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CITY OF OXNARD

PUBLIC WORKS INTEGRATED MASTER PLAN

RECYCLED WATER

PROJECT MEMORANDUM 4.6
PATHOGEN ANALYSIS FOR DIRECT POTABLE REUSE

REVISED FINAL DRAFT
September 2017

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PREFACE

The analysis and evaluations contained in these Project Memorandum (PM) are based on data and information available at the time of the original date of publication, December 2015. After development of the December 2015 Final Draft PMs, the City continued to move forward on two concurrent aspects: 1) advancing the facilities planning for the water, wastewater, recycled water, and stormwater facilities; and 2) developing Updated Cost of Service (COS) Studies (Carollo, 2017) for the wastewater/collection system and the water/distribution system. The updated 2017 COS studies contain the most recent near-term Capital Improvement Projects (CIP). **The complete updated CIP based on the near-term and long-term projects is contained in the Brief History and Overview of the City of Oxnard Public Works Department's Integrated Planning Efforts: May 2014 – August 2017 section.**

At the time of this Revised PWIMP, minor edits were also incorporated into the PMs. Minor edits included items such as table title changes and updating reports that were completed after the December 2015 original publication date.

City of Oxnard

Public Works Integrated Master Plan

RECYCLED WATER SYSTEM

PROJECT MEMORANDUM 4.6
PATHOGEN ANALYSIS FOR DIRECT POTABLE REUSE

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PATHOGEN ANALYSIS FOR DIRECT POTABLE REUSE

1.0 BACKGROUND

Pathogen concentrations and removal is the single most important component of potable water reuse purification, as pathogens represent an acute risk to human health. Indirect potable water reuse (IPR) projects in California, per final regulations, are required to attain 12-log removal (99.9999999999 percent) of virus and 10-log removal of protozoa from the point of raw wastewater to the point of potable water consumption (CDPH, 2014). Direct potable reuse (DPR) projects will be required to attain a similar or even higher level of pathogen removal. This section focuses on pathogen levels in the Oxnard secondary effluent and methods link pathogen concentrations to reliable surrogates.

1.1 State of California DPR Expert Panel

The State of California, working with the National Water Research Institute, completed an exhaustive analysis of research on potable water reuse, documented in *Evaluation of the Feasibility of Developing Uniform Water Recycling Criteria for Direct Potable Reuse* (September, 2016). That work determined that it is technically feasible to develop uniform water recycling criteria (regulations) for DPR that would incorporate a level of public health protection as good as or better than what is currently provided in California by conventional drinking water supplies, IPR systems using groundwater replenishment, and proposed IPR projects using surface water augmentation. As part of that work, the Expert Panel recommended areas of research that could be developed in concert with projects and with regulations. These include:

- Pathogen Monitoring and Qualitative Microbial Risk Assessments (QMRA).
- Multiple and Diverse Barriers for Chemicals and Pollutants.
- Critical Control Point Based Monitoring Systems.
- Engineered Storage.
- Enhanced Source Control Programs.

This review deals only with the removal of pathogens through primary and secondary treatment, addressing an important component of item No. 1 above (QMRA is a follow on effort). This new work is due to research funding from Oxnard and based upon collaborative research on Water Environment & Reuse Foundation (WE&RF) project 14 to 16.

1.2 Relevant Existing Literature on Pathogen Removal through Primary and Secondary Treatment

Pathogen removal will come through various treatment processes, including primary/secondary treatment, low pressure membrane filtration (MF or UF), reverse osmosis, and UV advanced oxidation. This review, and this new work, focuses entirely upon the removal of pathogens through the primary and secondary treatment process.

The existing data on pathogen levels in raw wastewater and the removal through primary and secondary treatment is limited, as is the ability to continuously or periodically monitor treatment process performance using online or grab-sample monitoring methods. Understanding pathogen concentrations prior to advanced treatment is a critical step in safely implementing potable water reuse. One relatively recent California Division of Drinking Water (DDW) approval of pathogen removal credits for combined primary and secondary treatment was obtained by the Water Replenishment District of Southern California (WRD, 2013). That document relied upon risk analysis data presented by Olivieri *et al.* (2007) which was developed based upon research by Rose *et al.* (2004). Rose *et al.* (2004) defined the range of bacteria, enterovirus, *Cryptosporidium*, and *Giardia* removal through six different full-scale wastewater treatment plants. The raw data from that work is reported by Olivieri *et al.* (2007) and shown in Table 1.1. At WRD (2013), the secondary process pathogen removal credits were based upon the data from two of the six tested secondary process configurations. Specifically, two of the secondary process trains (Facilities C and D, with solids retention times (SRTs) of 1.6 to 2.7 days and 3 to 5 days, respectively) had SRT values less than the secondary process feeding the WRD advanced treatment system (>9 days), and thus are presumed to be conservative estimates of performance. Per DDW request, WRD (2013) used the lower 10th percentile values calculated for each pathogen, resulting in 1.9-log reduction of enterovirus, 1.2-log reduction of *Cryptosporidium*, and 0.8-log reduction of *Giardia*.

Table 1 Pathogen Reduction Values Through Primary and Secondary Treatment (from Rose *et al.*, 2004) Public Works Integrated Master Plan City of Oxnard				
Lower 10th Percentile Values		**Log Reduction**		
SRT	**Facility**	**Enterovirus**	***Giardia***	***Crypto***
1.6-2.7	C	1.8	2.6	1.25
3-5	D	2.05	1.35	1.4
3.5-6	B	1.95	2.45	1.6
6-8	A	1.65	0.8	0.7
8.7-13.3	E	1.75	2.6	1.9
8-16	F	2.6	0.9	0.25
1.6-16	**ALL**	**1.85**	**0.8**	**1.2**
50th Percentile Values		**Log Reduction**		
SRT	**Facility**	**Enterovirus**	***Giardia***	***Crypto***
1.6-2.7	C	2.05	3.05	1.65
3-5	D	2.5	1.9	2.6
3.5-6	B	2.25	2.6	1.9
6-8	A	2.1	1.6	1.1
8.7-13.3	E	2.2	2.8	2.1
8-16	F	2.75	1.1	0.95
1.6-16	**ALL**	**2.3**	**2.6**	**1.6**

2.0 PATHOGEN REMOVAL RESEARCH PLAN

This new research with the City of Oxnard examines pathogen concentrations in raw wastewater and the removal of pathogens through primary and secondary treatment at the Oxnard Wastewater Treatment Plant (OWTP). This new research also considers potential surrogates for pathogen removal, measurements that can demonstration pathogen removal performance without the expense or time requirements for actual pathogen sampling and analysis. Table 1.2 summarizes the research.

Table 2 Removal Through Primary and Secondary Treatment Test Plan
Public Works Integrated Master Plan
City of Oxnard

Analyte	Method	Lab	Raw Wastewater, Oxnard	Secondary Effluent, Oxnard	No. of Samples per Month	No. Months of Testing
Male Specific Phage and Somatic Phage	Double Layer (Adams,'59)	BioVir	X		2	3
Male Specific Phage and Somatic Phage	US EPA 1602	BioVir		X	2	3
Enterococci	US EPA 1600 (MF)	BioVir	X	X	2	3
Giardia/Cryptosporidium	EPA 1626	SNWA	X	X	2	3
Virus: Norovirus, Enterovirus, total culturable virus (Coxsackievirus, Echovirus, Rotavirus, Reovirus)	EPA 1615 Tissue Culture with RTQPCR	BioVir	X	X	2	3
BOD	BOD5	Oxnard	X	X	2	3
TOC	SM 5310B	SNWA	X	X	2	3
UVA	SM 5910B	SNWA	X	X	2	3
Fluorescence	McKnight *et al.*, 2001	SNWA	X	X	2	3

3.0 RESEARCH RESULTS

The work shown below includes data from the Upper Occoquan Services Authority (UOSA), a partner on WE&RF Project 14-16. Research at UOSA followed an identical test plan to Oxnard, allowing for broader analysis and more robust conclusions. Two key findings can be taken from this new work:

- There is a qualitative relationship between secondary effluent water quality and pathogen concentrations, with higher water quality resulting in less pathogens. For a particular treatment plant looking to implement potable water reuse, such a facility could reasonably develop their own specific water quality and pathogen profile, documenting the range of pathogen concentrations in secondary effluent and linking those concentrations within a range of effluent water quality surrogates (such as TOC, BOD, TSS, UVT, *E. coli*, enterococci).
- This work also demonstrated relatively high removal (2 to 4+ log) of a broad range of pathogens through the primary/secondary process, with limited variability.

