Exhibit Y

ANDREA PLEVAN ET AL., MICROBIAL SOURCE TRACKING PILOT STUDY (Jan. 2013)

Microbial Source Tracking Pilot Study

Developed for the Upper Mississippi River Bacteria TMDL and Protection Plan

January 2013

Primary Authors

Emmons & Olivier Resources, Inc.
Andrea Plevan
Nancy-Jeanne LeFevre
Pat Conrad

Prepared for

Minnesota Pollution Control Agency
Barb Peichel
Phil Votruba

Minnesota Department of Health
Art Persons Mark Wettlaufer

CLEAN WATER LAND & LEGACY AMENDMENT

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Executive Summary

A pilot study was undertaken to investigate the use of microbial source tracking of fecal contamination for Total Maximum Daily Load (TMDL) studies in Minnesota. The Minnesota Pollution Control Agency (MPCA) and Minnesota Department of Health (MDH) collaborated with Dr. Michael Sadowsky from the University of Minnesota's Department of Soil, Water and Climate, and The BioTechnology Institute. Three *Bacteroides* primers were used to identify the source of fecal contamination: bovine (cow), porcine (swine), and humans (and cats/dogs[1]).

For the pilot study, 19 sites were selected and a total of 50 samples were taken from the sites over four sampling days (Table 1). Sites were selected to provide a cross-section of rural and urban drainage characteristics, and samples from the Mississippi River, tributaries, and stormsewer. MDH source water protection areas were also targeted. Up to four samples were collected from each site, to include a stormflow and baseflow sample from both June and fall. At least one liter of surface water was collected for each sample. Samples were collected from the middle of the river/stream/stormsewer at most of the sites. Four of the 19 sites entailed collecting the sample from off of boat launches or loading docks using a 12 foot dip pole extended towards the center of the river/stream, ultimately collecting the sample at minimum 25 to 50 feet from shore in flowing water. This sampling approach provides data on which sources were found at the monitoring site. However, the limited number of samples taken does not provide information about which sources were not found at the monitoring site during that time period. Relative concentrations of markers were compared in the data evaluation.

The bovine marker was found at twelve of the nineteen monitoring sites. Four of the five Mississippi River sites tested positive for the bovine marker, and six of the eleven tributary sites tested positive. Two of the three stormsewer sites also tested positive for the bovine marker. There were several sites that tested positive for the bovine marker that do not have any obvious sources of fecal contamination from cattle. The porcine marker was found at only one site. The humans (and cats/dogs) marker was found at thirteen of the nineteen monitoring sites. Two of the five Mississippi River sites tested positive for the humans (and cats/dogs) marker, and five of the eleven tributary sites tested positive. All of the samples taken from stormsewers showed the presence of human (and cat/dog) sources.

The following conclusions are based on the observations from this monitoring:

- Bovine and human (and cat/dog) sources of fecal contamination are present throughout the project area in both the mainstem Mississippi River, tributaries, and stormsewer.
- Unexpected results suggest areas of future investigation: are there bovine sources in the watersheds of County Ditch 17, 65th Avenue outfall in Brooklyn Center, and Shingle Creek?
- Stormsewers show a consistent presence of the human (and cat/dog) marker suggesting areas of future investigation: are there sources of human fecal contamination such as leaking sanitary sewers?

[1] The primer for humans also reacts with *Bacteroides* from cats/dogs; however, the primer is much more reactive to *Bacteroides* from humans than from cats/dogs. To represent this relative reactivity, the humans and cats/dogs marker is referred to as "humans (and cats/dogs)" throughout this report.

Approach

The source assessment component of the Upper Mississippi River Bacteria TMDL identifies sources and delivery of bacteria based on characteristics of the watershed. This is a common approach used for source assessments of multiple pollutant types (e.g. bacteria, sediment, phosphorus). With bacteria contamination associated with fecal matter, there are additional source tracking options available. The microbial community, which includes bacteria, is composed of different types of bacteria depending on what type of organism the fecal matter came from. This study used bacteria from the genus *Bacteroides* to investigate the use of microbial source tracking to track the source of the fecal contamination as a pilot study for TMDL studies in Minnesota. Refer to the U.S. Environmental Protection Agency's report *Using Microbial Source Tracking to Support TMDL Development and Implementation* (2011)[2] for further discussion regarding microbial source tracking methods, study design, and use for TMDL development and implementation.

Bacteroides are anaerobic bacteria that are abundant in human and animal feces and have a high degree of host specificity. This means that certain types of *Bacteroides* colonize the digestive system of specific types of animals (i.e. hosts). Since they do not survive under aerobic conditions, their presence in surface waters indicates recent fecal contamination.

The MPCA and MDH collaborated with Dr. Michael Sadowsky from the University of Minnesota's Department of Soil, Water and Climate, and The BioTechnology Institute. Three primers were used to identify the source of fecal contamination: bovine, porcine, and humans (and cats/dogs). The primer for humans also reacts with *Bacteroides* from cats/dogs; however, the primer is much more reactive to *Bacteroides* from humans than from cats/dogs. To represent this relative reactivity, the humans and cats/dogs marker is referred to as "humans (and cats/dogs)" throughout this report. Markers have varying levels of sensitivity and specificity; the selection of a marker balances the marker's sensitivity (the proportion of samples that *are* from a source that *can* be correctly classified to that source) with the marker's specificity (the proportion of samples that are *not* from a source that is correctly *not* classified as being from that source). The markers used in this study were CowM3F, Pig-2-Bac41F, and HF183f. More detailed information on methods can be found in the appendix.

