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Investigation of Endocrine Disrupting Compounds (EDCs) with an additional focus on Constituents of Emerging Concern (CECs) in Wastewater and Groundwater

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Abbreviation Index

1eff	Primary Effluent
2eff	Secondary Effluent
3AWRP	3A Wastewater Reclamation Plant
3eff	Tertiary Effluent
CEC	Constituent of Emerging Concern
CSU	California State University
CWRP	Chiquita Wastewater Reclamation Plant
DEET	N, N-Dimethyl-Meta-Toluamide
EDC	Endocrine Disrupting Compound
IIRMES	Institute for Integrated Research in Materials, Environments, and Society
MW	Monitoring Well
OCP	Organochlorine Pesticide
OWRP	Oso Wastewater Reclamation Plant
PAH	Polycyclic Aromatic Hydrocarbon
PCB	Polychlorinated Biphenyls
QA	Quality Assurance
QC	Quality Control
RS	Raw Sewage
SAT	Soil Aquifer Treatment
SCWD	South Coast Water District
SJBA	San Juan Basin Authority
SMWD	Santa Margarita Water District
USDA	United States Department of Agriculture
WRP	Wastewater Reclamation Plant
WRPI	Water Resources and Policy Initiative

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Section 1: Acknowledgements

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Section 1.A: Acknowledgements for Organizations

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Orange County Sanitation District
Orange County Water District
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Refer to Appendices for Organizations' Logos

Section 1.B: Acknowledgements for Individuals

Pitiporn Asvapathanagul, Ph.D.; *Mentor & Principal Investigator*
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Section 2: Executive Summary

This is the investigation of endocrine disrupting compounds (EDCs) with an additional focus on constituents of emerging concern (CECs) in wastewater and groundwater. Within the EDCs class, there are three types of chemicals: (1) organochlorine pesticides, (2) polychlorinated biphenyls, and (3) polycyclic aromatic hydrocarbons. The project is a local strand from the Santa Margarita Water District (SMWD) and is an attempt to create a list of contaminants that would be targeted in a future wastewater remediation research regarding soil aquifer treatment. All samples involved were from the Southern Orange County area that Santa Margarita Water District services and were sampled from August 2016 to July 2017. Techniques involved in the extraction of these micropollutants includes liquid-liquid and solid-phase extractions. Analysis was conducted using an Agilent Gas Chromatograph coupled with a Mass Spectrometer. Quantifications of the chemicals were done on the system's program, Agilent ChemStation. Confirmation of the chemicals' identities was accomplished based on the largest ion with some chemicals having additional confirmation from one to three other ions. Examination of the chemical results suggested that chemicals widely used in consumer products tends to have higher concentrations than those used in industrial settings. Chemicals like organochlorine pesticides and polychlorinated biphenyls, which were primarily used in the 1900's, are less likely to appear in the water system. Naturally occurring chemicals like polycyclic aromatic hydrocarbons do appear in the water system but were only detected in trace amounts. Moreover, polycyclic aromatic hydrocarbons of lighter molecular weights appeared more frequently. Beyond those three types, constituents of emerging concern raised concern with concentrations and detection likelihood being the greatest of all the chemicals analyzed. All data, in regards to groundwater contaminants, indicate low levels of all analyzed compounds. From the trend analyses of the three wastewater reclamation plants that Santa Margarita Water District operates, all three wastewater reclamation plants, Chiquita, Oso, and 3A, demonstrated abilities to remove some degree of the organochlorine pesticides, polychlorinated biphenyls, and polycyclic aromatic hydrocarbons analyzed. Examination of plants and groundwater suggests that chemicals found in groundwater appear to be sourced from wastewater. Additionally, a correlation between extraction data and weather patterns suggested that the appearance of groundwater polycyclic aromatic hydrocarbons increases with rainfall and varies by seasons. The seasonal association between chemical variety can also be found in wastewater reclamation plant intake.

Section 3: Project Objectives

Section 3.A: Background

Water demands in Southern California has been heightened by the on-going droughts throughout the early 21st century. As Southern California's population continues to grow, so does its demands for water. This demand for water followed by the lack of existing water supplies in Southern California has prompted local water agencies to seek neighboring Southern California water resources for the supplies they need.

Seeking to lessen their dependency on imported water, Santa Margarita Water District garnered interest in utilizing soil aquifer treatment (SAT) to improve tertiary effluents to reclaimable levels. The process of SAT takes advantage of the concept, adsorption, in which compounds adhere to the surface of another specie based on their polarity. If the treatment was proven to be highly successful at removing contaminants, the district predicted that the treated water could potentially be used to recharge their groundwater basin. However, one concern they had with repurposing tertiary effluent was with the potentially hazardous concentration levels of endocrine disrupting compounds (EDCs) and constituents of emerging concern (CECs). Many of these compounds tended to linger in water cycles because wastewater treatment processes, specifically, biological processes, were not designed for EDCs or CECs removal.

EDCs are chemicals that alter the way the endocrine system operates. The endocrine system or more commonly, the hormone system, is a series of glands within the human body that regulate the production and flow of hormones throughout the body. Hormones are made and released by these glands into the bloodstreams or into the fluids surrounding the cells ("What is the Endocrine System", 2017). In doing so, these hormones meet the receptors of the body's tissues and organs at their connection point and induce bodily responses based on the chemical interaction between them. EDCs are unique in this case because their chemical structures allow them to interfere with these interactions. When EDCs enter the human body, they can either mimic these hormones, block the binding sites of the hormones and receptors, or interfere with the production of hormones or receptors ("Endocrine Disrupters", 2017). The results of these interactions between the EDCs and the endocrine system cause the increased risks of cancer, developmental disorders, reproductive interference, and hindrance of the immune and nervous systems ("What is Endocrine Disruption?", 2017).

On the other hand, CEC is a class of chemicals that has been gaining attention due to its recent detection in the water system; hence the name, constituents of emerging concern. They are a group of compounds that are widely used amongst consumers and can originate from items such as pharmaceuticals, personal care products, and chemical additives. Due to their lack of monitoring in the 1900's, CECs has become a topic of concern amongst the water industry because there is little historical data of their past existence in water systems and resources.

As such, there are growing concerns about releasing reclaimed water due to the potentially high levels of these trace organics that may exist in wastewater reclamation plants' final effluents. Research needed to be conducted to identify these compounds and determine their concentrations in the water system before further investigations on SAT could begin.

Section 3.B: Goals

The goal of this study was to identify and monitor EDCs and CECs in wastewater and groundwater of the southern region of Orange County. The EDCs of interest included organochlorine pesticides (OCPs), polychlorinated biphenyls (PCBs), and polycyclic aromatic hydrocarbons (PAHs). The compounds investigated under these groups were primarily from the 1900's and were mostly banned due to their potential linkages to health risks such as cancer. These chemicals can be found in pesticides, electrical appliances, and petroleum refinery byproducts. The CECs included caffeine, triclosan, and n, n-dimethyl-meta-toluamide (DEET). Caffeine is found in coffee. Triclosan is used in soaps and sanitizers. DEET is used in insect repellants. All three chemicals are known to be consumed or used widely and frequently throughout the late 20th and early 21st century. Table 1, 2, 3 and 4 display the lists of OCPs, PCBs, PAHs and CECs analyzed in this study.

Table 1 Thirty organochlorine pesticides analyzed in this study.

2, 4' -DDE
2, 4' -DDT
2, 4'-DDD
4, 4' - DDT
4, 4' -DDD
4, 4' -DDE
4, 4' -DDMU
Aldrin
BHC-alpha
BHC-beta
BHC-delta
BHC-gamma
Chlordane-alpha
Chlordane-gamma
cis-Nonachlor
Dieldrin
Endosulfan I
Endosulfan II
Endosulfan sulfate
Endrin
Endrin aldehyde
Endrin ketone
Heptachlor
Heptachlor epoxide
Hexachlorobenzene
Methoxychlor
Mirex
Oxychlordane
Perthane
trans-Nonachlor

Table 2 Fifty-three polychlorinated biphenyls analyzed in this study.

PCB003
PCB008
PCB018
PCB028
PCB031
PCB033
PCB037
PCB044
PCB049
PCB052
PCB056 (060)
PCB066
PCB070
PCB074
PCB077
PCB081
PCB087
PCB095
PCB097
PCB099
PCB101
PCB105
PCB110
PCB114
PCB118
PCB119
PCB123
PCB126
PCB128
PCB138
PCB141
PCB149
PCB151
PCB153
PCB156
PCB157
PCB158
PCB167
PCB168+132
PCB169
PCB170
PCB174
PCB177
PCB180
PCB183
PCB187
PCB189
PCB194
PCB195
PCB199 (200)
PCB201
PCB206
PCB209

Table 3 Twenty-five polycyclic aromatic hydrocarbons analyzed in this study.

