Assessment of Microbial Source Tracking Markers and Tracers at Wastewater Treatment Facilities in Florida

Division of Environmental Assessment and Restoration
Florida Department of Environmental Protection
December 2018

2600 Blair Stone Road
Mail Station 6515
Tallahassee, FL 32399-2400
www.floridadep.gov

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Executive Summary

Florida Department of Environmental Protection (FDEP) Laboratory performed a study to better understand the effect of treatment process on the concentration of human-associated fecal indicators, pharmaceuticals and pesticides in raw/treated wastewater in Florida's domestic wastewater treatment facilities (WWTF). The knowledge of the concentration, degradation, and reduction of various indicators in raw wastewater and treated effluent helps the department identify potential anthropogenic inputs in ambient water and prioritize restoration of water bodies impacted by biological and chemical contaminants that poses the greatest risk to human health. Previous studies have shown that in general, removal efficiencies of bacteria, pharmaceutical compounds and pesticides during wastewater treatment are variable, often resulting in contamination of the aquatic environment. For this survey, primary-influent and treated-effluent samples were collected from twenty-three WWTF during the wet and dry season from November 2016 to September 2017. These samples were analyzed for pharmaceuticals and chemicals compounds such as sucralose, acetaminophen, carbamazepine, primidone, fluridone, linuron, diuron, fenuron, imidacloprid. In addition, microbiological indicators of fecal pollution like enterococci, *E. Coli*, fecal coliform and the human-associated (HF183) genetic maker targeting *Bacteroides* were examined.

Based on the analyses, we observed that:

- Human specific HF183 marker was present in consistently high concentrations in influent (1.90E6 – 1.39E8 GEU $100mL^{-1}$) and was either eliminated completely (no-detect) or reduced (6.09E5 GEU $100mL^{-1}$) in effluent. No differences in HF183 concentrations were detected in samples collected in dry and wet season.
- Acetaminophen was eliminated completely during the treatment process while sucralose, carbamazepine, and primidone passed through unaffected.
- Rainfall, disinfection type, and treatment type did not show consistent effects on removal outcomes, though some analytes displayed subtle differences.
- The strongest predictors of the presence of raw sewage were acetaminophen and HF183.

Introduction

Domestic wastewater treatment facilities are designed to collect and treat human fecal wastewater so that it can safely be discharged to surface waters or reused for purposes such as irrigation. Water or sewage that arrives at the WWTF is referred to as influent. Influent contains high concentrations of suspended solids and microorganisms, including pathogens. A combination of physical, chemical, and biological methods is used to systematically treat wastewater and sludge. After treatment, discharged wastewater is referred to as effluent.

The wastewater treatment process typically involves pretreatment to remove large solids and grits, followed by primary settling of organic solids and oil and grease removal. Secondary treatment involves the removal of nutrients through biologically mediated processes, using microorganisms to break down large molecular weight compounds into small molecules. This treatment removes biochemical oxygen demand (BOD) constituents to prevent oxygen depletion in receiving waters. There are multiple biological treatment technologies used in WWTFs, and the most commonly used is activated sludge. Tertiary, or advanced, treatment is used by some facilities to further improve the effluent quality by adding filtration and additional nutrient removal steps. The final step is disinfection to reduce microbiological load, including pathogenic organisms, in the effluent prior to discharge. The prerequisites of an effective disinfection treatment to inactivate viruses, bacteria, and other eukaryotic organisms include enough contact time, concentration, and water clarity. The most common disinfection methods used are chlorine, ultraviolet (UV) radiation, and ozonation. Chlorine has historically been the most widely used disinfection technology and is effective against a wide spectrum of pathogenic organisms. It destroys organisms by oxidizing cellular material, however the chlorine residual is toxic to aquatic life and the effluent must be dechlorinated before discharging into the environment. In recent years, the use of ozonation and UV radiation for disinfection has increased. Ozone gas causes direct oxidation of the cell wall, leading to cell lysis. UV light damages deoxyribonucleic acid (DNA) which disrupt cellular reproduction but does not destroy the DNA or the organism directly. With the proper dose and contact time, it is effective at inactivating most pathogens and leaves no residue. After UV disinfection, “remnant” DNA associated with dead cells may still be detected, making it important to distinguish between living and dead microbial cells to determine disinfection effectiveness (Weigel et al. 2017). Culturable indicator bacteria counts or alternative PCR methods, including Propidium Monoazide (PMA) treatment and molecular viability testing, can be used to determine cell survival after disinfection. PMA treatment has been identified as a method to distinguish between live and dead cells

in quantitative polymerase chain reaction (qPCR) analyses for microbial source tracking (MST) studies. This can help distinguish between recent and past fecal contamination.

After treatment and disinfection, effluent is often discharged into surface waters where it is further diluted and becomes part of the water cycle. Biological and chemical characteristics of the effluent can help to distinguish it from other contamination sources within the receiving waters. Human contact with wastewater constituents can occur during swimming, ingesting seafood, or contact with water from reuse applications. Therefore, it is important to characterize effluent, so it can be distinguished from other contamination sources and determine what effects it may have on human health and receiving waters. Compounds such as acetaminophen, ibuprofen, and naproxen exhibit nearly complete removal during wastewater treatment, while sucralose has negligible removal. Some substances including carbamazepine can even have negative removal (Kostich et al. 2014). Higher concentrations in effluent than influent is also known to occur when conjugated forms of the drug are transformed back into the parent drug during the wastewater treatment process (Sedlak and Pinkston 2001).

The artificial sweetener sucralose is often used as an indicator of domestic wastewater influence in the US because it is consistently found in effluent samples and source waters with known wastewater discharges (Oppenheimer et al. 2011). Sucralose has a half-life of up to several years in water and its degradation is influenced by physical factors such as the pH and temperature (Lubick 2008). Although sucralose is ubiquitous in WWTF effluents, it is typically found at trace-level concentrations in ambient waters. Sucralose exhibits low toxicity and does not bioaccumulate (Lubick 2008).

There is regional variation in the concentrations of analytes found in wastewater influent due to differences in pharmaceutical use, eating habits, and endemic illness. There is also a wide range of removal efficiencies for different compounds, depending on properties of the compound, treatment process used, capacity of facility, temperature, pH, dissolved oxygen concentration, and microbial populations (Subedi and Loganathan 2016). Variation in influent concentrations and removal efficiency makes it necessary to study the effects of treatment at a local level and include measurements of multiple tracers. Human qPCR marker persistence in water varies based on several parameters including temperature, sunlight, oxygen, and salinity (Boehm et al. 2015). The qPCR-measured human-associated *Bacteroides* bacterial markers have reported T_{90} values, the time in which the concentration of indicator

bacterial DNA is reduced by 90%, between 1 and 47 days (Boehm et al. 2015). Another study showed in marine waters, the average time for 99% reduction in human MST marker concentration is approximately 3 days, with detectable amounts seen up to 6 days after a raw sewage spill (Mattioli et al. 2017). *E. coli*, enterococci and fecal indicator bacteria (FIB) decay constants vary significantly based on season and depth of waters. Recent study showed that culture-based FIB decay rate constants were significantly larger than those of the human MST markers (Mattioli et al. 2017). Culturable bacteria and qPCR-detectable bacterial DNA can remain in the environment for different amounts of time, so it is important to analyze both to determine the timing of potential fecal contamination.

Human and animal waste can enter surface waters through various means. Sources include aged or leaking sanitary sewer collection lines, failing or improperly installed or maintained onsite sewage treatment and disposal systems (OSTDSs), urban runoff, and feces from livestock, wildlife, and pets. Counts of FIB are often used to determine the extent of fecal pollution in a water body. These bacteria are found in high numbers in the waste of humans and animals but can also naturally occur in plants, soil, and sediments. Elevated levels of FIB lack specificity about potential sources and do not always indicate the presence of fecal material. There are many water bodies throughout Florida that are impaired for FIB, but their levels not necessary equate to risk.