For the pilot study, 19 sites were selected (Figure 1) and a total of 50 samples were taken from the sites over four sampling days (Table 1). At least one liter of surface water was collected for each sample. Samples were collected from the middle of the river/stream/stormsewer at most of the sites. Four of the 19 sites entailed collecting the sample from off of boat launches or loading docks using a 12 foot dip pole extended towards the center of the river/stream, ultimately collecting the sample at minimum 25 to 50 feet from shore in flowing water.

This sampling approach provides data on which sources were found at the monitoring site. However, the limited number of samples taken does not provide information about which sources were not found at the monitoring site. For example, if *Bacteroides* with the humans (and cats/dogs) marker were found at one site, but not *Bacteroides* with the cattle marker, one can

[2] U.S. Environmental Protection Agency. April 2011. *Using microbial source tracking to support TMDL development and implementation.*

conclude that humans (and cats/dogs) are a source of fecal contamination, but one cannot conclude that cattle are not a source of fecal contamination for that particular time period.

Although the results are quantitative in nature, they should be interpreted semi-quantitatively. Relative concentrations of markers were compared in the data evaluation.

Another marker that identifies the presence of human wastewater is fluoride. Fluoride is added to Minnesota municipal drinking water supplies in the United States and is present in municipal wastewater. Water samples were taken at the same sites and schedule identified in Table 1 and analyzed for fluoride concentration.

Water samples were also analyzed for *E. coli* concentration, on which the water quality standard is based. Again, water samples were taken at the same sites and schedule identified in Table 1.

Table 1. Microbial source tracking monitoring sites; samples were taken for *Bacteroides*, fluoride, and *E. coli*.

Site #[1]	AUID[2]	Reach Description	Location within Source Water Protection Area[3]	Site Type	June 2011 base[4]	June 2011 storm[5]	Sept 2011 base[4]	April 2012 storm[5]
S004-653	07020106-509	Mississippi River - Coon Creek to Upper St. Anthony Falls (site at Shingle Ck outlet)	Priority B	Mississippi River	ü	ü	ü	ü
S006-163	07010201-502	Mississippi River - downstream MN-15 Bridge in Sauk Rapids	St. Cloud Priority A & B	Mississippi River	ü	ü	ü	ü
S006-735	07020106-505	Mississippi River - MN River to Metro WWTP	--	Mississippi River	ü			ü
S000-025	07010206-568	Mississippi River - at US-169 Bridge at Anoka (NW city limits of Anoka to Rum River)	St. Paul Priority A & B	Mississippi River		ü	ü	
S004-655	07020106-503	Mississippi River - Lower St. Anthony Falls to L&D#1	--	Mississippi River	ü			ü
S003-370	07010203-562	Johnson Ck btwn CR-75 and I-94, 5 mi S of St. Cloud (St. Augusta Ck)	Priority B	Tributary	ü	ü	ü	ü
S002-948	07010201-525	Spunk Creek	Priority B	Tributary	ü	ü	ü	ü
S006-162	07010201-516	Little Two River	Priority B	Tributary		ü	ü	
S006-148	07010203-528	Unnamed Creek	Priority B	Tributary	ü			ü
S002-949	07010201-523	Two River	Priority B	Tributary		ü	ü	
S003-995	07010206-594	Unnamed ditch (Pleasant Creek)	Mpls-St Paul Priority A & B	Tributary	ü			ü
S002-947	07010201-528	Watab River	St. Cloud Priority A & B	Tributary		ü	ü	

Site #[1]	AUID[2]	Reach Description	Location within Source Water Protection Area[3]	Site Type	June 2011 base[4]	June 2011 storm[5]	Sept 2011 base[4]	April 2012 storm[5]
S006-140	07010206-557	County Ditch 17	Mpls-St Paul Priority A & B	Tributary	ü	ü	ü	ü
S006-139	01070206-542	Unnamed Creek (downstream of MN River confluence with Mississippi River)	--	Tributary	ü			ü
S001-946	07010206-506	Shingle Creek	Mpls Priority A & B	Tributary	ü	ü	ü	ü
S005-017	07010206-538	Bassett Creek	--	Tributary		ü	ü	
SS00043	07010206-503[6]	MWMO 6UMN	--	Storm sewer		ü		ü
SS00010	07010206-509[6]	West Miss WMO - 65th Ave outfall in Brooklyn Center	Mpls Priority A & B	Storm sewer		ü		ü
SS00009	07010206-503[6]	St. Anthony, CRWD	--	Storm sewer		ü		ü

[1] Station IDs that begin with "S" are MPCA IDs. Station IDs that begin with "MCES" were generated for this project for Metropolitan Council sites.
[2] AUID = MPCA's Assessment unit identification number.
[3] The cities of St. Cloud, St. Paul, and Minneapolis have State endorsed Source Water Protection Plans following the Minnesota Department of Health guidance for surface water intakes from the Mississippi River. In each of these plans, cities have designated priority areas for drinking water protection, called Source Water Protection Areas.
[4] 'base' means that the sample was taken under baseflow conditions.
[5] 'storm' means that the sample was taken under stormflow conditions.
[6] Indicates that the storm sewer outfalls to the listed AUID