1-Methylnaphthalene
1-Methylphenathrene
2, 3, 5-Trimethylnapthalene
2, 6-Dimethylnaphthalene
2-Methylnaphthalene
Acenaphthene
Acenaphthylene
Anthracene
Benz[a]anthracene
Benzo[a]pyrene
Benzo[b]fluoranthene
Benzo[e]pyrene
Benzo[g,h,i]perylene
Benzo[k]fluoranthene
Biphenyl
Chrysene
Dibenz[a,h]anthracene
Dibenzothiophene
Fluoranthene
Fluorene
Indeno[1,2,3-c,d]pyrene
Naphthalene
Perylene
Phenathrene
Pyrene

Table 4 Three constituents of emerging concern analyzed in this study.

Caffeine
Diethyltoluamide (DEET)
Triclosan

Section 3.C: Project Development and Future Connection

This research originally set out to investigate *Adsorption of Endocrine Disrupting Compounds and Constituents of Emerging Concern through Soil Aquifer Treatment* as the second phase of the project. Sampling delays occurred due to the Southern California storms in January and March 2017. The working hours for the project were exhausted before the team could begin researching the treatment; thus, this report only contains content from the first phase. The investigation into SAT for tertiary effluents will be continued indefinitely beyond this internship.

Additionally, the initial priority of the project was to solely investigate EDCs' concentrations in wastewater and groundwater. However, data from the first sampling event showed that the detection of the observed EDCs in tertiary effluent were significantly lower than the overall quantity of observed compounds. For the first sampling set of tertiary effluents, 2 out of the observed 30 compounds for OCPs were detected, no traces of the 53 PCBs were detected, and 12 out of the observed 25 compounds for PAHs were detected. Overall, only 14 out of the observed 108 compounds were detected. In addition, only 6 out of the 14 detected compounds were found to be over 10 ng/L. Since the SAT research was dependent on the number of contaminants and their concentrations found in tertiary effluent, the research needed to be expanded to find other contaminants in high concentrations for existing and incoming samples.

The project expanded to identifying and monitoring CECs; more specifically, the chemicals introduced into the study were caffeine, triclosan, and DEET. Under Dr. Pitiporn Asvapathanagul, these chemicals were suggested due to their frequent appearance in research papers and confirmed into the study by Dr. Varenka Lorenzi, the laboratory coordinator of IIRMES. Dr. Varenka Lorenzi's previous work with water quality indicated that the three compounds were known to be frequently occurring in the water cycle in recent years. When reexamining the first set of wastewater samples for these chemicals, traces of all three compounds were found in all samples along with their respective chromatographs suggesting high concentrations. Thus, the project shifted its focus to include caffeine, triclosan, and DEET as part of the analytes list.

Section 4: Project Approach

Section 4.A: Quality Assurance/Quality Control

To ensure all extractions and extraction techniques were up to par, QA/QC mechanisms were put into place. One set of QA/QC samples was employed per sampling event.

For quality assurance purposes, recovery surrogates were injected into every sample prior to the extraction. Because of the surrogate's similarity to the analytes, percent recovery of the surrogates in the analysis allowed the team to estimate the percent recovery of the actual analytes. For example, if only 50% of the surrogates were detected in the final analysis, it was assumed that there was a high possibility that the concentration feedback may be an underestimated value of the true concentration. Four recovery surrogates were utilized for OCPs and PCBs while five recovery surrogates were used for PAHs and CECs.

In addition, blanks were also employed to detect possible contaminations from the extraction. Blanks were ultrapure water combined with recovery surrogates that underwent the same extraction procedure as their counterpart. If any analytes other than the recovery surrogates were detected in the blanks' extract, it indicated possible contaminations. Consequently, samples belonging to that specific blank's set had their analyte concentrations subtracted from the blank's analyte concentrations to account for these possible contaminations.

For quality control purposes, blanks spikes were used at the beginning of every extraction set to validate the effectiveness of the extraction methods. Blank spikes were ultrapure water combined with recovery surrogates and the targeted analytes. They also underwent the same extraction procedure as their counterpart. If low recoveries of the targeted analytes were found, it was assumed that the extraction method was ineffective at extracting the desired chemicals. If high recoveries of the targeted analytes were found, the extraction method was assumed to be effective at extracting these chemicals and were thus established as a viable method.

Section 4.B: Methodology

Three sampling events for wastewater and groundwater were performed. For each event, ten to eleven composite wastewater samples were collected for 4-liter volume per sample from three wastewater treatment plants (WRPs), including Chiquita (CWRP), Oso (OWRP) and 3A (3AWRP), in SMWD. The samples were taken from raw sewage, primary effluent, secondary effluent, and tertiary effluent from each WRP, except one plant without primary clarifiers. For groundwater, 9 grabbed samples were taken from 9 different wells of the San Juan Basin for each event. Sample size for groundwater ranged from 4L to 20L due to variations in extraction methodology. Table 5 and 6 lists the sampling dates and sites for wastewater and groundwater.

Table 5 Sampling schedule for wastewater.

Sampling Event	**Plant**	**Raw Sewage**	**Primary Effluent**	**Secondary Effluent**	**Tertiary Effluent**
1. September 2016	**CWRP**	x	x	x	x
	OWRP	x		x	x
	3AWRP	x	x	x	x
2. November 2016	**CWRP**	x	x	x	x
	OWRP	x		x	x
	3AWRP	x	x	x	
3. June 2017	**CWRP**	x	x	x	x
	OWRP	x		x	x
	3AWRP	x	x	x	x

Table 6 Sampling schedule for groundwater.

Sampling Event	**SJBA**						**SCWD**		
	MW-01S	**MW-03**	**MW-04**	**MW-05**	**MW-06**	**MW-08**	**MW-03**	**MW-4D**	**MW-2S**
1. September 2016	x	x	x	x	x	x	x	x	x
2. November 2016	x	x	x	x	x	x	x	x	x
3. January and March 2017	x	x	x	x	x	x	x	x	x

Section 4.B.I: Wastewater Extractions

For the first set of wastewaters, the extraction followed EPA method 625 for liquid-liquid extraction. The samples were split in half and each half was extracted in a different 2L separatory funnel. Extractions were conducted using three rinses of dichloromethane (DCM) in amounts of 100 mL, 50 mL, and 50 mL, with each rinse accompanied by a vigorous shaking for an even mixing. Settling of the post-shook samples was allowed for a separation of the DCM and water from the emulsion formed. After each rinse, filtration of the organic extract layer was conducted through layers of sodium sulfate inside a glass funnel.

For the second and third set of wastewaters, the samples were kept whole and the extractions were conducted inside the 4L amber glass jars that they came in with the three rinses of DCM similarly to the first set. The methodology involved also followed EPA method 625 for liquid-liquid extraction. Once extracted, 2L of the mixture in the amber glass jar was poured out into 2 different 1L beakers and the rest was poured out into a 2L separatory funnel. Caution during pouring was taken to ensure that all DCM and DCM/water emulsion ended in the 2L separatory funnel. Settling and filtering conditions followed the same procedure as the first set of samples.

Section 4.B.II: Groundwater Extractions

For the first set of groundwater samples, extractions were done through a C-18 disk, solid-phase extraction with a 10mL elution mixture of 1:1 dichloromethane to ethyl acetate. The elution was conducted under a soaking time of 1 minute and then repeated three times. Sample size was around 20L with one solid-phase extraction per 10L.

For the second set of groundwater samples, 5 out of the 9 samples were extracted through a C-18 disk, solid-phase extraction. 3 out of the 5 extractions were performed with a 10mL elution of dichloromethane. The remaining 2 out of the 5 extractions were performed with a 30mL elution mixture of 1:1:1 dichloromethane to ethyl acetate to hexane. Sample size, soaking and rinsing conditions were kept the same as the first groundwater sampling set.

For the remaining samples of the second set and all of third set of groundwater samples, extractions followed EPA method 625 for liquid-liquid extraction were performed. Sample size and extraction procedures were the same as the second and third set of wastewaters.

Section 4.B.III: Evaporation and Cleaning of the Extracts

Once completed, the extracts were concentrated down to 1 mL using a rotary evaporator and a nitrogen gas stream. Wastewater samples from the first set were joined together after evaporation. Groundwater samples from EPA method 625 were additionally concentrated down to 250 μL and injected into a 250 μL microvolume insert. Samples containing ethyl acetate as the solvent were dried completely under the nitrogen gas stream and solvent switched to 1mL of DCM.

Except for the third wastewater sampling event, all raw sewages and primary effluents extracts were cleaned through column chromatography. Prior to being cleaned, the extracts were solvent switched to 1mL of hexane by injection and evaporation on the rotary evaporator. The column was packed with a 12cm bottom layer of silica gel and a 6cm top layer of alumina gel. Eluting of the column was done in the order of 30mL hexane, 15mL 2:1 hexane to DCM mixture, then 15mL of DCM. Once completed, the extracts were re-concentrated down to 1 mL using a rotary evaporator and a nitrogen gas stream.