3.1 Pathogens and Surrogates

Table 1.3, below, lists the potential surrogates and pathogens investigated for this project. Laboratory methods are defined in Table 1.4.

Table 3 Surrogates and Pathogens Under Analysis
Public Works Integrated Master Plan
City of Oxnard

Potential Surrogates	Pathogens
Turbidity	Giardia
TSS	Cryptosporidium
COD	Total Culturable Virus
UVA/UVT	Enterovirus
DOC	Norovirus Type G1A
TOC	Norovirus Type G1B
BOD	Norovirus Type G2
Total Coliform	
E. coli	
Enterococci	
Male Specific Coliphage	
Somatic Coliphage	
Excitation Emission Spectrum (EEM, Fluorescence)	

Table 4 Analytical Methods Public Works Integrated Master Plan City of Oxnard		
Parameters	**Units**	**Method**
Bulk Analysis Methods		
Turbidity	NTU	2310
Total Suspended Solids (TSS)	mg/L	2540D
Biological Oxygen Demand (BOD)	mg/L	5210B
Total Organic Carbon (TOC)	mg/L	5310
Dissolved Organic Carbon (DOC)	mg/L	5310D
Chemical Oxygen Demand (COD)	mg/L	5220
Microbiological		
Total Coliform	Count/100 mL	9223 – Colilert
E. coli	Count/100 mL	9223 – Colilert
Enterococci	Count/100 mL	EPA 1600
Giardia/Cryptosporidium	cysts/L or oocysts/L	EPA 1623
Enterovirus and Norovirus	units/L	EPA 1615
Male Specific & Somatic Coliphage (method depends on sample type)	PFU/mL or PFU/100 mL	Adams, 1959 or EPA 1602
Other Parameters		
UVA and UVT	**A/cm, %**	Spectrophotometer
Fluorescence	**nm**	Fluorometer (SNWA)

3.2 Pathogen and Surrogate Organism Data

Six sampling events were over a 16-month time frame (from August 2015 to December 2016). The data is presented below through a series of figures below. The results suggest the following:

- UOSA has a generally better secondary effluent water quality compared to Oxnard.
- UOSA and Oxnard have reasonably similar concentrations of pathogens, both influent and secondary effluent. However, as shown in Table 1.5, UOSA has a consistently higher reduction of all pathogens through primary/secondary treatment, with the single exception of *Giardia*. This suggests that the better effluent quality from UOSA correlates to greater pathogen removal.
- Log reduction of pathogens was consistently high, 2 to >4-log, with little variability with several exceptions, as shown in Table 1.5 below. The log reduction for both facilities exceeds the pathogen removal documented in Rose et al. (2001).
- For UOSA and Oxnard, the pathogen data provides an important starting point for evaluation of pathogen concentrations, removal of pathogens by subsequent treatment processes, and determination of risk in the finished water.

Table 5 Log Removal of Pathogens Through Primary and Secondary Treatment at Oxnard and UOSA
Public Works Integrated Master Plan
City of Oxnard

Location and Sample Number	MS Phage	Somatic Phage	*Giardia*	Total Cultural Virus	Entero	Noro GIA	Noro GIB	Noro GII	TCV
#1 Ox	2.93	2.26	3.18		3.2	2.6	2.0	2.0	
#2 Ox	1.60	2.26	3.52	3.63	2.7	3.4	4.1	3.7	3.6
#3 Ox	2.98	1.80	2.38	2.11	3.0	2.6	2.6	3.2	2.1
#4 Ox	2.38	2.08	3.13	3.22	2.9	3.3	1.9	3.2	3.2
#5 Ox			2.44	2.87	2.2	3.0	2.8	2.4	
#6 Ox			2.19	2.56	2.8	2.8	2.9	2.8	
Average Oxnard	**2.47**	**2.10**	**2.81**	**2.88**	**2.81**	**2.94**	**2.71**	**2.87**	**2.99**
#1 UOSA	4.65	2.86	2.90	2.68					2.7
#2 UOSA	3.84	2.69	2.09	4.26	3.7	2.9	7.4	7.4	4.3
#3 UOSA	4.83	2.26	3.64	2.11	8.3	3.4	3.5	3.5	2.1
#4 UOSA	3.92	2.00	1.03	3.01	2.7	3.5	3.5	3.5	3.0
#5 UOSA	4.34	3.09	2.32	3.35	4.3	4.1	4.2	4.2	3.4
#6 UOSA	1.46	0.64	2.11	3.28	3.7	4.1	4.4	4.4	3.3
Average UOSA	**3.84**	**2.26**	**2.35**	**3.12**	**4.56**	**3.61**	**4.60**	**4.60**	**3.12**

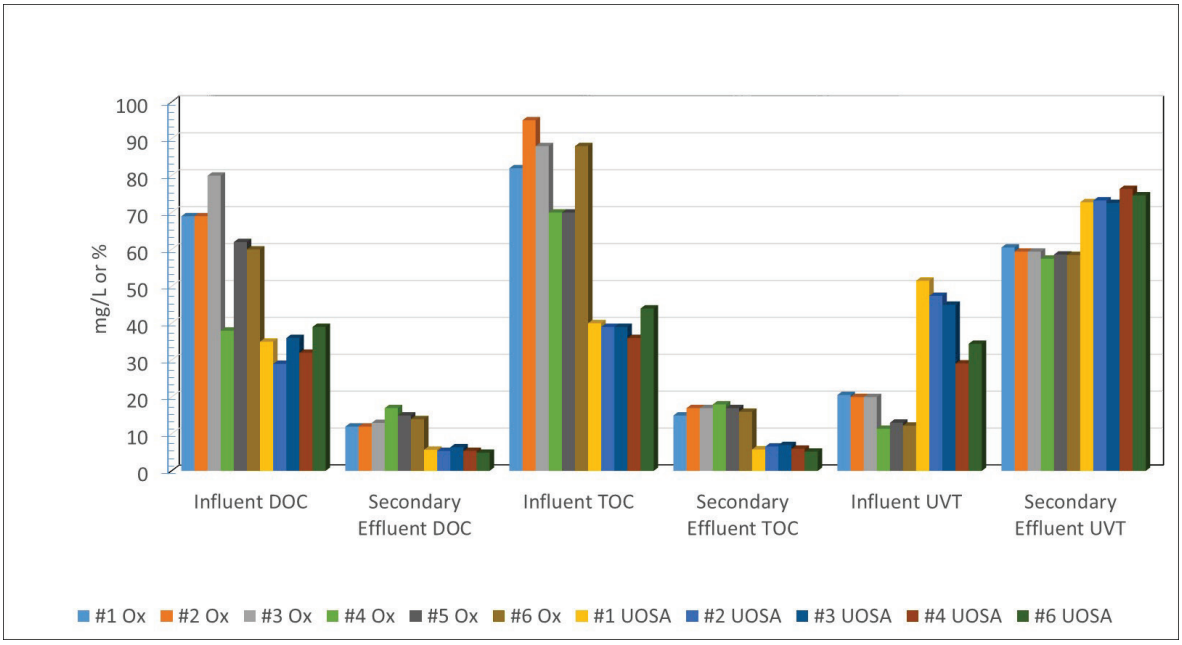


Figure 1 Primary Influents and Secondary Effluent TOC, DOC, and UVT for Oxnard and UOSA