MST is a rapidly growing field of study whereby various biological and chemical analytes are used to identify and potentially quantitate contaminant inputs in water. MST methods have been developed to distinguish origin of sources of fecal contamination, particularly to differentiate human from animal waste. This is important for prioritizing high risk areas for restoration, as human waste is a significant source of infectious viruses, bacteria, and protozoa, and it represents the highest manageable pathogen risk to public health (McMinn et al. 2017). DEP has employed several biological and chemical MST tools since 2011 to aid in this approach. These tools were used in this study to characterize raw and treated wastewater to improve the interpretation of fecal source identification in Florida's surface waters. The lab is currently adding additional molecular markers and chemical tracers to its MST toolbox. New molecular markers will provide information about fecal contamination from animals, including birds, ruminants, and dogs. Upcoming pharmaceuticals include ibuprofen, naproxen, duloxetine, hydrocodone, metformin, and simvastatin.

Study Design

A list of Florida National Pollution Discharge Elimination System (NPDES)-permitted domestic wastewater facilities was extracted from DEP's Wastewater Facility Regulation (WAFR) database and categorized by region, permitted capacity, treatment method, and disinfection type. The facilities were grouped by DEP district: Central (CD), Northeast (NE), Northwest (NW), South (SD), Southeast (SE), and Southwest (SW). Facilities were categorized into Type I (≥500,000 gallons/day), Type II (100,000-500,000 gallons/day), and Type III (2000-100,000 gallons/day) based on their permitted capacity. Since there are very few Type III facilities in Florida, they were excluded from this study. Most facilities chosen were characterized as Type I and were further subcategorized into Type I (<5), Type I (≥5), and Type I (≥10) if the permitted capacity was less than 5 MGD, greater than 5 MGD, or greater than 10 MGD, respectively. The facilities were also categorized into treatment and disinfection types. The secondary treatment facilities were defined based on the treatment type such as Activated Sludge, Sequencing Batch Reactor, and 5 Stage Bardenpho. The advanced treatment facilities met the Florida Statute 403.086 (4) requirements, which provides permitting limits for CBOD5 (5 mg/L), Suspended Solids (5 mg/L), Total Nitrogen (3 mg/L), and Total Phosphorus (1 mg/L). Only Chlorine and UV disinfection were considered for this study.

Twenty-three facilities were selected from the list to represent the domestic wastewater facilities in Florida (Table 1 and Figure 1). Primary settled influent and final effluent samples were collected from each facility. To include possible storm water influence, two sampling events were conducted. For the dry event, there was less than one inch of rain within 24 hours prior to collection. For the rainfall event, there was at least one inch of rain within 24 hours prior to collection.

Microbiological, molecular and chemical analyses were conducted by the DEP laboratory on influent and effluent samples, and the amount of removal was determined. Microbiological analyses measure the amount of fecal indicator bacteria present and included tests for enterococci, *Escherichia coli*, and fecal coliforms. Molecular assays use qPCR to measure the amount of host-associated bacterial DNA present and samples were analyzed for HF183, a marker targeting *Bacteroides*, commonly found in human waste. Samples were also treated with PMA to distinguish live versus dead cells based on cell membrane integrity. The chemical analyses tested for various pharmaceuticals (acetaminophen,

primidone, carbamazepine), herbicides (fluridone, linuron, diuron, fenuron), pesticides (imidacloprid), and the artificial sweetener sucralose.

All field sampling and laboratory analyses were performed using DEP Standard Operating Procedures (https://floridadep.gov/dear/quality-assurance/content/dep-sops and https://floridadep.gov/dear/florida-dep-laboratory/content/dep-laboratory-quality-assurance-manual-and-sops).

Results and Discussion

DEP routinely monitors and assesses surface water quality to better prioritize and remediate pollution problems; however, there are no published guidelines for interpreting molecular markers and pharmaceutical and pesticide tracers' results. This makes it challenging to determine the extent of fecal contamination and the potential health risks based on the FIB numbers alone. Also, considering the heterogeneity of the natural systems, there is no single method that can sufficiently identify sources, so an MST "tool box" approach is used. The utilization of multiple laboratory analyses along with field surveys, GIS mapping, and communication with local stakeholders, can provide evidence of contamination that can be used to prioritize restoration. With the overarching effort to improve the interpretation of available biological and chemical analytical tools for wastewater and human fecal source identification in Florida's surface waters, DEP laboratories conducted the year-long study testing influent and effluent from twenty-three WWTF during wet and dry seasons. The facilities were chosen to represent different regions, disinfection types, capacity, and treatment technologies allowing comparisons to be made across different parameters that can affect the contaminant concentrations.

The results suggest variation amongst the influent samples between facilities, but the effluent concentrations were more consistent for all analytes (Table 2). With the exception of acetaminophen, most of the chemical analytes measured, showed little evidence of removal during treatment, while the molecular and microbiological values were reduced by several orders of magnitude (Table 3). The results are summarized with the following parameters: non-detects were set to 0 in the graphs and for calculating mean. Mean concentration and percent removal were not calculated for analytes in which more than half of the influent samples were non-detects. Influent samples were diluted for microbiology and molecular assays because of turbidity and high concentration of bacteria. Dilution increases the method detection limit (MDL) for the assays and decreases sensitivity, if the influent samples contain very low amounts of fecal indicator bacteria or HF183 DNA. There were several molecular samples with detects below the MDL. To avoid assigning higher values to non-detects or excluding low level detects, non-detects for all assays were set to zero. MDL values for chemical analyses are sufficiently

low, hence did not affect mean concentration (Detailed results and data files are available on request from the Biology Program).

The removal of pharmaceuticals varied greatly, with acetaminophen being nearly completely removed and carbamazepine concentrations higher or unchanged after treatment (Table 3, Figure 2). These results are consistent with published studies that have shown that acetaminophen is very susceptible to removal during wastewater treatment and carbamazepine is resistant to degradation or removal due to the absence of electron donating groups (Tran and Gin 2017; Kostich et al. 2014). Some studies have found a higher concentration of carbamazepine in effluent samples than in influent samples, and another demonstrated that carbamazepine is resistant to biological treatment but sensitive to removal by granular activated carbon (GAC) (Barbash et al. 2015; Yang et al 2011). The results for primidone were more variable, with 9 out of 44 effluent samples showing evidence of partial removal while the rest suggested that this pharmaceutical is resistant to degradation. Several samples had primidone detected in the effluent but not influent samples. The literature has shown that primidone is resistant to breakdown with some treatment technologies, including activated sludge and GAC, but is partially removed with treatment by membrane bioreactor (MBR) (Barbash et al. 2015; Yang et al. 2011). Since there were no facilities using MBR included in this study and most of facilities use activated sludge, the primidone results observed were expected.

The artificial sweetener sucralose is ubiquitously found in WWTF effluents and receiving waters, as it is resistant to breakdown or removal during treatment. Published reports have shown effluent concentrations slightly below 30 µg/L in the United States (Oppenheimer et al. 2011; Subedi and Kannan 2014). All the influent and effluent samples for this study had detectable levels of sucralose. Average concentration of 37 µg/L in influent samples and 41 µg/L, for effluent is slightly higher than some published studies. The herbicides fluridone, linuron, diuron, and fenuron were undetected or detected below the PQL in most influents. The pesticide imidacloprid was detected at low levels in most samples and appears resistant to degradation or removal. The herbicides measured are not currently used as tracers for WWTF discharges and were excluded from subsequent analyses.