All concentrated extracts were injected into an Agilent gas chromatograph (GC; 6890N series) equipped with a mass selective detector (MSD; Agilent 5973 inert series). Data was acquired by the software in the GC/MS system. All concentration quantification from the GC/MS was based off the largest single ion. In most chemicals, an additional one to three other ions were available to assist with the confirmations of the chemical identification.

Section 4.C: Method Development

Many changes in extraction methodology occurred over the span of this project. This section explains the reasoning behind those changes.

Section 4.C.I: Wastewater Extractions

For a quick review, the first set of wastewaters underwent an extraction technique following EPA method 625 for liquid-liquid extraction. The samples were split into half (approximately 2L per half) and each half was individually extracted. Both halves were eventually reunited after one round on the rotary evaporator.

Feedback analysis data from this set showed that the surrogate recovery was at an average of 28.82±7.87%, for OCPs and PCBs, and 26.95±7.30%, for PAHs and CECs. On the other hand, feedback from the blank spikes (quality control) indicated that the EPA 625 extraction method was effective at extracting the targeted analytes. One of the issues that the team ran into with the wastewater samples was the excessive amount of emulsion from the vigorous shaking. To reduce the amount of emulsion, the extraction was then conducted inside the 4L amber jars they came in. Rather than having a 2L of samples to 100 or 50mL of solvent, this new technique had a ratio of 4L of samples to 100 or 50mL of solvent. By extracting them together, there were less emulsions to encounter for one sample. Average recovery surrogate percentage from this new extraction technique was 146.16%, for OCPs and PCBs, and 114.97%, for PAHs and CECs, higher than the previous one. These significant increases in recoveries prompted the change from individually extracting halves of the sample to extracting the entire sample in one process.

Section 4.C.II: Groundwater Extractions

For a quick review, the first set of groundwaters underwent a C18, solid-phase extraction for trace organics. Sample sizes were 20L with one extraction conducted per 10L. The samples' halves were joined together during the elution stage.

Feedback analysis data from this set showed that the OCP's and PCBs' surrogate recovery was at an average of 47.56±22.1% and the PAHs' and CECs' surrogate recovery was at an average of 38.73±29.23%. After receiving these responses, the research entered a method development phase for groundwater extractions. Several tests were conducted on the C18 disk extractions by changing elution solvent mixtures, soaking time, and elution method. During these tests, none of the combinations produced adequate average recoveries above 50%. Ultimately, an analysis was conducted on a C18 filtered blank spike which indicated that the C18 disk was ineffective at capturing the targeted compounds. As a result, the C18 solid-phase extraction method was discontinued and the groundwater extractions proceeded with EPA method 625 for liquid-liquid extraction. The procedure conducted was the same as the wastewater from the 2nd and 3rd sampling events; however, to accommodate for the large loss in the amount of contaminants from switching from a 20L to 4L sample, microvolume inserts were utilized to increase the concentration of the end extract. In turn, this allowed for final extracts to be better meet the detection limit of 0.1 ng/L. Average percentages from this new extraction technique was 39.16% higher than the 1st sampling's OCPs and PCBs and 91.18% higher than the 1st sampling's PAHs and CECs recovery surrogates' percentage. These significant increases in recoveries prompted the change in the extraction of groundwater from solid-phase extraction to liquid-liquid extraction with a microvolume insert.

Section 5: Project Outcomes

The following section is organized by wastewater results then discussion followed by groundwater results then discussion. Detection limits for wastewater was 1.0 ng/L and for groundwater was 0.1 ng/L.

Section 5.A: Wastewater Extraction Results and Discussion

Section 5.A.I: Organochlorine Pesticides and Polychlorinated Biphenyls

Section 5.A.I.1: 1st Wastewater Sampling Event Results and Discussion

Seven of eleven wastewater samples from the 1st wastewater sampling event contained organochlorine pesticides below the minimum detection limit of 1.0 ng/L. All wastewater samples (raw sewage, primary effluent, secondary effluent, and tertiary effluent) from 3AWRP detected of the organochlorine pesticides: chlordane-gamma, chlordane-alpha, and trans-nonachlor (Table 6). The concentrations of chlordane-gamma, chlordane-alpha and trans-nonachlor were around 2.60-7.13 ng/L, 2.79-8.69 ng/L and 1.11-4.95 ng/L, respectively. The concentrations of each compound across 3AWRP are displayed in Table 6. The highest concentrations of these compounds were measured in raw sewage, while the lowest concentrations were found in the tertiary effluent. The treatment processes demonstrated the average removal effectiveness of the three compounds (chlordane-gamma, chlordane-alpha and trans-nonachlor) was 69.67%±7.19% when tertiary treatment was included (Figure 1). Without tertiary treatment, the average removal efficiency for the OCPs was lower at 17.95%, 16.92% and 34.95%, respectively (Figure 1).

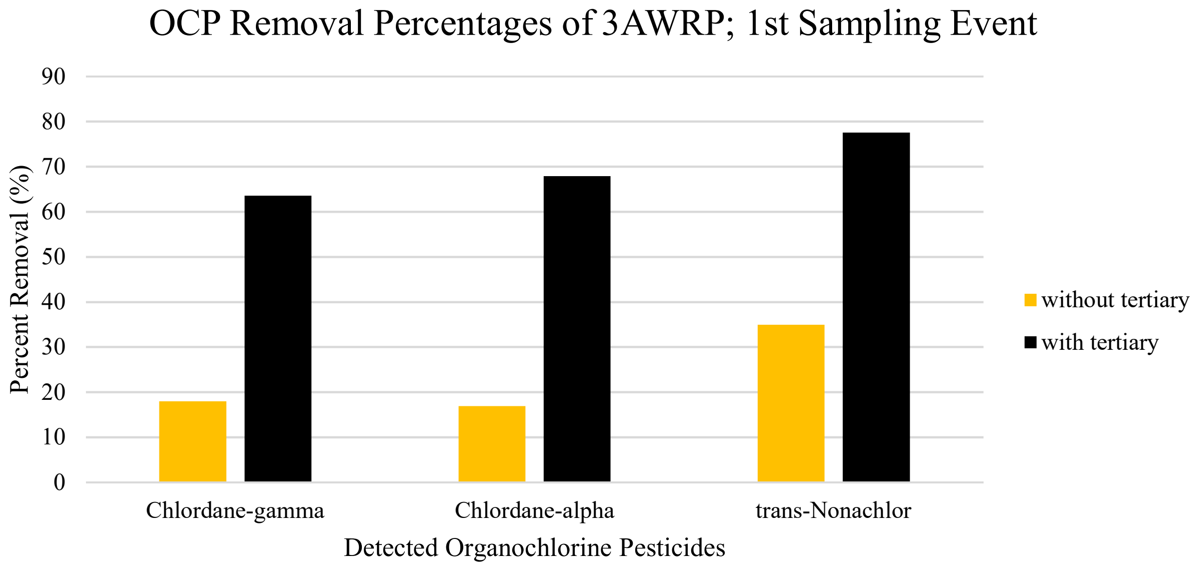


Figure 1: Removal efficiency for chlordane-gamma, chlordane-alpha and trans-nonachlor from 3AWRP

Table 6 Organochlorine pesticides types and concentrations in 3AWRP samples

	Chlordane-gamma (ng/L)	Chlordane-alpha (ng/L)	trans-Nonachlor (ng/L)
Raw Sewage	7.13	8.69	4.95
Primary Effluent	7.11	5.87	4.14
Secondary Effluent	5.85	7.22	3.22
Tertiary Effluent	2.60	2.79	1.11

Section 5.A.I.2: 2nd and 3rd Wastewater Sampling Events Results

Tertiary effluent from 3AWRP of the 2nd wastewater sampling event was not sampled. The remaining twenty-one wastewater samples from the 2nd and 3rd wastewater sampling events were undetected of any organochlorine pesticides and polychlorinated biphenyls.

