Achromobacter	455.84
-	Alcaligenes faecalis subsp. faecalis 5659-H.	455.84
-	Bordetella	455.84
-	uncultured bacterium	455.84
Uncultured Bacterium	uncultured bacterium	455.84
OTU16 - Betaproteobacteria	Achromobacter	489.44
-	Alcaligenes	489.44
-	Bordetella	489.44
-	Burkholderia	489.44
-	Cupriavidus necator	489.44
-	Kinetoplastibacterium crithidii.	489.44
-	Mitsuaria chitosanitabida 12.	489.44
-	Rubrivivax sp. 3016.	489.44
-	uncultured bacterium	489.44

MspI OTU LABEL with Highest common taxonomic unit among species returned	Species	T-RF
-	Achromobacter	489.44
-	Alcaligenes	489.44
-	Bordetella	489.44
-	Kinetoplastibacterium crithidii.	489.44
-	uncultured bacterium	489.44
OTU17 - Beta and Gammaproteobacteria	Achromobacter	489.44
-	Acidovorax	489.44
-	Alcaligenes	489.44
-	Bordetella	489.44
-	Burkholderia	489.44
-	Comamonas	489.44
-	Cupriavidus necator	489.44
-	Delftia	489.44
-	Diaphorobacter	489.44
-	Kinetoplastibacterium crithidii.	489.44
-	Leptothrix sp. S1.1.	489.44
-	Malikia granosa (T) type strain: P1.	489.44
-	Mitsuaria chitosanitabida 12.	489.44
-	Moritella	489.44
-	Rubrivivax sp. 3016.	489.44

MspI OTU LABEL with Highest common taxonomic unit among species returned	Species	T-RF
-	uncultured bacterium	489.44
-	Achromobacter	489.44
-	Alcaligenes	489.44
-	Bordetella	489.44
-	Glaciecola sp. BSi20138.	489.44
-	Kinetoplastibacterium crithidii.	489.44
-	Marinomonas sp. BSw10005.	489.44
-	Nitrosomonas sp. JL21.	489.44
-	Psychromonas sp. Ant5-5.	489.44
-	uncultured bacterium	489.44
OTU18 - Gammaproteobacteria	Dyella	489.44
-	Lysobacter sp. BBCT65.	489.44
-	Pseudomonas sp. An18.	489.44
-	Stenotrophomonas	489.44
-	Xanthomonas	489.44
-	Xylella fastidiosa	489.44
-	uncultured bacterium	489.44
OTU19 - Bacteria	Acidovorax	495.51
-	Comamonas sp. NSP4.	495.51
-	Delftia sp. ZM-1.	495.51
-	Nostoc sphaeroides HBHF0604.	495.51

MspI OTU LABEL with Highest common taxonomic unit among species returned	Species	T-RF
-	uncultured bacterium	495.51
-	dibenzofuran-degrading bacterium DBF-MAK.	495.51
-	Glaciecola sp. BSi20138.	495.51
-	Marinomonas	495.51
-	Nitrosomonas sp. JL21.	495.51
-	Piscirickettsia salmonis	495.51
-	Psychromonas sp. Ant5-5.	495.51
-	Serratia sp. EP26.	495.51
-	uncultured bacterium	495.51
OTU20 - Beta and Gammaproteobacteria	Achromobacter	495.51
-	Alcaligenes	495.51
-	Burkholderia sp. AK-5.	495.51
-	Cupriavidus necator	495.51
-	Rubrivivax sp. 3016.	495.51
-	Xylella fastidiosa	495.51
-	uncultured bacterium	495.51
OTU21 - Burkholderiales (Beta)	Achromobacter xylosoxidans	495.51
-	Bordetella	495.51
-	Burkholderia sp. PSB10.	495.51
-	Herbaspirillum	495.51

MspI OTU LABEL with Highest common taxonomic unit among species returned	Species	T-RF
-	uncultured bacterium	495.51

