Detection of Caffeine in the Streams and Rivers within the San Diego Region

Pilot Study

SWAMP
Surface Water
Ambient Monitoring
Program

December 22, 2015

Prepared by:

Lilian Busse, Environmental Scientist
Healthy Waters Branch

and

Carey Nagoda, WRC Engineer
Monitoring Assessment and Research Unit

CALIFORNIA REGIONAL WATER QUALITY CONTROL BOARD
SAN DIEGO REGION
2375 Northside Drive, Suite 100, San Diego, California 92108
Phone (619) 516-1990 • Fax (619) 516-1994
http://www.waterboards.ca.gov/sandiego/

Table of Contents

Introduction

Surface and ground waters that are contaminated by anthropogenic waste sources often contain detectable amounts of caffeine (Bradley et al., 2007). Caffeine is the most frequently consumed non-prescription drug and is found in foods, beverages, and pharmaceuticals. Caffeine originates from plant species that are primarily tropical. The only plant native to North America that contains caffeine is Yaupon (*Ilex vomitoria*), which is found in well-drained sandy soils in the Coastal Plain of the southeastern United States (PLANTS, 2015). The presence of caffeine in water bodies in the northern hemisphere, and more specifically in southern California streams and rivers, strongly suggests that the predominant sources are associated with human activities (Bradley et al., 2007).

Caffeine in the environment can originate from a variety of potential sources. Wastewater treatment plants have the ability to essentially completely remove caffeine before discharging into the environment (Buerge et al., 2003; Phillips and Chalmers, 2009; Froehner et al., 2011). The majority of the caffeine (51-99%) is removed during secondary treatment, where biological processes are often stimulated with the presence of oxygen, and microbes use caffeine as a carbon source during respiration (Thomas and Foster, 2005). Comparisons of typical septic treatment systems with centralized municipal treatment and decentralized advanced aerobic treatment showed that, in fall and winter, septic systems remove significantly less (approximately half) caffeine than the other treatment systems (Du et al., 2014). A recent southern California study showed that failing sanitary sewer system infrastructure can leak into stormwater conveyance systems leading to the discharge of caffeine contaminated into the environment (Sercu, 2001). Similarly, the California Microbial Source Identification Guidance Manual recommends that an evaluation of aging sanitary sewer infrastructure and septic systems should be included in microbial source identification surveys of surface waters (SCCWRP, 2013).

Caffeine's high aqueous solubility allows it to move with water flows rather than partitioning into sediment phase (Bradley et al., 2007), and current technologies enable low (ng/L) concentration detection in stream, wetland, estuarine, and groundwater systems (Peeler et al., 2006). A brief list of caffeine concentration ranges measured in wastewater sources and in the environment is presented in Table 1.

Table 1. Caffeine concentration ranges found in wastewater sources and measured in the environment.

Description	Concentration (μg/L)		Reference
	Max	Min	
Waste Stream Source			
Raw Sewage	300	20	Sauvé et al. (2012)
Septic Tanks	120	100	Seiler et al. (1999)
Treated Effluent (varying treatment levels, from primary to tertiary)	20	0.1	Sauvé et al. (2012)
Surface water downstream of municipal wastewater discharge	1.3 – 2.4 μg/L		Seiler et al. (1999)
Environmental Ranges			
Rivers, lakes, and seawaters	1.5	0.003	Sauvé et al. (2012)
Ground waters	0.08	0.01	Sauvé et al. (2012)
Mainstem of Mississippi River (highest concentrations associated with population centers)	0.07	0.01	Seiler et al. (1999)

Caffeine has been established as a suitable surrogate marker for untreated wastewater contamination of surface waters (Buerge et al., 2003; Buerge et al., 2006; Hillebrand et al., 2012) and is considered one of several reliable surrogate indicators for evaluating the advanced wastewater treatment efficiencies used for producing recycled water (SAWPA, 2014). Caffeine has also been demonstrated as a successful predictor of specific contaminants, such as fecal coliform (Daneshvar et al, 2012; Sauvé et al., 2012) and nitrate (Henjum et al., 2010; Peeler et al., 2006). In addition to sewage spills, leaky sewer pipes, poorly maintained septic systems, and other means of sanitary sewer flows, the presence of caffeine in surface waters may be attributable to stormwater runoff containing wastewater influences, food waste or beverage containers from trash receptacles, recycled water over-irrigation (e.g., urban landscape irrigation), human waste at homeless encampments, or other anthropogenic activities.

The concentration of caffeine may be influenced by environmental conditions. For example, caffeine may undergo sorption, chemical transformations, phototransformations, and biotransformations under aerobic and anaerobic environments (Bradley et al., 2007; Daneshvar et al., 2012; Seiler et al., 1999). Its half-life in surface waters has been reported to range from 5.3 to 24 hours (Bradley et al., 2007). Because these transformations have the potential to occur, the concentration of caffeine detected in samples may be a conservative estimate of the source contributions. Additionally, temporal variations of caffeine concentrations have been observed in rivers and streams, which means that flow-proportional sampling is required for robust quantitative assessments (Buerge, 2006).

According to traditional toxicity tests, caffeine alone does not appear to have toxic effects on aquatic organisms at the typical concentrations found thus far in the environment. However, environmentally relevant concentrations (e.g., 0.05 μg/L and 0.2 μg/L caffeine) have been shown to affect gill tissue of the California mussel (*Mytilus californianus*) at the molecular level, and little is known about effects of long-term exposure (Rodriguez del Rey et al., 2011). While caffeine most likely does not bioaccumulate and is not considered an acute threat, the detection of caffeine in water bodies often means the co-occurrence of organic wastewater compounds, including pharmaceuticals, pesticides, plasticizers, and other emerging chemicals of concern (Moore et al., 2008; Quinn et al., 2009; Richards and Cole, 2006; Smith and Burgett, 2005). Pharmaceuticals are of particular concern since they are designed to react biologically at low concentrations and are continuously being added to aquatic environments at rates often higher than their rate of transformation (Waiser et al., 2011). The persistence of such chemicals in aquatic ecosystems results in chronic exposure to organisms that can lead to detrimental effects in a species. Although particular chemicals may not produce toxic responses individually, aquatic organisms are constantly exposed to a combination of compounds. This suite of chemicals can have additive effects, producing greater risks that should be considered (Quinn et al., 2009, Waiser et al., 2011).

The presence of wastewater compounds in surface waters contributes to the degrading quality of inland and coastal waters and threatens human and ecosystem health. The presence of wastewater sources in surface waters may also result in economic losses when recreational and/or commercial areas need to be closed due to elevated pollution levels. Preventing untreated wastewater from entering water sources is important, especially where drinking water sources are limited and surface waters are used for recreational purposes.

The purpose of this study was to evaluate the presence of caffeine in San Diego Region surface water and establish a preliminary understanding of the extent that human activities are having on the stream systems in the San Diego Region. The study goals included:

1) Determining caffeine presence at randomly selected (probabilistic) and targeted surface water sites throughout the region
2) Evaluating if differences in caffeine presence and concentrations are associated with different site types, including:
    1. Treated effluent discharges (not surface waters)
    2. Developed areas within a wastewater treatment service area
    3. Developed areas near septic system(s)
    4. Open space
    5. Agricultural lands
    6. Sites receiving raw sewage

The San Diego Region Surface Water Ambient Monitoring Program conducted this study using a cooperative approach with regional and ambient monitoring programs. The samples collected for this study were collected through the in-kind services of the project participants and the data provided have been used to answer the study questions to the extent practical.

Study Area

The San Diego Region is classified as semi-arid, with a Mediterranean climate. Lower elevations are characterized by chaparral, oak woodlands, and sage scrub. Higher elevations are characterized by pine forests, where the mountain ranges include peaks that exceed 6,000 feet. The typical rainy season is October to April, with an average annual rainfall of about 10 inches. Snow is common only in the high mountains.   Wildfires and drought are frequent in the San Diego Region, with extensive fires occurring in 2003 and 2007 throughout much of the area (SCCWRP, 2011).

Much of the land at higher elevations in the San Diego Region is undeveloped and remains protected in national forests and a network of national, state, and county parks. Land at lower elevations has been pervasively altered by urbanization or conversion to agriculture. Urban development extends almost entirely along the coastal strip, with the exception of the Camp Pendleton Marine Corps Base in northern San Diego County. By area, the San Diego Region consists of undeveloped open space (68%), urbanization (23%), and agricultural use (9%).

This study focuses on the San Diego Region (California Regional Water Quality Control Board, Region 9), which includes all watersheds north of the Mexican border and south of the Santa Ana River, and west of the desert surrounding the Salton Sea and Imperial County border. The San Diego Region is bounded on the east by the Peninsular Range Mountains and spans westward to the Pacific Coast. It encompasses a total drainage area of about 4,000 mi^2 and includes most of San Diego County as well as portions of southern Orange and southwest Riverside Counties.

A map of the San Diego Region including the major watershed boundaries is shown in Figure 1.

Figure 1. Map of the San Diego Region watersheds.

Methods

Study Sites

A total of eighty-five (85) sample sites were included in this study, most of which are streams throughout the San Diego Region considered as waters of the State. Samples obtained for caffeine analysis were collected opportunistically from 2008 to 2015 in conjunction with three projects that are a part of the region-wide Surface Water Ambient Monitoring Program (SWAMP). These projects include:

1) Bioassessment Monitoring Program
2) Pilot Study on Pharmaceutical and Personal Care Products (PPCP) in the San Diego Region
3) Stormwater monitoring Coalition (SMC) Regional Monitoring Program

Caffeine samples results were collected during each of these projects in order to increase the geographic distribution of sites and to capture a more robust spatial and temporal representation of the San Diego Region.

A brief description of each monitoring project is provided in the following sections. Sample sites included in this study are located in all sub-watersheds except Pueblo San Diego (908). A map of the sampling locations is provided in Figure 2, which shows the spatial distribution of sample sites on per study basis.

Bioassessment Monitoring

The Bioassessment Monitoring is a San Diego Regional Water Quality Board sponsored project to evaluate the biological condition of streams within the region. The water quality sample effort included as part of the targeted site assessments provided twenty-eight (28) samples for this study in 2008. The Bioassessment Monitoring project included a wide range of environmental conditions consisting of reference condition streams to streams heavily influenced by anthropogenic activies.

PPCP

The Pilot Study on Pharmaceutical and Personal Care Products (PPCP) is a San Diego Regional Water Quality Control Board sponsored project to evaluate the extent to which a wide range of PPCPs chemicals, in addition to caffeine, occur within the region's streams. The PPCP project contributed twenty-three (23) samples in 2010, 2011, and several more in the 2015 follow-up survey (Busse, 2010). Sites used in the PPCP study were specifically chosen to fall into one of the following three categories: 1) untreated waste or wastewater treatment plant effluent, 2) influenced by the presence of septic tanks, or 3) low human activities/impact. A targeted approach was used to select sampling sites, focusing on three major rivers plus reference conditions sites and additional sites based on specific discharge attributes that could be used to extend the screening process throughout the Region. The project sites with specific discharge attributes include 1) the San Diego River with the Padre Dam Water Recycling Facility, 2) a site located on the Santa Margarita River due to a large number of on-site sewage treatment facilities (septic systems) that exist in its watershed, and 3) the Tijuana River due to its well-known history of receiving wastewater containing untreated human waste in the flows which transcend the international border.

SMC

The Stormwater Monitoring Coalition Regional Monitoring Program (SMC) is a collaborative effort of federal, state, and local agencies to evaluate the water quality and biological conditions of southern

California streams. The SMC regional program includes each of the hydrologic units in the San Diego Region. The SMC program provided forty-four (44) samples from 2009 through 2012. The sampling sites used by the Stormwater Monitoring Coalition were randomly distributed and spatially-balanced. A statistically-driven technique was used for sufficient distribution throughout the Region, with the purpose of evaluating perennial, wadeable streams (Strähler second-order or higher). The three land use categories used included urban, agricultural, and open space. A more detailed description on how the Region was stratified may be found in Technical Report 639 (SCCWRP, 2011).