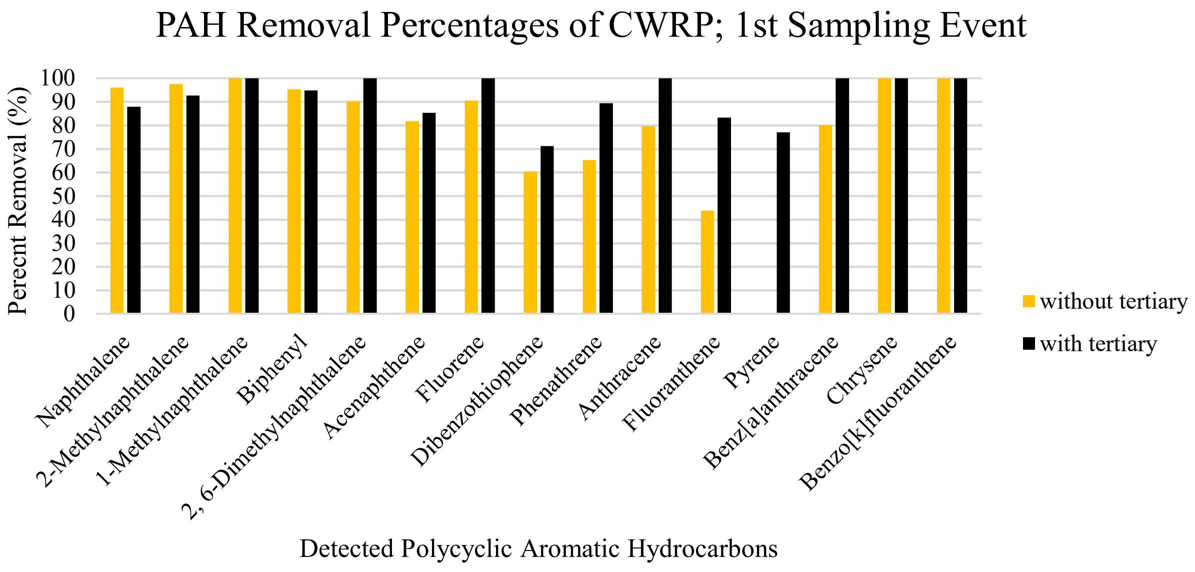
Section 5.A.II: Polycyclic Aromatic Hydrocarbons

Section 5.A.II.1: 1st Wastewater Sampling Event

From 25 PAH analysis, seven PAHs, including naphthalene, 2-methylnaphthalene, acenaphthene, dibenzothiophene, phenanthrene, fluoranthene and pyrene, were detected in all samples across three WRPs. 1-Methylnaphthalene, perylene and dibenz[a,h]anthracene were below the minimum detection limits in all 11 wastewater samples. The highest concentrations of PAHs in raw sewage were frequently observed in the 3AWRP and CWRP samples. The three maximum PAH concentrations observed were 138.19, 86.90, and 78.81 ng/L for pyrene, phenanthrene, naphthalene, respectively, which were from 3AWRP raw sewage. Generally, PAH occurrences and concentrations from 3AWRP were similar to those of CWRP. Concentration differences between shared PAH occurrences in their raw sewages ranged from 0.29-3.24 ng/L with an average percent difference of 32.72±16.80%. On the other hand, the concentration of 2, 6-dimethylnaphthalene, acenaphthene, fluorene, anthracene at 35.92, 14.26, 13.88 and 8.24 ng/L, respectively, in CWRP raw sewage were approximately twice to three times higher than other plants. However, these PAH concentrations from CWRP raw sewage were significantly lower than those of 3AWRP reported earlier. Additionally, phenanthrene, fluoranthene, and pyrene concentrations in secondary effluent had an increase of 19.61%, 95.04% and 667.03%, respectively, compared to the raw sewage concentrations at OWRP. This suggested that PAHs had accumulated occurred inside OWRP.

The PAH concentration in tertiary effluent ranged 0.93-4.72 ng/L, 0.42-24.67 ng/L, and 1.47-16.07 ng/L for CWRP, OWRP, and 3AWRP, respectively. The PAH removal efficiency of treatment processes was also investigated in the same manner of OCP removal. The removal efficiency for each PAH varied (Figure 2). The mean±standard deviation of PAH removal efficiency for all compounds was 92.08±9.48%, 69.97±26.93%, and 84.68±21.16% for CWRP, OWRP, and 3AWRP, respectively, when tertiary treatment was included. Excluding tertiary treatment, the PAH removal performance were lower in all plants, except OWRP, which were 84.28±17.27%, 75.06±28.73%, and 66.81±27.60% for CWRP, OWRP, and 3AWRP, respectively. The removal efficiency in WRPs for PAHs was greater than the percent removal of OCPs as stated earlier. However, there were a few compounds where the PAH concentrations in tertiary effluent were slightly higher than the concentrations in secondary effluent such as naphthalene and 2-methylnaphthalene. The minimum removal performance that measured the difference between raw sewage and tertiary effluent observed at each WRP were 71.21% (dibenzothiophene), 30.19% (phenanthrene), and 44.53% (acenaphthene) for CWRP, OWRP, and 3AWRP, respectively.

(a)



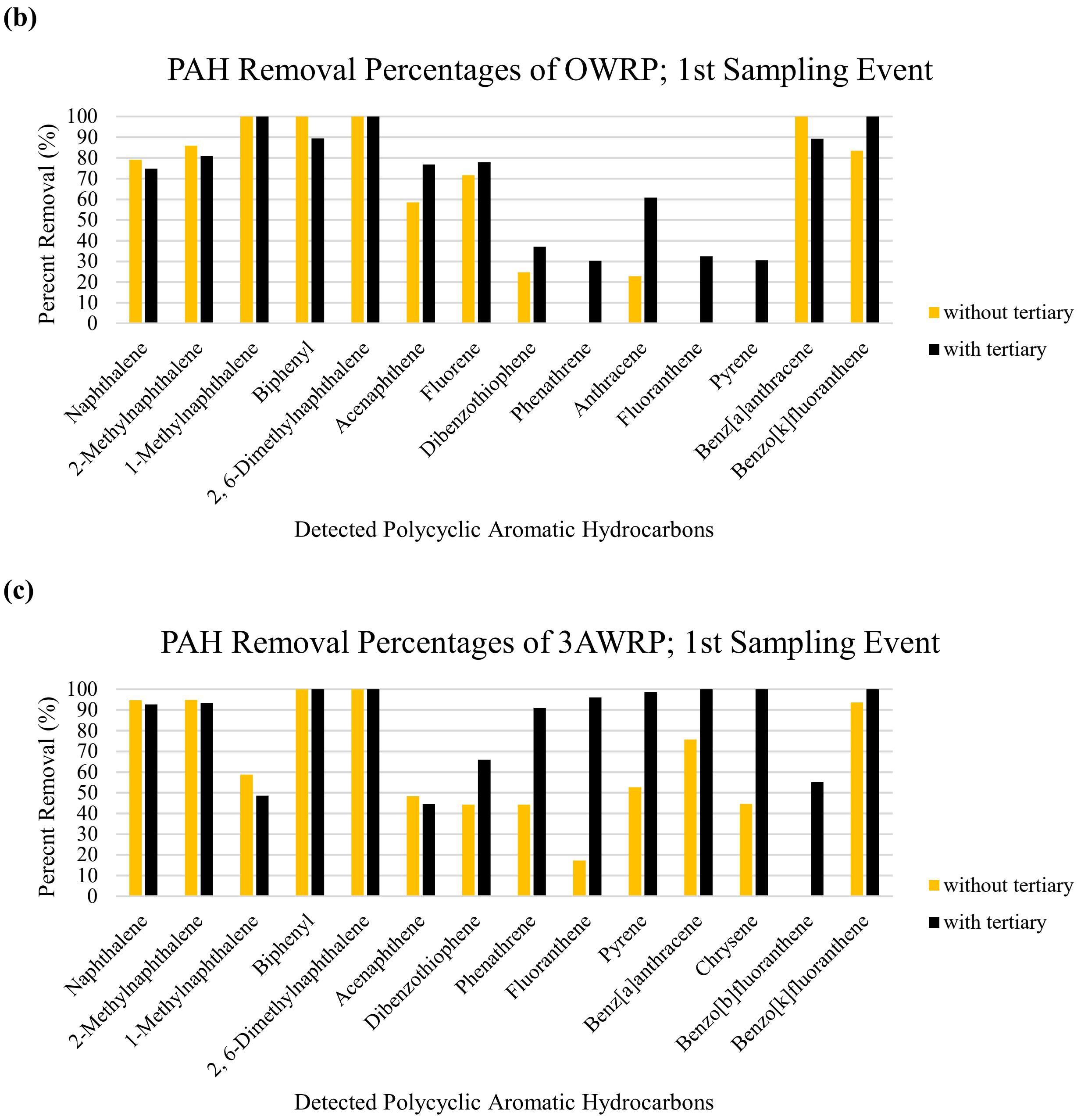


Figure 2: Removal efficiency for PAHs from three WRPs; 1st sampling event.