In accordance with the newly adopted water quality standards for bacteria, *E. coli* and enterococci concentrations were determined along with fecal coliform. The concentrations of microbiological

indicators found in influent were consistent with other published studies (McMinn et al. 2017; Mayer et al. 2016). Although substantial reduction of bacterial numbers was observed, there was considerable variation with many non-detects and a few high outliers in effluent samples (Table 3, Figures 3 and 4). All *E. coli* and enterococci effluent samples were in compliance with the water quality standards for bacteria. The average log reduction for FIB was higher than the published literature. Previous studies reported a reduction of approximately 2.3 log for enterococci and *E. coli* and approximately 3.0 for fecal coliform, while our results indicated a log reduction of 4.4-5.5 for all indicators, indicating more robust disinfection (McMinn et al. 2017; Mayer et al. 2016).

The molecular marker HF183 detects the DNA from several *Bacteroides* species commonly found in human waste and is widely considered the top performing molecular marker. In this study, high levels of HF183 marker (10^6 – 10^8 GEU/ 100mL) were detected in influent samples (Figure 5) consistent with a US study that reported HF183 concentration of approximately 10^7 copies/100 mL in raw sewage (Shanks et al. 2010). Studies have shown more variation in the amount of HF183 DNA present after treatment. Previous reports have found non-detectable or non-quantifiable densities of HF183 in many effluent samples with an LOQ of aproximatelty10^4 copies/100 mL of effluent (Chern et al. 2014; Boehm et al. 2015). A European study showed 100% occurrence of HF183 in effluent samples after tertiary treatment with a mean concentration of 10^6 copies/100 mL and approximately 2.3 log reduction after treatment (Mayer et al. 2016). While most of the effluent samples in this study had detectable levels of HF183, the values were mostly near or below the LOD and LOQ (Table 2 and Figure 5). There was approximately 3-log reduction in HF183 concentration after wastewater treatment.

However, this DNA-based method does not distinguish live versus dead cells, so PMA treatment was added to samples to determine the live fraction of total *Bacteroides* cells. PMA is a photoreactive membrane-impermeant DNA binding dye that intercalates into double-stranded DNA upon exposure to intense visible light. The resulting chemically modified DNA strongly inhibits PCR. Live cells with intact membranes are not affected by PMA treatment, so the corresponding PCR signal from PMA-treated cells corresponds to cells with intact membranes. In this study, fewer samples showed amplification after PMA treatment with concentrations of PMA HF183 approximately two orders of magnitude lower than the total HF183 signal, indicating that the majority of the cells detected were dead (Figure 6). The live HF183 results were consistent with the microbiological results, providing additional support that these varied tests provide accurate counts for live fecal indicator bacteria. A recent study,

from 54 geographic locations in the US, linked enterococci and HF183 levels in determining risk of illness (Boehm et al. 2015). The study reported that the risk of 30 gastrointestinal illness per 1000 swimmers, as established in the new EPA Recreational Water Criteria, occurred at median concentrations of 4200 copies of HF183/100 mL (Boehm et al. 2015). Another recent study determined that 3220 HF183 copies/100 mL of raw sewage or 3660 HF183 copies/100 mL of secondary treated sewage posed a significant health risk to swimmers (Ahmed et al. 2018). While these numbers can vary due to water conditions, these studies provide a starting point for comparing microbiological and molecular results when determining health risk.

Regression analysis to assess the relationship among acetaminophen, sucralose, HF183 and *E.coli* revealed high correlation (R^2= 0.5634) between acetaminophen and the total Bacteroides cells as assessed by HF183 (Figure 7). These data suggest that elevated levels of HF183 and acetaminophen in water samples can indicate an untreated wastewater source or very recent human fecal contamination. Correlation between other analytes such as HF183, sucralose, and *E. coli* were weak and indicated that they should not be used independently to indicate wastewater contamination (Figure 7).

This study also looked at the efficacy of disinfection by UV light versus chlorine by examining the biological markers in influent and effluent samples. Previous published data showed no significant differences between the type of disinfection used and the reduction in culturable fecal indicator bacteria or genetic markers (Chern et al. 2014). Our data also revealed no noteworthy differences in disinfection efficacy between chlorine and UV radiation, as the reductions of *E. coli*, enterococci, and fecal coliforms were similar between disinfection types. There were more HF183 non-detects in effluent samples after chlorine disinfection (9/31) than UV disinfection (1/14), suggesting that HF183 DNA might be more readily destroyed during chlorine treatment compared to UV (Figure 5). This is not surprising, given that UV light damages DNA without degrading it and may not damage the cell membrane. These results suggest that both UV light and chlorine inactivate indicator bacteria to a similar degree, but chlorine may cause more DNA degradation. One caveat to this finding is that most of the facilities with undetectable HF183 (qPCR crude) in effluent samples were also advanced treatment facilities, making it difficult to distinguish differences from disinfection type or treatment technologies. It is possible that the reduction in HF183 concentration occurred during the advanced treatment before the disinfectant was applied.

None of the other chemical analytes studied showed obvious differences in degradation or removal based on secondary versus tertiary treatment. This may be because these analytes are either quite susceptible or hardily resistant to treatment regardless of treatment type used. It could also indicate that the facilities using secondary treatment technologies are as effective as advanced facilities at removing these analytes from wastewater. Another possibility is that there is too much variation in the facilities or the number of facilities or analytes tested is not sufficiently large to tease out small differences in effectiveness.

Wet and dry sample events were analyzed to observe the influence of storm water in primary-influent and treated-effluent samples. The dry sampling event occurred when there was less than one inch of rain in the 24 hours prior to collection, and the wet sampling event occurred when there was at least one inch of rain in the 24 hours prior to collection. This sampling strategy could potentially reveal the effects of dilution or the addition of pesticides or animal waste flushing into nearby water bodies. There were no clear differences in herbicides, pesticides, microbiological indicators, or molecular markers when comparing wet versus dry sampling events (Figure 2, Figure 4, Figure 5). There was a slight decrease in sucralose and acetaminophen concentrations in wet sampling events, suggesting a small amount of dilution from the storm water (Figure 2). Acetaminophen is typically completely removed during wastewater treatment, but it was detected in effluent samples from three facilities during this study. These samples were all collected after a rain event, and the corresponding dry effluent samples from these facilities were all below detection. This suggests that the extra storm water influx may lead to shorter treatment time or less effective treatment, leaving small but detectable amounts of acetaminophen in the final effluent.

Conclusions

Characterization of the human fecal source contamination markers and tracers in domestic wastewater treatment facilities was done to improve their interpretation in Florida's surface waters. The aim of the study was to determine the type and concentration of contaminants that are discharged and may result in harmful impacts when disseminated in the surface environment. This report provides measurements to determine a typical Florida wastewater profile (Tables 2 and 3, Figure 8) using MST markers and chemical tracers.

Key observations of this study:

1. This report provides baseline measurements for the concentrations of various analytes in WWTF influent and effluent samples in Florida.

2. No dramatic differences were detected between wet and dry sampling events, disinfection efficacy for chlorine vs UV light, or secondary versus advanced treatment.
3. Sucralose, carbamazepine, and primidone are detected at similar levels in both raw and treated sewage. These analytes are known to persist in the environment. While their presence indicates human influence, it does not necessarily indicate raw sewage or a recent discharge event.

4. High levels of acetaminophen, HF183, and fecal indicator bacteria were found in raw sewage, and wastewater treatment process efficiently reduced or eliminated these markers and traces in treated wastewater.

5. PMA treatment reveals influent samples have greater concentration of live *Bacteroides* cells in comparison to effluent water after the disinfection process.

6. There is a strong positive correlation between total *Bacteroides* cells as assessed by HF183 and acetaminophen concentrations, suggesting that these two analytes are the strongest predictors of the presence of raw sewage.

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Table 1. Overview of the WWTF used in the study.