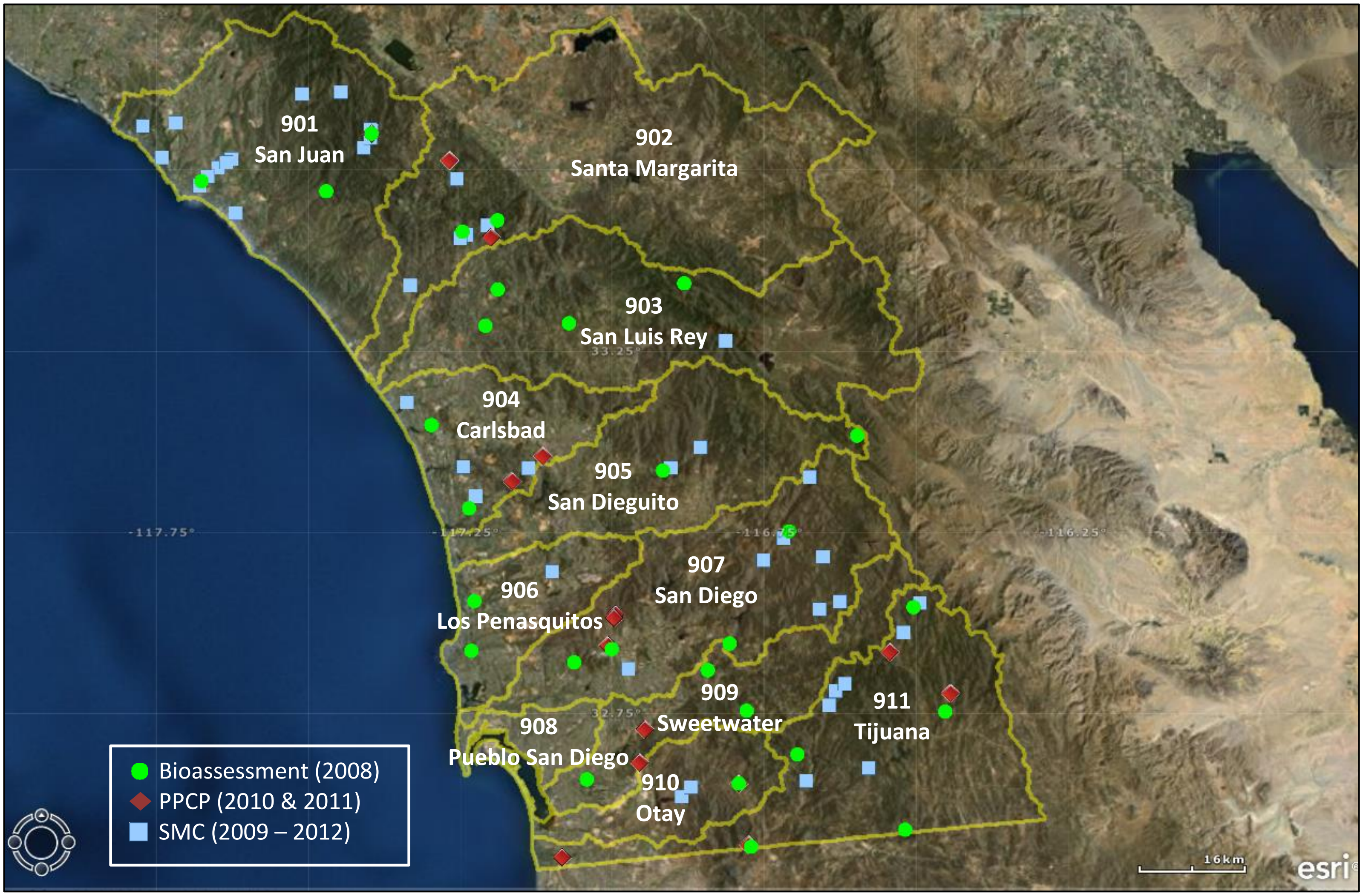


Figure 2. Study map of sites sampled for caffeine.

Sample Collection Timing

The majority of sampling for the three studies occurred during the spring months, with some sampling events taking place from late February (in 2011) through early November (in 2010). The Bioassessment Monitoring and SMC Regional Monitoring Program samples were collected during dry-weather non-storm event based conditions when normal base flow conditions were present in the streams. In contrast, the PPCP project conducted sampling during both winter/wet and summer/dry seasons. The monthly and annual time periods in which samples were collected are provided in Table 2 to show the temporal distribution of sites per study and year.

Rainfall occurrence was also investigated with the caffeine data generated by the PPCP study. Rainfall data was obtained from the Western Regional Climate Center data (http://www.wrcc.dri.edu/). Caffeine concentrations were compared with total inches of rainfall the 24 hours, 48 hours, week, and month prior to sample collection.

Table 2. Monthly time periods for sampling events on an annual and per study basis.

YEAR	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
2008 (Bioassessment)					28							
2009 (SMC)				2	17							
2010 (PPCP & SMC)					15	4				6	2	
2011 (PPCP)		5	10									
2012 (SMC)					6							
Total	0	5	10	2	66	4	0	0	0	6	2	0

Sample Collection Procedures

Clean techniques were used to minimize sample contamination. The sampling procedures for caffeine adopted ambient monitoring sample collection procedures based on guidance provided by USEPA Method 1669 (EPA 1669) and Standard Operating Procedures (SOPs) developed by SWAMP(MPSL-DFG, 2007). This study's reliance on field teams to apply EPA and SWAMP sampling procedures was intended to generate study data that minimizes the potential for opportunities for sample contamination in the field.

Samples were collected at approximately mid-stream locations, at least 0.3 meters from the bank and at a depth of 0.1 meter. Pre-cleaned polyethylene or Teflon containers were filled and stored at 4°C in the dark (holding time of 48 hours) until arrival at the laboratory for caffeine extraction. Caffeine was extracted from the samples within seven days of collection, and the samples were analyzed within forty days of extraction.

Caffeine Extraction and Laboratory Analysis

The caffeine extractions and analyses via liquid chromatography were conducted by the CA Department of Fish and Wildlife, Water Pollution Control Laboratory in Rancho Cordova. Caffeine was extracted from the samples and analyzed following a modified version of EPA Method 1694 (EPA, 2007). A brief description of the modified extraction procedure is provided in this section.

The extraction procedure included the following steps:

1) Condition extraction cartridges (Waters Oasis® HLB 6cc) with 5 mL of methyl alcohol (MeOH) and then 5 mL of deionized (DI) water by gravity.
2) Pass 100 mL of the sample water through the cartridge under a vacuum rate of 5 mL per minute and dry cartridge using Kimwipes®.
3) Continue drying the cartridge for about 3 minutes under a vacuum.
4) Elute cartridge with 2 mL of 90%MeOH/10%HPLC with 0.1% formic acid (FA).
5) Filter the collected eluate through an Acrodisc® CR 13-mm syringe filter with 0.45-μm PTFE membrane into gas chromatography (GC) vials.

The extracted samples were analyzed using an Agilent 1200 liquid chromatograph with an Agilent Eclipse Plus C18 column connected to a triple quadrupole tandem mass spectrophotometer 6410A (Agilent Technologies, Santa Clara, CA). Samples were injected into the column with 20 μL of standard solution at a flow rate of 0.2 mL/minute. The mobile phase was initiated and then brought back to initial conditions for a 5-minute equilibrium. The mass conditioning was followed by High Performance Liquid Chromatography (HPLC), using an electron spray ionization source (ESI) at 350°C and a flow rate of 12 L/min. The instrument was adjusted when necessary and calibrated appropriately to achieve proper detection of standards with high sensitivity. Data was analyzed using Mass Hunter Workstation, Version 4.0quantitative analysis software.

The caffeine extraction and sample analysis methods used for this study resulted in a method detection limit (MDL) of 0.020 micrograms per liter (μg/L), or parts per billion, with the reporting limit set at a value of 0.050 μg/L.

Summary of Quality Assurance and Quality Control (QA-QC) Procedures for this Study

The data presented in this report includes project-specific and study-based quality assurance and quality control procedures. A set of specific protocols were followed for each of the projects providing data to this study to ensure quality results are used in the data analyses and to support the interpretations developed and the conclusions assessed about the conditions of the San Diego Region surface waters.

The protocols used for the Bioassessment study are detailed in the Quality Assurance Project Plan (QAPP) for SWAMP Monitoring Project 2007/2008 (Busse, 2007). The data quality evaluation and data reporting, procedures specified in SWAMP QAPrP (2008), were employed for the PPCP and SMC studies. The PPCP project includes a QAPP for the sampling activities that generate the additional data included in this study (PPCP QAPP (2010)).

Sample results that did not conform to QA-QC guidelines detailed in the above documents were not included in this study. The sample results collected as part of the PPCP project on September 29, 2011 were suspected to have contamination issues and as a result did not meet the QA-QC requirements for this study and were excluded from the dataset included with this report.

Land Use Analyses

The land uses within the coastal rivers and streams of the San Diego Region grouped into the 11 hydrologic units (Hus) are listed in Table 3. Each hydrologic unit listed includes the watershed name, corresponding hydrologic unit codes (HUC), and the distribution of major land uses within the drainage area. As shown by the land use information, many of the smaller coastal watersheds are completely urbanized, while open space areas predominates the inland watersheds of the region. Agricultural land use is primarily contained within the San Luis Rey, San Dieguito, Carlsbad, and Otay hydrologic units, which represents 67 percent of the overall agricultural areas within the San Diego Region.

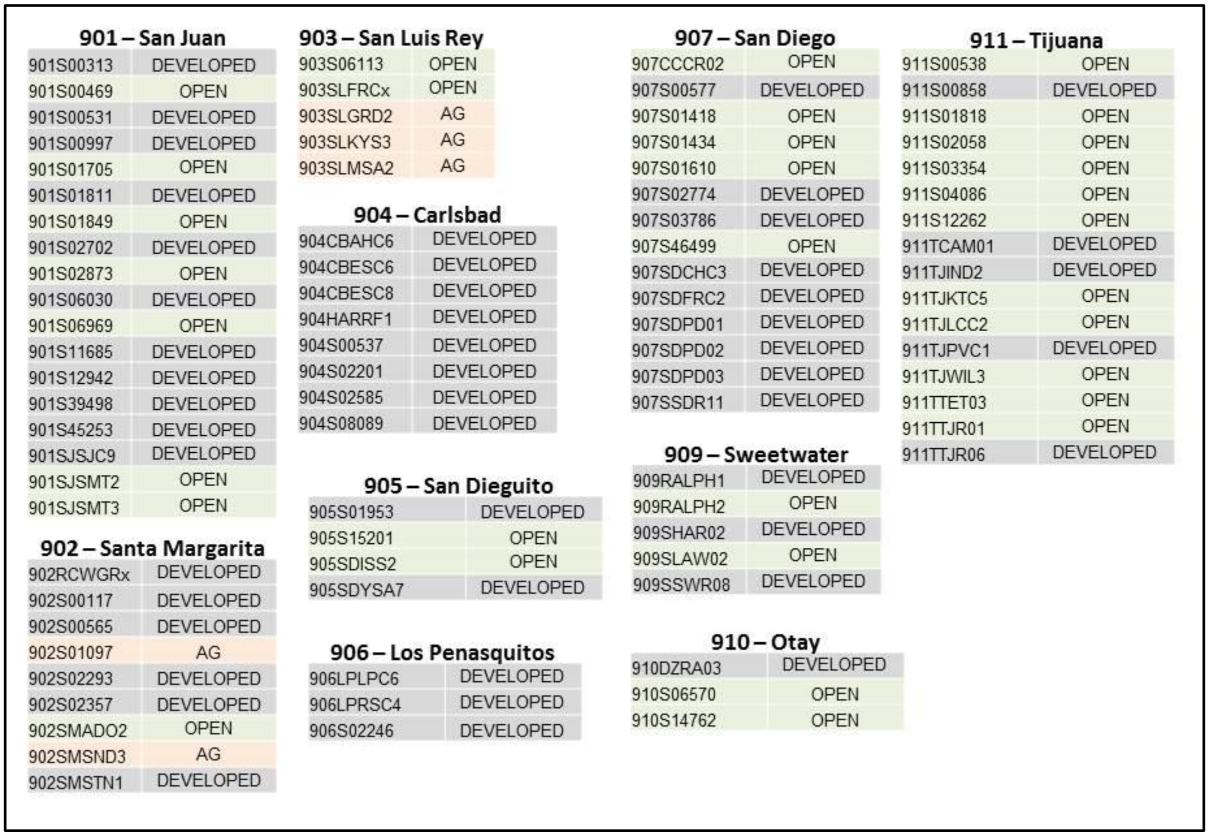
Table 3. Land uses for Region 9 hydrologic units (data from SANDAG, 2008 and SCCWRP, 2008).

Watershed	HUC	Area (mi^2)	% Open	% Developed	% Agricultural
San Juan	901	496	92	7	1
Santa Margarita	902	750	81	13	6
San Luis Rey	903	560	61	15	24
Carlsbad	904	211	38	50	12
San Dieguito	905	346	18	61	21
Los Peñasquitos	906	162	43	53	4
San Diego	907	440	72	26	2
Pueblo San Diego	908	56	12	88	0
Sweetwater	909	230	67	29	4
Otay	910	154	70	20	10
Tijuana	911	463	90	6	4
TOTAL		3868	68	23	9

Study Site Categorization

Each of the sample sites used for this study were categorized by its dominant land use type of open space, agricultural, or developed (urbanized). The dominant land use category was determined using the methods adopted by the Stormwater Monitoring Coalition Regional Monitoring Program (SCCWRP, 2007 and Mazor et al., 2011) for regional consistency purposes. Five-kilometer (5K) buffers were drawn around National Hydrography Dataset (NHD) Plus stream segments, and percentages of land cover types were determined using the National Oceanic and Atmospheric Administration (NOAA) 1996 Coastal Change Analysis Program (C-CAP) land cover data layer. The land cover types considered in this exercise included urban, agricultural, roadways (Code 21), and open. To determine the percentage of development in the watershed at a 5K-scale, the following percentages of land cover types were added: urban, agricultural, and roadways (Code 21). If the total percentage of development was less than 5%, then the sample site's 5K watershed was classified as open. If the total percentage of developed land was greater than 5% and the percentage of land used for agricultural purposes was greater than 25%, then the sample site's 5K watershed was classified as agricultural. The remaining 5K watersheds were classified as developed. The 5K-scale watershed determinations for each sample site used in the study are provided below in Table 4.

