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March 9, 2010

Ms. Pat Boldt
Milk Producers Council
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BROWN AND CALDWELL

Subject: Winter 2009-10 Results of Middle Santa Ana River Pathogen TMDL Agricultural Source Evaluation Plan Monitoring

Dear Ms. Boldt:

Thank you for the opportunity to assist you with storm water monitoring associated with the Middle Santa Ana River (MSAR) Pathogen Total Maximum Daily Load (TMDL) Agricultural Source Evaluation Plan (AgSEP) Monitoring effort. These monitoring activities were conducted in accordance with the MSAR Pathogen TMDL Monitoring Plan.

The Agricultural Representatives to the MSAR TMDL Task Force have agreed to conduct sampling for bacterial indicators, *Bacteroidales*, and total suspended solids (TSS) during two storm events in order to better understand the levels of these constituents in storm water runoff draining from agricultural land uses in the watershed. The group retained Brown and Caldwell to perform the sampling for this effort. Brown and Caldwell subcontracted with E.S. Babcock & Sons Laboratory in Riverside to perform laboratory analysis of TSS and bacterial indicators (*E. coli*, fecal coliform, and total coliform), and the University of California – Davis (Dr. Stefan Wuertz's laboratory) to perform *Bacteroidales* analyses. *Bacteroidales* is used to determine the host organism source(s) of *E.coli* (e.g., human, cow, dog).

During wet-weather season 2009-10, Brown and Caldwell successfully collected samples from one storm event, which occurred on December 12, 2009. The results of this storm event are presented below. This is the second of two storm events that was to be sampled for this project (the first storm was sampled on February 16, 2009).

Summary of Work Conducted During Storm Event of December 12, 2009

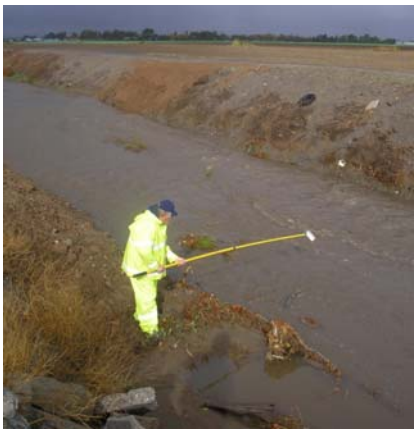
Storm water monitoring was conducted at 4 locations during a storm event that occurred on December 12, 2009. These sites included:

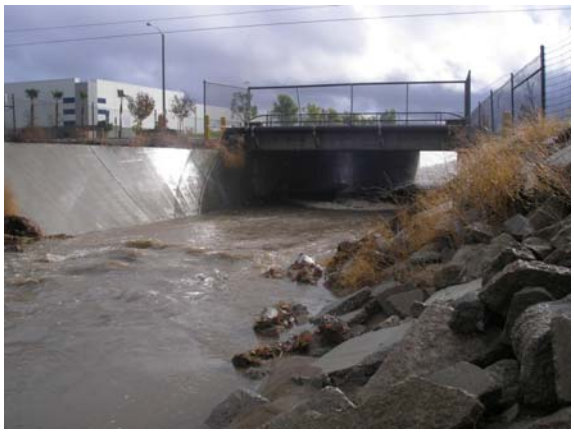
- AG-E2 – Euclid Avenue Channel at Pine Avenue
- AG-CYP1 – Cypress Channel at Kimball Avenue
- AG-G2 – Grove Avenue Channel at Merrill Avenue
- AG-CL1 – Eucalyptus Avenue at Cleveland Avenue

Flow conditions at the previously sampled site location AG-G1 (Eucalyptus Avenue at Walker Avenue) were insufficient due to standing water; thus, the secondary site AG-CL1 was sampled instead.

During this storm, Brown and Caldwell staff proceeded to each site and collected two samples (one sample upon arrival at the site and a second sample 30 minutes following the first sample). Field measurements (temperature, conductivity, pH, dissolved oxygen, and turbidity) were recorded for each sample using a portable Horiba U-10 meter. A Marsh-McBirney Flo-Mate flowmeter was used to record the relative flow rate at each site. The flowmeter requires a minimum depth of water in order to provide an accurate flow measurement. Under low flow conditions, flow was visually estimated by recording the time of travel of a floating object over a known distance. All measurements and observations were recorded on field data sheets. Water samples for laboratory analysis were collected using disposable sampling equipment to avoid the need for decontaminating collection equipment between sites. Additional duplicates and blanks were collected in accordance with the quality assurance/quality control (QA/QC) requirements listed in Table 9a of the MSAR Quality Assurance Project Plan (QAPP).