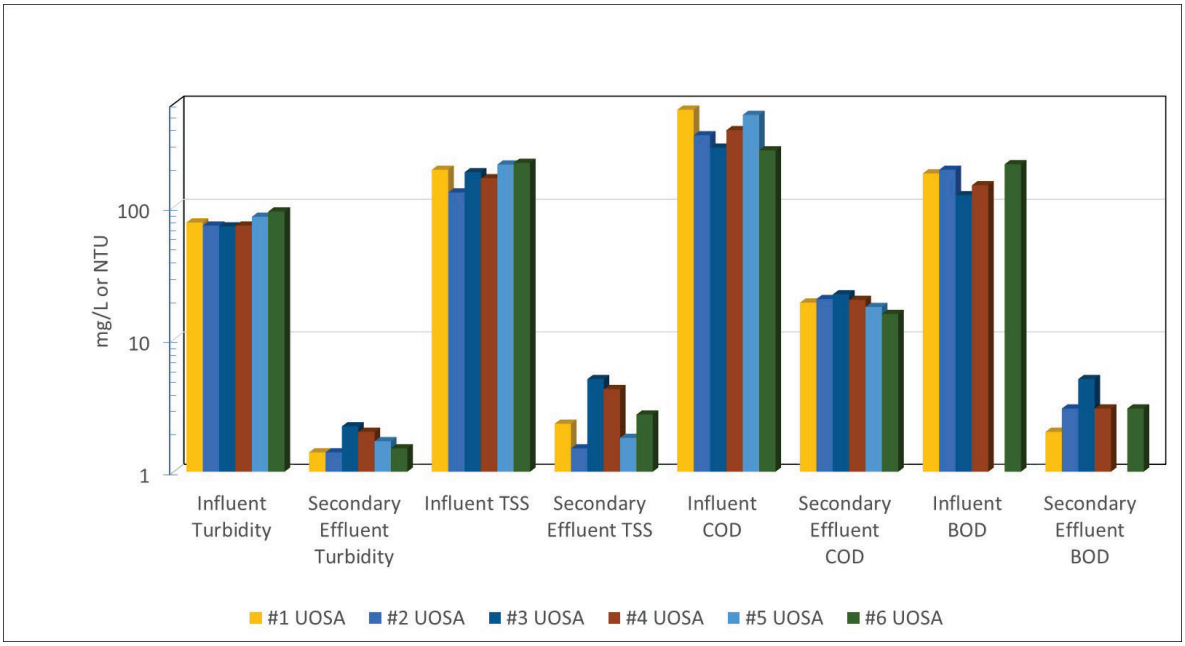


Figure 2 Primary Influents and Secondary Effluent Turbidity, TSS, COD, BOD for UOSA

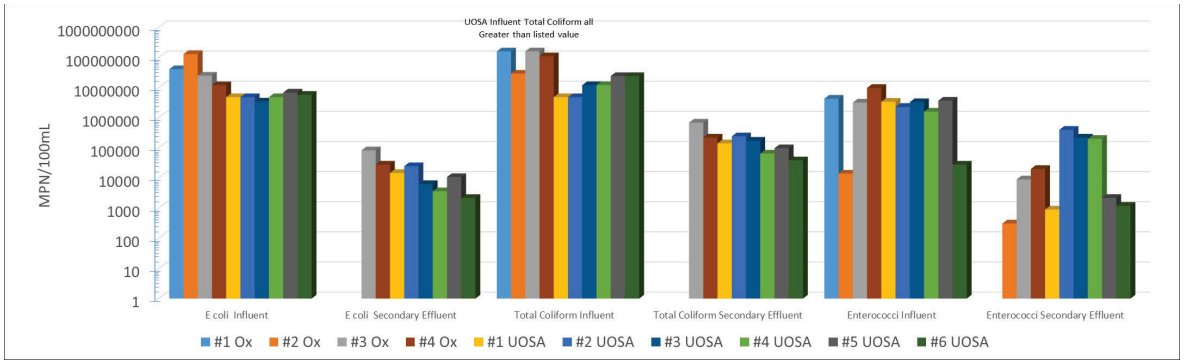


Figure 3 Primary Influents and Secondary Effluent E. coli , Total Coliform, and Enterococci for Oxnard and UOSA

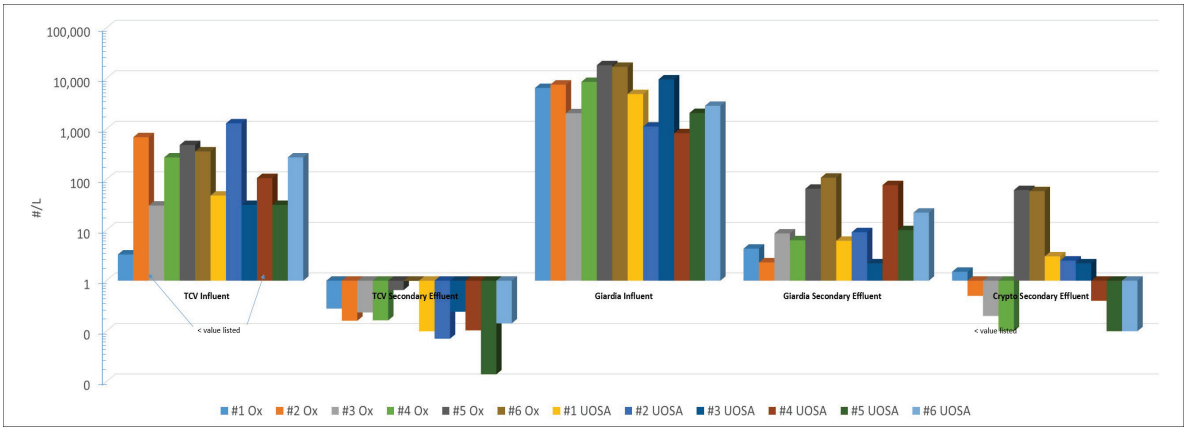


Figure 4 Primary Influents and Secondary Effluent Total Culturable Virus, Giardia, and Cryptosporidium for Oxnard and UOSA

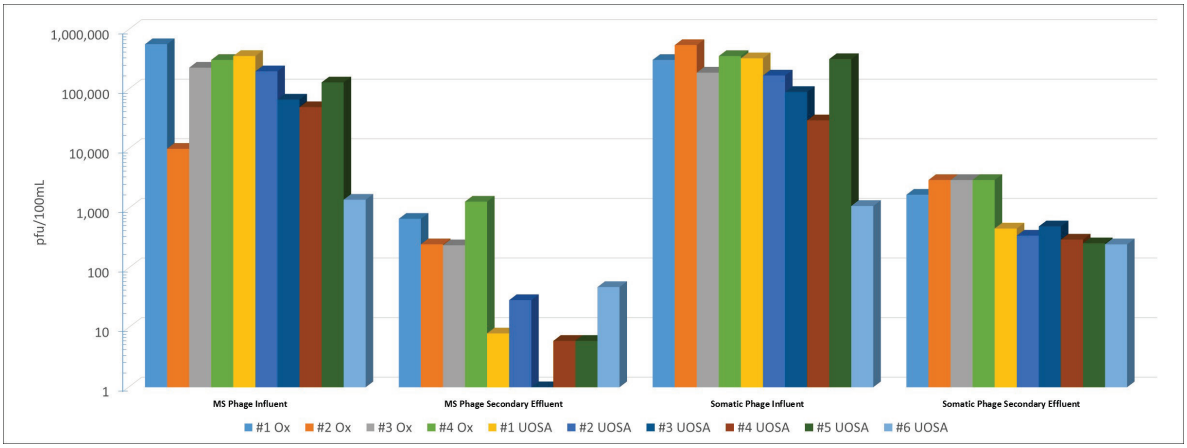


Figure 5 Primary Influents and Secondary Effluent MS Phage and Somatic Phage for Oxnard and UOSA