Table 4. Watershed determinations at the 5K-scale for each sample site (AG = agricultural).



901 – San Juan

901S00313	DEVELOPED
901S00469	OPEN
901S00531	DEVELOPED
901S00997	DEVELOPED
901S01705	OPEN
901S01811	DEVELOPED
901S01849	OPEN
901S02702	DEVELOPED
901S02873	OPEN
901S06030	DEVELOPED
901S06969	OPEN
901S11685	DEVELOPED
901S12942	DEVELOPED
901S39498	DEVELOPED
901S45253	DEVELOPED
901SJSJC9	DEVELOPED
901SJSMT2	OPEN
901SJSMT3	OPEN

902 – Santa Margarita

902RCWGRx	DEVELOPED
902S00117	DEVELOPED
902S00565	DEVELOPED
902S01097	AG
902S02293	DEVELOPED
902S02357	DEVELOPED
902SMADO2	OPEN
902SMSND3	AG
902SMSTN1	DEVELOPED

903 – San Luis Rey

903S06113	OPEN
903SLFRCx	OPEN
903SLGRD2	AG
903SLKYS3	AG
903SLMSA2	AG

904 – Carlsbad

904CBAHC6	DEVELOPED
904CBESC6	DEVELOPED
904CBESC8	DEVELOPED
904HARRF1	DEVELOPED
904S00537	DEVELOPED
904S02201	DEVELOPED
904S02585	DEVELOPED
904S08089	DEVELOPED

905 – San Dieguito

905S01953	DEVELOPED
905S15201	OPEN
905SDISS2	OPEN
905SDYSA7	DEVELOPED

906 – Los Penasquitos

906LPLPC6	DEVELOPED
906LPRSC4	DEVELOPED
906S02246	DEVELOPED

907 – San Diego

907CCCR02	OPEN
907S00577	DEVELOPED
907S01418	OPEN
907S01434	OPEN
907S01610	OPEN
907S02774	DEVELOPED
907S03786	DEVELOPED
907S46499	OPEN
907SDCHC3	DEVELOPED
907SDFRC2	DEVELOPED
907SDPD01	DEVELOPED
907SDPD02	DEVELOPED
907SDPD03	DEVELOPED
907SSDR11	DEVELOPED

909 – Sweetwater

909RALPH1	DEVELOPED
909RALPH2	OPEN
909SHAR02	DEVELOPED
909SLAW02	OPEN
909SSWR08	DEVELOPED

910 – Otay

910DZRA03	DEVELOPED
910S06570	OPEN
910S14762	OPEN

911 – Tijuana

911S00538	OPEN
911S00858	DEVELOPED
911S01818	OPEN
911S02058	OPEN
911S03354	OPEN
911S04086	OPEN
911S12262	OPEN
911TCAM01	DEVELOPED
911TJIND2	DEVELOPED
911TJKTC5	OPEN
911TJLCC2	OPEN
911TJPVC1	DEVELOPED
911TJWIL3	OPEN
911TTET03	OPEN
911TTJR01	OPEN
911TTJR06	DEVELOPED

The distribution of sample sites and land use in San Diego County on a HU basis is displayed in the map below (Figure 3) for comparison. SANDAG land use designations are shown in blue ("urban area") and orange ("agriculture commodity"). The agriculture-commodity layer was created to assist in queries to geographical location where pesticide may be used on crops. The map does not include the "agriculture preserve" layers since they include land preserved for both agriculture (plant and animal production for commercial purposes) and open space.

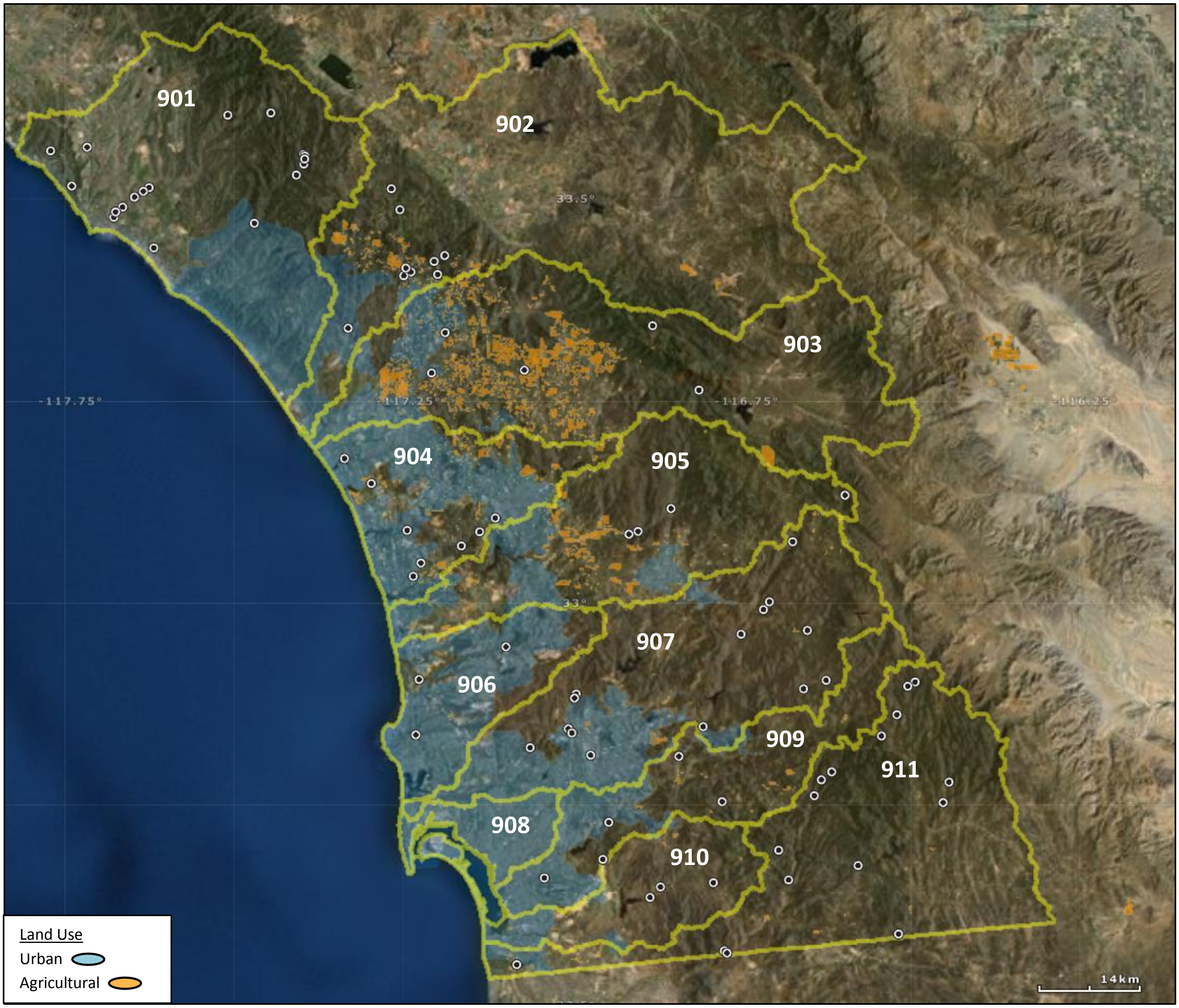


Figure 3. Regional distribution of sites in comparison to dominant land use classification (SanGIS layers).

Land uses within the group of developed category sites were further differentiated using a secondary set of criteria. Developed sites were further parsed into the following categories:

- Developed and within wastewater treatment plant (WTP) service area
- Developed with known septic systems
- Developed unknown (unable to confirm septic system presence or wastewater treatment plant service)

Wastewater treatment plant service areas were determined using GIS layers that were provided by SanGIS (sanitation district boundaries), Vallecitos Water District, Leucadia Water District, and the Cities of San Diego, Oceanside, Vista, Carlsbad, and Encinitas.

Septic site locations within the study area were determined several ways. The County of Orange provided a GIS layer with actual locations of septic systems. Some sites, used for the PPCP study, were previously determined as being near septic systems. The Carlsbad HU (904), is documented as having a high number of septic systems, so GIS layers from Leucadia Wastewater District (showing properties outside of the sewer district) and the City of Vista (showing properties with non-sewer permits) were used to confirm septic system presence. The portions of the watersheds outside of the incorporated zoning area in Riverside and San Diego Counties could potentially have septic systems present, but specific locations could not be confirmed. Therefore, these sites and others that did not fall into confirmed sewer service areas, were grouped together and classified as "developed unknown."

A watershed land use category of untreated waste water (raw sewage) was created for sites in the Tijuana River watershed with known or potential cross-border waste flows. An additional category, wastewater treatment plant effluent, was also created and used for the effluent data generated by the PPCP study. Table 5 provides a summary of the final land use categories used for analyses in this study.

Table 5. Land use categories used for analyses.

1	***Developed – Within Wastewater Treatment Service Area***
2	***Developed - Near Septic***
3	***Developed - Unknown***
4	***Agricultural***
5	***Open***
6	***Raw Sewage Impacted***
7	***Wastewater Treatment Plant Effluent***

Results

Temporal Detections of Caffeine

Caffeine was detected year-round in the San Diego Region (Table 6). Caffeine appears to be ubiquitous, present during both wet (October-April) and dry (June-September) seasons. Similarly, follow-up PPCP sampling events that were conducted in calendar year 2015 found that caffeine is nearly always present and can be regularly detected in stormwater runoff (Busse, 2010 and Yu, in progress).

Table 6. Number of caffeine detections per sample collected each month per year. Data are presented as detected over the number of samples collected (detected/collected).

YEAR	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
2008 (Bioassessment)					15/28							
2009 (SMC)				1/2	10/17							
2010 (PPCP & SMC)					7/15	2/4				1/6	2/2	
2011 (PPCP)		5/5	6/10									
2012 (SMC)					6/6							

Caffeine Concentration in Relation to Antecedent Rainfall

Caffeine concentrations from the PPCP project study were compared with rainfall totals for each of the sampling locations. No significant correlations were observed between the caffeine concentrations and total inches of rainfall. The rainfall analyses indicate that the ambient concentrations of caffeine increase as a function of increasing antecedent rainfall. The caffeine concentrations in relation to 24 hours, 48 hours, one week, and one month antecedent dry weather periods is shown in Figure 4.

The rainfall correlative analyses indicate that caffeine concentrations are found at maximum levels at approximately one month prior to the sampling event.

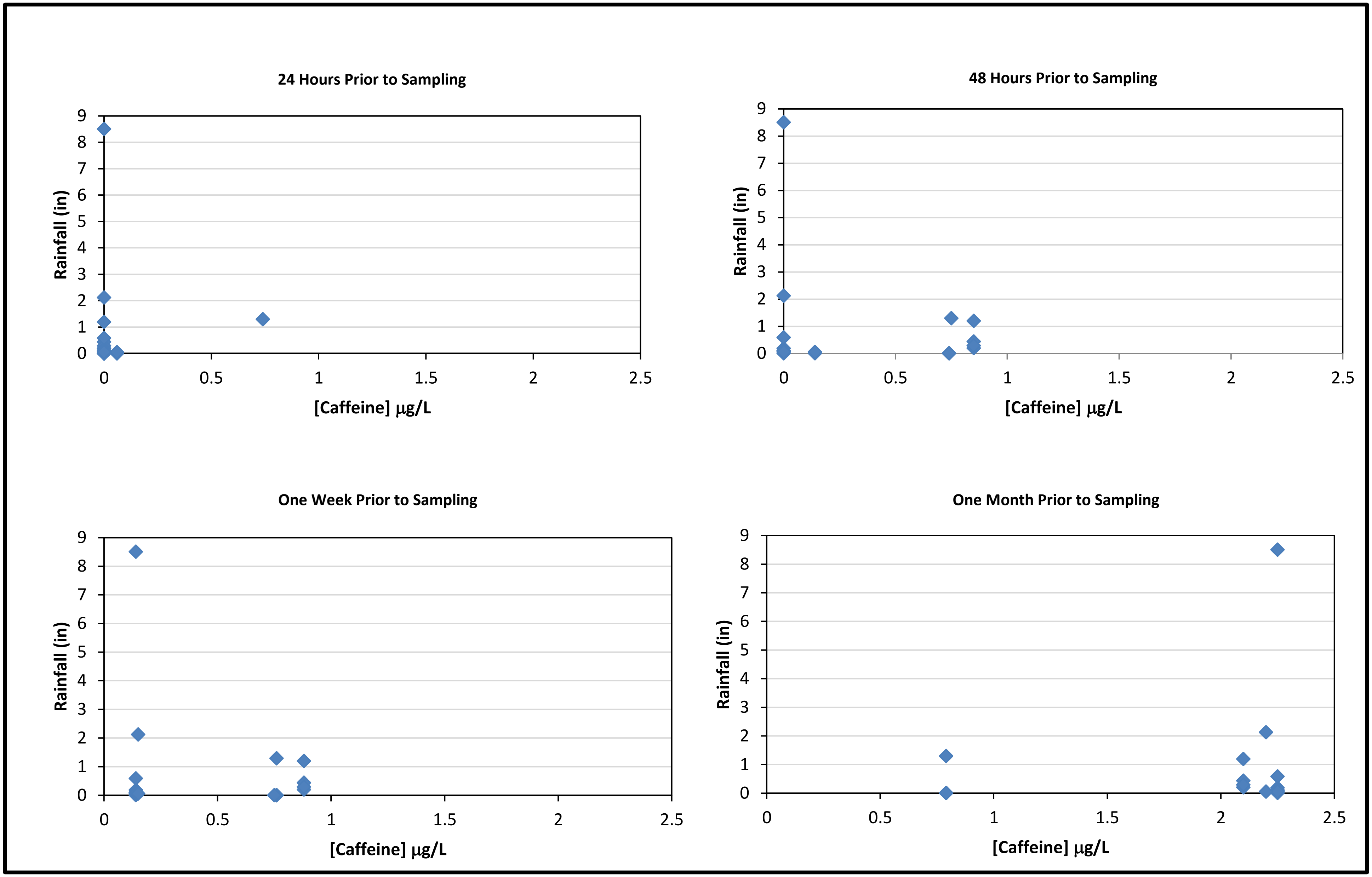


Figure 4. Rainfall total and antecedent dry weather time periods in relation to caffeine concentrations from the PPCP project.