Facility Name	Facility No. (refer in Fig. 1)	Facility ID	ROC	Treatment Type	Disinfection Type	Advanced?	Capacity
Baldwin WWTF	1	FL0027812	NED	Modified Ludzack-Ettinger (BNR)	UV	N	II
Blountstown WWTF	2	FL0026867	NWD	Sequencing batch reactor (SBR)	UV	Y	I (<5)
Callahan WWTF	3	FL0038407	NED	Activated Sludge	Chlorine	Y	II
Century, Town of - WWTF	4	FL0032468	NW	Extended aeration	Chlorine	N	I (<5)
Clearwater City of Marshall Street WRF	5	FL0021857	SWD	5 Stage Bardenpho (BNR)	Chlorine	Y	I (≥10)
Daytona Beach/Bethune Point WRF	6	FL0025984	CD	5 Stage Bardenpho (BNR)	UV	Y	I (≥10)
Florida State Hospital WWTF	7	FL0031402	NWD	Extended aeration	Chlorine	N	I (<5)
Fort Myers South AWWTF	8	FL0021270	SD	5 Stage Bardenpho (BNR)	Chlorine	Y	I (≥10)
Holly Hill, City of	9	FL0027677	CD	Oxidation ditch	Chlorine	Y	I (<5)
Howard F Curren AWTP	10	FL0020940	SWD	Activated Sludge (BNR)	Chlorine	N	I (≥10)
Hurlburt Field AWTP	11	FL0003174	NWD	Activated Sludge (BNR)	Chlorine	Y	I (<5)
Lee County Utilities - Fiesta Village	12	FL0039829	SD	Oxidation ditch	Chlorine	Y	I (≥5)
Mandarin WRF	13	FL0023493	NED	Modified Ludzack-Ettinger (BNR)	UV	Y	I (≥5)
Millville AWT Facility	14	FL0170909	NWD	Sequencing batch reactor (SBR)	UV	Y	I (≥5)
Orlando/Iron Bridge Regional WRF	15	FL0037966	CD	5 Stage Bardenpho (BNR)	Chlorine	Y	I (≥10)
Ormond Beach WWTF	16	FL0020532	CD	5 Stage Bardenpho (BNR)	Chlorine	Y	I (≥5)
Palatka WWTF	17	FL0040061	NED	Activated Sludge	Chlorine	N	I (<5)
Panama City Beach WWTF #1	18	FL0021512	NWD	Activated Sludge	UV	N	I (≥10)
Plant City WRF	19	FL0026557	SWD	Activated Sludge	Chlorine	N	I (≥10)
Sanford/North WWTF	20	FL0020141	CD	Activated sludge	Chlorine	Y	I (≥5)
South Cross Bayou WRF	21	FL0040436	SWD	Activated Sludge	UV	Y	I (≥10)
St Andrews WWTF	22	FL0020451	NWD	5 Stage Bardenpho (BNR)	Chlorine	Y	I (≥5)
St Augustine WWTF No 1	23	FL0021938	NED	Activated sludge	Chlorine	N	I (<5)

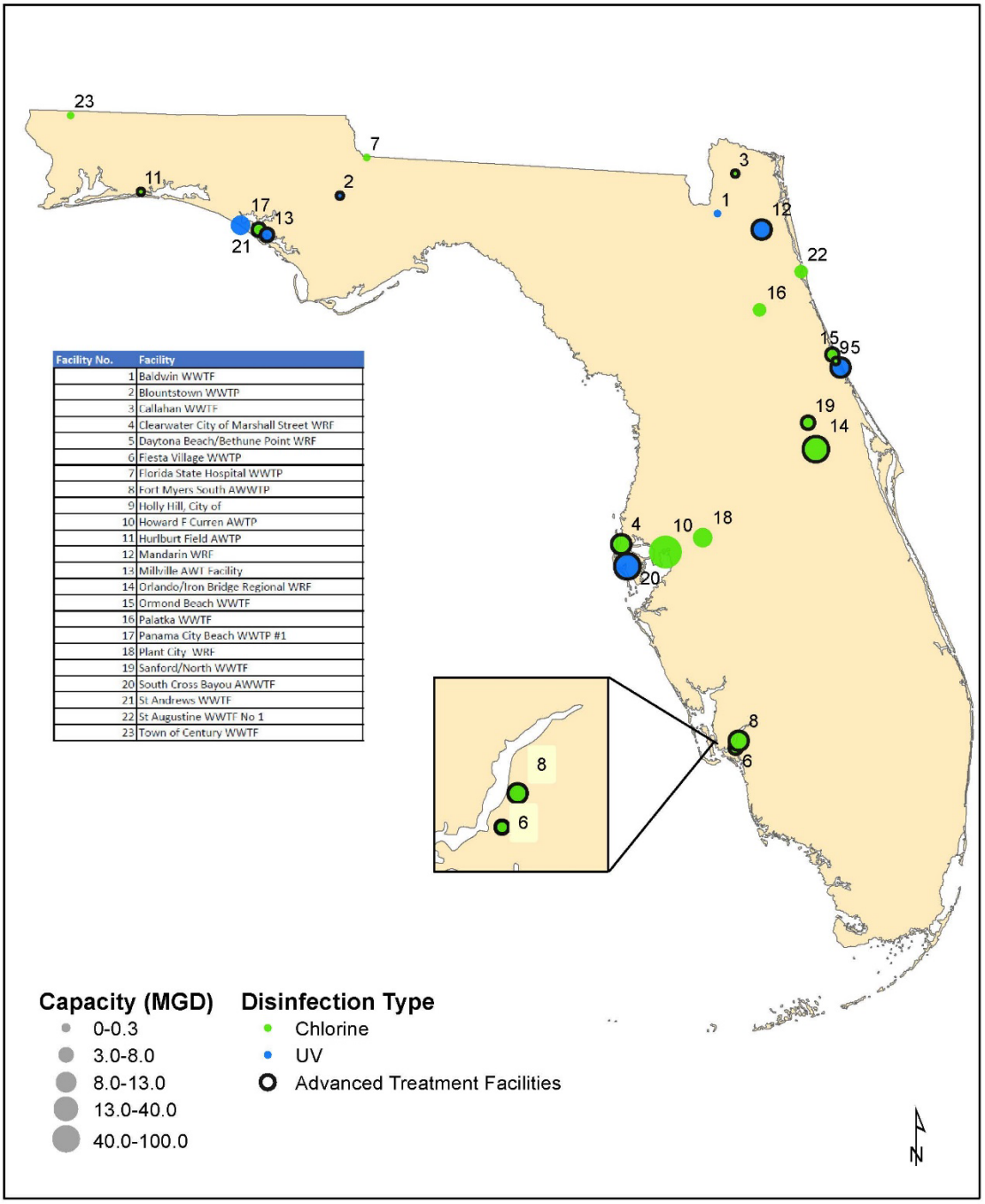
Table 2. Minimum and maximum measured concentrations of analytes in WWTF.