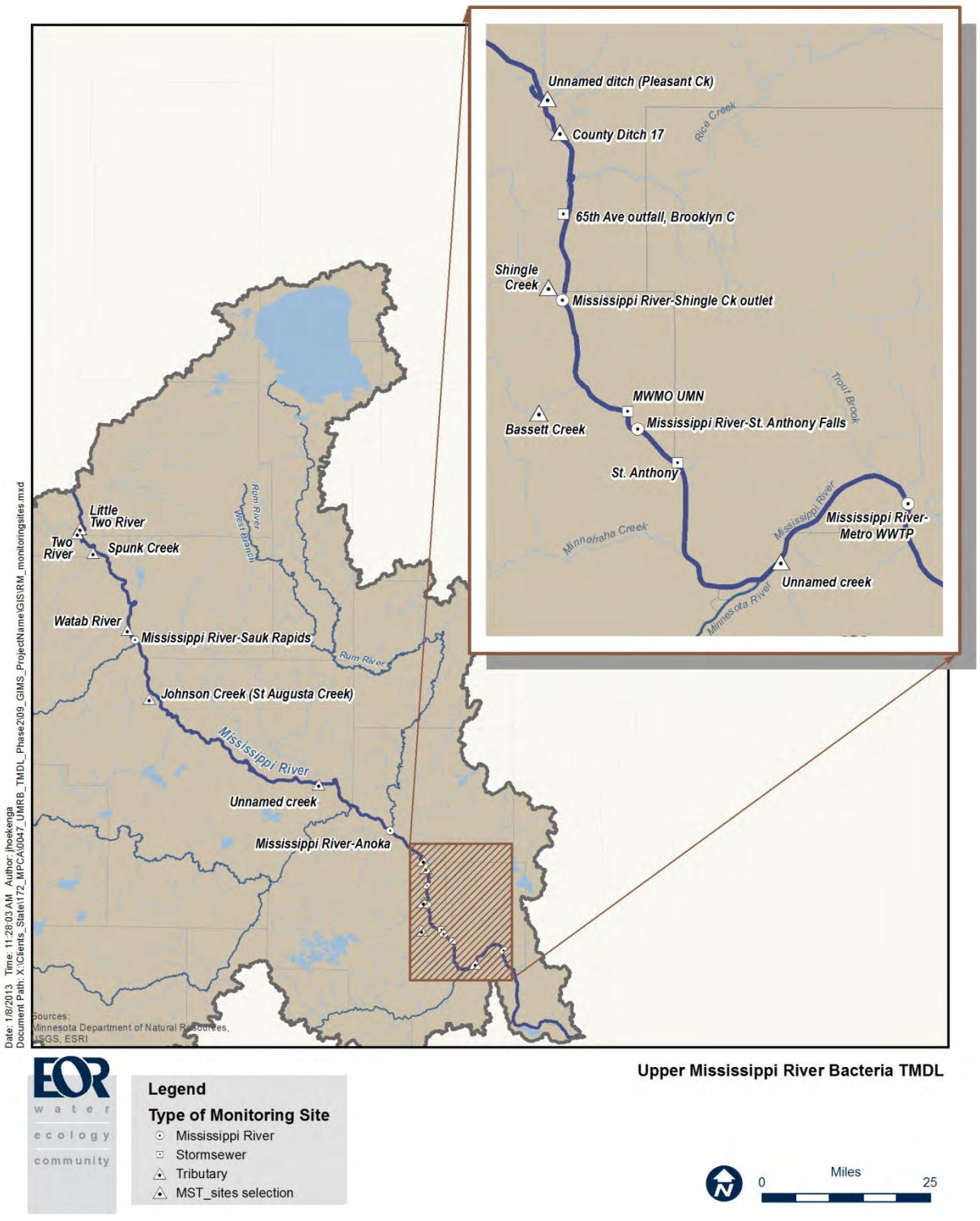

Figure 1. Microbial source tracking monitoring sites.

Results and Discussion

Bacteroides Markers

Table 2 reports the numeric results (gene copies/100mL) of the three *Bacteroides* markers for the detection of bovine, porcine, and human (and cat/dog) fecal contamination. In addition, Figure 2 through Figure 5 provides a graphical representation of these measurements (sites on the x-axis from left to right are organized from upstream to downstream). Remember, although the results are quantitative in nature, they should be interpreted semi-quantitatively. Therefore, relative concentrations of markers were compared in the data evaluation, which is summarized in Table 3. Table 3 provides the most appropriate summary information from this study given the limited sample size and analytical limitations.

The bovine marker was found at twelve of the nineteen monitoring sites (Table 2, Table 3, Figure 2). Of the positive results, concentrations ranged from 175 to 17,166 gene copies/100 mL. Concentrations were highest at Johnson Creek (S003-370) and at the St. Anthony stormsewer (SS00009). Four of the five Mississippi River sites tested positive for the bovine marker, and six of the eleven tributary sites tested positive. Two of the three stormsewer sites also tested positive for the bovine marker. There were several sites that tested positive for the bovine marker that do not appear to have cattle operations in the respective drainage areas: County Ditch 17, 65th Avenue outfall in Brooklyn Center, and Shingle Creek. Shanks et al. (2010)[3] reports that the bovine marker used in this study (Cow M3) has 100% specificity, which indicates that it should have no false positives. Shanks et al. (2010) also report that, while some bovine markers cross-react with other ruminants, the bovine marker used in this study does not cross-react with other ruminants. A potential source of bovine fecal contamination that might be considered is the use of improperly (or inadequately) processed compost. The stormsewer at St. Anthony has drainage that includes the MN State Fairgrounds and the University of Minnesota campus, both potential sources of fecal contamination from cattle.