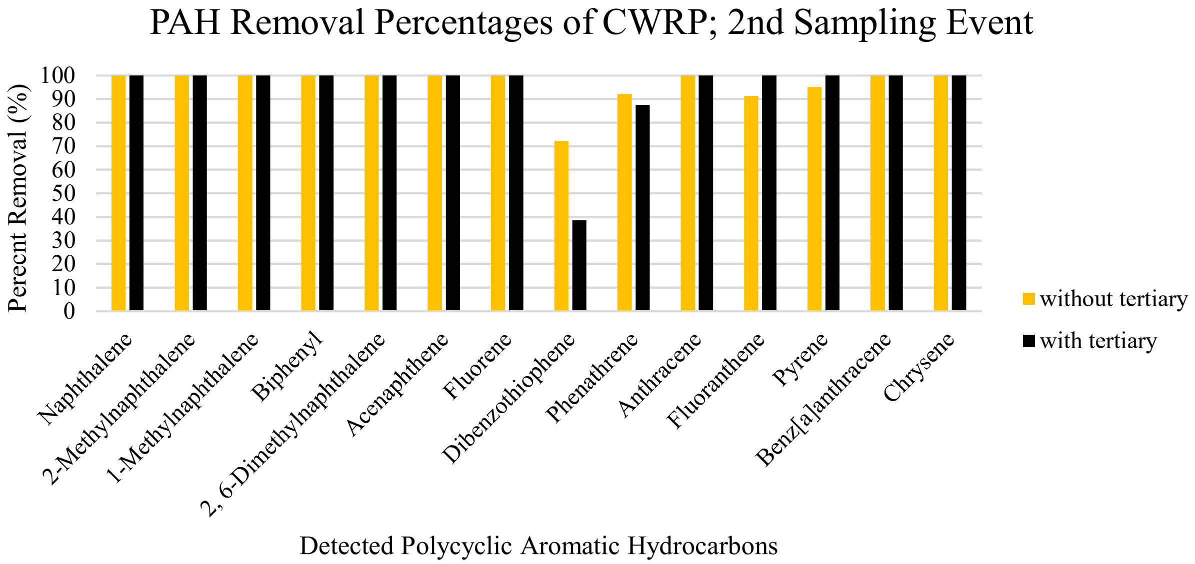
Section 5.A.II.2: 2nd Wastewater Sampling Event Results

3AWRP's tertiary effluent was not sampled for this event. From 25 PAH analysis, two PAHs, dibenzothiophene, and phenathrene, were detected in all samples across three WRPs. Benzo[k]fluoranthene, benzo[e]pyrene, benzo[a]pyrene, perylene, indeno[1,2,3-c,d]pyrene, dibenz[a,h]anthracene, and benzo[g,h,i]perylene were below the minimum detection limits in all 10 wastewater samples. The highest concentrations of PAHs in raw sewage were frequently observed in the 3AWRP and CWRP samples. Three maximum PAH concentrations observed were 62.01, 54.65, and 42.93 ng/L for naphthalene, 2, 6-dimethylnaphthalene, and phenathrene, respectively, from 3AWRP, OWRP, and CWRP, respectively. Generally, PAH occurrences and concentrations from 3AWRP were similar to those of CWRP. Excluding naphthalene, concentration differences between shared PAH occurrences in their raw sewages ranged from

0.02-7.56 ng/L with an average percent difference of 15.94±9.01%. It was also noted that the feed PAH concentrations from OWRP, the plant without a primary clarifier, were generally lower than those of CWRP and 3AWRP. Contrary to the 1st sampling, no PAH concentration increases were detected from OWRP's secondary treatment of raw sewage. The minimum removal performance measuring the difference between raw sewage and tertiary effluent observed at each WRP was 72.14% (dibenzothiophene), 69.22% (phenanthrene), and 47.08% (dibenzothiophene) for CWRP, OWRP, and 3AWRP, respectively.

The PAH concentration in tertiary effluent ranged 2.36-5.38 ng/L and 1.00-10.10 ng/L for CWRP, and OWRP, respectively. Again, the removal efficiency for each PAH varied (Figure 3). The mean±standard deviation of PAH removal efficiency for all compounds were 94.71±16.51%, and 92.18±13.48% for CWRP, and OWRP, respectively, when tertiary treatment was included. Excluding tertiary treatment, the PAH removal performances were 96.47±7.67%, 94.62±11.31%, and 92.20±16.28% for CWRP, OWRP and 3AWRP, respectively (Figure 3). Again, there were a few PAH concentrations in tertiary effluent that were slightly higher than the concentrations in secondary effluent such as dibenzothiophene, and phenathrene.

(a)



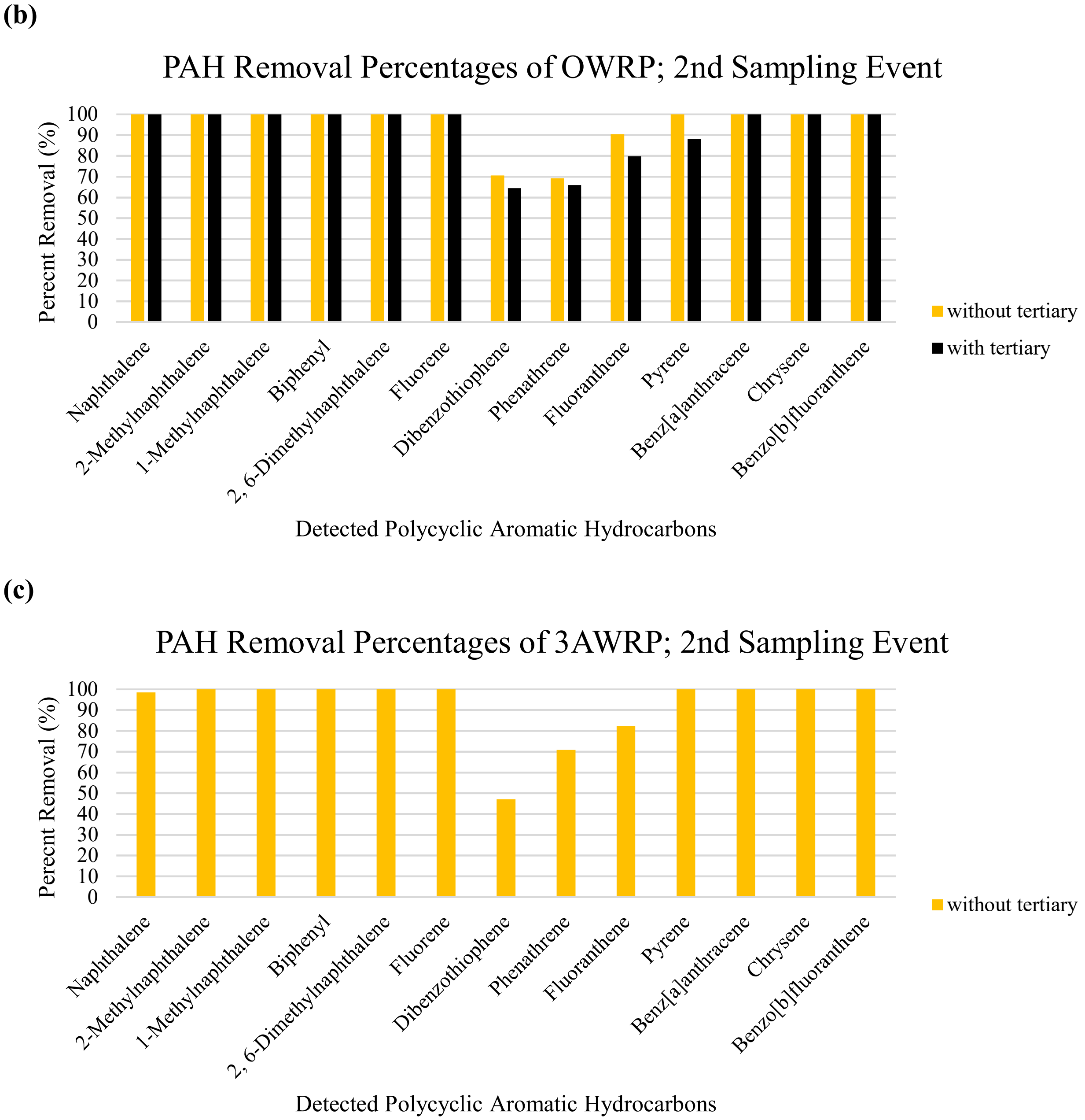


Figure 3: Removal efficiency for PAHs from three WRPs; 2nd sampling event.

Section 5.A.II.3: 3rd Wastewater Sampling Event Results

Unique to this wastewater sampling event, raw sewages and primary effluents were analyzed without going through column chromatography. Because they were not cleaned, the chromatographs were unclear and could only produce results that were indicative of the chemicals' presence. As such, all PAH concentrations from this set were inaccurate and are thus, omitted from this report.