Analyte	Type	Sampling	# detects	# samples	Min	Max	Units
Acetaminophen	Influent	Dry	23	23	3.6 J	280	µg/L
		Wet	21	22	0.04 U	200	
	Effluent	Dry	0	23	0.004 U	0.008 U	
		Wet	3	22	0.004 U	0.56	
Carbamazepine	Influent	Dry	23	23	0.0046	1.3	µg/L
		Wet	20	21	0.004 U	0.4	
	Effluent	Dry	23	23	0.005	2.3	
		Wet	21	21	0.0016	0.66	
Primidone	Influent	Dry	20	23	0.008 U	3.5	µg/L
		Wet	17	21	0.008 U	1.2	
	Effluent	Dry	22	23	0.008 U	0.38	
		Wet	21	21	0.023 I	0.44	
Sucralose	Influent	Dry	23	23	15	88	µg/L
		Wet	22	22	8.5	54	
	Effluent	Dry	23	23	11	100	
		Wet	22	22	13	71	
Fluridone	Influent	Dry	4	23	0.0004 U	0.0053	µg/L
		Wet	4	21	0.0004 U	0.0046	
	Effluent	Dry	11	23	0.0004 U	0.0084	
		Wet	10	21	0.0004 U	0.0093	
Linuron	Influent	Dry	0	23	0.004 U	0.04 U	µg/L
		Wet	0	21	0.004 U	0.04 U	
	Effluent	Dry	0	23	0.004 U	0.004 U	
		Wet	0	21	0.004 U	0.04 U	
Diuron	Influent	Dry	11	23	0.004 U	0.14 I	µg/L
		Wet	11	21	0.004 U	0.2	
	Effluent	Dry	18	23	0.004 U	0.037	
		Wet	15	21	0.004 U	0.043	
Fenuron	Influent	Dry	2	23	0.008 U	0.15	µg/L
		Wet	1	21	0.008 U	0.051	
	Effluent	Dry	0	23	0.004 U	0.008 U	
		Wet	0	21	0.008 U	0.08 U	
Imidacloprid	Influent	Dry	21	23	0.004 U	0.15	µg/L
		Wet	17	21	0.004 U	0.14	
	Effluent	Dry	21	23	0.004 U	0.18	
		Wet	20	21	0.004 U	0.2	
enterococci	Influent	Dry	21	22	49.6 Q	2.42E7 JQ	MPN/100 mL
		Wet	21	22	2.42E3 J	6.24E5 Q	
	Effluent	Dry	11	23	1 U	2.42E3 JQ	
		Wet	10	22	1 U	2.42E3 J	
E. coli	Influent	Dry	22	23	410.6 Q	2.42E7 Q	MPN/100 mL
		Wet	21	22	410.6	1.05E7 Q	
	Effluent	Dry	14	23	1 U	2.42E3 JQ	
		Wet	8	22	1 U	2.42E3 J	
Fecal coliform	Influent	Dry	22	23	2.00E4 UQ	2.40E7 BQ	CFU/100 mL
		Wet	22	22	6.20E4 BQ	7.80E6 BQ	
	Effluent	Dry	11	23	1 UQ	3.50E2 Q	
		Wet	15	22	2 U	1.40E4 BQ	
HF183	Influent	Dry	23	23	1.90E6	1.39E8 Q	GEU/100 mL
		Wet	21	22	1.40E5 U	9.56E7	
	Effluent	Dry	16	23	14 T	1.53E5	
		Wet	19	22	15 T	6.09E5	
HF183 purified	Influent	Dry	23	23	2.20E5 J	4.47E7 J	GEU/100 mL
		Wet	22	22	3.80E3 TJ	2.10E7 AJ	
	Effluent	Dry	18	23	2.9 TJ	5.19E4 J	
		Wet	18	22	2.3 TJ	1.66E5 J	
HF183 PMA	Influent	Dry	22	23	7.10E3 TJ	2.85E6 J	GEU/100 mL
		Wet	21	22	2.40E4 UJ	1.92E6 J	
	Effluent	Dry	13	23	2.3 TJ	2.51E3 J	
		Wet	14	22	1.1 TJ	3.37E3 J	

Table 3. Average influent and effluent concentrations for measured analytes.

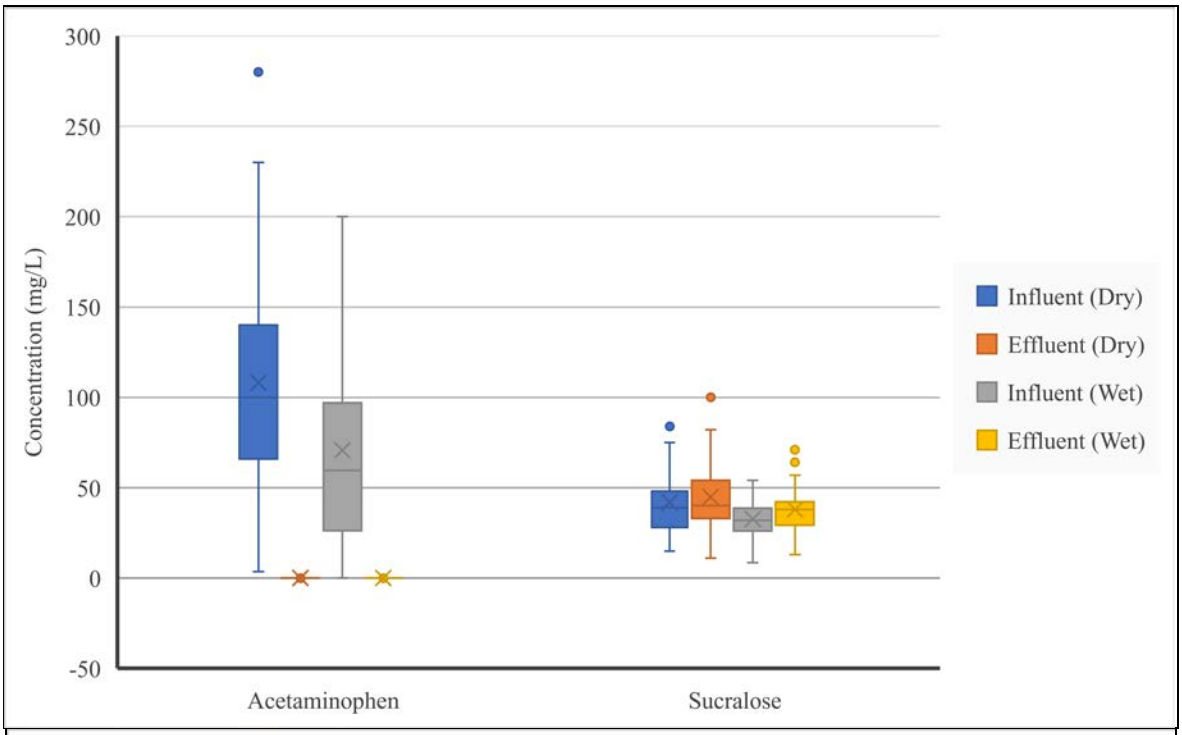
Analyte	Units	Influent mean	Effluent mean	% Removal (log reduction)
Acetaminophen	µg/L	89.8	0.01	>99.9%
Carbamazepine	µg/L	0.12	0.21	≤0%
Primidone	µg/L	0.24	0.18	≤0%
Sucralose	µg/L	37.32	41.42	≤0%
Imidacloprid	µg/L	0.05	0.10	≤0%
enterococci	MPN/100 mL	9.24E5	2.24E2	>99.9% (4.43)
E. coli	MPN/100 mL	5.57E6	1.78E2	>99.9% (5.36)
Fecal coliform	CFU/100 mL	4.35E6	4.34E2	>99.9% (5.56)
HF183	GEU/100 mL	4.45E7	3.10E4	>99.9% (3.16)
HF183 purified	GEU/100 mL	1.00E7	9.98E3	>99.9% (3.00)
HF183 PMA	GEU/100 mL	5.50E5	3.97E2	>99.9% (3.14)

Figure 1. Location of wastewater treatment facilities.



Facility No.	Facility
1	Baldwin WWTF
2	Blountstown WWTP
3	Callahan WWTF
4	Clearwater City of Marshall Street WRF
5	Daytona Beach/Bethune Point WRF
6	Fiesta Village WWTP
7	Florida State Hospital WWTP
8	Fort Myers South AWWTP
9	Holly Hill, City of
10	Howard F Curren AWTP
11	Hurlburt Field AWTP
12	Mandarin WRF
13	Millville AWT Facility
14	Orlando/Iron Bridge Regional WRF
15	Ormond Beach WWTF
16	Palatka WWTF
17	Panama City Beach WWTP #1
18	Plant City WRF
19	Sanford/North WWTF
20	South Cross Bayou AWWTF
21	St Andrews WWTF
22	St Augustine WWTF No 1
23	Town of Century WWTF

Figure 2. Concentration of chemical tracers with different disinfection types.