The porcine marker was found at only one site (Table 2, Figure 3), Johnson Creek (S003-370). The positive result was found during the June 2011 runoff event, but not on the other three sampling days. Johnson Creek drainage is known to have hog operations according to county-based data from the National Agricultural Statistics Service[4]. A potential source of fecal contamination might be methods used for land application of manure and/or stockpiling.

The humans (and cats/dogs) marker was found at thirteen of the nineteen monitoring sites (Table 2, Table 3, Figure 4, and Figure 5). Of the positive results, concentrations ranged from 162 to 195,676 gene copies/100 mL. Concentrations were highest at the UMN stormsewer (SS00043) and the St. Anthony stormsewer (SS00009) sites. Two of the five Mississippi River sites tested positive for the humans (and cats/dogs) marker, and five of the eleven tributary sites tested positive. All of the samples taken from stormsewers showed the presence of human (and cat/dog) sources. Scientific literature supports findings that as a result of aging or damaged infrastructure,

[3] Shanks, O. C. et al. 2010. "Performance Assessment PCR-Based Assays Targeting Bacteroidales Genetic Markers of Bovine Fecal Pollution." Applied and Environmental Microbiology 76(5):1359–1366.
[4] USDA NASS (U.S. Department of Agriculture National Agricultural Statistics Service). 2009. *2007 Census of Agriculture: United States – Summary and State Data*. Volume 1, Geographic Area Series, Part 51, Updated December 2009. AC-07-A-51. Washington, D.C.: United States Department of Agriculture.

impervious landscapes can be characterized by chronic contamination of stormsewer systems that convey raw sewage originating from leaking sanitary sewers[5,6,7].

Fluoride

Mean fluoride concentrations at each site are illustrated in Figure 6. In addition, fluoride concentrations for each individual sampling event are illustrated in Figure 7. As was done in the graphs of *Bacteroides* marker result, sites on the x-axis from left to right are organized from upstream to downstream. The majority of the fluoride samples were found to have concentrations below the detection limit of the laboratory analytical method; the detection limit was 0.2 mg/L and, per standard protocol, these samples were evaluated in this data analysis as if at a value of half the detection limit (at 0.1 mg/L). The right-hand column of Table 2 illustrates the samples that had fluoride concentrations above detection limits.

Fluoride is expected to be present in Minnesota's municipal wastewater as a result of drinking water fluoridation requirements. Minnesota Rule 4720.0030 Subp. 2 sets the required average fluoridation level to 1.2 mg/L (0.9 mg/L – 1.5 mg/L). Therefore, the presence of fluoride in surface waters is an indicator of possible human fecal contamination. With a robust sampling regime, we would anticipate a) measureable fluoride concentrations to appear at sites exhibiting human fecal contamination derived from municipal wastewater breeches, and b) sites exhibiting measurable fluoride concentrations to also have tested positive for the human (and cat/dog) *Bacteroides* marker. Of the four sites exhibiting fluoride concentrations above the detection limit (SS00010, SS00043, S006-735, and S006-148), only the two stormsewer sites tested positive for the human (and cat/dog) *Bacteroides* marker (SS00010, SS00043). Data are inconclusive but warrant further investigation into the possibility of human fecal contamination at sites exhibiting fluoride concentrations above detection limits. In addition, several sites that did *not* exhibit fluoride concentrations above the detection limit *did* test positive for the human (and cat/dog) marker (refer to Table 2), which may be an indicator that human fecal contamination is from sources other than municipal wastewater leakage, e.g. failing septic systems.

E. coli

Results from water samples analyzed for *E. coli*, on which the water quality standard is based, are illustrated in Figure 8 and Figure 9. Figure 8 illustrates geometric mean concentrations at each site across all sampling events for the site. Figure 9 illustrates *E. coli* concentrations for each individual sampling event. The quantity of *E. coli* cannot be directly compared to the *Bacteroides* source tracking results since the source tracking results merely identify *what* is there, not *how much* is there. However, data are presented in order to provide the known water quality characteristics at each site at the time of the sampling events.

[5] Sauer, P.S., VandeWalle, J.S., Bootsma, M.J., McLellan, S.L. 2011. Detection of the human specific *Bacteroides* genetic marker provides evidence of widespread sewage contamination of stormwater in the urban environment. *Water Research*, 45:4081-4091.
[6] Sercu, B., L.C. Van de Werfhorst, J. Murray, and P. 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.
[7] Sercu, B., Van De Werfhorst, L.C., Murray, J.L.S., Holden, P.A. 2011. Sewage exfiltration as a source of storm drain contamination during dry weather in urban watersheds. *Environmental Science & Technology*, 45:7151-7157.

MPCA assessment for *E. coli* impairments requires at least five samples per month, typically over a two-year period or more, during the period from April through October. Since the sampling regime of this study does not meet MPCA assessment protocol, comparing *E. coli* data from this project to the standard (126 org/100 mL) provides merely a reference point with respect to water quality at the sampling site. The majority of samples exhibit *E. coli* concentrations above the water quality standard (Figure 8 and Figure 9). By far, the highest *E. coli* concentrations were exhibited at Johnson Creek (S003-370), which also tested positive for the bovine marker (all four sampling events) and the porcine marker (one sampling event). The Mississippi River sites tended to exhibit lower *E. coli* concentrations than tributary sites with two exceptions; Unnamed Creek (S006-148) and Pleasant Creek (S003-995) also exhibited relatively low *E. coli* concentrations.

Table 2. *Bacteroides* and fluoride monitoring results.
See Table 1 for site location descriptions.