From 25 PAH analysis, only the chemical, naphthalene was detected in all samples across three WRPs. Unique to this sampling event, naphthalene and the rest of the chemicals, 2-methylnaphthalene, 2, 6-dimethylnaphthalene, fluorene, phenathrene, and fluoranthene, were detected in at least one sample from each WRP. In addition, all quantifiable PAH concentrations above 10 ng/L were all observed to be from naphthalene, the lightest PAH compound analyzed.

Furthermore, naphthalene and 2-methylnapthalene were the only two identifiable PAHs in raw sewages from this sampling set.

The PAH concentration in tertiary effluent ranged 2.74-13.67 ng/L, 2.45-15.25 ng/L, and 1.65-15.61 ng/L for CWRP, OWRP, and 3AWRP, respectively. Due to the unclear chromatographs, the WRPs' PAHs removal efficiencies were unavailable for this set. Again, there were a few PAH concentrations in tertiary effluent that were slightly higher than the concentrations in secondary effluent such as fluorene and phenathrene.

Section 5.A.III: Constituents of Emerging Concern

Section 5.A.III.1: 1st Wastewater Sampling Event Results

CECs analyses for these samples were conducted in March, six months after the first analysis. The calibration curve was unavailable during the first analysis due to the late addition of CECs into the research. The samples were only analyzed with an old CECs method from the GC/MS. Thus, the analyses were unable to accurately quantify the concentrations and could only indicate the targeted chemicals' presence. As a result, CECs concentrations were unavailable for this set.

Due to the removal of the targeted CECs during the column chromatography process, raw sewage and primary effluents of this event were unable to detect the three CECs. Chemical analyses for this wastewater sampling event indicated the presence of CECs across all three WRPs in their respective secondary and tertiary effluent.

Section 5.A.III.2: 2nd Wastewater Sampling Event Results

CECs analyses for these samples were conducted in March, two months after the first analysis. The calibration curve was unavailable during the first analysis due to the late addition of CECs into the research. The samples were only analyzed with an old CECs method from the GC/MS. Thus, the analyses were unable to accurately quantify the concentrations and could only indicate targeted chemicals' presence. As a result, CECs concentrations were unavailable for this set.

Due to the removal of the targeted CECs during the column chromatography process, raw sewage and primary effluents of this event were undetected of the three CECs. Chemical analyses for this wastewater sampling event indicated the presence of CECs across all three WRPs in their respective secondary and tertiary effluent, similar to the first sampling.

Section 5.A.III.3: 3rd Wastewater Sampling Event Results

The raw sewage and primary effluents of the third sampling event did not undergo column chromatography. Since they were not cleaned, their chromatographs were unclear. Therefore, the feedback results were only indicative of the chemical presence. All quantified concentrations of raw sewage and primary effluents were inaccurate and are thus, omitted from this report. On the other hand, analysis of the chromatographs indicated that all three CECs were present throughout each of the WRP. The analyzable CECs' concentration ranges were 15.61-584.70 ng/L, and 10.79-478.19 ng/L for secondary and tertiary effluents, respectively. All three WRPs demonstrated the ability to remove triclosan through their tertiary treatment with an average percent removal of 72.15±4.48%.

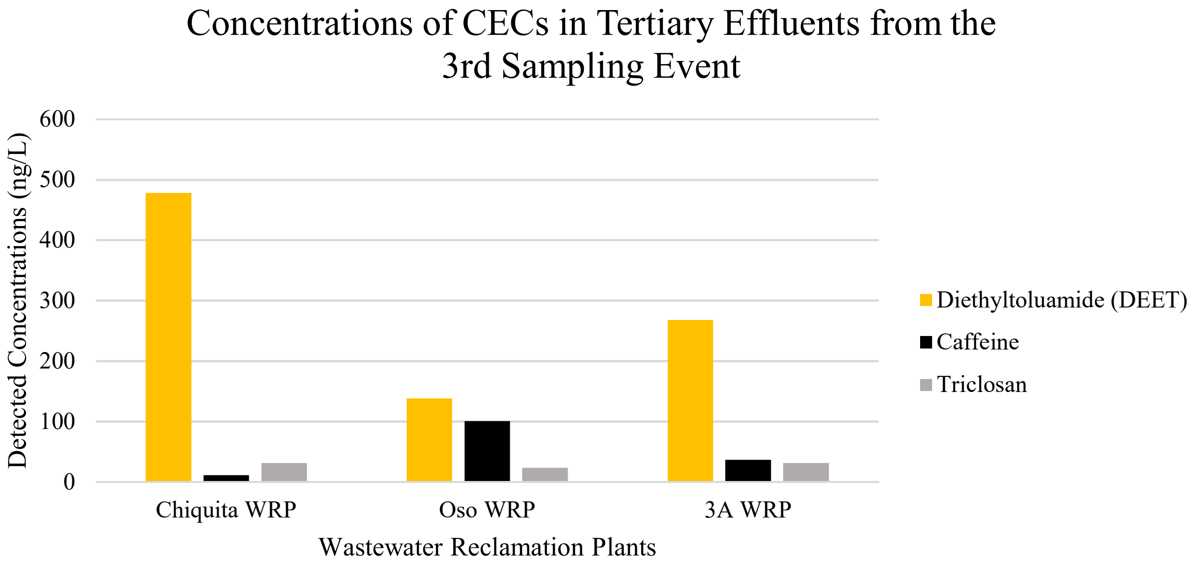


Figure 4: Constituents of emerging concern concentrations in tertiary effluent; 3rd sampling event

Section 5.A.IV: Wastewater Discussion

These results indicated that SMWD's wastewater reclamation plants generally did not receive influents containing organochlorine pesticides. Although some organochlorine pesticides were detected, their concentrations were significantly low. Additionally, biological nutrient removal activated sludge (partially nitrifying) could remove up to 67.67%±7.19% of the organochlorine pesticides. Moreover, tertiary treatment showed consequential impacts of the removal organochlorine pesticides. For PCBs, the results obtained from GC-MS analysis did not detect any of the 53 types of PCBs in the 32 wastewater samples from all three wastewater sampling events.

For PAHs, results indicated that PAH removal with tertiary treatment was the most prominent in the CWRP, followed by 3AWRP then OWRP. Across the three sampling events, CWRP showed the highest PAH removal percentage amongst the three WRP with higher percentage quantities of 2.53 to 22.11. Generally, the PAH concentrations from OWRP were lower than those of CWRP and 3AWRP. Additionally, raw sewage from all three plants generally shared similar PAH chemicals. The largest variety in PAHs observed in any two given raw sewage samples in the same sampling event were four chemicals. In that case, twelve of the same chemicals were detected in both samples. This implied that although each plant received their raw sewage from a different source, the PAH chemicals detected tended to be the same from plant to plant. Intake PAHs appeared to be dependent on the WRPs' service area. Since all three WRP serve suburban areas within Southern Orange County, it is expected that the PAH chemical intake from all three plants would be relatively similar. Additionally, although there were increases in PAH concentrations for chemicals such as naphthalene from the third sampling event post-tertiary effluent, decreases in the quantity of PAH was apparent throughout the three sampling events. Samples that entered tertiary treatment detected at least one less PAH. Furthermore, PAHs with lower molecular weight were observed more than the PAHs with higher molecular weight. More PAH compounds were found in the samples than OCPs and PCBs because PAHs can be originated from both nature and urbanization. Consequently, PAHs naturally occur in a variety of sediment, these increases in PAHs concentration due to tertiary treatment could be due to anthracite coal or other material incorporated into the tertiary treatment filters in the SMWD WRPs. Depending on the condition of the filters, tertiary treatment could either reduce the concentration of all PAHs or reduce all PAHs except for those naturally occurring in the filters' sediments. This is shown by the fluctuations in secondary versus tertiary treatment removal of PAHs when examined across all three sampling events. However, it is important to note that these increases or decreases in PAH concentrations from tertiary treatment was always accompanied by an apparent decrease in the quantity of PAHs detected. These comparisons suggest that tertiary treatment by sediment filtration has a varying removal efficiency that is dependent on which specific PAHs are targeted. As such, a unanimous decrease in all PAHs post-tertiary effluent was unnoted across all sites and throughout all sampling events.

There were apparent influents CECs into the WRPs across all the samples. Despite the CECs removal of raw sewage and primary effluent of the 1st and 2nd sampling events, CECs concentrations were still identifiable in their secondary and tertiary effluents. Coupled with the available CECs detection data from the 3rd sampling event, deductions can be made that all raw sewages and primary samples contained levels of all three CECs. With available CEC data from the 3rd sampling event, DEET was suggested to be the more concerning chemical of the all compounds analyzed due to its high concentrations in tertiary effluent. Caffeine and DEET showed fluctuations between removal and accumulation when subjected to tertiary treatment. Additionally, all three WRPs can remove triclosan.