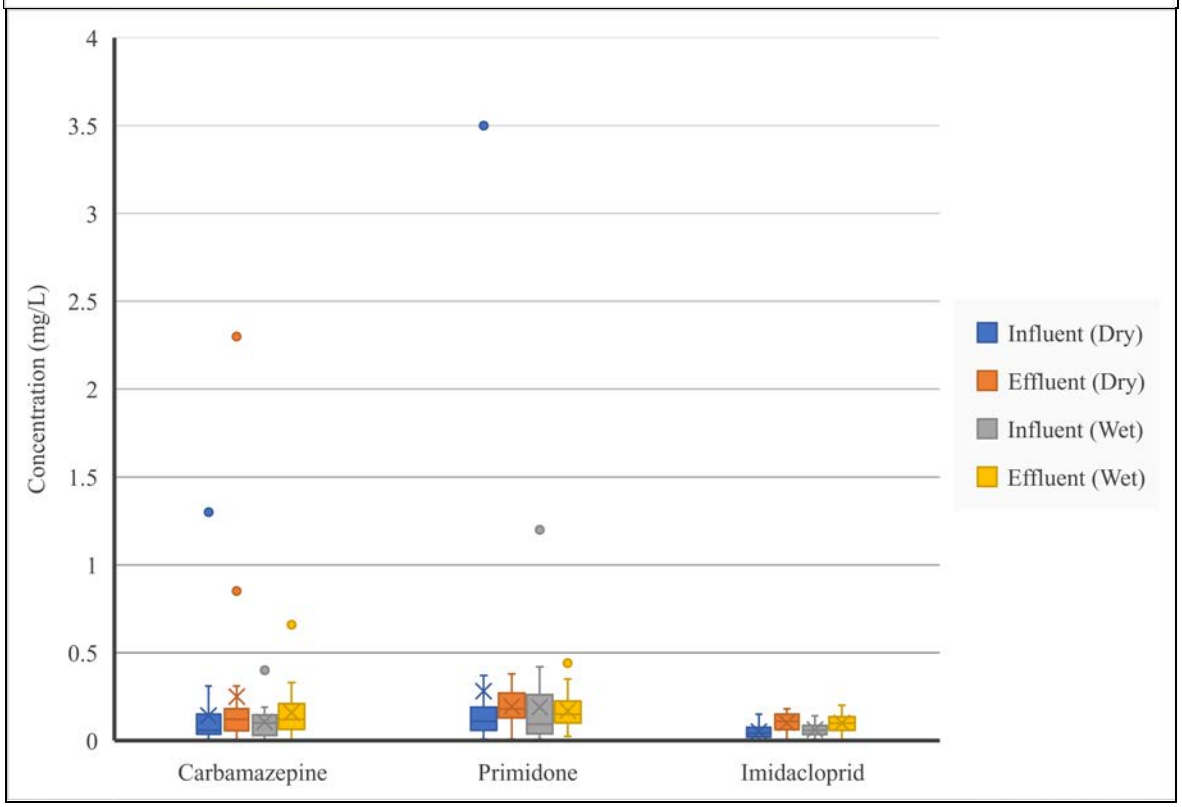


Figure 3. Concentration of microbiological indicators Vs disinfection type.

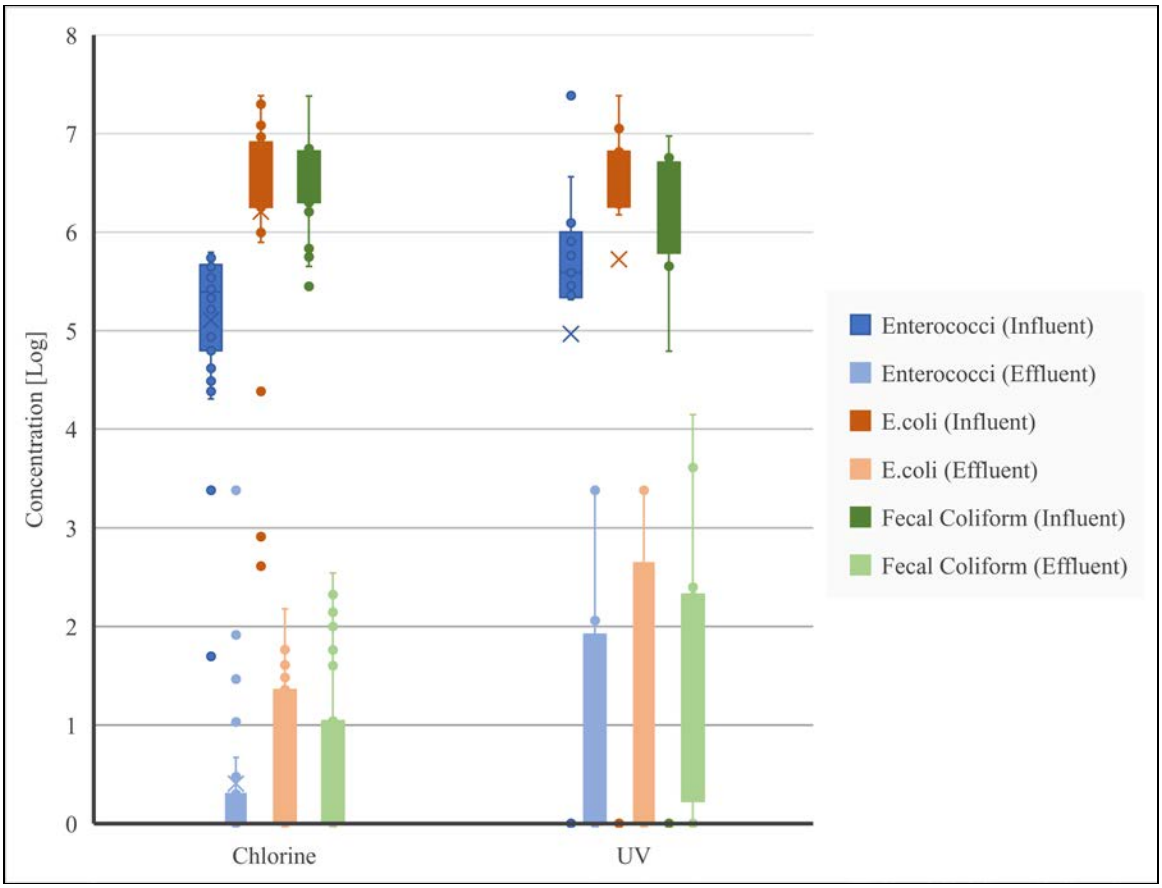


Figure 4. Reduction of microbiological indicators Vs sampling seasons.