Site #	Date	Time	Storm or Baseflow	Bovine (gene copies/100 ml)		Porcine (gene copies/100 ml)		Human (and cat/dog) (gene copies /100 ml)		Fluoride (mg/L)
				Mean	SE[1]	Mean	SE[1]	Mean	SE[1]	
S000-025	6/15/2011	13:32	Storm	-	-	-	-	306	66	-
	9/29/2011	11:15	Base	-	-	-	-	-	-	-
S001-946	6/15/2011	5:30	Storm	-	-	-	-	311	57	-
	6/28/2011	12:37	Base	-	-	-	-	-	-	-
	9/29/2011		Base	189	41	-	-	-	-	-
	4/16/2012	8:00	Storm	-	-	-	-	-	-	-
S002-947	6/15/2011	11:05	Storm	-	-	-	-	-	-	-
	9/29/2011	9:01	Base	-	-	-	-	-	-	-
S002-948	6/15/2011	10:35	Storm	1,700	96	-	-	-	-	-
	6/28/2011	8:50	Base	-	-	-	-	-	-	-
	9/29/2011	8:32	Base	-	-	-	-	-	-	-
	4/16/2012	10:00	Storm	802	149	-	-	-	-	-
S002-949	6/15/2011	10:15	Storm	193	9	-	-	-	-	-
	9/29/2011	8:17	Base	-	-	-	-	-	-	-
S003-370	6/15/2011	12:15	Storm	2,272	329	261	66	-	-	-
	6/28/2011	10:06	Base	2,536	400	-	-	-	-	-
	9/29/2011	10:05	Base	3,854	147	-	-	-	-	-
	4/16/2012	11:30	Storm	17,166	1,590	-	-	-	-	-
S003-995	6/28/2011	11:40	Base	-	-	-	-	574	38	-
	4/16/2012	13:06	Storm	-	-	-	-	-	-	-
S004-653	6/15/2011	15:20	Storm	-	-	-	-	190	39	-
	6/28/2011	12:58	Base	285	66	-	-	-	-	-
	9/29/2011	12:45	Base	-	-	-	-	-	-	-
	4/16/2012	7:45	Storm	-	-	-	-	-	-	-
S004-655	6/28/2011	13:36	Base	278	68	-	-	-	-	-
	4/16/2012	7:10	Storm	-	-	-	-	-	-	-

Site #	Date	Time	Storm or Baseflow	Bovine (gene copies/100 ml)		Porcine (gene copies/100 ml)		Human (and cat/dog) (gene copies /100 ml)		Fluoride (mg/L)
				Mean	SE[1]	Mean	SE[1]	Mean	SE[1]	
S005-017	6/15/2011	16:30	Storm	-	-	-	-	485	82	-
	9/29/2011	5:52	Base	-	-	-	-	-	-	-
S006-139	6/28/2011	6:35	Base	-	-	-	-	-	-	-
	4/16/2012	6:15	Storm	-	-	-	-	-	-	-
S006-140	6/15/2011	14:17	Storm	-	-	-	-	162	23	-
	6/28/2011	12:00	Base	1,868	163	-	-	-	-	-
	9/29/2011	11:57	Base	-	-	-	-	-	-	-
	4/16/2012	13:30	Storm	-	-	-	-	-	-	-
S006-148	6/28/2011	10:55	Base	296	61	-	-	-	-	0.25
	4/16/2012	12:16	Storm	-	-	-	-	-	-	0.45
S006-162	6/15/2011	10:03	Storm	-	-	-	-	520	33	-
	9/29/2011	6:00	Base	-	-	-	-	-	-	-
S006-163	6/15/2011	11:32	Storm	175	40	-	-	-	-	-
	6/28/2011	9:23	Base	-	-	-	-	-	-	-
	9/29/2011	9:25	Base	-	-	-	-	-	-	-
	4/16/2012	10:43	Storm	-	-	-	-	-	-	-
S006-735	6/28/2011	6:05	Base	653	31	-	-	-	-	0.28
	4/16/2012	5:40	Storm	-	-	-	-	-	-	-
SS00009	6/15/2011	17:25	Storm	11,564	270	-	-	5,227	265	-
	4/16/2012	6:45	Storm	-	-	-	-	163	34	-
SS00010	6/15/2011	14:40	Storm	503	107	-	-	213	43	-
	4/16/2012	8:20	Storm	-	-	-	-	357	59	0.25
SS00043	6/15/2011	16:38	Storm	-	-	-	-	195,676	5,687	0.21
	4/16/2012	14:40	Storm	-	-	-	-	998	106	-