Table 13 - OTUs for RE RsaI

RsaI OTU LABEL with Highest common taxonomic unit among species returned	Species	T-RF
Burkholderiaceae bacterium	Burkholderiaceae bacterium	109.39
OTU1 - Burkholderiales (Beta)	Achromobacter	425.57
-	Acidovorax	425.57
-	Alcaligenes	425.57
-	Bordetella	425.57
-	Burkholderiaceae bacterium	425.57
-	Comamonas	425.57
-	Delftia	425.57
-	Diaphorobacter	425.57
-	Leptothrix sp. S1.1.	425.57
-	Malikia granosa (T) type strain: P1.	425.57
-	Mitsuaria chitosanitabida 12.	425.57
-	Moritella	425.57
-	uncultured bacterium	425.57
OTU2 - Beta and Gammaproteobacteria	Achromobacter	473.99
-	Alcaligenes	473.99
-	Bordetella	473.99
-	Burkholderia	473.99

RsaI OTU LABEL with Highest common taxonomic unit among species returned	Species	T-RF
-	Cupriavidus	473.99
-	Frateuria aurantia IFO3249.	473.99
-	Kinetoplastibacterium crithidii.	473.99
-	Mitsuaria chitosanitabida 12.	473.99
-	Rubrivivax sp. 3016.	473.99
-	uncultured bacterium	473.99
OTU3 - Betaproteobacteria	Achromobacter	473.99
-	Alcaligenes	473.99
-	Bordetella	473.99
-	Burkholderia	473.99
-	Cupriavidus necator	473.99
-	Herbaspirillum sp. CHNTR44.	473.99
-	Kinetoplastibacterium crithidii.	473.99
-	Mitsuaria chitosanitabida 12.	473.99
-	Rubrivivax sp. 3016.	473.99
-	uncultured bacterium	473.99
-	Achromobacter	473.99
-	Alcaligenes	473.99
-	Bordetella	473.99
-	Burkholderia	473.99
-	Cupriavidus necator	473.99

RsaI OTU LABEL with Highest common taxonomic unit among species returned	Species	T-RF
-	Kinetoplastibacterium crithidii.	473.99
-	Mitsuaria chitosanitabida 12.	473.99
-	Rubrivivax sp. 3016.	473.99
-	uncultured bacterium	473.99
OTU4 - Bacteria	Acidovorax	473.99
-	Alcaligenes sp. 3013.	473.99
-	aniline-degrading bacterium HY99.	473.99
-	Burkholderia sp. AK-5.	473.99
-	Comamonas	473.99
-	Cupriavidus necator	473.99
-	Delftia	473.99
-	Diaphorobacter	473.99
-	Kinetoplastibacterium crithidii.	473.99
-	Leptothrix sp. S1.1.	473.99
-	Mitsuaria chitosanitabida 12.	473.99
-	Nostoc sphaeroides HBHF0604.	473.99
-	Rubrivivax sp. 3016.	473.99
-	uncultured bacterium	473.99
OTU5 - Beta and Gammaproteobacteria	Burkholderia	473.99
-	Dyella	473.99

RsaI OTU LABEL with Highest common taxonomic unit among species returned	Species	T-RF
-	Frateuria aurantia IFO3249.	473.99
-	Kinetoplastibacterium crithidii.	473.99
-	Lysobacter sp. BBCT65.	473.99
-	Mitsuaria chitosanitabida 12.	473.99
-	Pseudomonas sp. An18.	473.99
-	Stenotrophomonas	473.99
-	uncultured bacterium	473.99
-	Xanthomonas	473.99
-	Xylella fastidiosa	473.99
OTU6 - Burkholderiales (Beta)	Achromobacter	477.87
-	Alcaligenes	477.87
-	Bordetella	477.87
-	Herbaspirillum sp. CHNTR44.	477.87
-	uncultured bacterium	477.87
OTU7 - Gammaproteobacteria	Pseudomonas sp. An18.	479.06
-	Stenotrophomonas	479.06
-	uncultured bacterium	479.06
-	Xanthomonas	479.06
OTU8 - Beta and Gammaproteobacteria	Achromobacter	479.06
-	Alcaligenaceae	479.06

RsaI OTU LABEL with Highest common taxonomic unit among species returned	Species	T-RF
-	Bordetella	479.06
-	Burkholderia sp. AK-5.	479.06
-	Cupriavidus necator	479.06
-	Pseudoxanthomonas mexicana (T) AMX 26B.	479.06
-	Rubrivivax sp. 3016.	479.06
-	uncultured bacterium	479.06
-	Xylella fastidiosa	479.06
OTU9 - Bacteria	Alkalilimnicola ehrlichei MLHE-1.	882.63
-	dibenzofuran-degrading bacterium DBF-MAK.	882.63
-	Glaciecola	882.63
-	Marinomonas	882.63
-	Nitrosomonas sp. JL21.	882.63
-	Piscirickettsia salmonis	882.63
-	Psychromonas	882.63
-	Serratia sp. EP26.	882.63
-	uncultured bacterium	882.63

Appendix 6 – Original Phylogenetic Trees (before removing duplicate samples)

The following is a listing of all sequences from clones used to create the trees above. Some clones represent identical species, and so were removed from the above trees. Sampling names are as follows: Abbreviation for WWTP name; month and year sample was taken; location in 96 well plate for record keeping and distinguishing samples.