San Diego Region Detections of Caffeine

Detections occurred throughout the region and in each HU, except for San Luis Rey (903), where the majority of the watershed is open space and agricultural land uses.

Caffeine was detected in a little over half of the samples (56 percent), collected from the eighty-five (85) sampling sites located throughout the San Diego Region. Caffeine was present at forty-nine (49) sample sites (57.6 percent) and in fifty-three (53) of the ninety-four (94) total samples (56.4 percent) analyzed. Caffeine concentrations throughout the San Diego Region ranged from less than 0.050 µg/L (<0.050 µg/L) to 8.50 µg/L as shown in Table 7. The highest caffeine concentration of 8.5 µg/L was detected in a sample from the Tijuana River watershed, at a location where the presence of untreated sewage is highly likely. Figure 5 below shows the distribution of caffeine detections and non-detections throughout the study area.

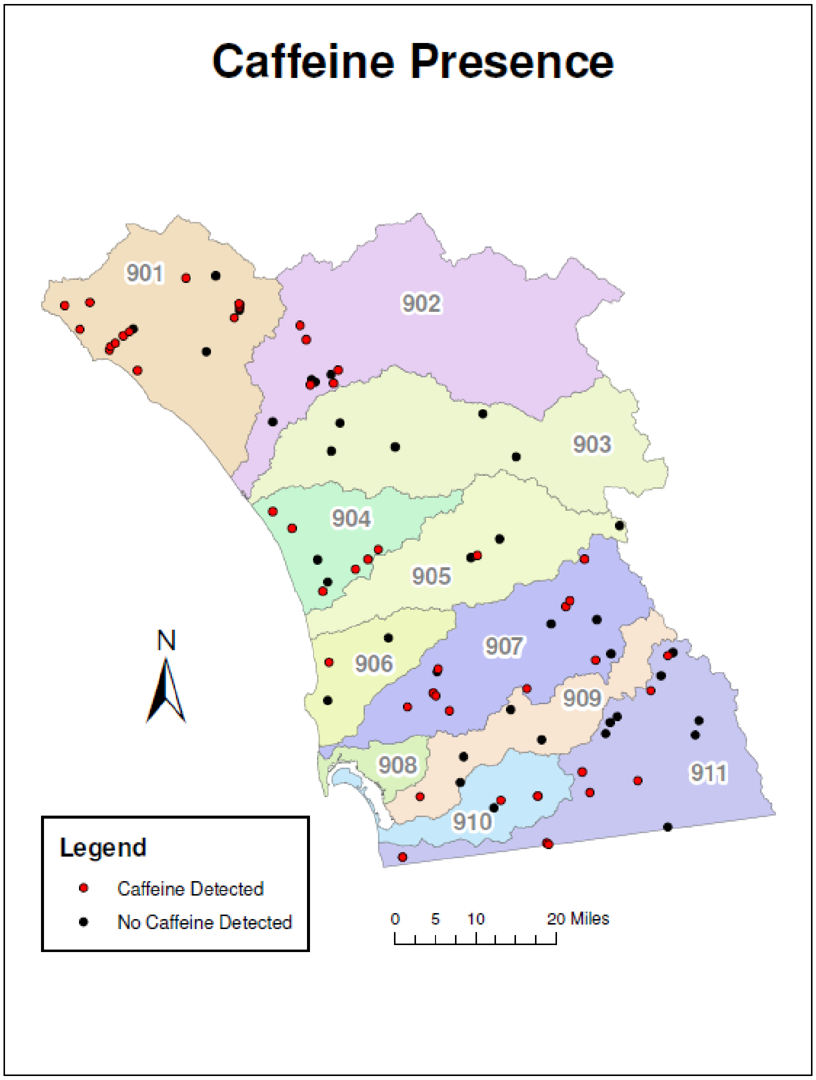


Figure 5. Caffeine detections throughout the San Diego Region.

Table 7. Caffeine detections and ranges of concentrations per San Diego Region hydrologic unit.

HU	Samples per HU (#)	Samples with Caffeine Detected above RL (#)	Sample Detections (%)	Sites per HU (#)	Sites with Positive Caffeine Detections (#)	Site Detections (%)	Caffeine Concentration Range (µg/L)
San Juan 901	18	15	83%	18	15	83%	ND – 0.662
Santa Margarita 902	11	5	45%	9	5	56%	ND – 0.432
San Luis Rey 903	5	0	0%	5	0	0%	ND
Carlsbad 904	9	6	67%	8	6	75%	ND – 1.19
San Dieguito 905	4	1	25%	4	1	25%	ND – 0.113
Los Penasquitos 906	3	1	33%	3	1	33%	ND – 0.296
San Diego 907	16	11	69%	14	10	71%	ND – 1.29
Sweetwater 909	5	1	20%	5	1	20%	ND – 0.084
Otay 910	4	3	75%	3	2	67%	ND - 0.236
Tijuana 911	19	10	53%	16	8	50%	ND – 8.50
ALL	94	53	56%	85	49	58%	ND – 8.50

Notes: Results measured below the detection limit are shown as ND = non-detect. The detection limit for caffeine analyses is 0.020 µg/L.

Land Use Patterns of Caffeine Detections

Caffeine detections were determined on land use category basis following the classifications presented in Table 5. The prevalence of caffeine detections by land use category ranked in order of highest percent detection to lowest percent detection is shown in Table 8. As anticipated, caffeine was detected in every sample (n = 5) collected from the Tijuana River sites that are potentially receiving raw sewage from cross-international border flows originating in Mexico. The majority of the samples collected in developed areas (66%, 32 out of 44) contained caffeine at detectable concentrations. Fewer samples contained detectable levels of caffeine from sites located in open spaces (39%, 14 out of 36), agricultural areas (20%, 1 out of 5), and in wastewater treatment plant effluent discharges (20%, 1 out of 5).

Table 8. Prevalence of caffeine detections per land use category.

Land Use Category	**Detected**		**Not Detected**		**Total (n)**
	Count	**Percent**	**Count**	**Percent**	
Raw Sewage	5	100%	0	0%	5
Developed - Near Septic	12	75%	4	25%	16
Developed - Unknown	11	73%	4	27%	15
Developed – Within Wastewater Treatment Plant (WTP) Service Area	9	69%	4	31%	13
Open	14	39%	22	61%	36
Agricultural	1	20%	4	80%	5
Wastewater Treatment Plant Effluent	1	20%	4	80%	5

Figure 6 shows the range of caffeine concentrations present per land use category when caffeine was detected above the method detection limit (0.020 µg/L) in the samples. Sample results measured below the detection limit have been omitted from the boxplots. The highest concentration and largest range of caffeine concentrations were observed in the samples collected from the sites that potentially receive raw sewage from cross-border flows. The range of caffeine concentrations observed in the samples collected from developed and open lands are comparable.

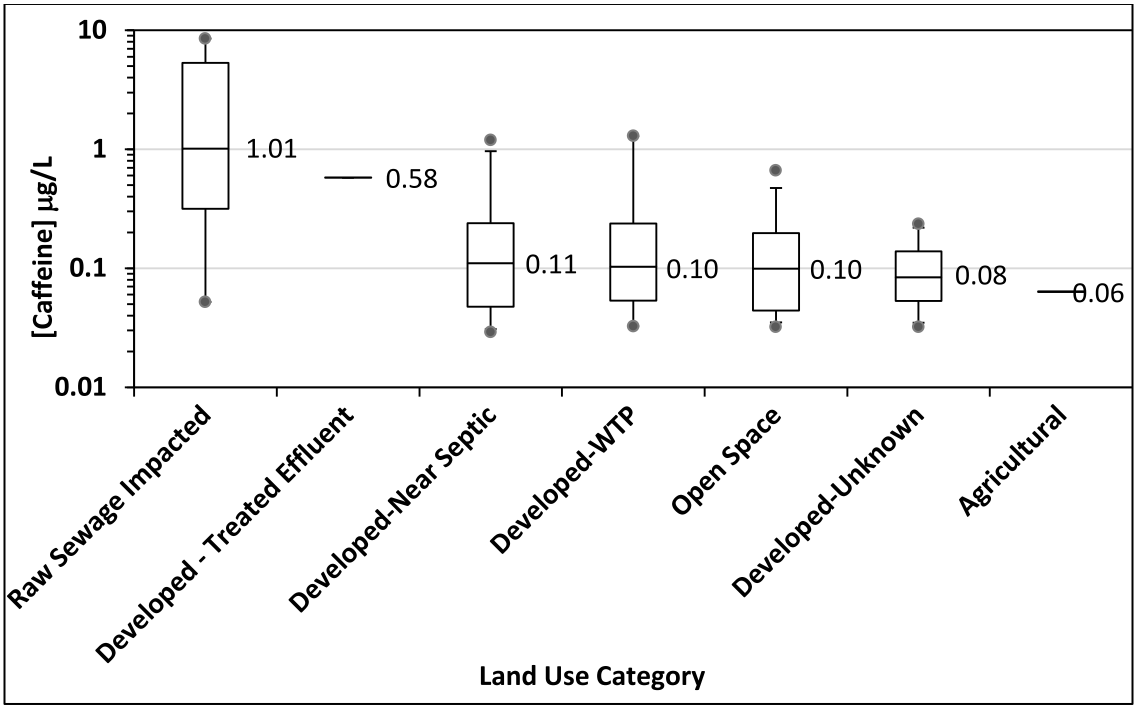


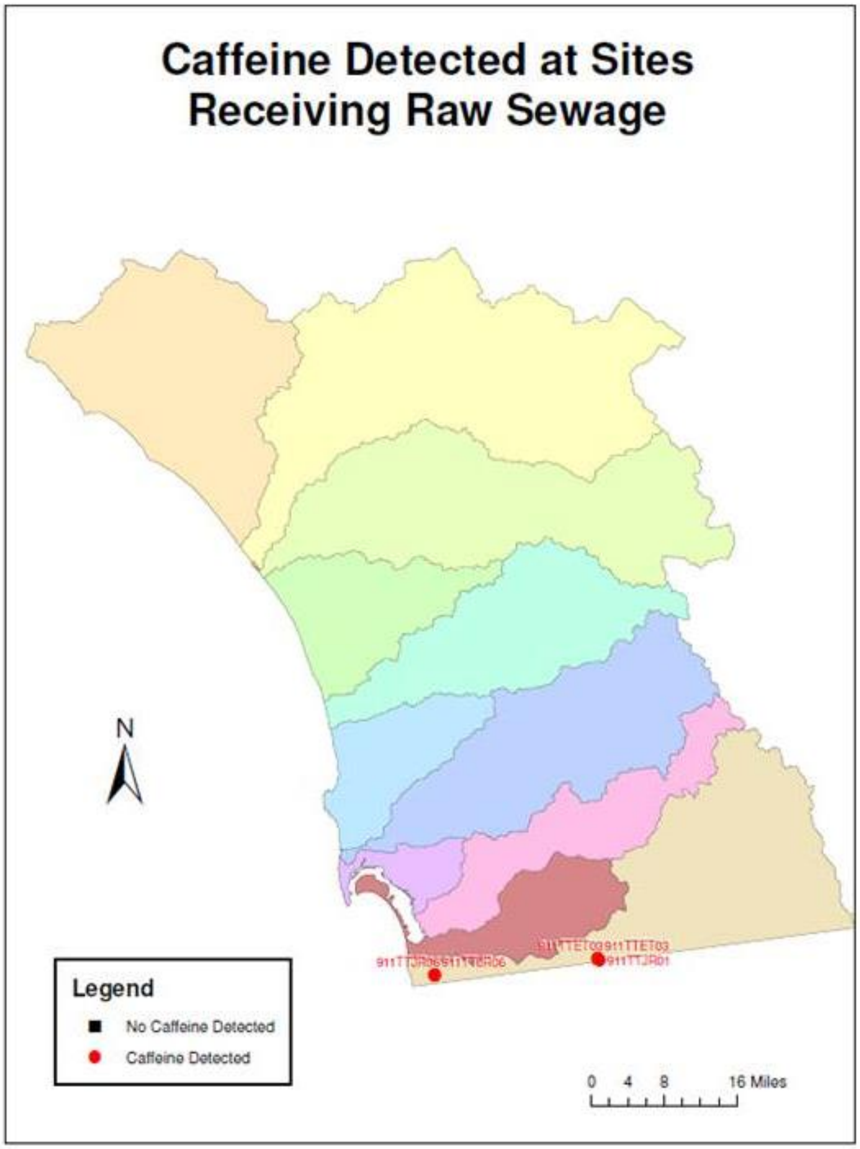
Figure 6. Box plots of caffeine concentrations in samples for each land use category. Non-detects not included.