Once samples were successfully collected from each of the four sites, Brown and Caldwell staff placed the sample bottles in coolers on ice and completed the proper Chain-of-Custody forms. The samples were delivered to E.S. Babcock Laboratories for analysis of *E. coli*, fecal coliform, and total suspended solids (TSS) in time to meet the strict 6-hour bacteria holding time limits. We also shipped samples for analysis of *Bacteroidales* (via overnight mail) to Dr. Stefan Wuertz's laboratory at the University of California – Davis.





Photos of AG-CYP1 site during storm event of December 12, 2009

Discussion of Results from the December 12, 2009 Storm Event

Laboratory Results – Field Sampling

The results of the laboratory analyses for bacterial indicators and total suspended solids are summarized in Table 1. The results of the QA/QC samples are also provided in this table. The table organized as Tables 1A and 1B to present the data from the first storm (February 16, 2009) and the second storm (December 12, 2009) so that the results can be compared.

Table 1B shows that the bacterial indicators in the samples from the December 12, 2009 storm were at their highest levels at sites AG-CYP1 and AG-G2 Cypress Channel at Kimball Avenue and Grove Avenue Channel at Merrill Avenue). The land uses in this area include dairies and agriculture. Bacterial counts for *E. coli* and fecal coliforms at both sites were at or in excess of 130,000 MPN/100 mL (most probable number per 100 milliliters of water). The total coliform count at both sites was in excess of 1,600,000 MPN/100 mL. These counts are higher than those typically observed in storm water samples, and are considerably higher than the water quality objective of 200 MPN/100 ml listed in the Regional Water Quality Control Board's Basin Plan. The bacterial counts in samples from sites AG-E2 and AG-CL1 (Euclid Avenue Channel at Pine Avenue and Eucalyptus Avenue at Cleveland Avenue) were also elevated, but not as high as those from the other two sites. All samples from all sites exceeded the Basin Plan standard during the sampled storm event.

In comparing the bacterial results between Sample 1 and Sample 2 at all sites, only at site AG-E2 are the values numerically identical or within close range of each other. AG-E2 is geographically separated from the other sites such that runoff from the dairies and agricultural areas discharges across pavement and other land uses prior to reaching the sampling point. At all other sites, the levels of *E. coli* and fecal coliform either substantially decreased (site AG-G2, from 130,000 to 30,000 MPN/100 mL *E. coli*) or substantially increased (site AG-CYP1, from 80,000 to 170,000 MPN/100 mL *E. coli*) between Sample 1 and Sample 2. At site AG-CL1 both the *E. coli* and fecal coliform counts decreased (from 7,000 to 2,000 MPN/100 mL), whereas the level of total coliform increased (from 35,000 to 70,000 MPN/100 mL) between Sample 1 and Sample 2. The levels of the three indicator bacteria were lowest at the AG-CL1 site (both *E. coli* and fecal coliform counts were 2,000 MPN/100 mL) and at the AG-E2 site (total coliform count was 30,000 MPN/100 mL). While the data demonstrate the volatile nature of bacteria counts in stormwater samples, the conclusion is that indicator bacteria counts in all samples from all sites exceeded the Basin Plan standard.

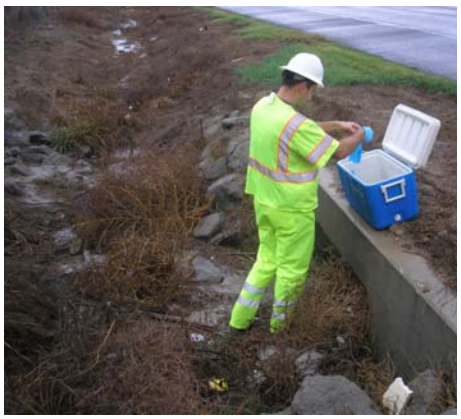
Unlike the bacterial indicators, total suspended solids (TSS) results were fairly consistent between Samples 1 and 2 at all sites. TSS concentrations were lowest at site AG-E2, with TSS measuring 38 and 36 mg/L, respectively. TSS results were highest at the AG-G2 site (570 and 800 mg/L, respectively). TSS concentrations at three of the four sites exceeded the EPA Multi-sector General Permit Water Quality Objective of 100 mg/L for both samples.