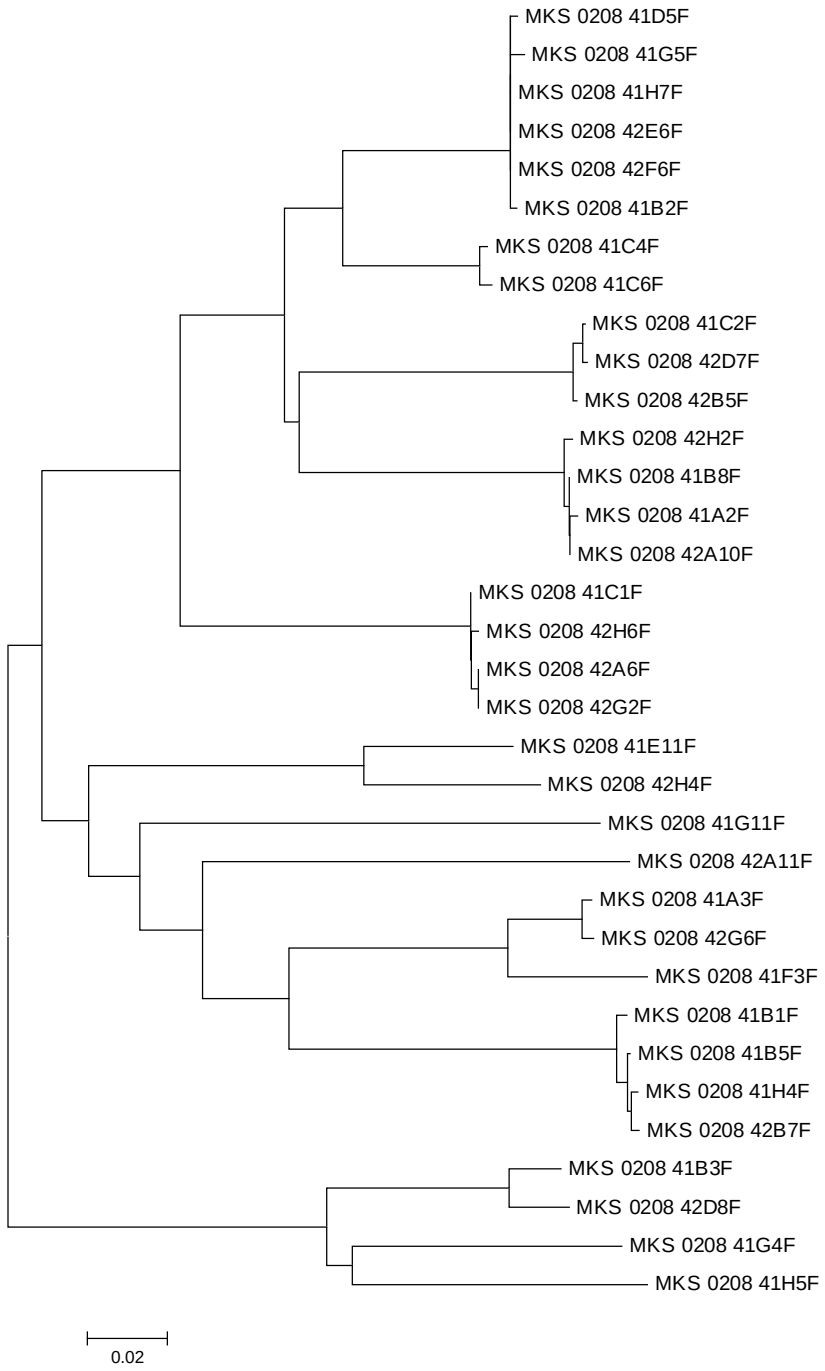
MKS 0208 41D5F
MKS 0208 41G5F
MKS 0208 41H7F
MKS 0208 42E6F
MKS 0208 42F6F
MKS 0208 41B2F
MKS 0208 41C4F
MKS 0208 41C6F
MKS 0208 41C2F
MKS 0208 42D7F
MKS 0208 42B5F
MKS 0208 42H2F
MKS 0208 41B8F
MKS 0208 41A2F
MKS 0208 42A10F
MKS 0208 41C1F
MKS 0208 42H6F
MKS 0208 42A6F
MKS 0208 42G2F
MKS 0208 41E11F
MKS 0208 42H4F
MKS 0208 41G11F
MKS 0208 42A11F
MKS 0208 41A3F
MKS 0208 42G6F
MKS 0208 41F3F
MKS 0208 41B1F
MKS 0208 41B5F
MKS 0208 41H4F
MKS 0208 42B7F
MKS 0208 41B3F
MKS 0208 42D8F
MKS 0208 41G4F
MKS 0208 41H5F
0.02