Section 5.B: Groundwater Extraction Results and Discussion

Section 5.B.I: Organochlorine Pesticides and Polychlorinated Biphenyls

Section 5.B.I.1: 1st Groundwater Sampling Event Results

All nine groundwater samples from the 1st groundwater sampling event did not detect any organochlorine pesticides and polychlorinated biphenyls.

Section 5.B.I.2: 2nd Groundwater Sampling Event Results

Seven out of nine wells showed no indication of any OCPs. Wells SCWD MW-03 and SCWD MW-4D all contained an amount of chlordane-gamma and chlordane alpha. Chlordane-gamma detected amounts were 0.47 and 0.14 ng/L for SCWD MW-03 and SCWD MW-4D, respectively. Chlordane-alpha detected amounts were 0.23 and 0.14 ng/L for SCWD MW-04 and SCWD MW-4D, respectively.

All nine groundwater samples from the 1st groundwater sampling event were undetected of polychlorinated biphenyls.

Section 5.B.I.3; 3rd Groundwater Sampling Event Results

From the 3rd groundwater sampling event, only chlordane-gamma was detected amongst the nine samples. The detected amounts for chlordane-gamma were 0.90, 0.40, and 0.59 ng/L for SJBA MW-01S, SJBA MW-05, and SCWD MW-2S, respectively.

All nine groundwater samples from the 1st groundwater sampling event were undetected of the polychlorinated biphenyls.

Section 5.B.II: Polycyclic Aromatic Hydrocarbons

Section 5.B.II.1: 1st Groundwater Sampling Event Results

PAHs were detected in all samples from the 1st groundwater sampling event. The PAH concentrations in groundwater were one to one hundred folds lower than the PAH concentrations found in wastewater. Although several PAHs were measurable, their concentrations were below the minimum detection limit of 0.1 ng/L. Among all PAHs measured, the concentrations were remarkably low with concentrations below 0.5 ng/L. There were no PAHs shared amongst the 9 wells. Most PAH compounds in groundwater samples had lower molecular weight, similar to the wastewater samples.

Section 5.B.II.2: 2nd Groundwater Sampling Event Results

Four out of nine wells did not detect any of the 53 analyzed PAHs. Although several PAHs were measurable, their concentrations were below the minimum detection limit of 0.1 ng/L. In all PAHs measured, all concentrations were remarkably low. Most PAH compounds in groundwater samples had low molecular weights, which was similar to the wastewater samples. Of the 12 PAHs measured, only 4 were detected in concentrations above 1.00 ng/L.

Section 5.B.II.3: 3rd Groundwater Sampling Event Results

All nine wells detected at least one of the 53 analyzed PAHs. Although several PAHs were measurable, their concentrations were below minimum the detection limit of 0.1 ng/L. SJBA MW-01S contained the largest PAH variety with 19 PAH appearances out of 25 analyzed PAHs. Furthermore, among all PAHs measured, phenathrene from SJBA MW-01S showed the highest concentration in the amount of 39.12 ng/L. No specific chemical was detected across all nine wells. Most PAH compounds in groundwater samples had low molecular weights, which was similar to the wastewater samples. SJBA MW-03, SJBA MW-06, and SCWD MW-4D of the 3rd sampling event also showed similarities in their PAH detections of naphthalene, 2-methylnaphthalene, and 1-methylnaphthalene, the three lightest PAH compounds analyzed. These three wells were a part of the third sampling event but were sampled separately in March, after the storm. All three wells detected the three chemicals with a concentration gradient of high to low based on molecular weights.

Section 5.B.III: Constituents of Emerging Concern

Section 5.B.III.1: 1st Groundwater Sampling Event Results

None of the three CECs were detected in the samples from the 1st groundwater sampling event.

Section 5.B.III.2: 2nd Groundwater Sampling Event Results

Five out of nine samples did not detect the three analyzed CECs. SJBA MW-01S, SJBA MW-03, SJBA MW-08, and SCWD MW-2S were detected of DEET in the amounts of 1.04 ng/L, 1.13 ng/L, 0.94 ng/L, and 1.25 ng/L, respectively. Furthermore, SJBA MW-08 and SCWD

MW-2S were also detected of caffeine in amounts of 1.29 ng/L and 0.37 ng/L. Triclosan was not detected across all nine samples.

Section 5.B.III.3: 3rd Groundwater Sampling Event Results

All nine samples did not detect the three analyzed CECs. SJBA MW-01S, SJBA MW-04, and SCWD MW-4D were detected of DEET in the amounts of 1.01, 1.08, and 0.98 ng/L, respectively. Additionally, caffeine was also detected in SJBA MW-01S, SCWD MW-03, and SCWD MW-2S in the concentrations of 9.18 ng/L, 0.49 ng/L, and 0.92 ng/L. Triclosan was not detected across all nine samples.

Section 5.B.IV: Groundwater Discussion

For organochlorine pesticides, only chlordane-gamma and chlordane-alpha were detected across all three samples. In addition, all concentrations regarding these two compounds were below 1.0 ng/L and out of the seven appearances, four were a part of the second sampling and three were a part of the third sampling. This suggests that organochlorine pesticides were not an immediate issue in the San Juan Basin groundwater supply. Additionally, these low concentrations of chlordane-gamma and chlordane-alpha were detected after the first detection of chlordane-gamma and chlordane-alpha in 3AWRP's tertiary effluent from the 1st wastewater sampling event. It is possible that these trace levels for chlordane-gamma could be traced back to the 1st sampling of wastewater when wastewater was released into the environment for recycling or reused as irrigation water. In turn, the appearance of chlordane-gamma and chlordane-alpha in groundwater could have been a result of released water seeping into the San Juan groundwater basin. These chronologically ordered detections of two chemicals suggests that OCP occurrences in groundwater are indicative of the OCP occurrences in tertiary effluent.

Among all PAHs measured across all three sampling events, all their concentrations were remarkedly low. This indicated that the groundwater in the San Juan basin was of high quality. As noted previously, PAHs found in the groundwater of San Juan Basin were generally chemicals of low molecular weight. Of the 27 total groundwater samples that were extracted across all three events, only the 4 samples, SJBA MW-01S of the 3rd sampling event, SJBA MW-08 of the 2nd sampling event, SJBA MW-08 of the 3rd sampling event, and SCWD MW-04 of the 2nd sampling event detected PAHs beyond pyrene, the 15th heaviest PAH of the 25 analyzed. Additionally, the variety of PAHs detected tended to be consistent throughout all wells for any given sampling event. Consequently, because PAHs are chemicals that are found to be naturally occurring in sediments, these trace detections of PAHs in the groundwater basin could be a result of the nature environment being comprised of the PAHs associated sediments. Moreover, the data suggests that samples received after rainfall tended to contain more PAHs. In these cases, chemicals of lower molecular weights were noted of having higher concentrations than those of higher molecular weights.

CECs were detected in groundwater amongst all samples at trace levels with a concentration maximum of 9.18 ng/L. Consistent across all samples, triclosan was undetected suggesting that triclosan was not a groundwater contamination concern. Additionally, the 1st sampling event for groundwater was undetected of any of the three CECs. Despite the high CECs concentrations range of 10.79-478.19 ng/L detected in tertiary effluent, all the CECs analyzed showed low concentration amounts indicating that CECs transfer into the groundwater basin as not an immediate issue. DEET also appeared to be the more frequent CEC indicated by its appearance ratio of 7:5 against caffeine.

Section 6: Conclusions

The micropollutants, organochlorine pesticides (OCPs), were detected only in wastewater from 3AWRP of the 1st wastewater sampling event, while polychlorinated biphenyls (PCBs) were below the minimum detection limit for all wastewater and groundwater samples. Polycyclic aromatic hydrocarbons (PAHs), were measurable in all samples, but the PAH concentrations in groundwater were significantly lower than those of wastewater samples. The PAH concentrations in both wastewater and groundwater were in a harmless range. Constituents of emerging concerns (CECs) showed the most significant concentrations with the highest values when compared to the endocrine disrupting compounds analyzed. Lower molecular weight PAHs were more commonly observed than higher molecular weight PAHs. Chemicals detected in groundwater appeared to be linked to those in wastewater. Additionally, chemical detections seemed to be dependent on seasons. Lastly, primary and secondary sedimentation associated with tertiary treatment demonstrated the ability to remove OCPs but had a varied removal efficiency based on the specific PAHs or CECs targeted.

Section 7: Closing Statement

Over the year I had spent on this project, I developed an immense interest in the water field, specifically water quality. Visiting the treatment plant for the samples, extracting them, quantifying them, and immersing myself in analyzing this data had taught me the exciting side of water chemistry. As part of my USDA internship, I had to choose a USDA career that I felt most confident in pursuing after college. I knew I wanted to be able to continue to do research in water beyond this project; so, for that reason, the obvious career choice for me was Forest Services: Hydrologist.