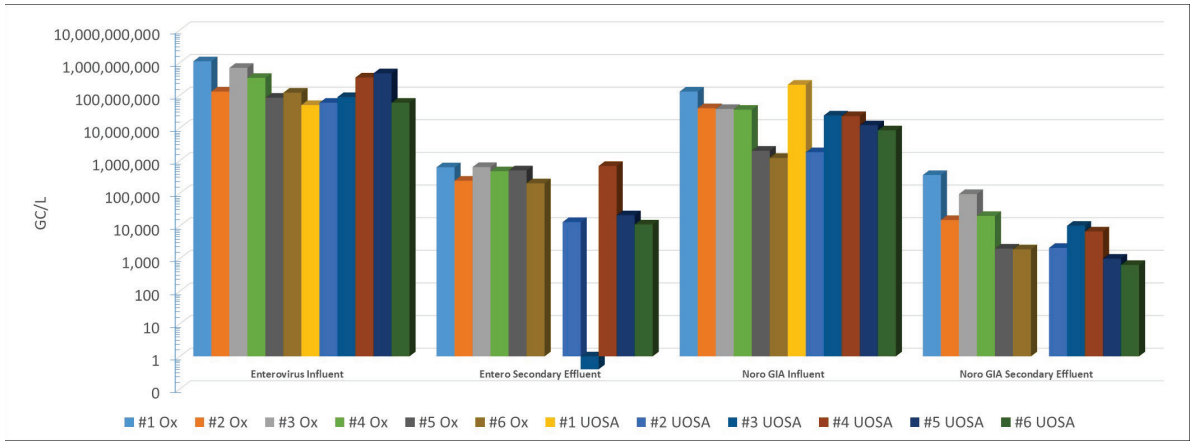


Figure 6A Primary Influents and Secondary Effluent Virus qPCR Results for Oxnard and UOSA (0.4 GC/L values below detection)

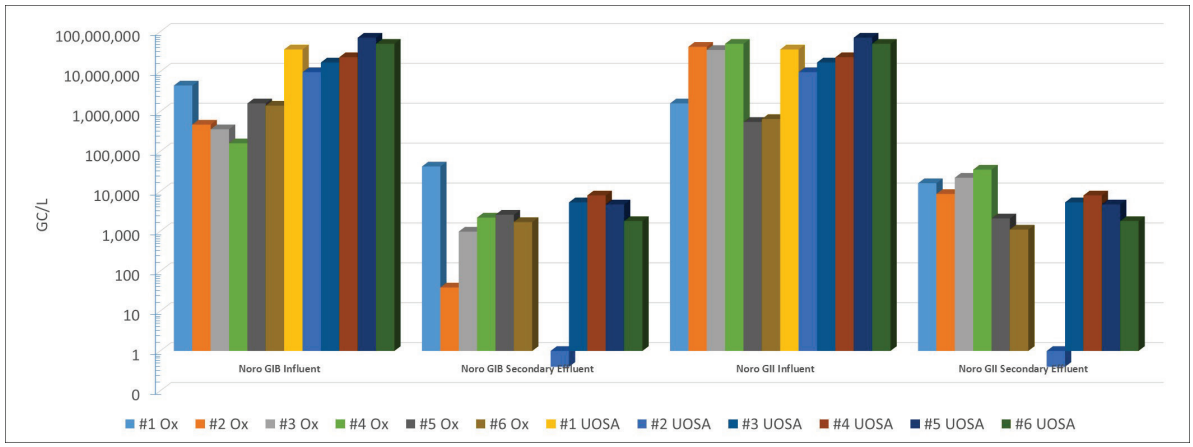


Figure 6B Primary Influents and Secondary Effluent Virus qPCR Results for Oxnard and UOSA. (0.4 GC/L values below detection)

3.3 Data Correlations

The concentrations of different parameters and pathogens were compared and plotted, in an effort to discern a correlation. Example plots are shown as figures below. These qualitative results suggest:

- Various surrogates (e.g., E. coli, TOC, TSS, UVT, BOD) provide some correlation with pathogen concentrations.
- Some surrogates appear to have a better correlation to pathogens than others, such as *E. coli* or enterococci.
- ***Repeatedly, higher quality effluent (lower BOD, TOC, TSS, surrogate organisms, higher UVT) correlate to less pathogens. Hence, setting a baseline range of primary/secondary treatment performance at a particular wastewater***

treatment plant will provide a degree of confidence in a stable effluent pathogen concentrations, plus or minus 1-log.