'-' indicates below detection limit

[1] SE = standard error

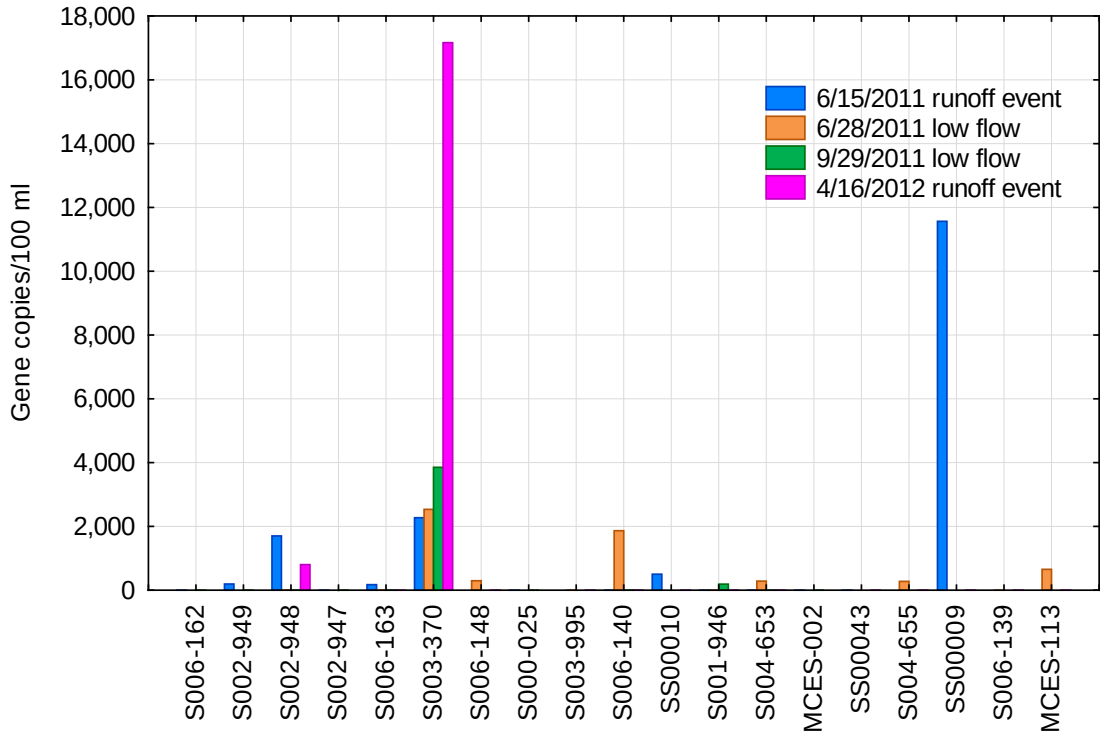

Figure 2. Results from *Bacteroides* marker for bovine fecal contamination.
Note that for each site label on the x-axis, there are four placeholders for bars of data, two on either side of the corresponding tick mark.

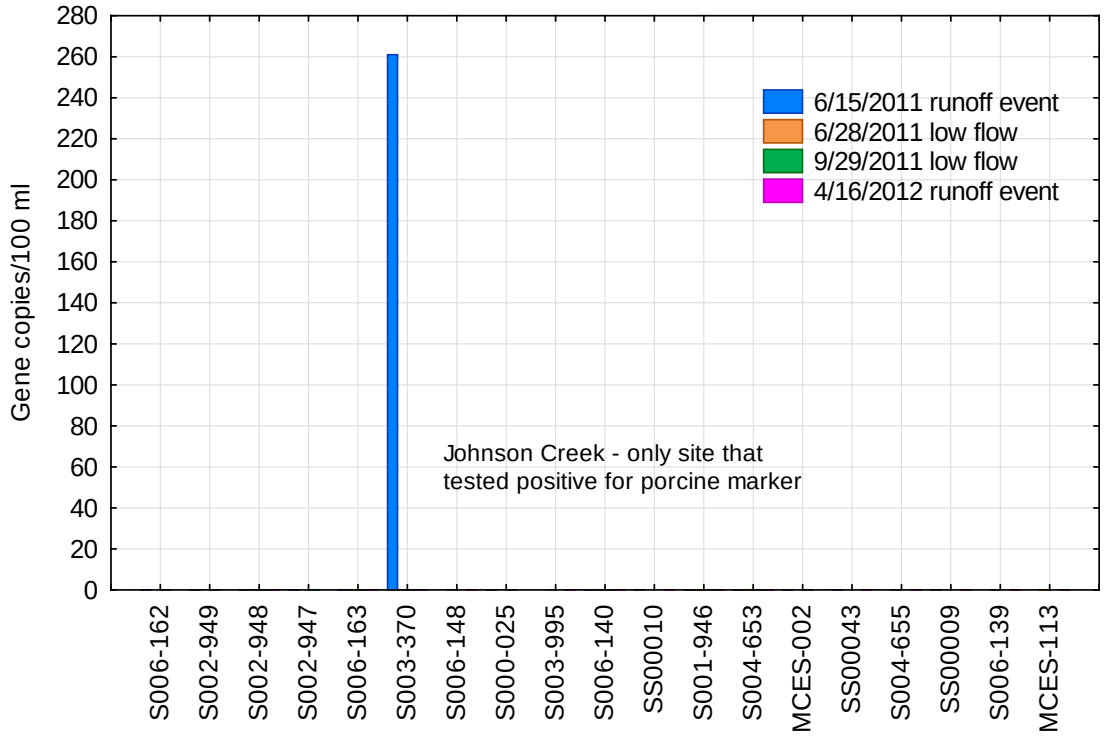

Figure 3. Results from *Bacteroides* marker for porcine fecal contamination.
The single porcine result applies to site S003-370.