Laboratory Results – QA/QC

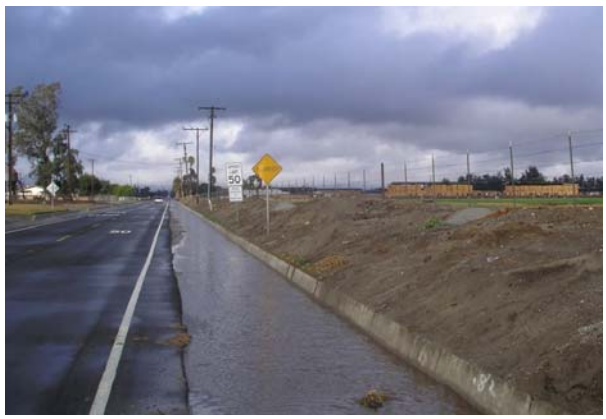
The QA/QC results indicate that the reported laboratory results are consistent, and that no outside contaminants were introduced into the samples in the course of sample handling and transport. Specifically, the results of the duplicate sample from site AG-E2 were either identical or near identical to those of the original sample for *E. coli*, fecal coliform and TSS. Though the results of the total coliform test differed between the two samples, the difference between them is within one order of magnitude. The results for the Blank sample were all below the Reporting Limits for all constituents analyzed.

Field Measurements

Field measurements are presented in Table 2. The table organized as Table 2A and 2B to present the data from the first storm (February 16, 2009) and the second storm (December 12, 2009). Table 2B shows that the results for conductivity and pH were within the ranges commonly observed in stormwater at all sites. The pH results at sites AG-CYP1 (pH = 8.8) and AG-G2 (pH = 8.68) are close to the upper Basin Plan objective (between 6 and 9 pH units). The dissolved oxygen level was depressed (3.45 mg/l) at the AG-E2 site. This measurement is consistent with field observations of low flow and abundant organic material. Bacteria in slowly moving water can consume oxygen as organic matter decays. Temperature was slightly elevated (17.1 ^{o}C) at the AG-G2 site relative to the other sites. Turbidity was high (685 NTU) at site AG-G2, consistent with the TSS concentration measured at the lab, and also consistent with the previous February 16, 2009 storm event.



event of December 12, 2009



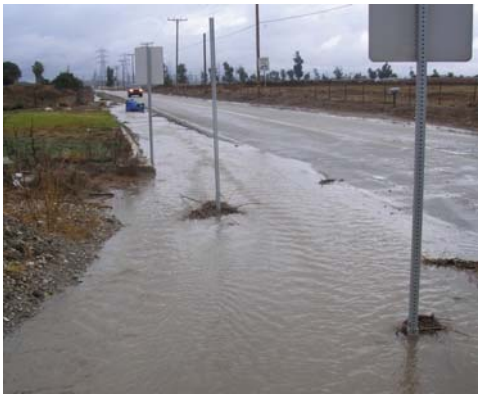
during storm event of December 12, 2009

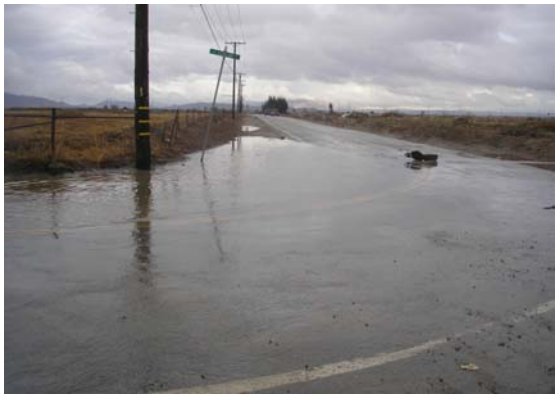
Bacteroidales Results

The objective of the *Bacteroidales* tests is to help identify the relative contributions of fecal pollution originatingfrom human, dog, and bovine (cow) sources by using a microbial source tracking assay approach. Some *Bacteroidales* are undifferentiated as to their host species and are identified as "universal." These organisms are ubiquitous in watersheds. However, specific genetic markers have been identified for human, dog, and cow species and can assist with source identification.