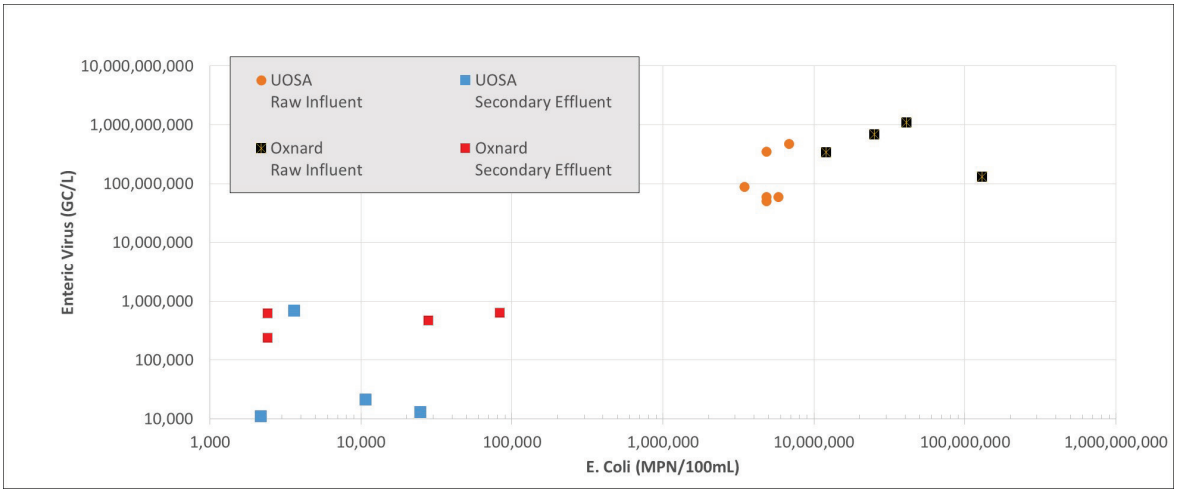


Figure 7 Correlation of *E. Coli* to Enteric Virus

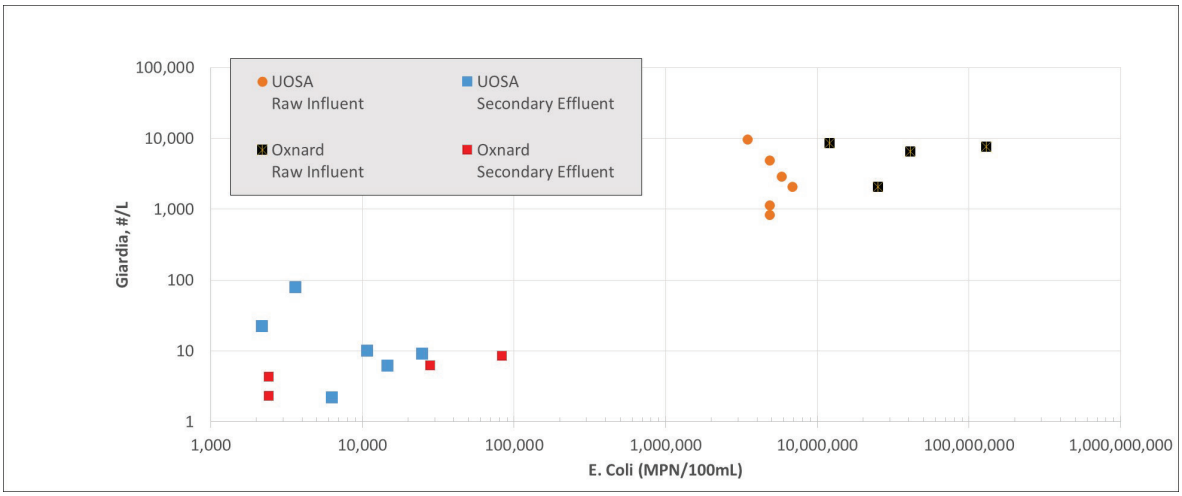


Figure 8 Correlation of *E. Coli* to *Giardia*

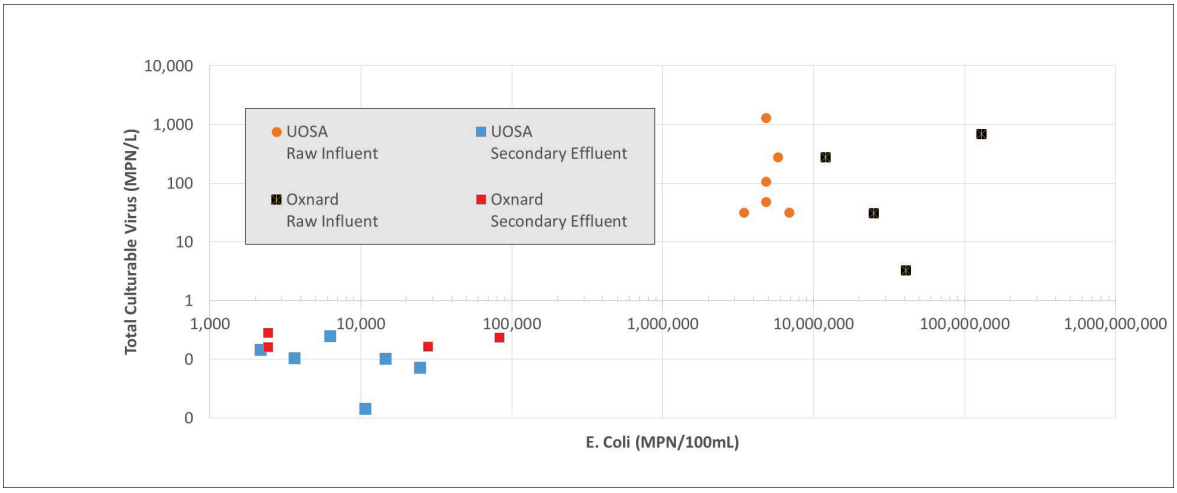


Figure 9 Correlation of *E. Coli* to Total Culturable Virus

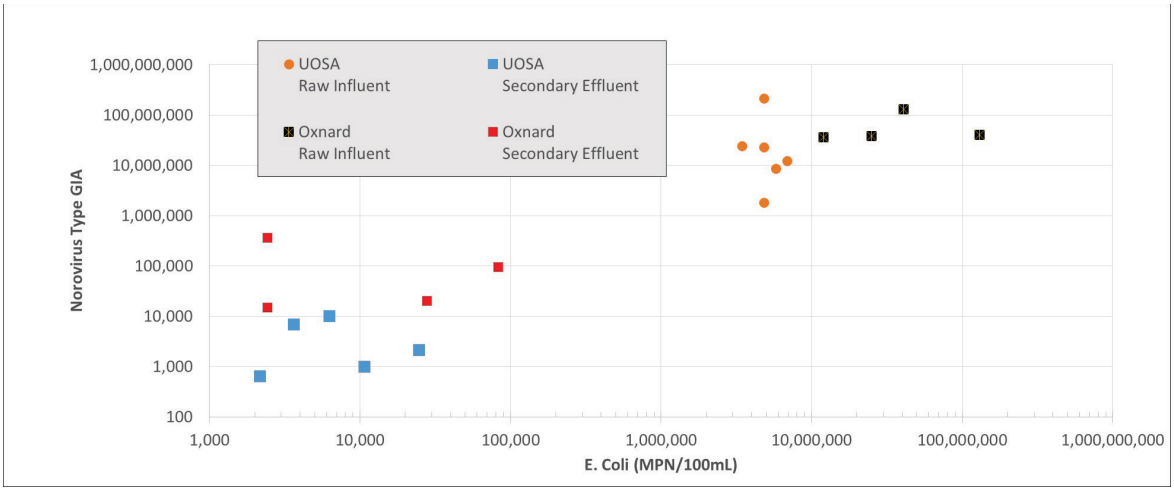


Figure 10 Correlation of *E. Coli* to Norovirus Type GIA

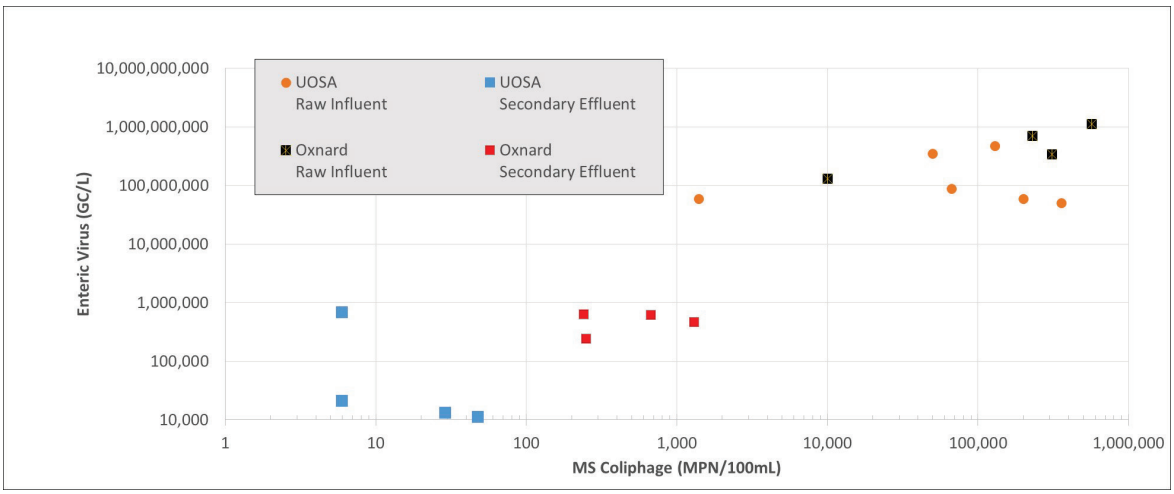


Figure 11 Correlation of MS Coliphage to Enteric Virus

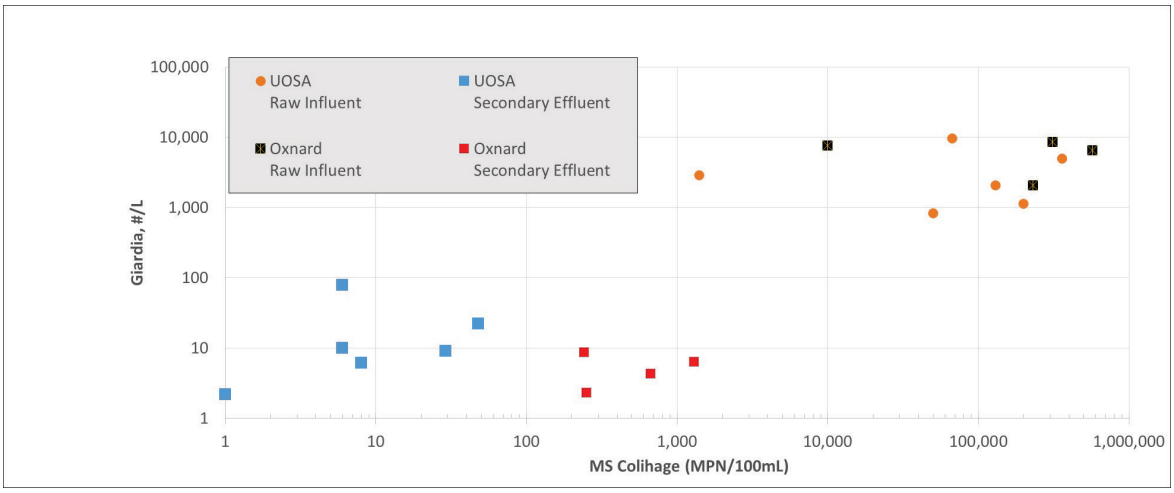


Figure 12 Correlation of MS Coliphage to *Giardia*

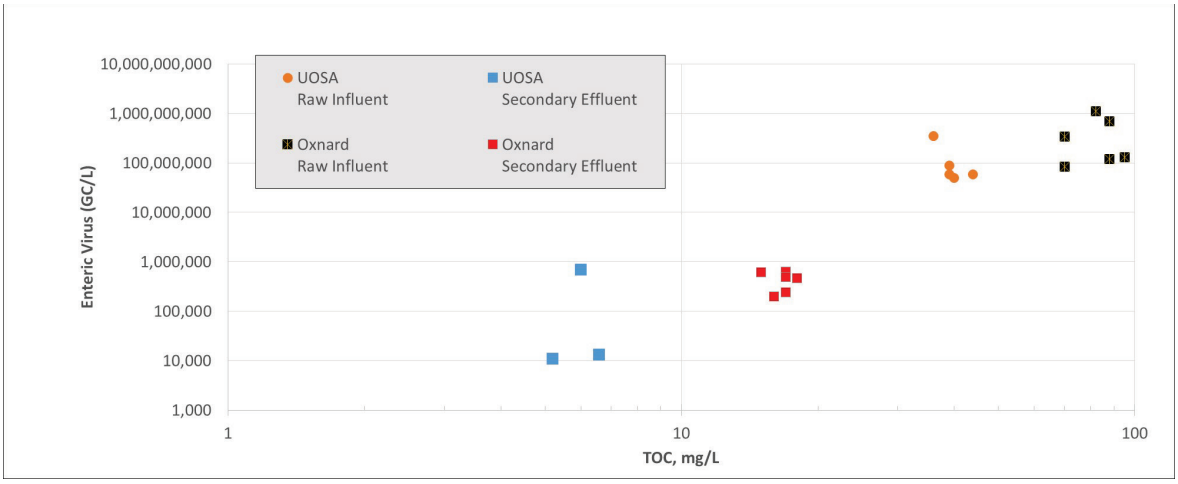


Figure 13 Correlation of TOC to Enteric Virus

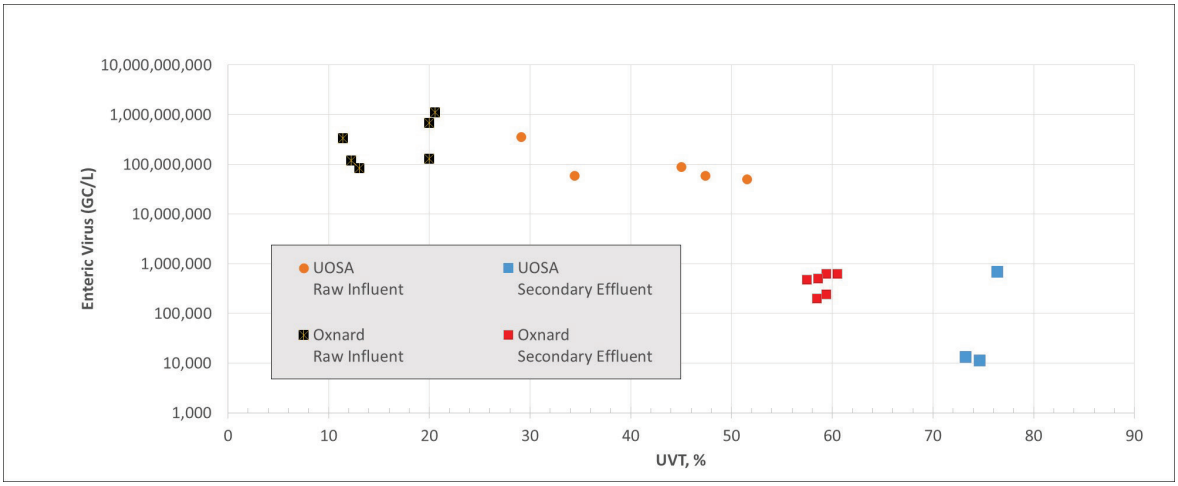