My potential career pathway as it pertains to the USDA career choice was to further my educational and practical knowledge of water related applications and issues. With the opportunity that the WRPI and USDA had given me, I was able to develop a passion for water chemistry and pursue it freely within the confines of the immense amount of resources given to me. In the year to come, I set my sights on pursuing more research opportunities as well as interning with the Metropolitan Water District of Southern California to diversify and advance my knowledge of current regional water issues and technology. Despite the ending of this internship, it had given light to other opportunities for me in research and in the public sector. The internship may be ending but the momentum to which it has guided me has not slowed down.

With all that said, I would like to give one final recognition to the United States Department of Agriculture and the Water Resources and Policy Initiative at CSU San Bernardino.

Thank you for allowing me to be a part of this wonderful internship.

Section 8: Appendices

Appendix A: Visuals

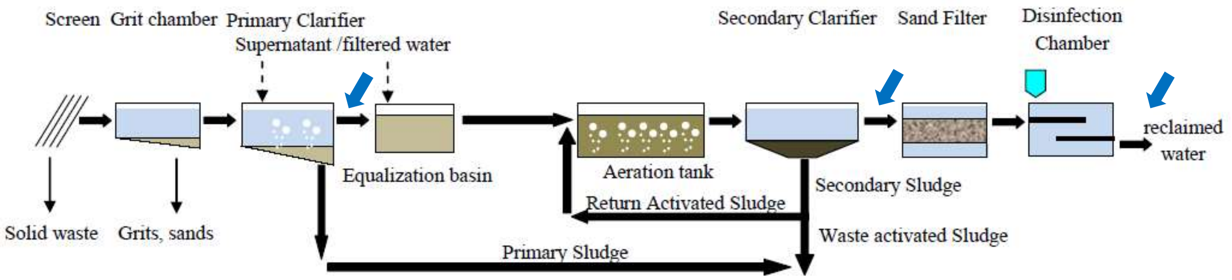


Figure 5: Process flow diagram for a general wastewater treatment plant.

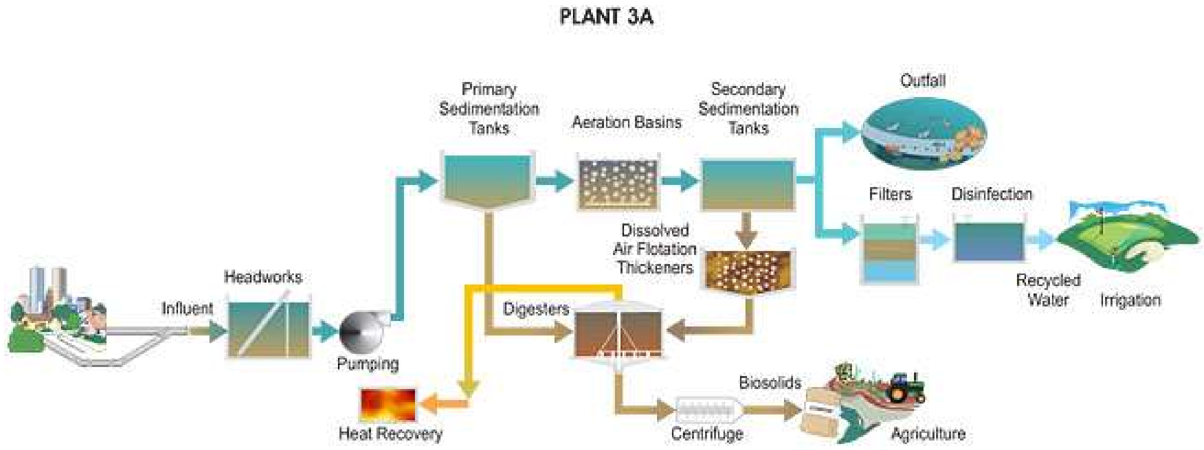


Figure 6: Process flow diagram of the 3A wastewater treatment plant.

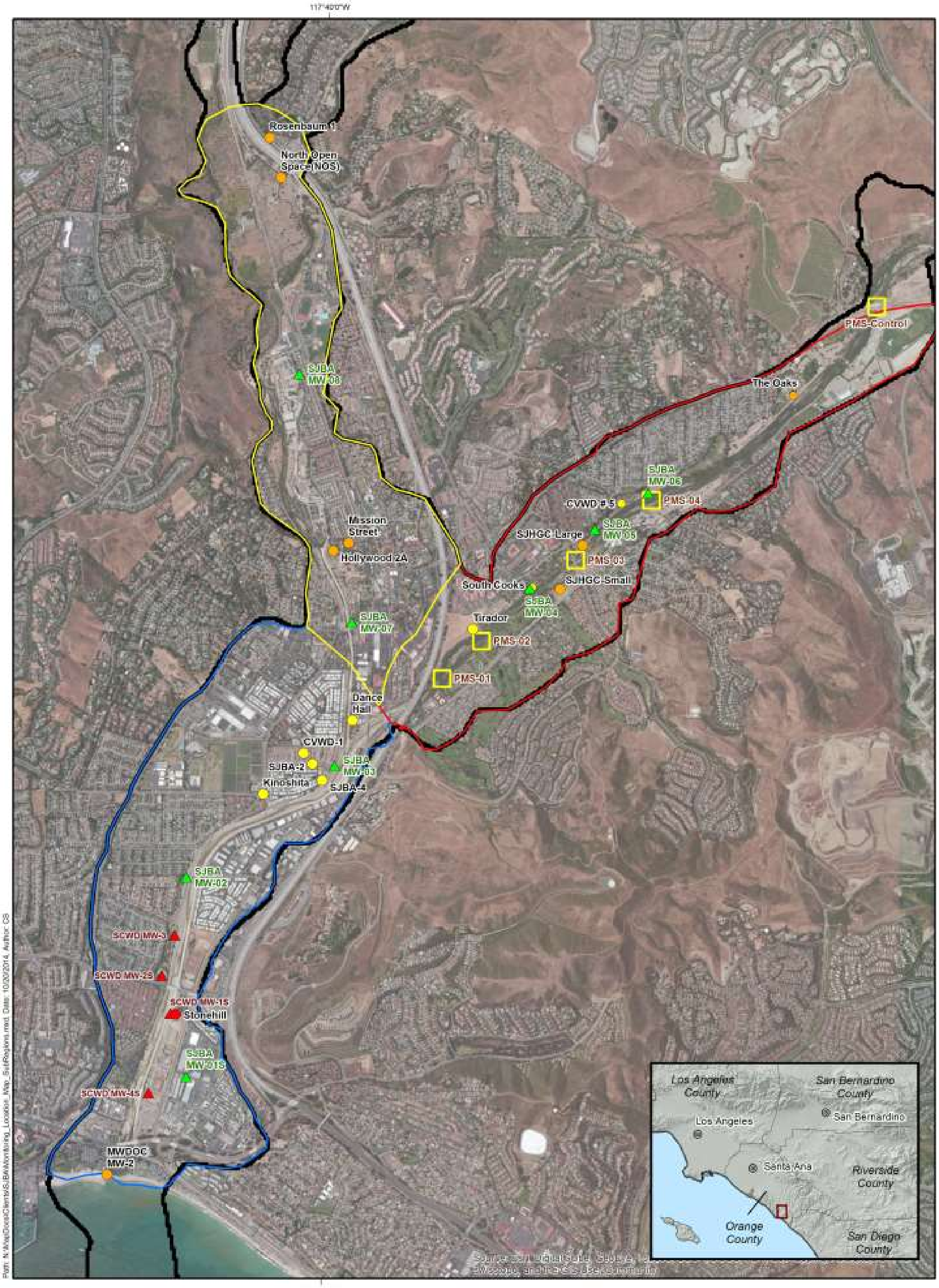


Figure 7: Satellite overview of the San Juan Groundwater Basin.

Appendix B: Acknowledged Organizations' Logo

WRPI

CALIFORNIA STATE UNIVERSITY
SAN BERNARDINO

USDA

THE METROPOLITAN WATER DISTRICT OF SOUTHERN CALIFORNIA

IIRMES

Santa Margarita
Water District

SANITATION DISTRICTS OF LOS ANGELES COUNTY

THE UNIVERSITY OF CALIFORNIA
LET THERE BE LIGHT
IRVINE

Friends of the United Nations

U.S. DEPARTMENT OF THE INTERIOR
BUREAU OF RECLAMATION

City of San Juan Capistrano
ESTABLISHED 1776
INCORPORATED 1961
California

ORANGE COUNTY
WATER DISTRICT
SINCE 1933

ORANGE COUNTY SANITATION DISTRICT
PROTECTING THE ENVIRONMENT

water for people

UCI WEX Center
Research at the Water-Energy Nexus

WEI
WILDERMUTH ENVIRONMENTAL, INC.

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