The following sections provide a brief discussion of the results for each land use category and present the caffeine detection in a graphical format on the hydrologic unit maps. The analyses presented reflect an initial attempt to identify potential sources of caffeine contamination in surface waters and provide context as to the level of significance for the impact that these sources have on San Diego Region surface waters.

1. Raw Sewage Impacted

Sites were selected in hydrologic units known to have previously documented raw sewage impacts. The intent of this sampling effort was to provide context within this study as to the upper limit of caffeine concentrations that are present in surface waters impacted by untreated waste.

Five (5) samples were collected from three (3) sites located on the Tijuana River (Figure 7), after it crosses the Mexican border and flows back into the United States. The Tijuana River is impacted by many anthropogenic activities and potentially contains untreated sewage from Mexico. All of the samples analyzed in this reach of the Tijuana River contained caffeine. Caffeine concentrations ranged from 0.052 to 8.5 μg/L, which is the highest concentration of caffeine detected in samples collected for this study. The maximum concentration detected in Tijuana River flows (8.5 μg/L) represents 57 to 97 percent dilution of the caffeine concentrations reported for raw sewage (see Table 1).



STATION CODE	DATE	[CAFFEINE] (ug/L)
911TTJR01	05-May-08	1.01
911TTET03	04-Nov-10	0.052
911TTET03	09-Mar-11	0.58
911TTJR06	04-Nov-10	2.12
911TTJR06	09-Mar-11	8.5

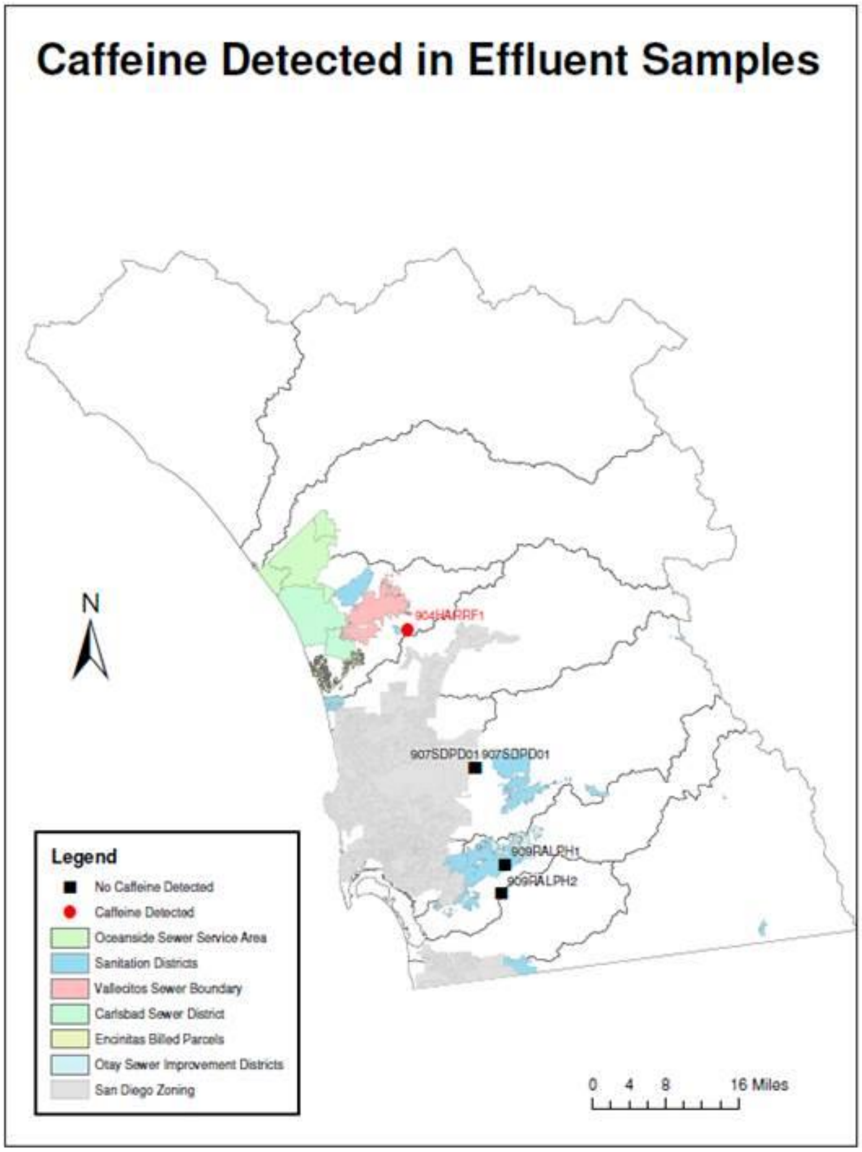
Caffeine detected in 5/5 samples.

Figure 7. Caffeine detected in samples collected from waters potentially containing untreated sewage.

2. Wastewater Treatment Plant Effluent

Two San Diego Region inland surface waters are permitted to receive treated wastewater effluent discharges. These surface waters include Escondido Creek and San Diego River.

Four (4) samples were collected from three (3) distinct wastewater treatment plant effluents. One site (at the Padre Dam effluent) was sampled twice, in 2010 and 2011. Of the four (4) samples analyzed, caffeine was detected in one (1) sample. The few samples that were collected are consistent with the literature, that wastewater treatment facilities, especially those employing treatment to a tertiary level, have the ability to completely remove caffeine. While the majority of caffeine is removed during secondary treatment, it may not be fully removed if adequate retention time is not provided. See Figure 8 for the effluent sampling locations and sewer service areas that were made available for this study. Table 9 provides information regarding the level of treatment provided by each of the facilities.



STATION CODE	DATE	[CAFFEINE] (ug/L)
904HARRF1	28-Feb-11	0.576
907SDPD01	06-Oct-10	ND
907SDPD01	07-Mar-11	ND
909RALPH1	07-Mar-11	ND
909RALPH2	07-Mar-11	ND

Caffeine detected in 1/5 samples.

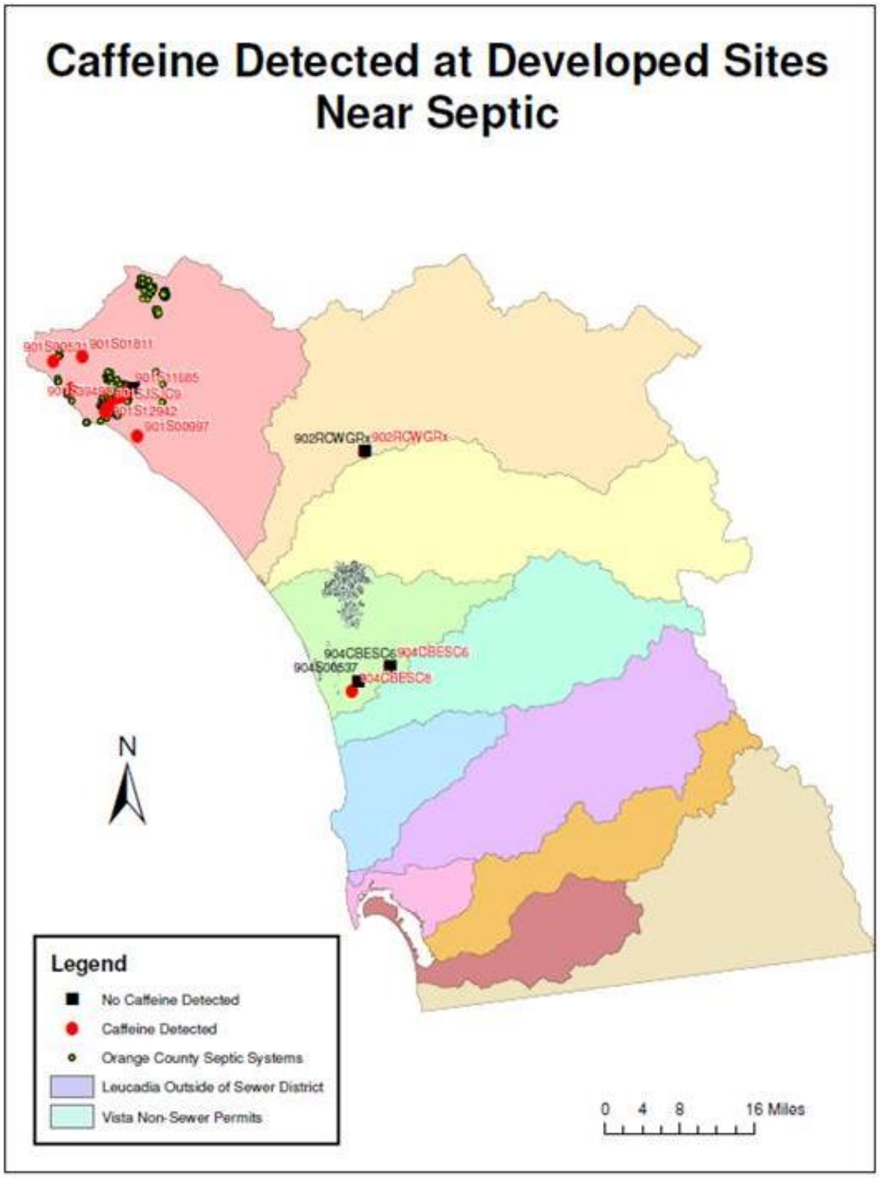
Figure 8. Caffeine detected in wastewater treatment plant effluent samples.

Table 9. Caffeine concentrations detected in samples collected from wastewater treatment plant effluents.

STATION	DATE	TREATMENT FACILITY	TREATMENT LEVEL	CAFFEINE (μg/L)
904HARRF1	2/28/2011	Hale Avenue Resource Recovery Facility	Secondary	0.576
907SDP01	10/6/2010	Padre Dam	Tertiary	ND
907SDP01	3/7/2011	Padre Dam	Tertiary	ND
909RALPH1	3/7/2011	Ralph W. Chapman Water Recycling Facility	Tertiary	ND

3. Developed – Near Septic

Sixteen (16) samples were analyzed from fourteen (14) sites that are known to be located near septic systems. Caffeine was detected in the majority (12 of 16, 75%) of these samples (Figure 9). Detection concentrations ranged from 0.029 to 1.19 μg/L. Many of the caffeine detections are co-located with previously documented septic systems, especially in the San Juan HU (901), within the County of Orange. Septic systems are likely sources of caffeine for these detections, but other potential wastewater sources may be present in these developed areas including leaking sanitary sewer infrastructure as previously described during the Source Identification Pilot Project (SCCWRP, 2013). The absence of specific details about the potential contributions of septic systems or leaking infrastructure as the primary source of caffeine in these hydrologic units suggests further studies are necessary to confirm the source.