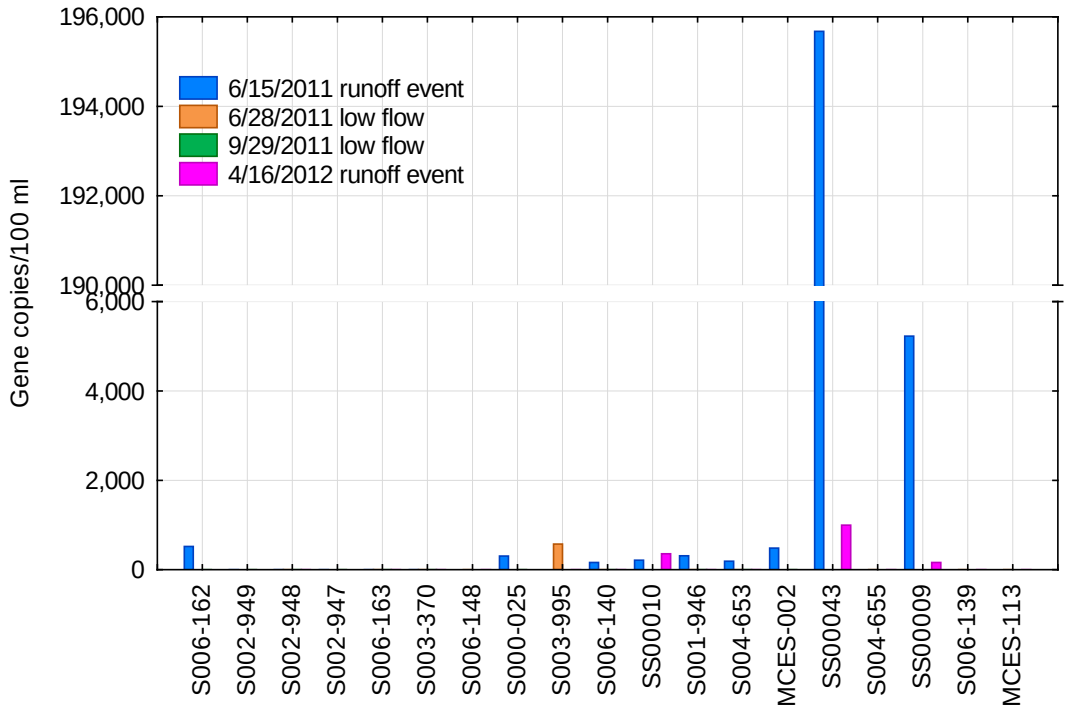

Figure 4. Results from *Bacteroides* marker for human (and cat/dog) fecal contamination, highlighting high concentrations.
Note y-axis scale break. Note, too, that for each site label on the x-axis, there are four placeholders for bars of data, two on either side of the corresponding tick mark.

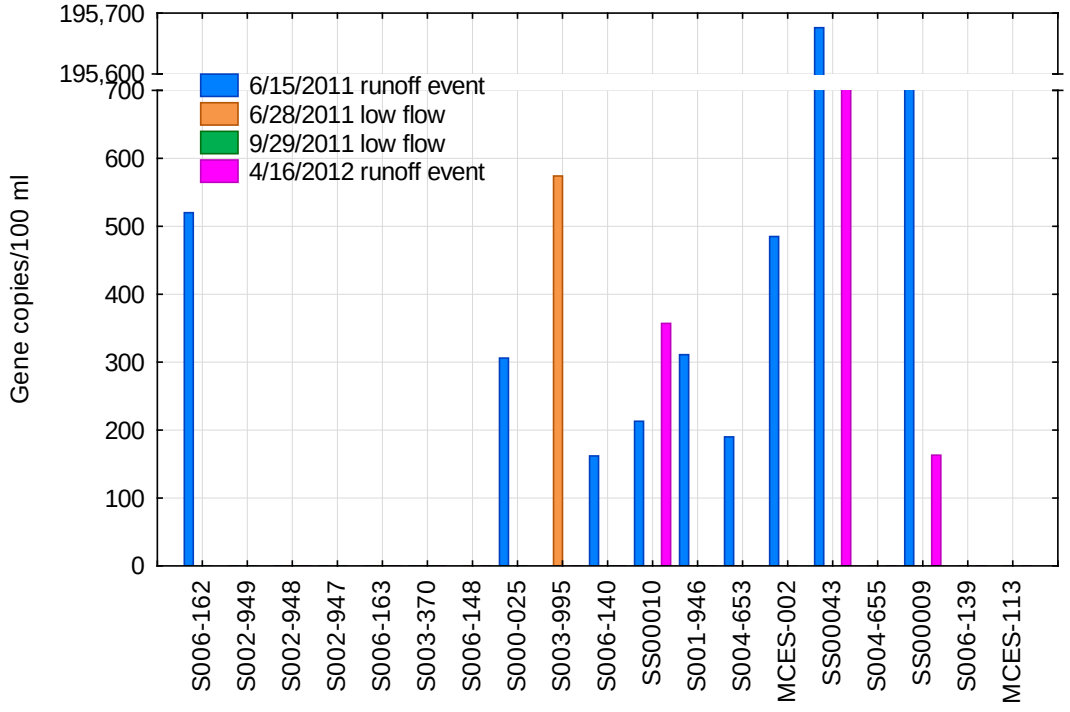

Figure 5. Results from *Bacteroides* marker for human (and cat/dog) fecal contamination, highlighting low concentrations.
Note y-axis scale break. Note, too, that for each site label on the x-axis, there are four placeholders for bars of data, two on either side of the corresponding tick mark.

Table 3. Microbial source tracking results summary.