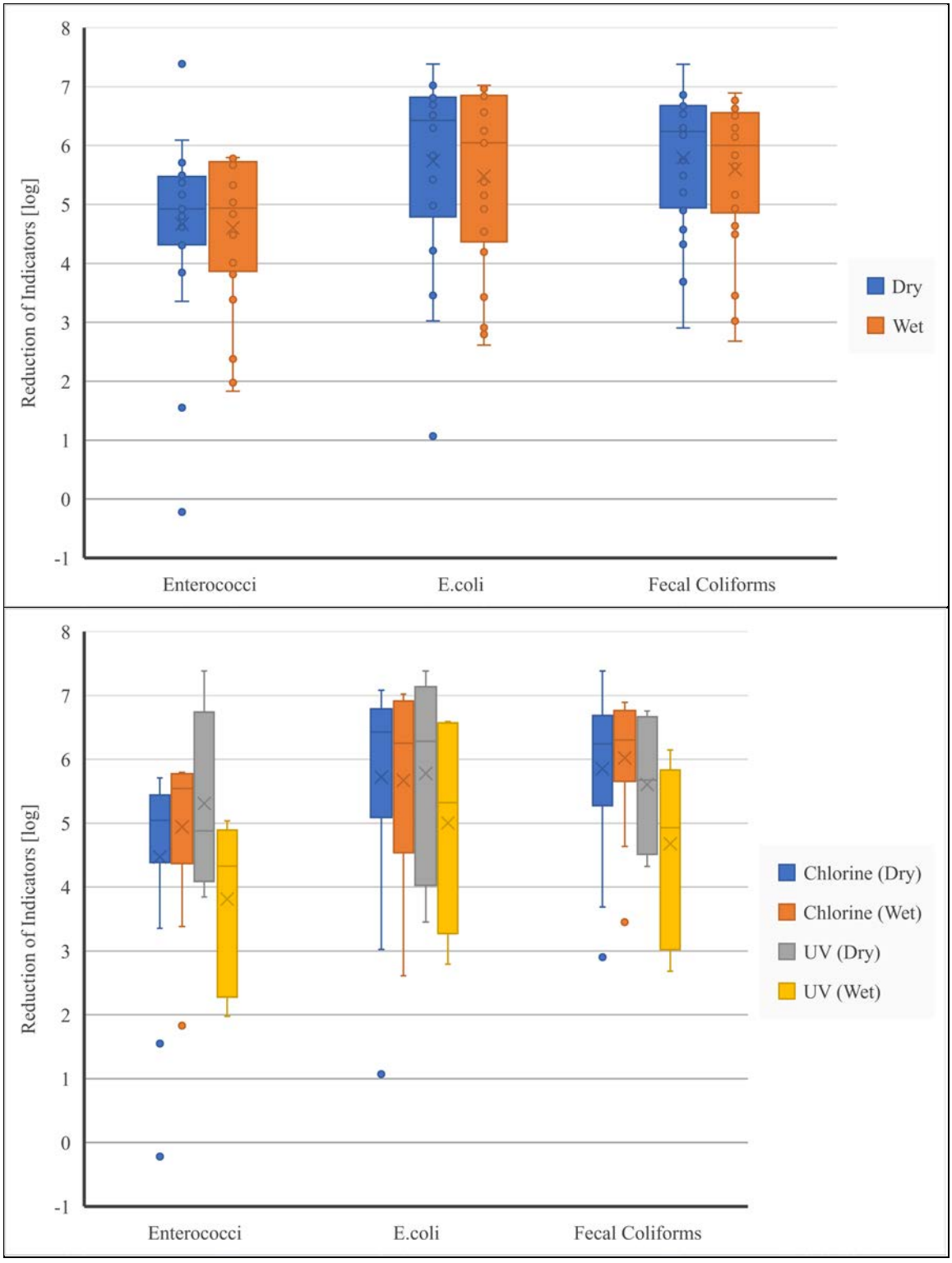
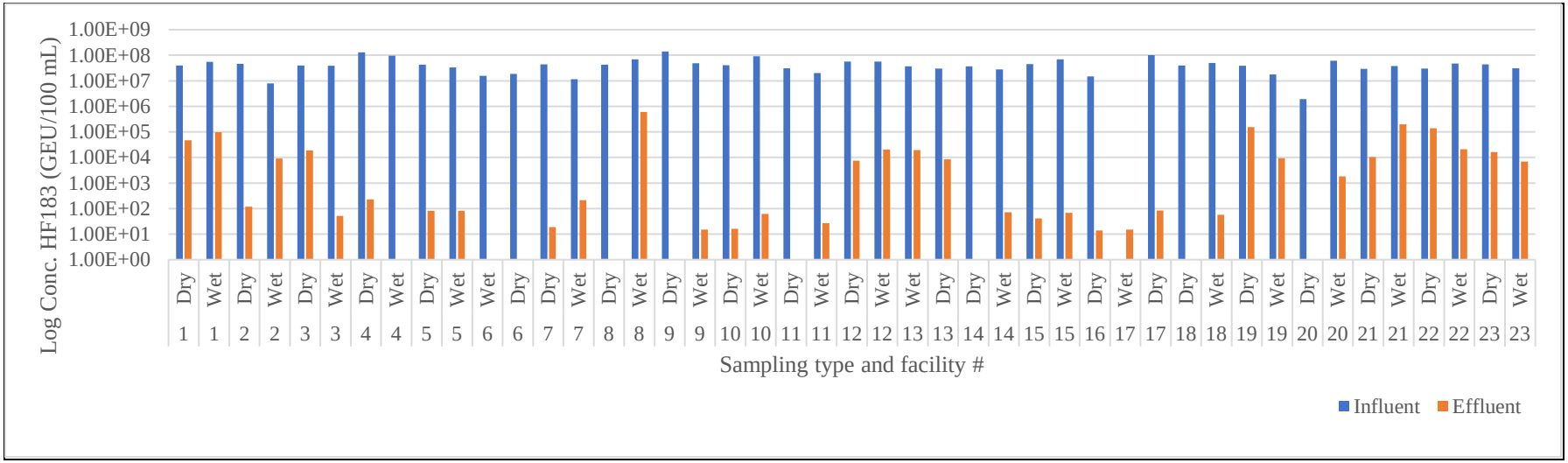


Figure 5. Concentration of molecular marker HF183.



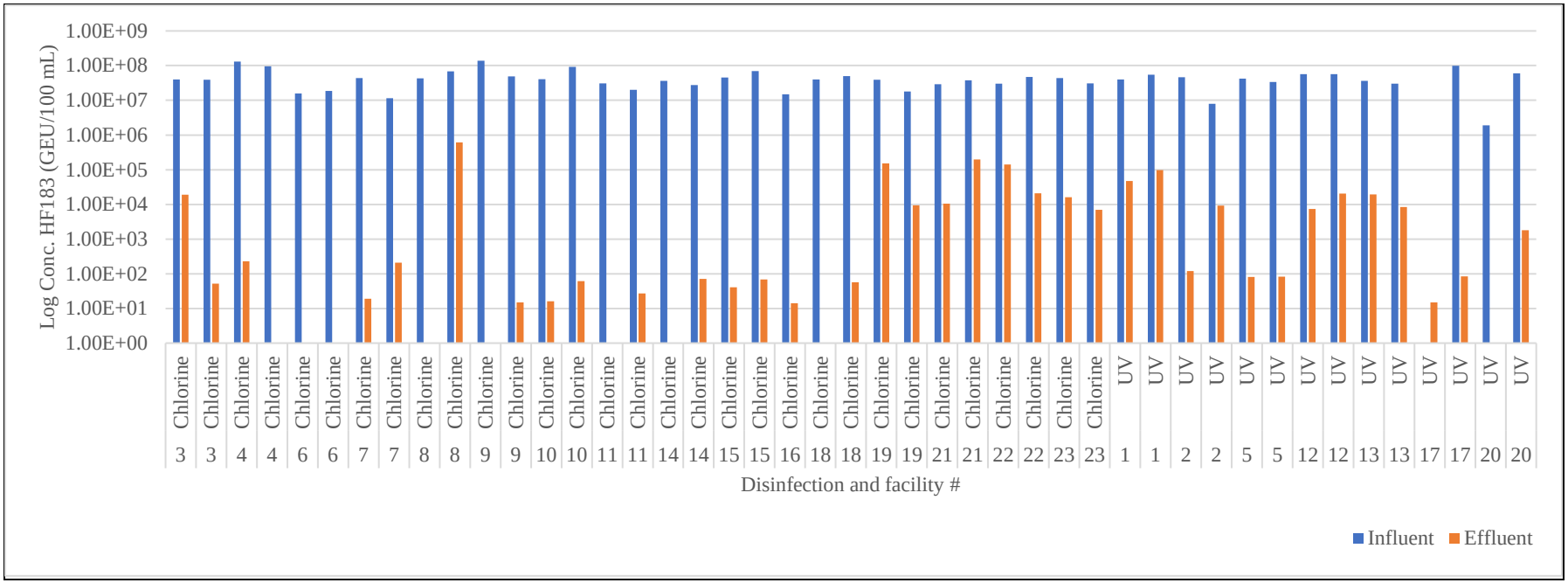


Figure 6. Concentration of molecular marker HF183 after PMA treatment.