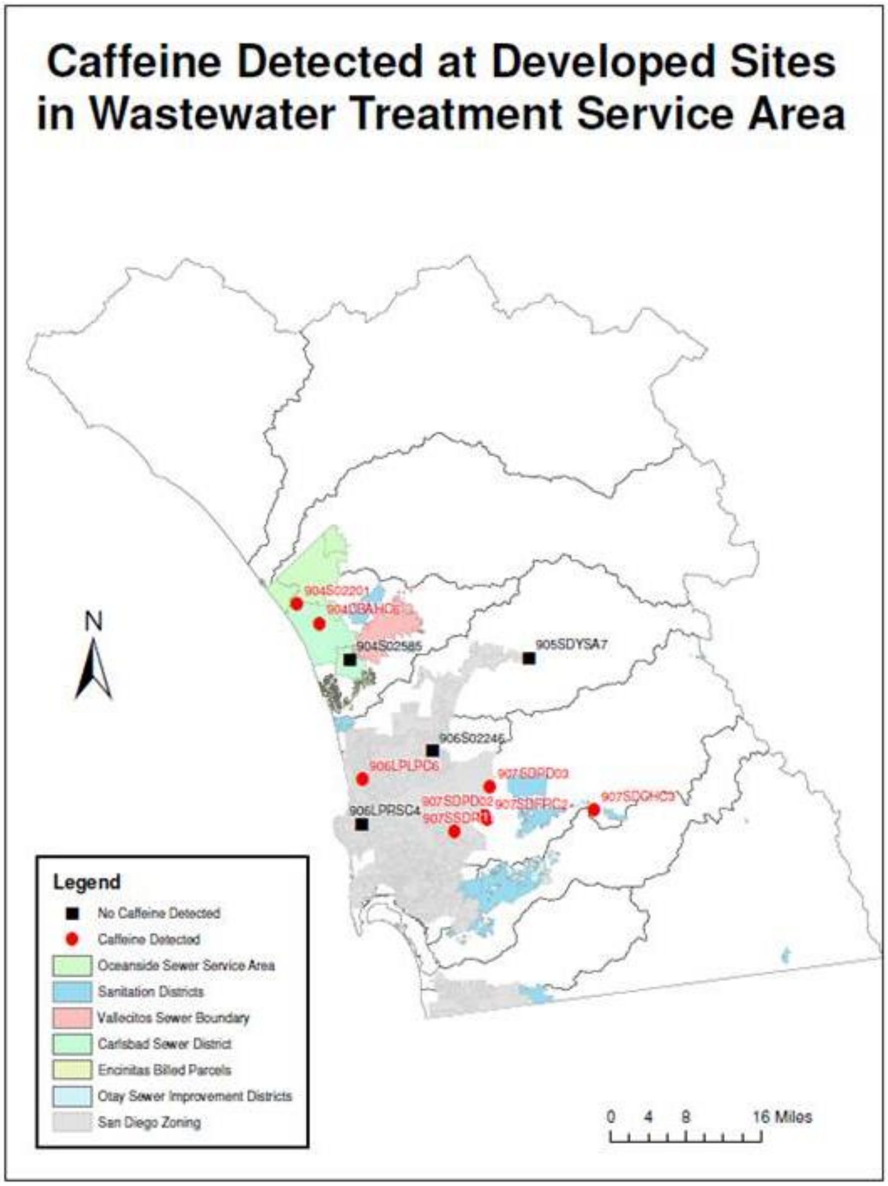
STATION CODE	DATE	[CAFFEINE] (ug/L)
901S00531	07-May-09	0.115
901S00997	14-May-09	0.258
901S01811	07-May-09	0.168
901S02702	02-Jun-10	0.029
901S06030	30-Apr-09	0.105
901S11685	31-May-12	0.063
901S12942	30-May-12	0.044
901S39498	18-May-10	0.035
901S45253	18-May-10	ND
901SJSJC9	8-May-08	0.184
902RCWGRx	28-Feb-11	0.432
902RCWGRx	07-Oct-10	ND
904CBESC6	28-Feb-11	1.19
904CBESC6	07-Oct-10	ND
904CBESC8	06-May-08	0.058
904S00537	04-May-09	ND

Caffeine detected in 12/16 samples.

Figure 9. Caffeine detections in samples collected from sites near known septic systems.

4. Developed – Within Wastewater Treatment Service Areas

Thirteen (13) samples were analyzed from twelve (12) developed area sites located within known wastewater treatment plant service areas. Caffeine was detected in the majority (9 of 13, 69%) of the samples, ranging from 0.0325 to 1.29 μg/L. Though elevated, these results are not necessarily unexpected due to the magnitude of continuous sources within these areas. Because there are a large variety of human-induced impacts in developed areas, potential sources are not limited to leaky sewer lines. Additional sources could include trash, stormwater runoff, recycled water used for irrigation, and others. Figure 10 presents the locations and concentrations of caffeine detections.



STATION CODE	DATE	[CAFFEINE] (ug/L)
904S02201	19-May-10	0.0325
904CBAHC6	07-May-08	0.05
904S02585	19-May-10	ND
905SDYSA7	06-May-08	ND
906LPLPC6	06-May-08	0.296
906LPRSC4	06-May-08	ND
906S02246	26-May-10	ND
907SDCHC3	06-May-08	0.057
907SDFRC2	06-May-08	0.103
907SDPD02	06-Oct-10	1.29
907SDPD02	09-Mar-11	0.18
907SDPD03	09-Mar-11	0.101
907SSDR11	06-May-08	0.147

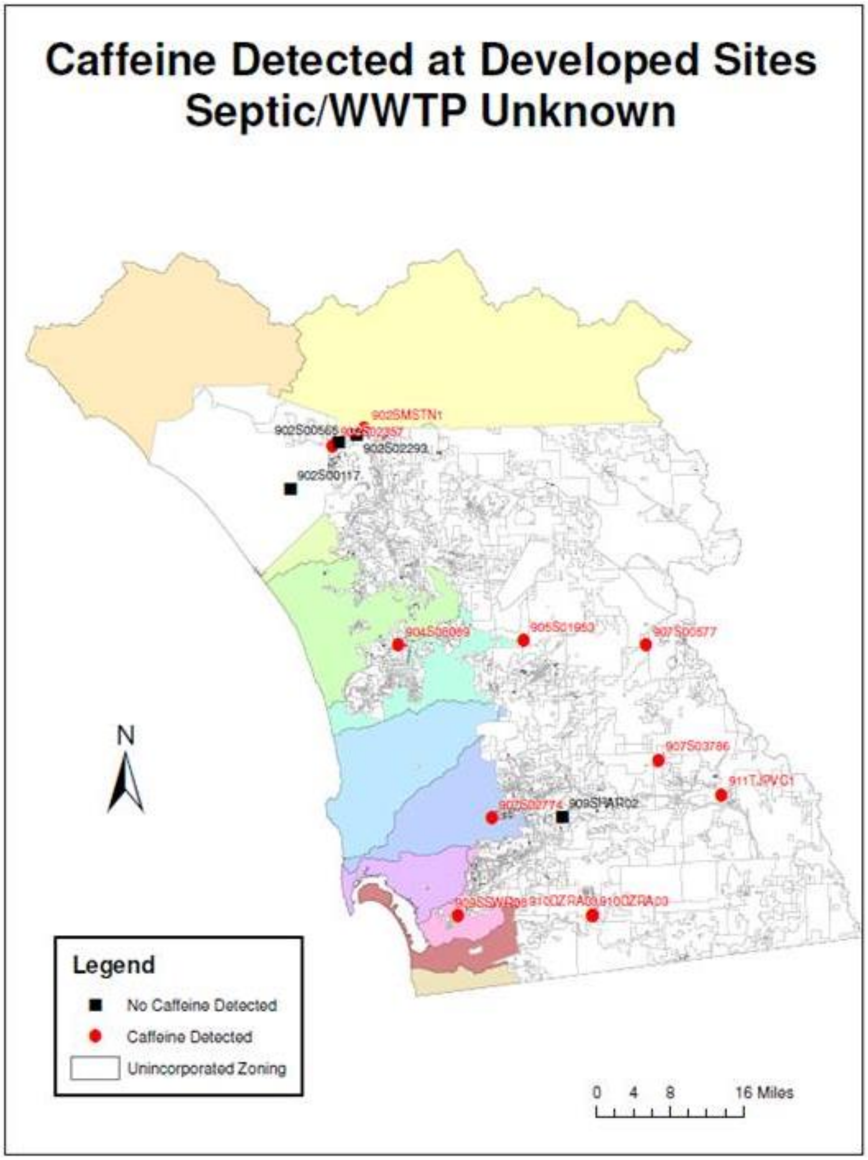
Caffeine detected in 9/13 samples.

Figure 10. Caffeine detections in samples collected from developed sites located within wastewater treatment service areas.

5. Developed - Unknown

A subset of samples were collected in developed areas, where determination of septic system presence could not be confirmed, and the sewer service area layers were unavailable or considered to be of questionable reliability by the data providers. Figure 11 shows the location of the samples collected where it is unknown whether a septic system is nearby, whether it is located within a wastewater treatment service area, or neither. Caffeine was detected in the majority of the samples (11 of 15, 73%), and caffeine detections ranged from 0.032 to 0.236 μg/L.

The white portion of the map in Figure 11 shows lands zoned as unicorporated. It is highly likely that septic systems could be present near some of the sample locations in the unincorporated zone. However, the 5K-scale watershed for those samples was classified as developed. Therefore, numerous anthropogenic influences could also be contributing factors. Further, detailed studies would be necessary to confirm the source of caffeine at those sites.



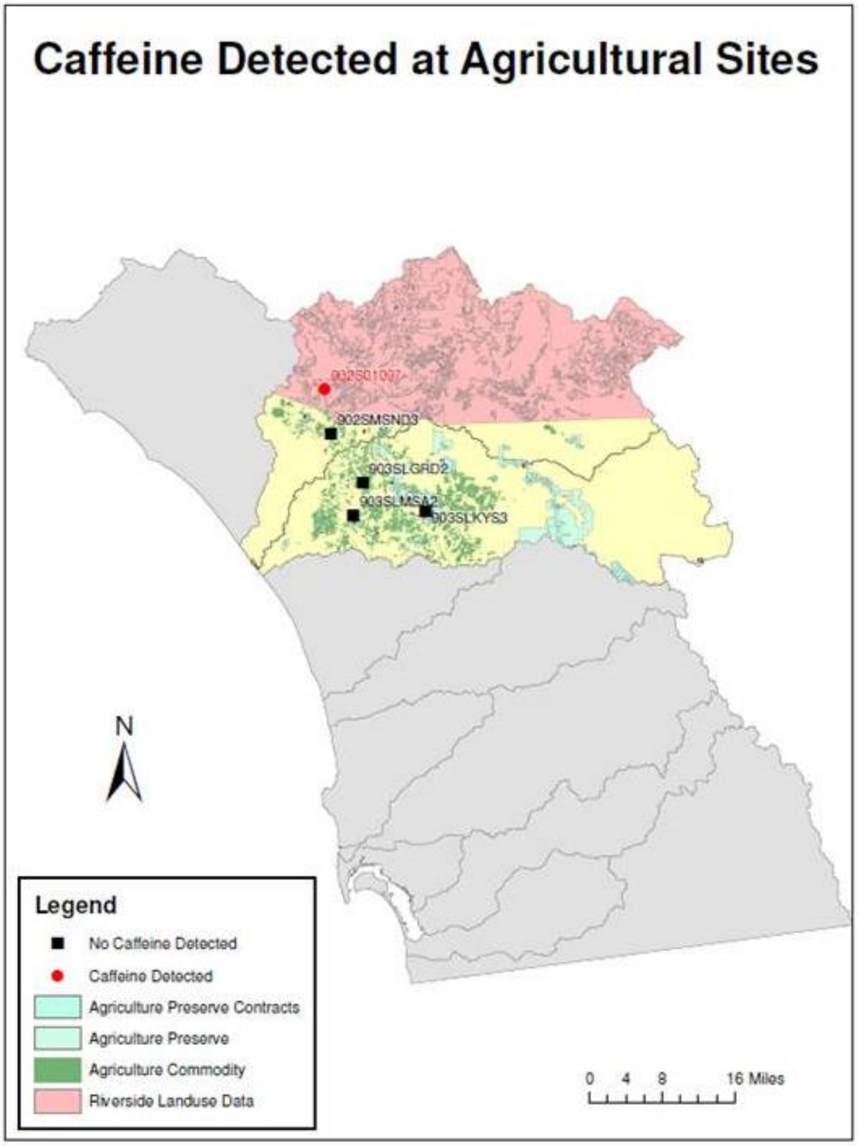
STATION CODE	DATE	[CAFFEINE] (ug/L)
902S02357	25-May-10	0.09
902S00117	25-May-10	ND
902S00565	06-May-09	ND
902S02293	27-May-10	ND
902SMSTN1	07-May-08	0.15
904S08089	20-May-10	0.046
905S01953	06-May-09	0.113
907S00577	12-May-09	0.032
907S02774	20-May-10	0.139
907S03786	30-May-12	0.063
909SHAR02	06-May-08	ND
909SSWR08	05-May-08	0.084
910DZRA03	09-Mar-11	0.08
910DZRA03	05-May-08	0.236
911TJPVC1	07-Mar-11	0.053

Caffeine detected in 11/15 samples.

Figure 11. Caffeine detections in samples collected from sites located in developed areas, where the presence of septic systems is unknown.

6. Agricultural

Five (5) samples were collected from five (5) distinct sites located within watersheds designated as agricultural. Caffeine was only detected in one (1) of the samples at a very low concentration (0.063 μg/L). Figure 12 shows the location of the sample sites in reference to lands used for agricultural purposes. Dark green represents San Diego County lands designated as agricultural commodity (created to determine where pesticides may be used on crops), and pink represents Riverside County lands designated for agricultural use. Minimal to no caffeine detections are expected, as caffeine is not a compound used in common practices for lands used for agricultural purposes.