Figure 14 Correlation of UVT to Enteric Virus

3.4 Fluorescence Excitation/Emission Matrices

Within this project, for the pathogen surrogate analysis, the project team closely examined the use of Excitation/Emission Matrices (EEM). EEM imaging was produced for both UOSA and Oxnard sampling events. Figure 15 is an example EEM graphic, depicting slight, but measurable change to EEM through Primary and Secondary treatment.

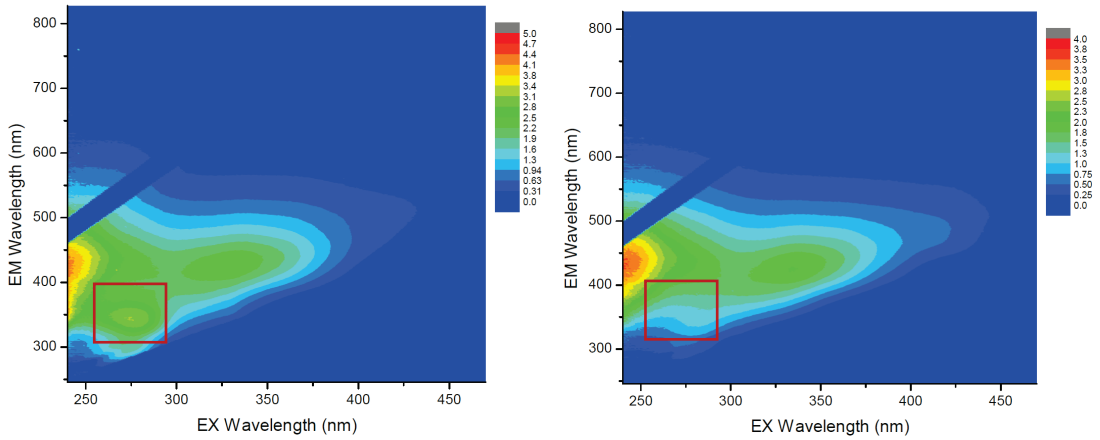


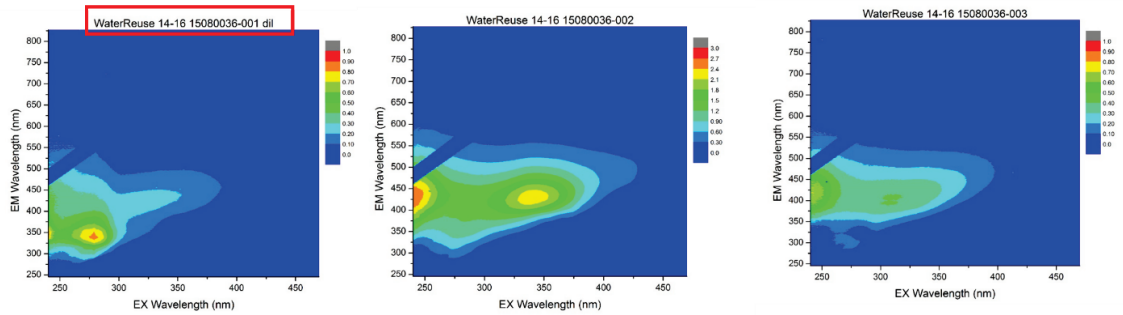
Figure 15 Example of influent (left) and effluent (right) EEMs

(collected as a part of this study with a red-box illustrating the decrease in intensity post-treatment in the protein-like or tryptophan-like region of the spectra).