Figure 145 – Original Tree for MKS sample before pruning duplicate samples.

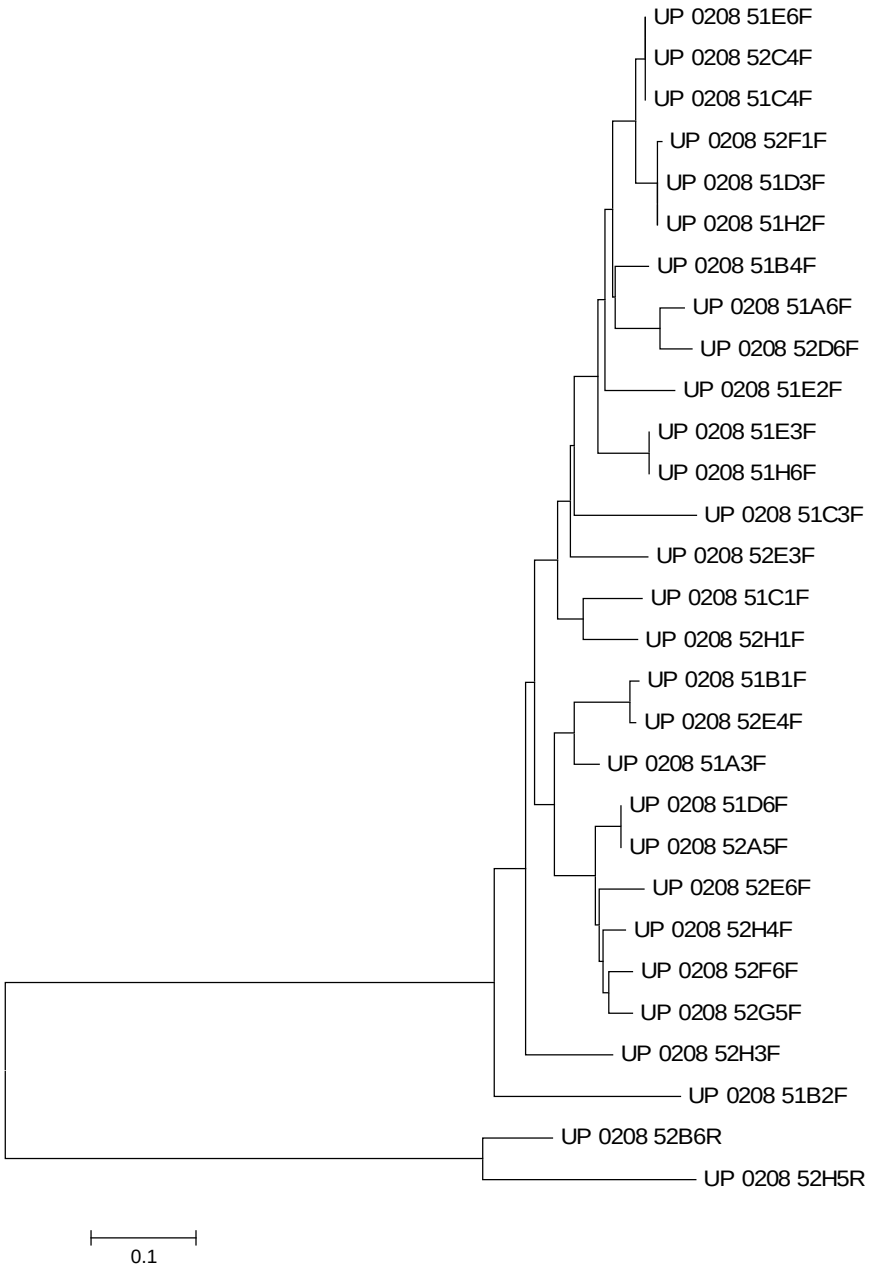

Figure 146 - Original Tree for UP sample before pruning duplicate samples.