STATION CODE	DATE	[CAFFEINE] (ug/L)
902S01097	11-May-09	0.063
902SMSND3	07-May-08	ND
903SLGRD2	07-May-08	ND
903SLKYS3	07-May-08	ND
903SLMSA2	07-May-08	ND

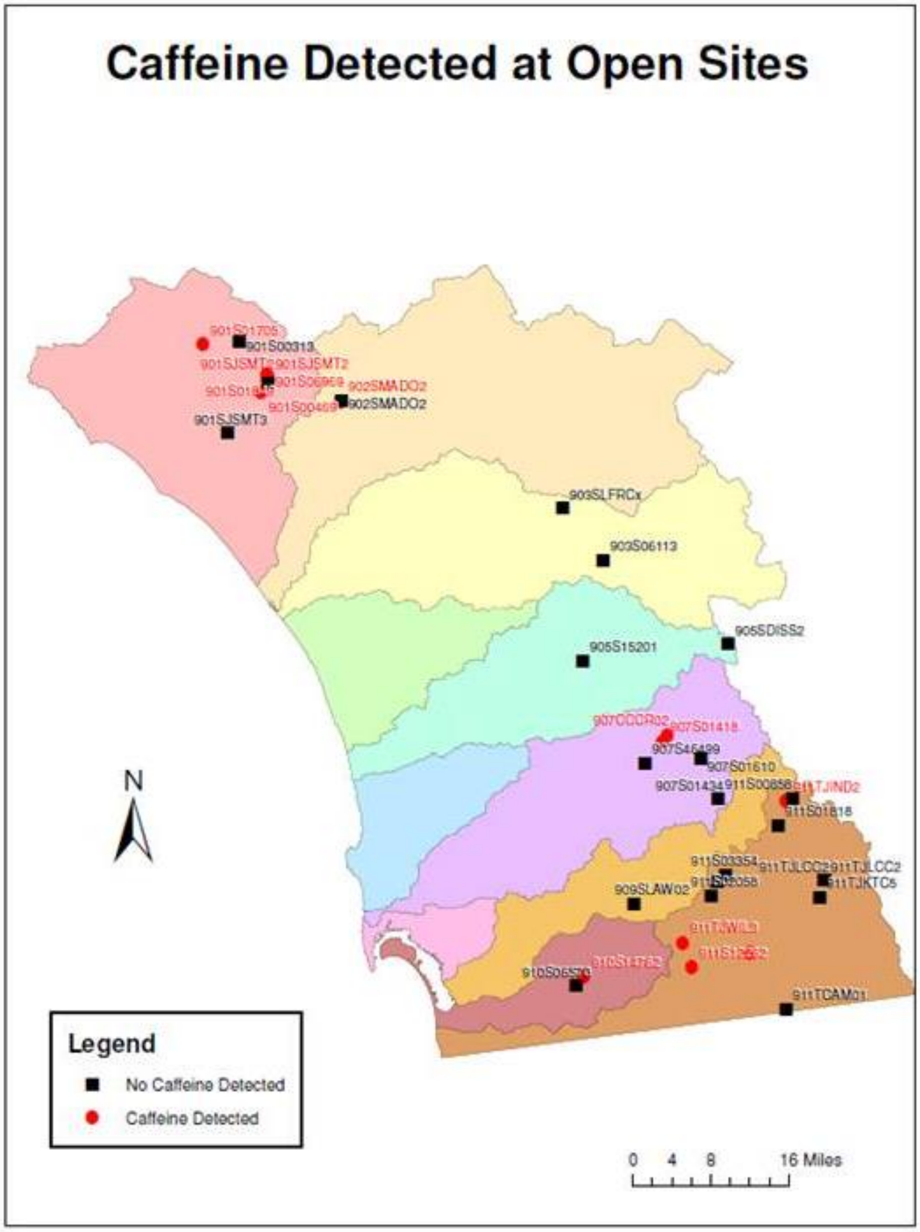
Caffeine detected in 1/5 samples.

Figure 12. Caffeine detections in samples collected from sites located within watersheds designated for agricultural uses.

7. Open

Thirty-six (36) samples were collected from thirty-three (33) sites within watersheds containing mostly open lands. All sites classified as "open" according to the methods employed by the SMC study were included in this grouping. An additional five (5) sites were added to this grouping, following to the SMC methods described above, and contained little to no development within the 5K-sale watershed.

Of all sample site groupings, the results from the open sites were contrary to the authors' expectations. Few to no caffeine detections were anticipated in the areas with little or no development. However, well over one third (14/36, or 39%) of the samples collected from open sites contained caffeine (Figure 13). Caffeine concentration detections ranged from 0.032 to 0.662 μg/L. This prompted further investigation into the site characteristics that could account for the presence of caffeine.



STATION CODE	DATE	[CAFFEINE] (ug/L)
901S00469	13-May-09	0.066
901S00313	30-Apr-09	ND
901S01705	14-May-09	0.148
901S01849	01-Jun-10	0.038
901S02873	01-Jun-10	ND
901S06969	31-May-12	0.132
901SJSMT2	07-May-08	0.662
901SJSMT2	28-Feb-11	0.196
901SJSMT3	8-May-08	ND
902SMADO2	28-Feb-11	0.286
902SMADO2	07-Oct-10	ND
903S06113	23-May-12	ND
903SLFRCx	07-May-08	ND
905S15201	23-May-12	ND
905SDISS2	07-May-08	ND
907CCCR02	06-May-08	0.195
907S01418	12-May-09	0.032
907S01434	13-May-09	ND
907S01610	31-May-10	ND
907S46499	02-Jun-10	ND
909SLAW02	05-May-08	ND
910S06570	24-May-10	ND
910S14762	26-May-10	0.045
911S00538	05-May-09	ND
911S00858	31-May-10	ND
911S01818	06-May-09	ND
911S02058	05-May-09	ND
911S03354	05-May-09	ND
911S04086	06-May-09	0.041
911S12262	24-May-10	0.0545
911TCAM01	05-May-08	ND
911TJIND2	06-May-08	0.202
911TJKTC5	05-May-08	ND
911TJLCC2	12-Oct-10	ND
911TJLCC2	07-Mar-11	ND
911TJWIL3	05-May-08	0.051

Caffeine detected in 14/36 samples.

Figure 13. Caffeine detections in samples collected from open sites.

Most of the sampling sites are located in truly remote areas, only accessible on foot or horseback. Therefore, we hypothesized that one of the only means of potential human impacts at these sites included recreational usage. Recreational uses at each site were determined based on site visits or knowledge of the areas. Recreational uses considered include hiking, camping, fishing, and hunting.

A pattern was observed when considering known recreational uses at or near each sample collection site. Table 10 shows the sampling events parsed into sites without and with known recreational use. While this study did not evaluate the intensity of recreational use, consultation with local agency staff found that sites where caffeine was detected have known recreational uses and public access. Only two (2) sites with known recreational uses did not have caffeine detected in at least one of the samples collected. Every site where little to no recreational usage is known to occur had no caffeine detected in the samples collected.

Table 10. Caffeine data for sampling sites in open areas, divided into recreational use patterns.

LITTLE TO NO KNOWN RECREATIONAL USE			HIGH RECREATIONAL USE		
Site	**Date**	**[Caffeine] (µg/L)**	**Site**	**Date**	**[Caffeine] (µg/L)**
901S00313	30-Apr-09	**ND**	901S00469	13-May-09	**0.066**
901SJSMT3	8-May-08	**ND**	901S01705	14-May-09	**0.148**
903S06113	23-May-12	**ND**	901S01849	01-Jun-10	**0.038**
903SLFRCx	07-May-08	**ND**	901S02873	01-Jun-10	**ND**
905S15201	23-May-12	**ND**	901S06969	31-May-12	**0.132**
905SDISS2	07-May-08	**ND**	901SJSMT2	07-May-08	**0.662**
907S01434	13-May-09	**ND**	901SJSMT2	28-Feb-11	**0.196**
907S01610	31-May-10	**ND**	902SMADO2	07-Oct-10	**ND**
907S46499	02-Jun-10	**ND**	902SMADO2	28-Feb-11	**0.286**
909SLAW02	05-May-08	**ND**	907CCCR02	06-May-08	**0.195**
910S06570	24-May-10	**ND**	907S01418	12-May-09	**0.032**
911S00538	05-May-09	**ND**	910S14762	26-May-10	**0.045**
911S00858	31-May-10	**ND**	911S04086	06-May-09	**0.041**
911S01818	06-May-09	**ND**	911S12262	24-May-10	**0.0545**
911S02058	05-May-09	**ND**	911TJIND2	06-May-08	**0.202**
911S03354	05-May-09	**ND**	911TJWIL3	05-May-08	**0.051**
911TCAM01	05-May-08	**ND**	Caffeine detected in **14/16** samples		
911TJKTC5	05-May-08	**ND**			
911TJLCC2	12-Oct-10	**ND**			
911TJLCC2	07-Mar-11	**ND**			
Caffeine detected in **0/20** samples					

The areas heavily used for hiking with designated trails, include Tenaja Falls, Tenaja Canyon, Bluewater Canyon, Adobe Creek, Cedar Creek Falls, and Indian Creek. There are no restroom facilities located at the trails, and trash was documented by field crews during sampling.

Three (3) of the samples collected near sites with known recreational uses are associated with reservoirs, Barrett Reservoir (samples collected upstream and downstream) and Morena Reservoir (sample collected downstream). Barrett Reservoir, especially, is heavily used for fishing. It is open for fishing from May through September and requires reservations to restrict the number of visitors. There is one portable restroom and several dumpsters located at the entrance. Field crew visits confirmed the presence of litter, including discarded caffeine drink containers (e.g., coffee cups).

Recreation is a relatively unstudied source of caffeine in the environment. Future studies should investigate the links between caffeine detections and recreation and potential means for eliminating its presence (and other associated contaminants) in the environment.

Conclusions and Recommendations

Caffeine was found to be ubiquitous in streams throughout the San Diego region and across land use types. Its existence in San Diego Region streams is an indicator of anthropogenic impacts and can be associated with other contaminants, such as untreated sewage or treated effluents. As expected, many of the surface water samples collected in developed watersheds contained caffeine, as caffeine is a good indicator of human influence. The source(s) of caffeine in these samples could be leaky sewer lines, septic systems, trash, recycled water used for irrigation, and stormwater runoff. Further studies are necessary to confirm caffeine sources at specific locations and the potential impacts of these sources.

In watersheds with little or no urban development, the data opportunistically collected for these efforts revealed differences between open lands and lands used for agricultural practices. While a small number of samples were analyzed from watersheds where the dominant land use is agriculture, caffeine detections in surface waters appear to be rare. This could be due to lack of human presence and limited use of septic systems in these areas. With increasing use of recycled water for irrigation, this is highly likely to change. In watersheds containing little or no development and mostly open space, a fair portion of surface water samples were found to contain caffeine. An association was found between caffeine detection and recreational use.

Future areas of study should include exploring the connection between recreational use and the presence of caffeine in surface waters:

1) What are the main pathways of caffeine delivery to surface waters in high recreational use areas, especially considering its short half-life (less than 24 hours) and its ability to biodegrade?
2) What are the impacts of caffeine presence in surface waters in these remote areas, many of which are considered reference due to lack of development?
3) What are potential means for preventing caffeine and associated contaminants from entering surface waters?

Additional understudied subjects include the presence of caffeine in recycled water used for agriculture, irrigation in urban landscapes, and groundwater recharge. Study design should include investigating its fate and transport, associated contaminants, and potential environmental impacts.

References

Bradley, P.M., L.B. Barber, D.W. Kolpin, P.B. McMahon and F.H. Chapelle. 2007. Biotransformation of caffeine, cotinine, and nicotine in stream sediments: Implications for use as wastewater indicators. *Environmental Toxicology and Chemistry* 26(6):1116-1121.

Buerge, I.J., T. Poiger, M.D. Müller and H.-R. Buser. 2003. Caffeine, and anthropogenic marker for wastewater contamination of surface waters. *Environmental Science and Technology* 37(4):691-700.

Buerge, I.J., T. Poiger, M.D. Müller and H.-R. Buser. 2006. Combined sewer overflows to surface waters detected by the anthropogenic marker caffeine. *Environmental Science and Technology* 40(13):4096-4102.

Busse, L. 2010. Surface Water Ambient Monitoring Program (SWAMP) Monitoring Plan for Region 9: Pilot Study on Pharmaceutical and personal Care Products in the San Diego Region, Fiscal Year 2008/2009 and Fiscal year 2009/2010. California Regional Water Quality Control Board, San Diego Region.

Daneshvar, A., K. Aboulfadl, L. Viglino, R. Broséus, S. Sauvé, A.-S. Madoux-Humery, G.A. Weyhenmeyer and M. Prévost. 2012. Evaluating pharmaceuticals and caffeine as indicators of fecal contamination in drinking water sources of the Greater Montreal region. *Chemosphere* 88:131-139.

Department of Fish and Game, Marine Pollution Studies Laboratory (MPSL-DFG). 2007. Standard Operating Procedures (SOPs) for Conducting Field Measurements and Field Collections of Water and Bed Sediment Samples in the Surface Water Ambient Monitoring Program (SWAMP).

Du, B., A. E. Price, W. C. Scott, L.A. Kristofco, A. J. Ramirez, C. K. Chambliss, J. C. Yelderman, B. W. Brooks. 2014. Comparison of contaminants of emerging concern removal, discharge, and water quality hazards among centralized and on-site wastewater treatment system effluents receiving common wastewater influent. *Science of the Total Environment* 466-467:976-984.