A Horiba Aqualog spectrofluorometer was used to collect 3D EEM and absorbance spectra for each water sample. The EEMs were run from an excitation (ex) of 240 to 600 nm at 10 nm increments and emission (em) data was collected from 245 to 800 nm. Post-processing included applying instrument specific correction factors, correcting for primary and secondary inner filter effects, subtracting a daily blank, and normalizing the data to the Raman area of the blank at 350 nm excitation. Total fluorescence intensity (TF) was calculated by summing the Raman normalized intensities over all wavelengths (Lawaetz and Stedmon, 2009). A visual analysis revealed a consistent decrease in the protein-like or tryptophan-like peak from the influent to the effluent, as shown above in Figure 15. To represent the change pre- and post treatment, a specific ex/em pair was selected within this peak at 275/350 nm TF and the ex/em pair at 275/350 nm of each sample along with the delta of the influent and effluent at 275/350 nm were used in regression analysis with the microbial parameters. 350 nm were used in regression analysis with the microbial parameters.

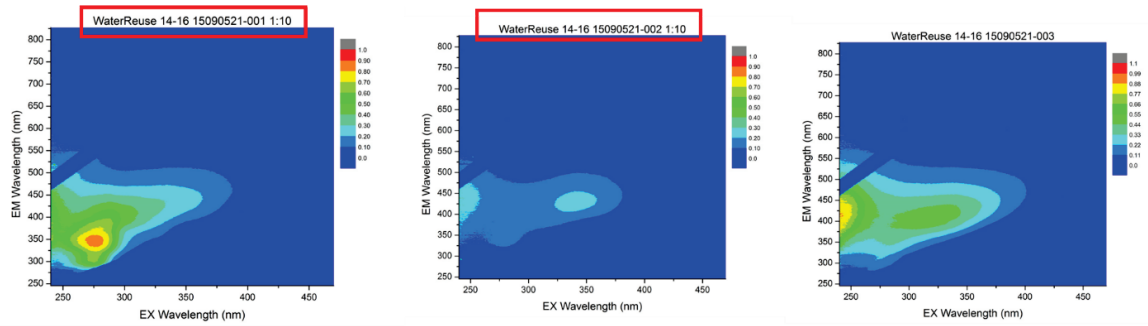
Three UOSA EEMs and four Oxnard EEMs have been produced (Figures 16 and 17). With this information and analysis, *based on a limited data set*, we can conclude similarly to the other surrogate analysis, that EEM does track changes to water quality, with better water quality correlating to reduce pathogen concentrations. However, these results are quantitative, and not statistically robust.

UOSA
08/18/15 – Round 1 Sampling Results



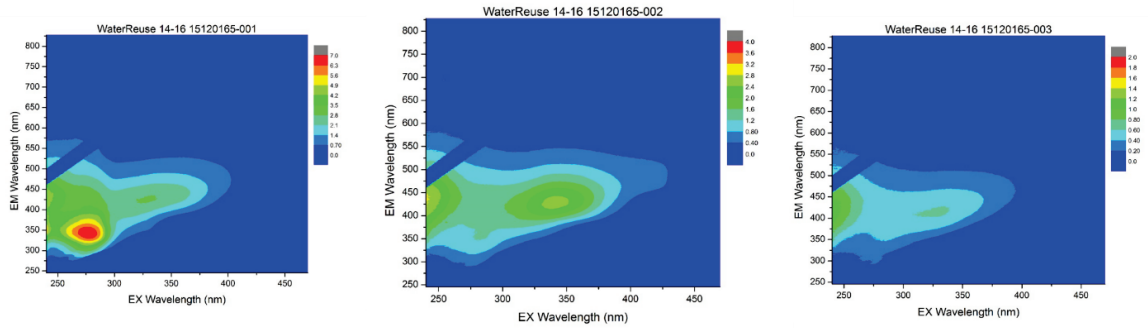
RAW Sewage Influent | Secondary Effluent | Finished Water

UOSA
10/20/15 – Round 2 Sampling Results



RAW Sewage Influent | Secondary Effluent | Finished Water

UOSA
12/15/15 – Round 3 Sampling Results

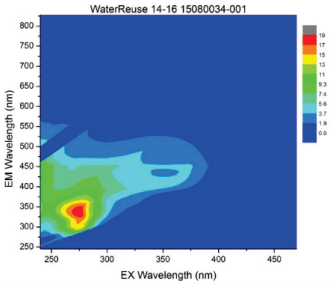


RAW Sewage Influent | Secondary Effluent | Finished Water

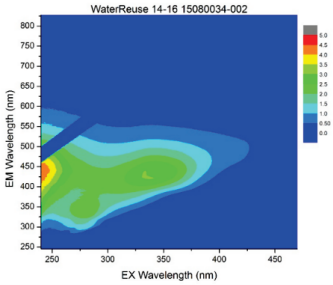
Figure 16 EEM data from UOSA rounds 1-3 Sampling Events

(including Primary influent, secondary effluent and finished water. Red boxes around the label indicate that this sample was a dilution that has been corrected for the 1:10 dilution value.)