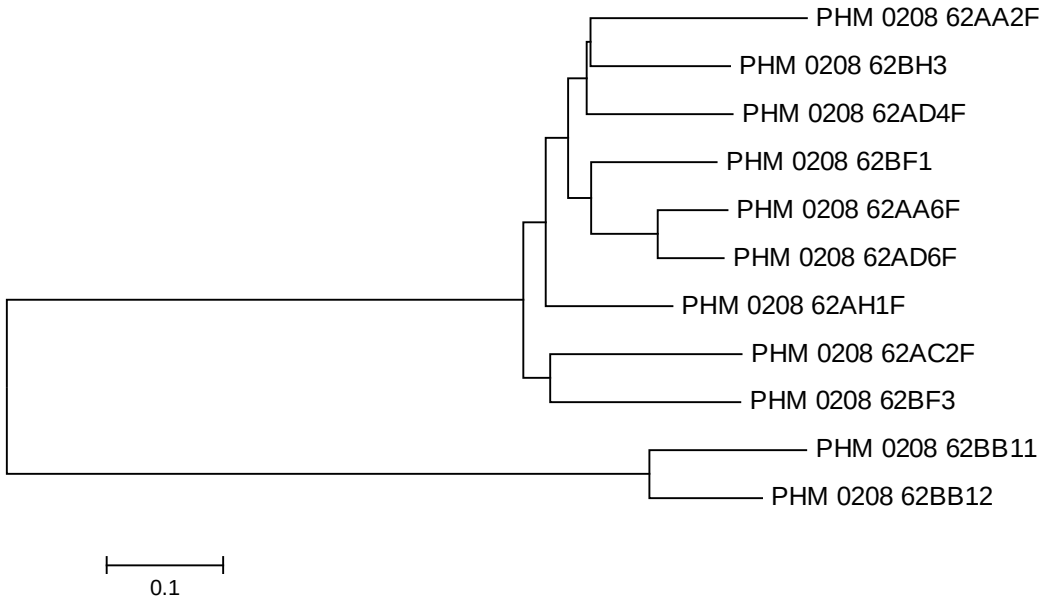

Figure 147 - PHM cloning results. No duplicates to remove.

Appendix 7 - Cloned Sequences

> MKS_0208_41A2F
TGCAGTCGACGGCAGCACGGACTTCGGTCTGGTGGCGAGTGGCGAACGGGTGAGTA
ATGTATCGGAACGTGCCCAGTAGCGGGGGATAACTACGCGAAAGCGTAGCTAATAC
CGCATACGCCCTACGGGGGAAAGCAGGGGATCGCAAGACCTTGCACTATTGGAGCG
GCCGATATCGGATTAGCTAGTTGGTGGGGTAACGGCTCACCAAGGCGACGATCCGT
AGCTGGTTTGAGAGGACGACCAGCCACACTGGGACTGAGACACGGCCCAGACTCCT
ACGGGAGGCAGCAGTGGGGAATTTTGGACAATGGGGGAAACCCTGATCCAGCCATC
CCGCGTGTGCGATGAAGGCCTTCGGGTTGTAAAGCACTTTTGGCAGGAAAGAAACG
TCATGGGTTAATACCCCGTGAAACTGACGGTACCTGCAGAATAAGCACCGGCTAACT
ACGTGCCAGCAGCCGCGGTAATACGTAGGGTGCAAGCGTTAATCGGAATTACTGGG
CGTAAAGCGTGCGCAGGCGGTTCGGAAAGAAAGATGCGAAATCCCAGAGCTTAACT
TTGGAACTGCATTTTTAACTACCGGGCTAGAGTGTGTCAGAGGGAGGTGGAATTCCG
CGTGTAGCAGTGAAATGCGTAGATATGCGGAGGAACACCGATGGCGAAAGCAGCCT
CCTGGGATAACACTGACGCTCATGCACGAAAGCGTGGGGAGCAAACAGGATTAGAT
ACCCTGGTAGTCCACGCCCTAAACGATGTC