The results of UC – Davis' *Bacteroidales* tests are summarized in Table 3. The table is organized asTable 3A and 3B to present the data from the first storm (February 16, 2009) and the second storm (December 12, 2009). Table 3B shows that the strongest signal observed in the samples from sites AG-CYP1 and AG-CL1 was human, suggesting that fecal contamination correlates with human host organisms. Possible sources for the human signal potentially include nearby homeless encampments, failing septic tanks, illegal cross connections, cracked or broken sanitary sewer lines or illegal dumping; however, no specific evidence of contamination was observed at the time of sampling. The strongest signal observed in the sample from site AG-E2 was from dog, suggesting that fecal contamination correlates with canine host organisms. Canine fecal matter was observed close to site AG-E2 at the time of sampling. At site AG-G2, the strongest signal was from the cow marker (1,676 gene counts per mL), suggesting that fecal contamination correlates with bovine host organisms. Site AG-G2 is directly adjacent to a cow pasture.

The *Bacteroidales* results of the second storm event differ significantly from the first sampling event (Table 3A), when the bovine genetic signal was more prevalent than the human or dog genetic signals. One possible reason for this difference may be the different sizes of the two storm events (1.28 inches on February 16-17 and 0.69 inches on December 12). As shown in Table 2, the measured flow rates were much higher in the first event, meaning that a greater amount of runoff was discharged from the adjacent diaries and agriculture open areas.





Photos of AG-CL1 site (though different streets, they are both upstream) during storm event of December 12. 2009

Table 1A. Analytical Results for AgSEP Storm Event of February 16, 2009

Parameter	Units	Reporting Limit	Method Detection Limit	AG-E2, Sample 1	AG-E2, Sample 2	AG-CYP1, Sample 1	AG-CYP1, Sample 2	AG-G2, Sample 1	AG-G2, Sample 2	AG-G1, Sample 1	AG-G1, Sample 2	AG-E2, Sample 1 (DUP)	AG-E2 (Blank)
E. coli	MPN/100 mL	200	-	3,000	5,000	17,000	24,000	≥160,000	≥160,000	≥160,000	≥160,000	3,000	<200
Fecal Coliform	MPN/100 mL	200	-	3,000	13,000	17,000	24,000	≥160,000	≥160,000	≥160,000	≥160,000	3,000	<200
Total Coliform	MPN/100 mL	200	-	24,000	50,000	≥160,000	≥160,000	≥160,000	≥160,000	≥160,000	≥160,000	24,000	<200
Total Suspended Solids	mg/L	5	3	43	25	200	210	5700	2600	600	1300	27	ND

Table 1B. Analytical Results for AgSEP Storm Event of December 12, 2009

Parameter	Units	Reporting Limit	Method Detection Limit	AG-E2, Sample 1	AG-E2, Sample 2	AG-CYP1, Sample 1	AG-CYP1, Sample 2	AG-G2, Sample 1	AG-G2, Sample 2	AG-CL1, Sample 1[1]	AG-CL1, Sample 2[1]	AG-E2 (DUP)	AG-E2 (Blank)
E. coli	MPN/100 mL	200	-	4,000	4,000	130,000	30,000	80,000	170,000	7,000	2,000	4,000	<2.0
Fecal Coliform	MPN/100 mL	200	-	4,000	8,000	240,000	130,000	130,000	210,000	7,000	2,000	4,000	<2.0
Total Coliform	MPN/100 mL	200	-	50,000	30,000	>1,600,000	300,000	500,000	1,700,000	35,000	70,000	90,000	<2.0
Total Suspended Solids	mg/L	5	3	38	36	270	360	570	800	350	350	34	ND

ND: Not detected.

Note: Basin Plan Objective for REC 1 = Shall not exceed 200/100mL (log mean for 5 samples over 30 days)

[1] Site AG-G1 was not sampled on December 12, 2009 due to insufficient flow. Therefore, the secondary site AG-CL1 was used.