OXNARD
08/18/15 – Round 1 Sampling Results

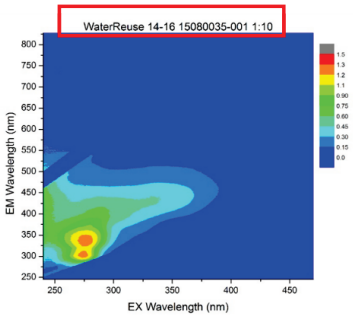


RAW Sewage Influent

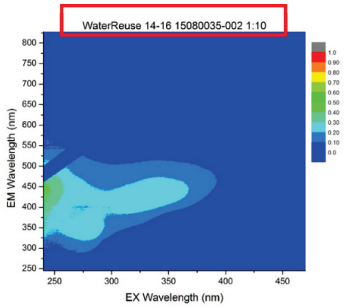


Secondary Effluent

OXNARD
11/03/15 – Round 2 Sampling Results

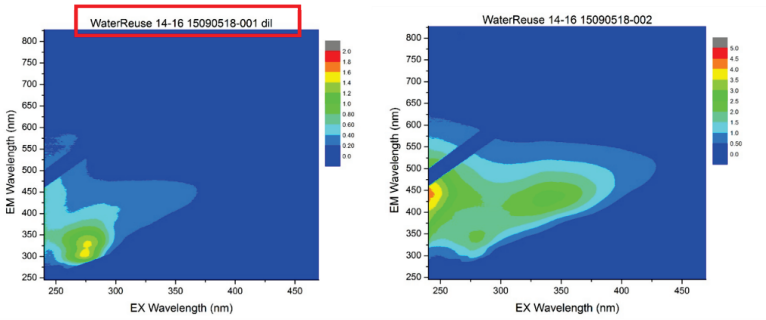


RAW Sewage Influent



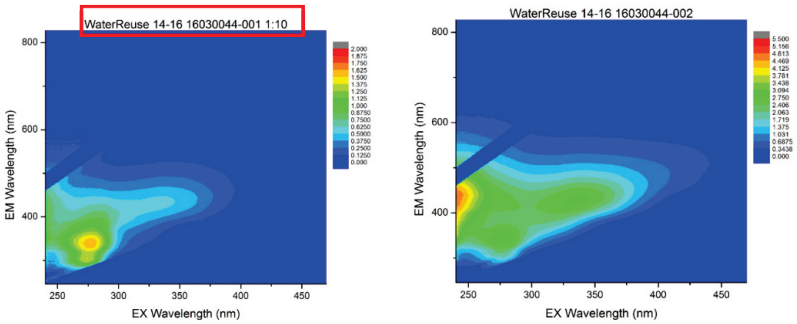
Secondary Effluent

OXNARD
11/18/15 – Round 3 Sampling Results



RAW Sewage Influent Secondary Effluent

OXNARD
3/7/16 – Round 4 Sampling Results



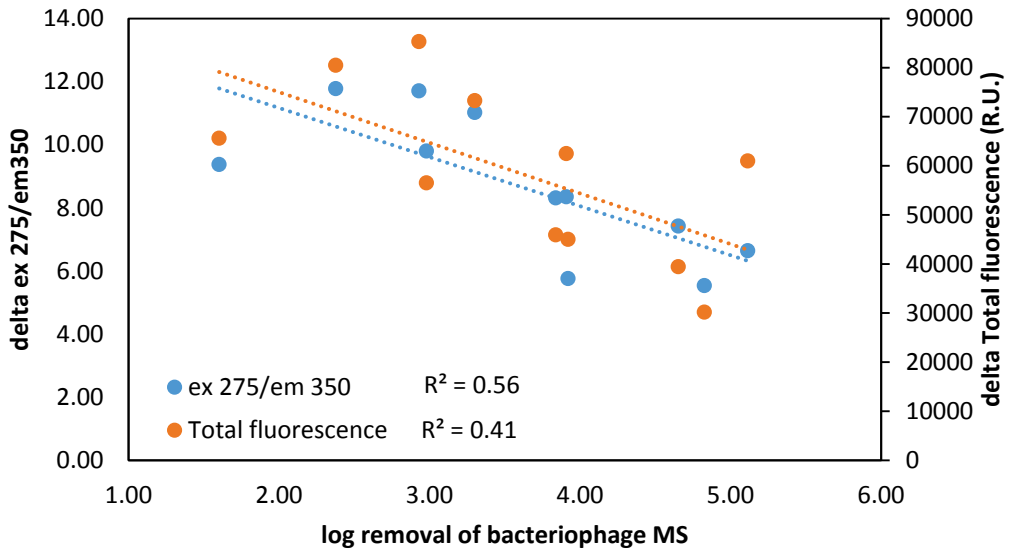
RAW Sewage Influent Secondary Effluent

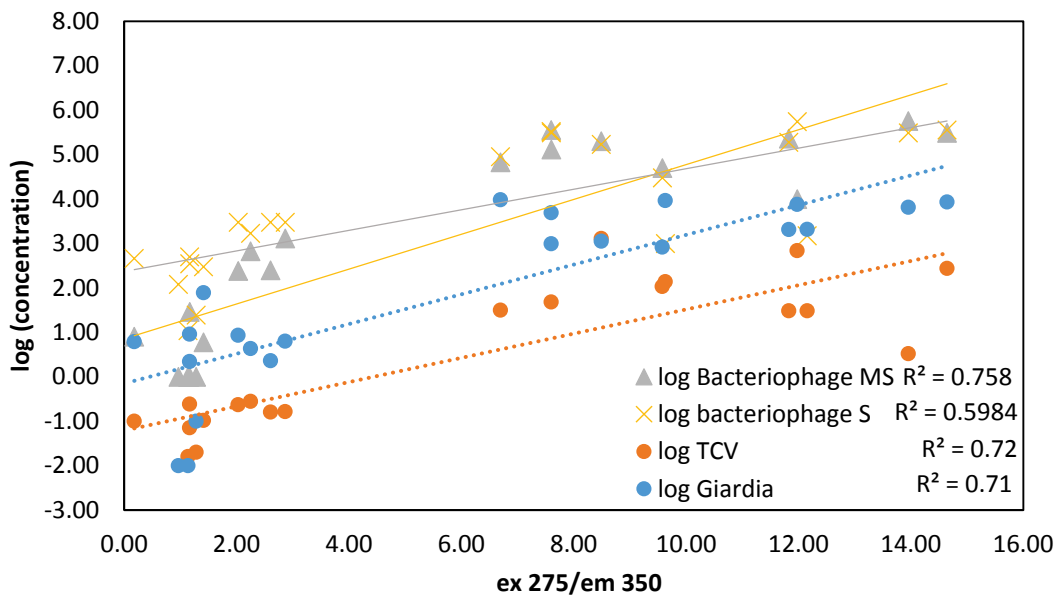
Figure 17 EEM data from Oxnard rounds 1-4 Sampling Events
(including primary influent and secondary effluent. Red boxes around the label indicate that this sample was a dilution that has been corrected for the 1:10 dilution value)

Several correlations were generated with varying degrees of statistical confidence, as shown below in Figure 18. In this study, the highest R^2 was generated between the log of bacteriophage MS and the ex/em intensity at 275/350 nm (R^2 = 0.75), but the log of giardia (R^2 = 0.72) and total culturable viruses (R^2 = 0.71) were also significant. Lower R^2 values were observed for norovirus (GIB, GIA, and GII) and enterovirus and the ex/em at 275/350 nm (R^2 between 0.52 – 0.63) and between the delta TF (R^2 = 0.41) and 257/350 nm (R^2 = 0.56) and the log removal of the bacteriophage MS. Note, this dataset is limited and more data must be generated to confirm the significance of these correlations.

Wastewater treatment concurrently reduces the concentration of bacteria and viruses and the intensity of the fluorescence. The protein-like or tryptophan-like fluorescent peak is commonly used as an identifier of wastewater-impact in a water sample and is reduced at

every step in wastewater treatment (Henderson et al., 2009). This is reflected by the possible correlations (R^2 or $\rho > 0.75$) observed with this peak and traditional wastewater treatment parameters, such as BOD, COD, TOC, and TN in previous studies (Henderson et al., 2009 and references therein).





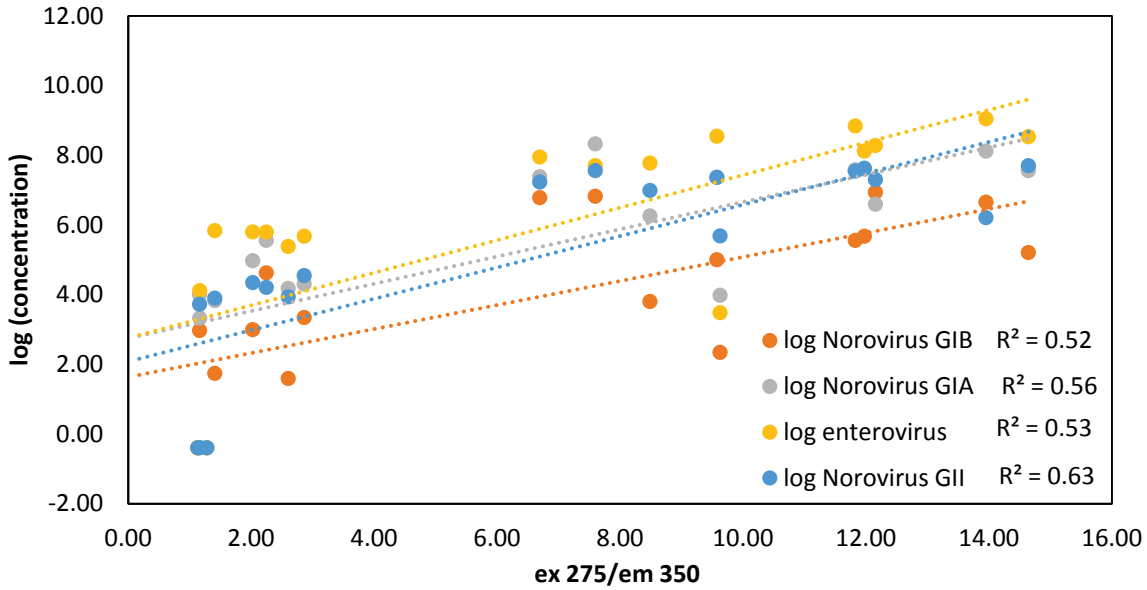


Figure 18 (a) regression of the delta between the influent and the effluent for total fluorescence intensity (R.U.) and ex/em at 275/350 nm and log removal for bacteriophage MS; (b). regressions observed between the intensity of the ex/em at 275/350 nm and the log concentration for bacteriophage MS, bacteriophage S, total culturable virus, and the giardia; (c) log concentration of norovirus GIB, norovirus GIA, enterovirus, and norovirus GII plotted against the ex/em at 275/350.