> MKS_0208_41A3F
TCTTCGGGAGTGAAGCTAGCGGCGGACGGGTGAGTAACACGTGGGCAACCTGCCTC
ATAGAGGGGAATAGCCTTTCGAAAGGAAGATTAATACCGCATAAGATTGTAGCTTC
GCATGAAGTAGCAATTAAAGGAGCAATCCGCTATGAGATGGGCCCGCGGCGCATTA
GCTAGTTGGTGAGGTAACGGCTCACCAAGGCGACGATGCGTAGCCGACCTGAGAGG
GTGATCGGCCACATTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGT
GGGGAATATTGCACAATGGGGGAAACCCTGATGCAGCAACGCCGCGTGAGTGATGA
CGGCCTTCGGGTTGTAAAGCTCTGTCTTCAGGGACGATAATGACGGTACCTGAGGAG
GAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCGAGCGTT
GTCCGGATTTACTGGGCGTAAAGGGAGCGTAGGCGGACTTTTAAGTGAGATGTGAA
ATACCCGGGCTCAACTTGGGTGCTGCATTTCAAACTGGAAGTCTAGAGTGCAGGAG
AGGAGAATGGAATTCCTAGTGTAGCGGTGAAATGCGTAGAGATTAGGAAGAACACC
AGTGGCGAAGGCGATCCTCTGGACTGTAACTGACGCTGAGGCTCGAAAGCGTGGGG
AGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGA

> MKS_0208_41B1F
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AACCTGCCCTGTACACACGGATAACATACCGAAAGGTATGCTAATACGAGATAATA
TGCTTTTATCGCATGGTAGAAGTATCAAAGCTTTTGCGGTACAGGATGGACCCGCGT

CTGATTAGCTAGTTGGTAAGGTAACGGCTTACCAAGGCGACGATCAGTAGCCGACCT
GAGAGGGTGATCGGCCACATTGGAACTGAGACACGGTCCAAACTCCTACGGGAGGC
AGCAGTGGGGAATATTGCACAATGGGCGAAAGCCTGATGCAGCAACGCCGCGTGAG
CGATGAAGGCCTTCGGGTCGTAAAGCTCTGTCCTCAAGGAAGATAATGACGGTACTT
GAGGAGGAAGCCCCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGGGCT
AGCGTTATCCGGAATTACTGGGCGTAAAGGGTGCGTAGGTGGTTTCTTAAGTCAGAG
GTGAAAGGCTACGGCTCAACCGTAGTAAGCCTTTGAAACTGGGAAACTTGAGTGCA
GGAGAGGAGAGTGGAATTCCTAGTGTAGCGGTGAAATGCGTAGATATTAGGAGGAA
CACCAGTTGCGAAGGCGGCTCTCTGGACTGTAACTGACACTGAGGCACGAAAGCGT
GGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAGTACTA
GCTG

> MKS_0208_41B2F
GCAGTCGACGGCAGCACGGGTGCTTGCACCTGGTGGCGAGTGGCGAACGGGTGAGT
AATACATCGGAACATGTCCTGTAGTGGGGGATAGCCCGGCGAAAGCCGGATTAATA
CCGCATACGATCTACGGATGAAAGCGGGGGACCTTCGGGCCTCGCGCTATAGGGTT
GGCCGATGGCTGATTAGCTAGTTGGTGGGATAAAGGCCTACCAAGGCGACGATCAG
TAGCTGGTCTGAGAGGACGACCAGCCACACTGGGACTGAGACACGGCCCAGACTCC
TACGGGAGGCAGCAGTGGGGAATTTTGGACAATGGGCGAAAGCCTGATCCAGCAAT
GCCGCGTGTGTGAAGAAGGCCTTCGGGTTGTAAAGCACTTTTGTCCGGAAAGAAATC
CTTGGCTCTAATACAGTCGGGGGATGACGGTACCGGAAGAATAAGCACCGGCTAAC
TACGTGCCAGCAGCCGCGGTAATACGTAGGGTGCAAGCGTTAATCGGAATTACTGG
GCGTAAAGCGTGCGCAGGCGGTTTGCTAAGACCGATGTGAAATCCCCGGGCTCAAC
CTGGGAACTGCATTGGTGACTGGCAGGCTAGAGTATGGCAGAGGGGGGTAGAATTC
CACGTGTAGCAGTGAAATGCGTAGAGATGTGGAGGAATACCGATGGCGAAGCAGCC
CCCTGGGCCAATACTGACGCTCATGCACGAAAGCGTGGGGAGCAAACAGGATTAGA
TACCCTGGTAGTCCACGCCCTAAACGATGTCAACTAG