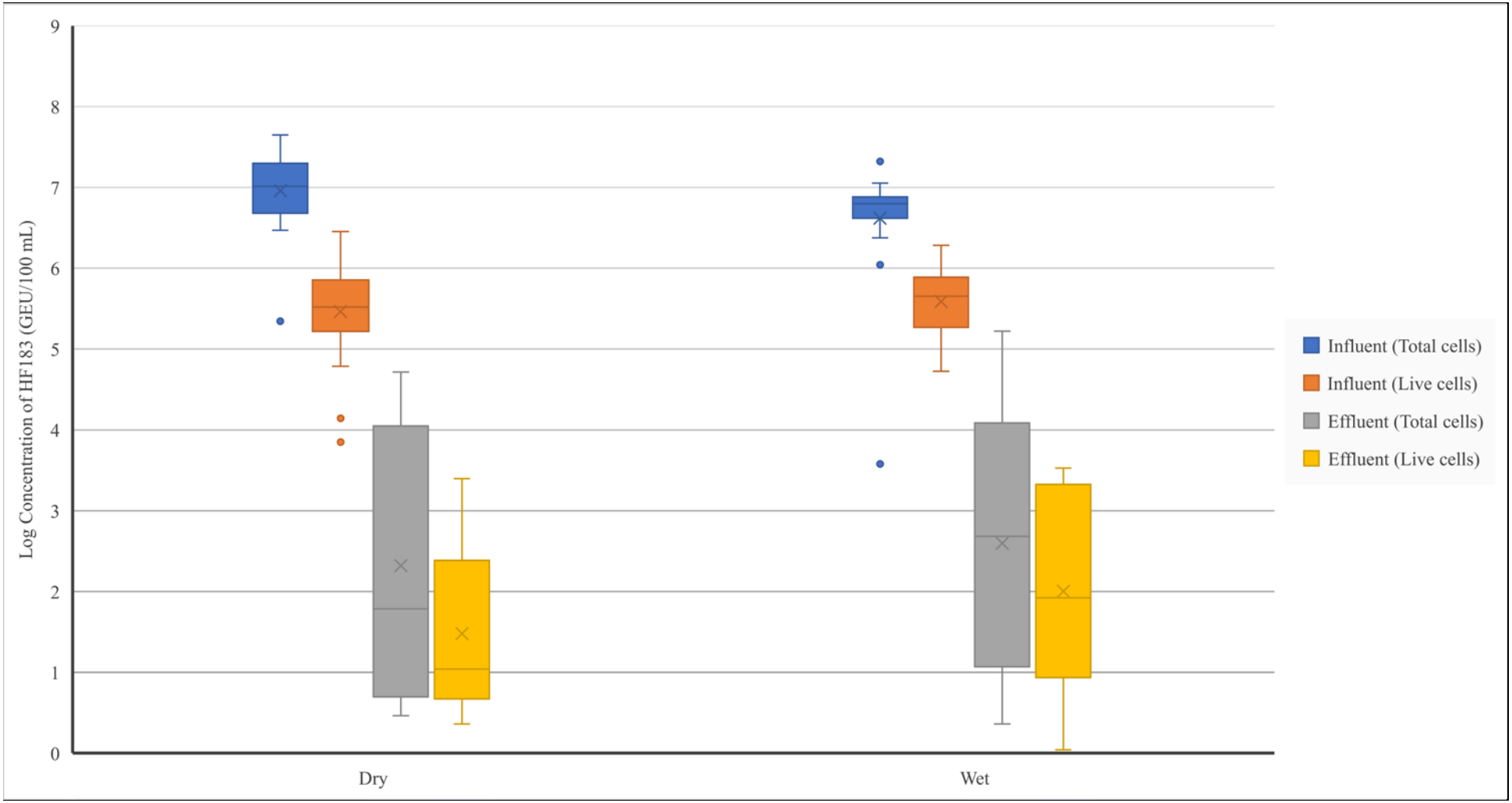


Figure 7. Regression analysis to estimate relationship between different analytes.

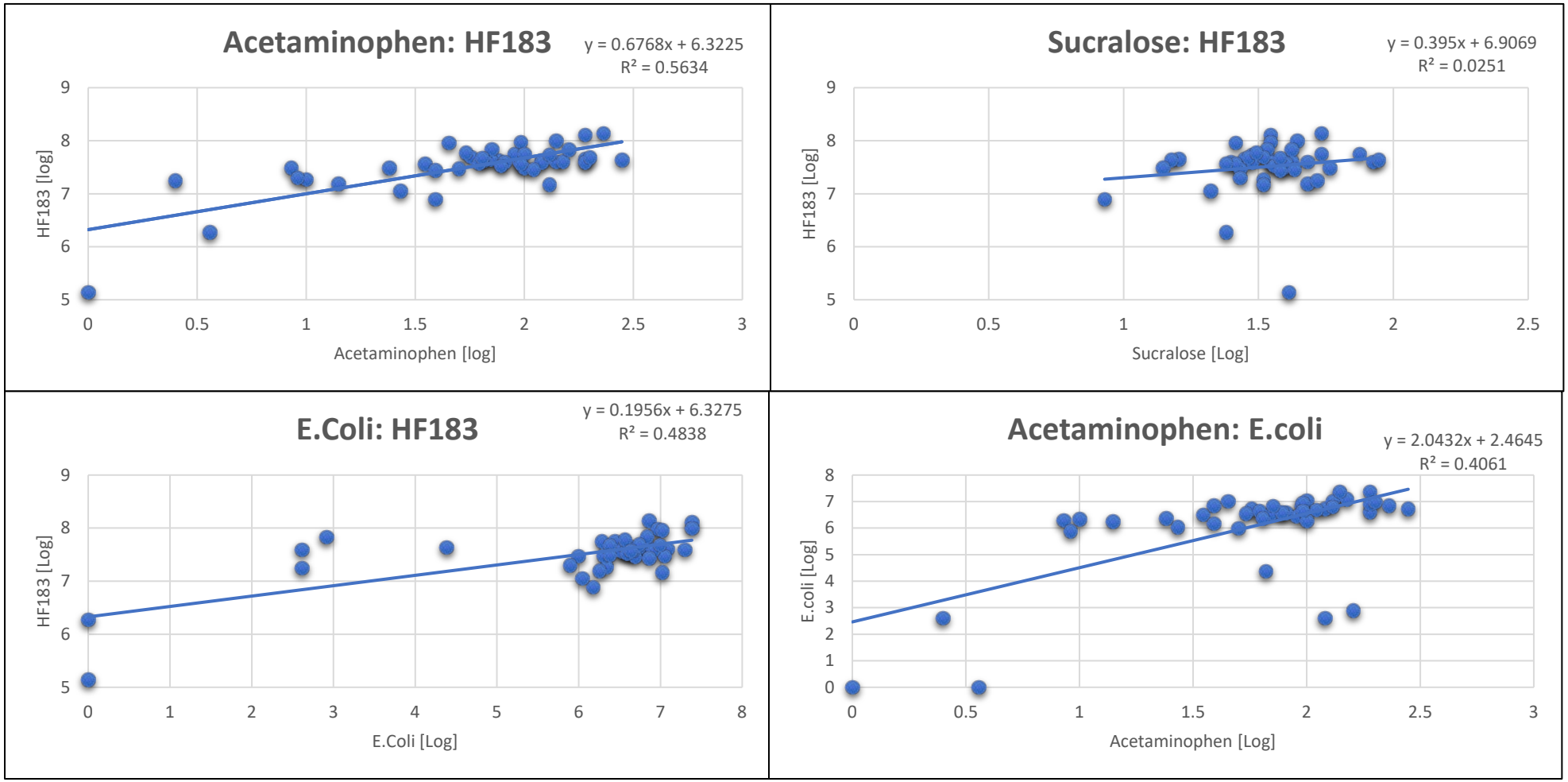


Figure 8. Interpretive wastewater profile based on this study.