Environmental Protection Agency (EPA). 1996. Method 1669: Sampling Ambient Water for Trace Metals at EPA Water Quality Criteria Levels.

Environmental Protection Agency (EPA). 2007. Method 1694: Pharmaceuticals and Personal Care Products in Water, Soil, Sediment and Biosolids by HPLC/MS/MS. (EPA-821-R-08-002).

Froehner, S., W. Piccioni, K.S. Machado and M.M. Aisse. 2011. Removal capacity of caffeine, hormones, and bisphenol by aerobic and anaerobic sewage treatment. *Water Air Soil Pollution* 216:463-471.

Henjum, M.B., R.M. Hozalski, C.R. Wennen, W. Arnold and P.J. Novak. 2010. Correlations between *in situ* sensor measurements and trace organic pollutants in urban streams. *Journal of Environmental Monitoring* 12:225-233.

Hillebrand, O., K. Nödler, T. Licha, M. Sauter and T. Geyer. 2012. Caffeine as an indicator for the quantification of untreated wastewater in karst systems. *Water Research* 46:395-402.

Mazor, R.D., D.J. Gillett, K. Schiff, K. Ritter, E. Stein. 2011. Ecological Condition of Watersheds in Coastal Southern California: Progress Report of the Stormwater Monitoring Coalition's Stream Monitoring Program First Year (2009). Southern California Coastal Water Research Project, Technical Report 639.

Moore, M.T., S.L. Greenway, J.L. Farris and B. Guerra. 2008. Assessing caffeine as an emerging environmental concern using conventional approaches. *Archives of Environmental Contamination and Toxicology* 54:31-35.

Peeler, K.A., S.P. Opsahl and J.P. Chanton. 2006. Tracking anthropogenic inputs using caffeine, indicator bacteria, and nutrients in freshwater and urban marine systems. *Environmental Science and Technology* 40(24):7616-7622.

Phillips, P. and A. Chalmers. 2009. Wastewater effluent, combined sewer overflows, and other sources of organic compounds to Lake Champlain. *Journal of the American Water Resources Association* 45(1):45-57.

PLANTS Web site. United States Department of Agriculture, Natural Resources Conservation Service. March 2015 <http://plants.usda.gov>

Quinn, B., F. Gagné and C. Blaise. 2009. Evaluation of the acute, chronic and teratogenic effects of a mixture of eleven pharmaceuticals on the cnidarian, *Hydra attenuate*. *Science of the Total Environment* 407: 1072-1079.

Richards, S.M. and S.E. Cole. 2006. A toxicity and hazard assessment of fourteen pharmaceuticals to *Xenopus laevis* larvae. *Ecotoxicology* 15:647-656.

Rodriguez del Rey, Z., E.F. Granek and B.A. Buckley. 2011. Expression of HSP70 in *Mytilus californicanus* following exposure to caffeine. *Ecotoxicology* 20:855-861.

SANDAG. 2008. GIS Data Downloads. Land Use 2008. http://www.sandag.org

Santa Ana Watershed Project Authority (SAWPA). 2014. 2013 Sampling Report for Emerging Constituents in the Santa Ana Region. California Regional Water Quality Control Board, Santa Ana Region.

Sauvé, S., K. Aboulfadl, S. Dorner, P. Payment and G. Deschamps. 2012. Fecal coliforms, caffeine and carbamazepine in stormwater collection systems in a large urban area. *Chemosphere* 86:118-123.

Secru, B., L.C. Van De Werfhorst, J. Murray and P.A. Holden. 2009. Storm Drains are Sources of Human Fecal Pollution during Dry Weather in Three Urban Southern California Watersheds. *Environmental Science and Technology* 43:293-298.

Seiler, R.L., S.D. Zaugg, J.M. Thomas and D.L. Howcroft. 1999. Caffeine and pharmaceuticals as indicators of waste water contamination in wells. *Ground Water* 37:405-410.

Smith, G.R. and A.A. Burgett. 2005. Effects of three organic wastewater contaminants on American Toad, *Bufo americanus*, tadpoles. *Ecotoxicology* 14:477-482.

Southern California Coastal Water Research Project (SCCWRP). 2007. Regional Monitoring of southern California's coastal watersheds, Stormwater Monitoring Coalition Bioassessment Working Group. Technical Report 539. Southern California Coastal Water Research Project. Costa Mesa, CA.

Southern California Coastal Water Research Project (SCCWRP). 2008. Surface Water Ambient Monitoring Program (SWAMP) synthesis report on stream assessments in the San Diego Region. Technical Report 527-Synthesis. Southern California Coastal Water Research Project. Costa Mesa, CA.

Southern California Coastal Water Research Project (SCCWRP). 2011. Ecological Condition of Watersheds in Coastal Southern California: Progress Report of the Stormwater Monitoring Coalition's Stream Monitoring Program First year (2009). Technical Report 639. Southern California Coastal Water Research Project. Costa Mesa, CA.

Southern California Coastal Water Research Project (SCCWRP). 2013. The California Microbial Source Identification Manual: A Tiered Approach to Identifying Fecal Pollution Sources to Beaches. Technical Report 804. Southern California Coastal Water Research Project. Costa Mesa, CA.

Thomas, P.M. and G.D. Foster. 2005. Tracking acidic pharmaceuticals, caffeine, and triclosan through the wastewater treatment process. *Environmental Toxicology and Chemistry* 24(1):25-30.

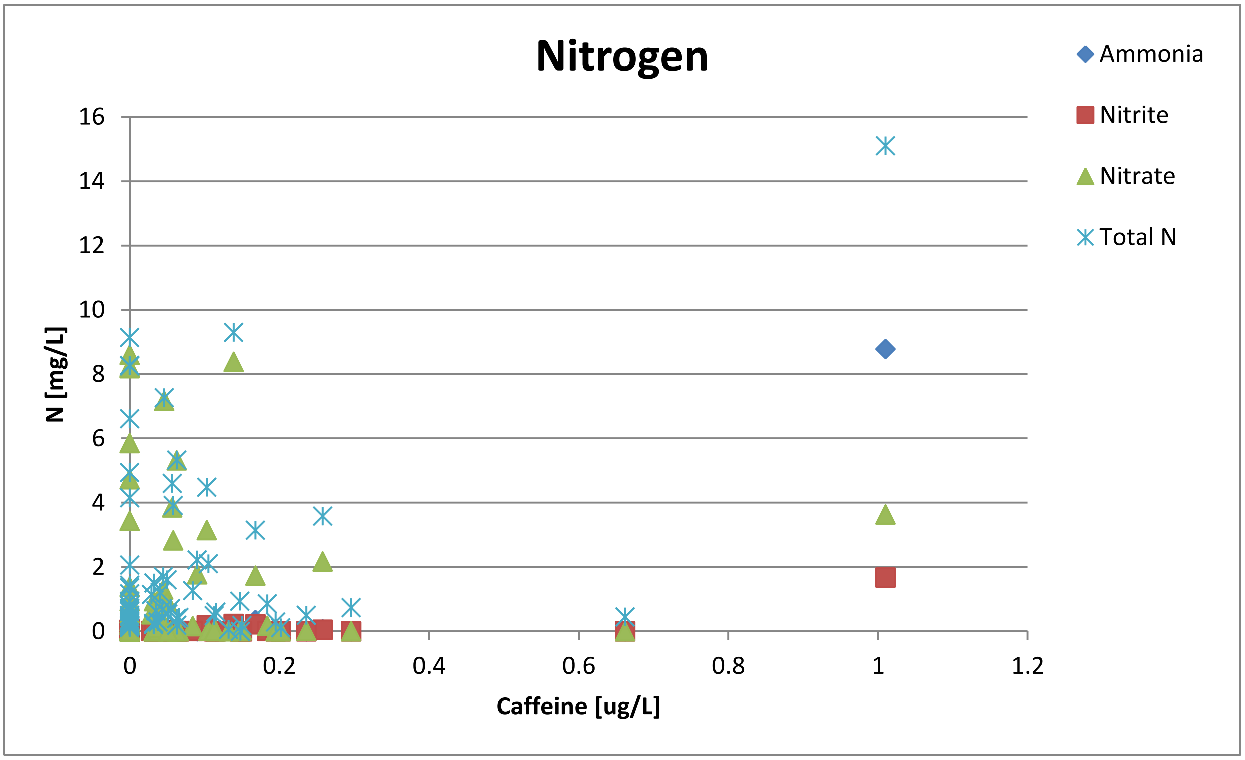
Waiser, M.J., D. Humphries, V. Tumber and J. Holm. 2011. Effluent-dominated streams. Part 2: Presence and possible effects of pharmaceuticals and personal care products in Wascana Creek, Saskatchewan, Canada. *Environmental Toxicology and Chemistry* 30(2):508-519.

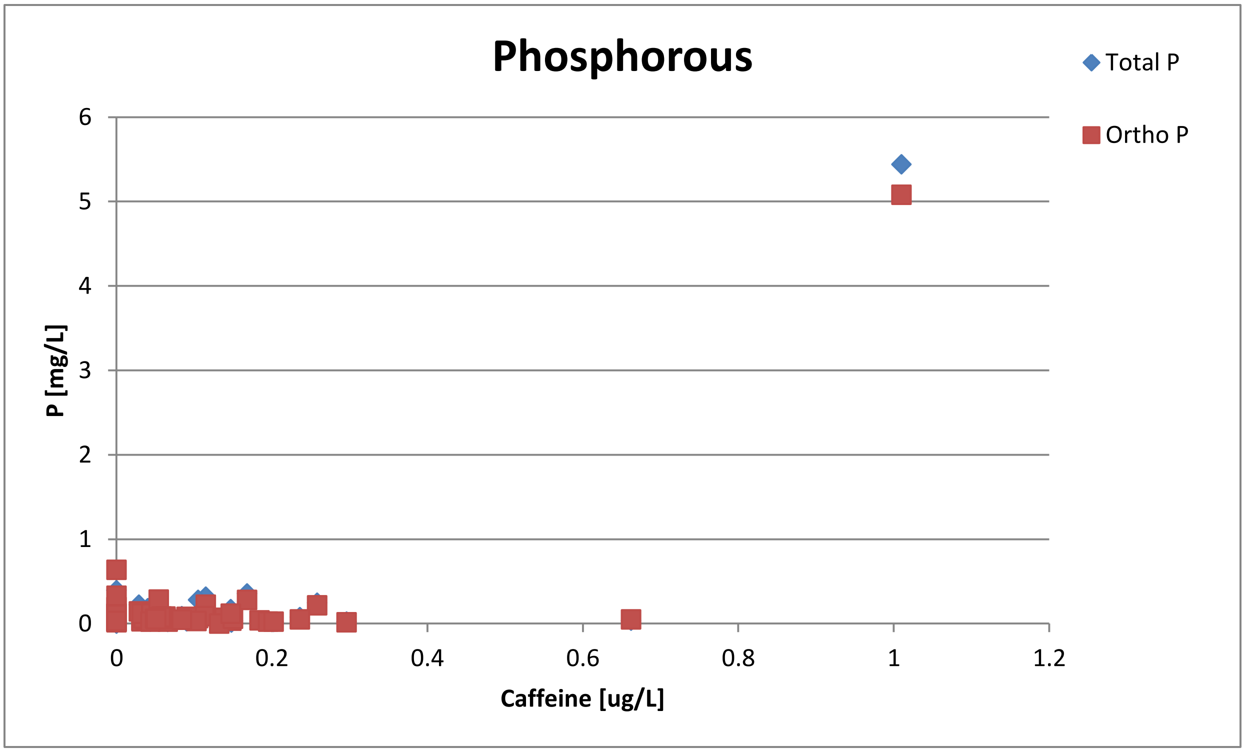
Yu, H. in progress. Pharmaceuticals and Personal Care Products in the San Diego Region – Results of Pilot Study 2010/2011.

Appendix A

Example Ancillary Data Plots

Nutrient concentrations vs. Caffeine Concentration





Appendix B

Photos of Example Sampling Sites

Developed – Within Wastewater Treatment Plant Service Area
Site 907SDFRC2 (Forester Creek)
Sampled on 5/6/2008, [caffeine] = 0.103 μg/L
Photo date: 11/24/2015

Developed – Near Septic
Site 904BESC6 (Escondido Creek)
Sampled on 10/7/2010, [caffeine] = ND
Sampled on 2/28/2011, [caffeine] = 1.19 μg/L
Photo date: 11/24/2015

Agricultural

Site 903SLMSA2 (Moosa Creek)
Sampled on 5/7/2008, [caffeine] = ND
Photo date: 10/13/2015

Open – High Recreational Use

Site 901S01849 (Tenaja Creek)
Sampled on 6/1/2010, [caffeine] = 0.038 μg/L
Photo date: 3/11/2015

Open – Little to No Known Recreational Use
Site 903SLFRCx (Fry Creek)
Sampled on 5/7/2008, [caffeine] = ND
Photo date: 5/22/2015

Raw Sewage Impacted
Site 911TTJR06 (Tijuana River)
Sampled on 11/4/2010, [caffeine] = 2.12 μg/L
Sampled on 3/9/2011, [caffeine] = 8.5 μg/L
Photo date: 11/2/2015