> MKS_0208_41B3F
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GTGCTCTTCGGAGTAAAGCTTTATGCGCTTTAGGAGTGGCCTGCGTCCGATTAGCTT
GTTGGTGAGGTAATAGCTCACCAAGGCGACGATCGGTAGCTGGTCTGAGAGGATGA
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ATTTTCCACAATGGGCGAAAGCCTGATGGAGCAACGCCGCGTGCAGGATGAATGCC
TTCGGGTTGTAAACTGCTTTTATGTGCGAAGAATTTGACTGTAACACATGAATAAGG
ATCGGCTAACTACGTGCCAGCAGCCGCGGTCATACGTAGGATCCAAGCGTTATCCG
GAATTACTGGGCGTAAAGAGTTGCGTAGGTGGATTGTTAAGTGGGGCGTGAAAGCG

TACGGCTCAACCGTATTATTGCGTTCCAAACTGACAGACTAGAGTATGAGAGAGGTA
GATGGAATTTCTAGTGTAGGAGTGAAATCCGTAGATATTAGAAGGAACACCGATGG
CGTAGGCAGTCTACTGGCTCATTACTGACACTAAGGCACGAAAGCGTGGGGAGCAA
ACGGGATTAGATACCCCGGTAGTCCACGCTGTAAACGATGGATGCTAGCTGTTACTC
ACATCGACCTGAGTAGTAGCGAA

> MKS_0208_41B5F
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ATACTTTTATCGCATGGTAGAAGTATCAAAGCTCCGGCGGTACAGGATGGACCCGCG
TCTGATTAGCTAGTTGGTAAGGTAACGGCTTACCAAGGCGACGATCAGTAGCCGACC
TGAGAGGGTGATCGGCCACATTGGAACTGAGACACGGTCCAAACTCCTACGGGAGG
CAGCAGTGGGGAATATTGCACAATGGGCGAAAGCCTGATGCAGCAACGCCGCGTGA
GCGATGAAGGCCTTCGGGTCGTAAAGCTCTGTCCTCAAGGAAGATAATGACGGTAC
TTGAGGAGGAAGCCCCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGGG
CTAGCGTTATCCGGAATTACTGGGCGTAAAGGGTGCGTAGGTGGTTTCTTAAGTCAG
AGGTGAAAGGCTACGGCTCAACCGTAGTAAGCCTTTGAAACTGGGAAACTTGAGTG
CAGGAGAGGAGAGTGGAATTCCTAGTGTAGCGGTGAAATGCGTAGATATTAGGAGG
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TA

> MKS_0208_41B8F
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CGCATACGCCCTACGGGGGAAAGCAGGGGATCGCAAGACCTTGCACTATTGGAGCG
GCCGATATCGGATTAGCTAGTTGGTGGGGTAACGGCTCACCAAGGCGACGATCCGT
AGCTGGTTTGAGAGGACGACCAGCCACACTGGGACTGAGACACGGCCCAGACTCCT
ACGGGAGGCAGCAGTGGGGAATTTTGGACAATGGGGGAAACCCTGATCCAGCCATC
CCGCGTGTGCGATGAAGGCCTTCGGGTTGTAAAGCACTTTTGGCAGGAAAGAAACG
TCATGGGTTAATACCCCGTGAAACTGACGGTACCTGCAGAATAAGCACCGGCTAACT
ACGTGCCAGCAGCCGCGGTAATACGTAGGGTGCAAGCGTTAATCGGAATTACTGGG
CGTAAAGCGTGCGCAGGCGGTTCGGAAAGAAAGATGTGAAATCCCAGAGCTTAACT
TTGGAACTGCATTTTTAACTACCGGGCTAGAGTGTGTCAGAGGGAGGTGGAATTCCG
CGTGTAGCAGTGAAATGCGTAGATATGCGGAGGAACACCGATGGCGAAGGCAGCCT
CCTGGGATAACACTGACGCTCATGCACGAAAGCGTGGGGAGCAAACAGGATTAGAT
ACCCTGGTAGTCCACGCCCTAAACGATG