Table 2A. Field Measurement Results for AgSEP Storm Event of February 16, 2009

Parameter	Units	AG-E2	AG-CYP1	AG-G1	AG-G2
Conductivity	mS/cm	0.152	0.078	0.213	0.32
Dissolved Oxygen	mg/L	8.66	8.36	6.75	6.85
pH	pH units	8.15	8.08	7.64	8.55
Turbidity	NTU	40.1	140	822	7314
Temperature	°C	14.3	12.8	11.9	11.6
Flow Rate	ft/sec	0.69	2.0	1.3	4.57

Table 2B. Field Measurement Results for AgSEP Storm Event of December 12, 2009

Parameter	Units	AG-E2	AG-CYP1	AG-G2	AG-CL1
Conductivity	mS/cm	0.155	0.171	0.414	0.085
Dissolved Oxygen	mg/L	3.45	7.32	6.54	7.43
pH	pH units	8.57	8.80	8.68	8.05
Turbidity	NTU	-	176	685	379
Temperature	°C	15.4	15.5	17.1	15.7
Flow Rate	ft/sec	-	0.347	0.292	1.7 – 2.2

Note: The flow rate at site AG-E2 was too low (near surface flow) to effectively determine; as a result, the Turbidity parameter could not be reliably measured.
The flow rate at AG-CL1 varied between 1.7 ft/sec and 2.2 ft/sec due to the influence of adjacent vehicle traffic splashing water into the sampling channel.
Rainfall precipitation in the first and second storm events were 1.28 inches (February 16-17) and 0.69 inches (December 12), repectively.

Table 3A. *Bacteroidales* Results for AgSEP Storm Event of February 16, 2009

Sample ID	Universal Bacteroidales		Human Bacteroidales		Cow Bacteroidales		Dog Bacteroidales	
	Total Concentration in Sample	SLOD	Total Concentration in Sample	SLOD	Total Concentration in Sample	SLOD	Total Concentration in Sample	SLOD
	(gc/ml)	(gc/ml)	(gc/ml)	(gc/ml)	(gc/ml)	(gc/ml)	(gc/ml)	(gc/ml)
AG-E2 Sample 1	12,750	7	398	12	228	12	491	2
AG-E2 Sample 2	63,815	29	427	51	84	54	765	8
AG-E2 Blank	ND	3	ND	5	ND	6	ND	1
AG-E2 Duplicate	6,461	9	ND	15	ND	16	215	2
AG-CYP1 Sample 1	23,859	2	48	4	861	4	466	1
AG-CYP1 Sample 2	28,156	1	86	2	588	3	215	0
AG-G2 Sample 1	4,460,455	154	ND	266	145,350	285	140	43
AG-G2 Sample 2	3,715,964	1	ND	2	346,301	2	44	0
AG-G1 Sample 1	12,788,700	7	376	12	935,638	13	461	2
AG-G1 Sample 2	8,323,365	82	239	141	632,373	151	530	23

Table 3B. *Bacteroidales* Results for AgSEP Storm Event of December 12, 2009

Sample ID	Universal Bacteroidales		Human Bacteroidales		Cow Bacteroidales		Dog Bacteroidales	
	Total Concentration in Sample	SLOD	Total Concentration in Sample	SLOD	Total Concentration in Sample	SLOD	Total Concentration in Sample	SLOD
	(gc/ml)	(gc/ml)	(gc/ml)	(gc/ml)	(gc/ml)	(gc/ml)	(gc/ml)	(gc/ml)
AG-G2 Sample 1	94,859	7	ND	40	1,676	2	418	6
AG-CYP1 Sample 1	19,484	5	1,269	32	39	1	716	5
AG-E2 Sample 1	3,854	15	ND	88	ND	4	532	13
AG-CL1 Sample 1	2,893	9	2,324	56	69	2	442	8

ND = Non Detect

gc = Gene Count

SLOD = Sample Limits of Detection

Thank you for the opportunity to provide storm water monitoring services for this important project. If you have any questions about this report, please contact Nancy Gardiner at (858) 514-8822.

Very truly yours,

BROWN AND CALDWELL

Nancy E. Gardiner

Nancy E. Gardiner
Project Manager

cc: Mr. Rick Whetsel, Santa Ana Watershed Project Authority