> MKS_0208_41C1F
GCACAGCAGAGCTTGCTCTGTGGGTGGCGAGTGGCGGACGGGTGAGGAATACGTGG
GAATCTGCCCAGTCGTGGGGGATAACGTAGGGAAACTTACGCTAATACCGCATACG
CCCTTCGGGGGAAAGCCGGGGACCTTCGGGCCTGGCGCGATTGGATGAGCCCACGT
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Appendix 8 – Assessment of Solid Waste Management Practices in GK Villages

About half of the sites visited had access to solid waste collection, with residents of other sites taking their solid waste to collection sites themselves, creating open dump sites, or burning their waste. Almost 40% of the residents interviewed indicated their solid waste management practices have improved since moving to GK villages, but 10% indicated their practices have deteriorated, typically due to relocating to an area away from city collection.

Assessment of whether practices had improved or deteriorated was based on answers to questions posed to beneficiaries including which of the following combinations of things they do with their waste: municipal collection, burning, recycling, composting. If residents burned their waste before and now send it for collection, that was deemed an improvement (the opposite a deterioration); recycling before but not after moving to the GK site was viewed as a deterioration (the opposite an improvement), etc. Raw data for these interviews is available in

Appendix 3.

Appendix 9 - Noted Problems and Suggestions – Solid Waste Management

Many beneficiaries interviewed did not understand reasons for their actions with regard to solid waste management. Some had access to city collection, but would still burn at least some of their garbage, often including plastics and other non-biodegradables, and would only give biodegradables to the city for collection. Many would burn leaves or yard trimmings when they had access to compost facilities. While this is certainly also partially due to a hurdle in changing beneficiaries' behaviors, and partially because residents would burn waste to ward off mosquitoes, general lack of understanding of their actions make decision-making confusing for many residents.

In cases where collection is unavailable or only rarely available, the local government may be able to offer better services if their help is solicited. If this help is unavailable, careful consideration should be given to determine the best disposal method at each individual site, working with the project director and the neighborhood association. Working together to decide what is best and making conscious decisions is more likely to produce agreed upon, understood results, which makes implementation more effective and sustainable. Figure 17 of GK DMC (Davao) shows the residents at this site burning yard clippings. Every afternoon, each family burns their yard trimmings, along with the rest of their trash, in the middle of the site, amongst their neighbors' homes. A better option would be to reduce the garbage by composting the yard clippings and burn the remaining waste on the edge of the site where there are no houses.

Composting is an option that should be explored at more GK sites. A large number of sites host small piles of burning yard clippings in the afternoons. Some of these sites are also hosts to productivity agriculture and/or compost piles. Many residents don't understand how composting works, or what materials can be composted. Composting reduces the volume of trash that must be collected or otherwise disposed of, which is especially important on sites with infrequent collection, and on agricultural sites, composting also has economic benefits. At GK San Juan in Batangas, for instance, the residents burn all of their biodegradable material and are simultaneously concerned that they cannot afford commercial fertilizer. Beginning a composting program will both make their food and yard waste available as fertilizer and ensuring that nutrients are not depleted from their land.

Removing biodegradable waste from the trash and ensuring that no degradable materials go into the trash bin has the added benefit that trash be stored on site for longer periods of time without attracting disease vectors and nuisances until collection is available.

Figure 148 – DMC (Davao). The residents at this site burn their waste individually.

Figure 18 of GK Brookside (Manila) shows areas near the GK village that are host to dump sites. Dump sites pose a significant threat to human health in terms of direct contact, attraction of disease vectors, and also contamination of surface and groundwater, and so should be planned or better controlled whenever possible.

Figure 149 - GK Brookside (Manila). Dump sites pose threat to human health and the environment.

Installing community-scale Material Recovery Facilities (MRFs) on sites is generally appropriate. Most recyclables can be easily bought and sold in the Philippines on a small scale, which makes the installation of onsite MRFs a good option to alleviate some of the stress of the waste produced by the village while also being a source of employment and income for several of the residents.

Many of the residents do not understand the why they should participate in appropriate waste management. Ensuring the residents understand the tradeoffs of burning or dumping their garbage and the benefits of composting will yield greater participation than macro-threats

like global warming. As an added benefit, if the residents truly understand their actions, they won't be ashamed of their practices, which aids in restoring dignity, one of the key focuses of GK.