Site Name	Site ID		Presence of Indicator+			Predominant Land Use		Site Type			Comments
		AUID	Bovine	Human (and cats/dogs)	Porcine	Rural	Urban	Mississippi River	Tributary	Storm-sewer	
Little Two River	S006-162	07010201-516		+		ü			ü		
Two River	S002-949	07010201-523	+			ü			ü		
Spunk Creek	S002-948	07010201-525	+			ü			ü		
Watab River	S002-947	07010201-528				ü			ü		
MR - downstream MN-15 Bridge in Sauk Rapids	S006-163	07010201-502	+			mixed		ü			
Johnson Ck btwn CR-75 and I-94, 5 mi S of St. Cloud (St. Augusta Ck)	S003-370	07010203-639	++		+	ü			ü		- 4 of 4 samples bovine presence, highest value observed of all bovine tests - Only site that showed the presence of porcine marker
Unnamed Creek	S006-148	07010203-528	+			ü			ü		
MR - at US-169 Bridge at Anoka (NW city limits of Anoka to Rum River)	S000-025	07010206-568		+		mixed		ü			
Unnamed ditch (Pleasant Creek)	S003-995	07010206-594		+			ü		ü		- High human (and cat/dog) presence during low flow
County Ditch 17	S006-140	07010206-557	+	+			ü		ü		- Bovine marker in predominantly urban land use
65th Ave outfall in Brooklyn Center	SS00010	07010206-509*	+	+			ü			ü	- 2 of 2 samples human (and cat/dog) presence - Bovine marker in predominantly urban land use
Shingle Creek	S001-946	07010206-506	+	+			ü		ü		- Bovine marker in predominantly urban land use
MR - Coon Creek to Upper St. Anthony Falls (site at Shingle Ck outlet)	S004-653	07010206-509	+	+		mixed		ü			
Bassett Creek	S005-017	07010206-538		+			ü		ü		
MWMO UMN	SS00043	07010206-503*		++			ü			ü	- 2 of 2 samples human (and cat/dog) presence, highest value observed of all human tests
MR - Lower St. Anthony Falls to L&D#1	S004-655	07010206-503	+			mixed		ü			
St. Anthony Ave, CRWD	SS00009	07010206-503*	++	++			ü			ü	- 2 of 2 samples human (and cat/dog) presence, second highest value observed of all human (and cat/dog) tests - Bovine marker in urban region; drainage area includes State Fairgrounds and U of MN campus
Unnamed Creek (downstream of MN River confluence with Mississippi River)	S006-139	07010206-542					ü		ü		
MR - MN River to Metro WWTP	S006-735	07010206-505	+			mixed		ü			

* indicates that the storm sewer outfalls to the listed AUID

\+ indicates presence of the marker; ++ indicates high marker concentrations relative to other results

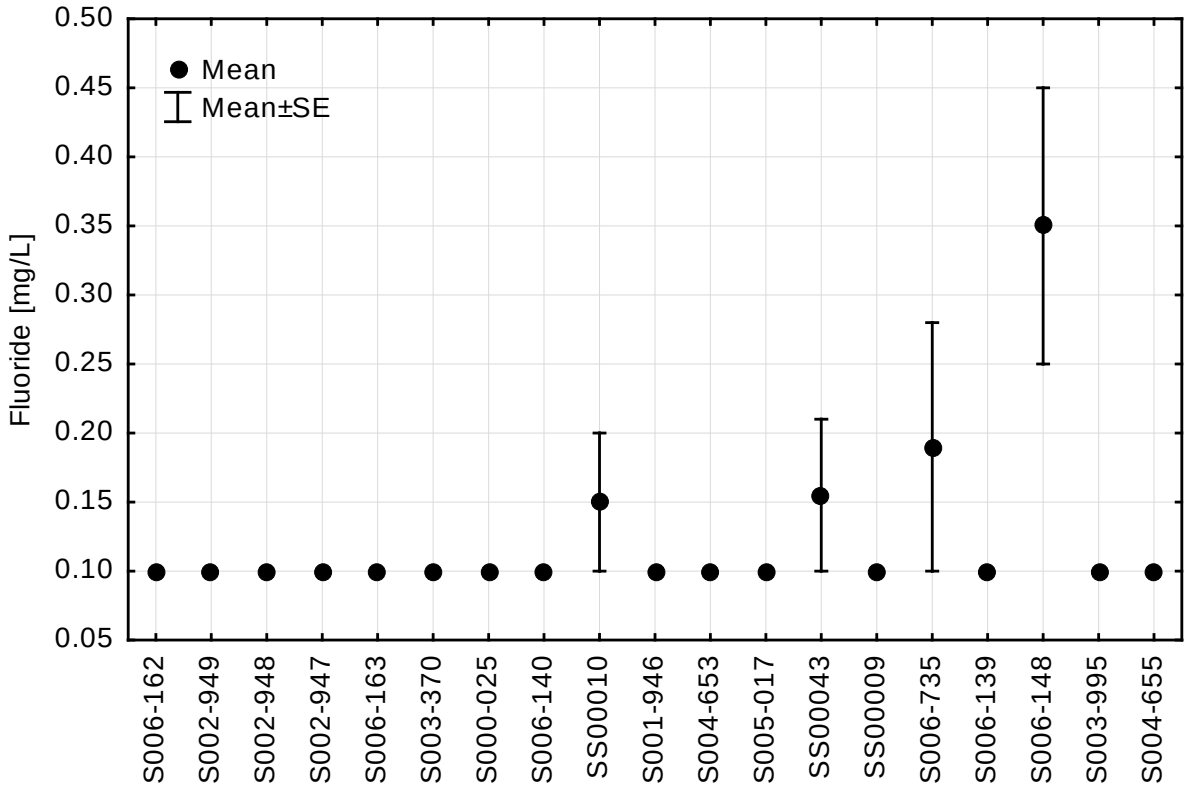

Figure 6. Mean fluoride concentrations from all sampling events at microbial source tracking monitoring sites.
The majority of the fluoride samples were found to have concentrations below the detection limit of the laboratory analytical method; the detection limit was 0.2 mg/L and, per standard protocol, these samples are included here assumed to be at a value of half the detection limit (at 0.1 mg/L).
SE = standard error.

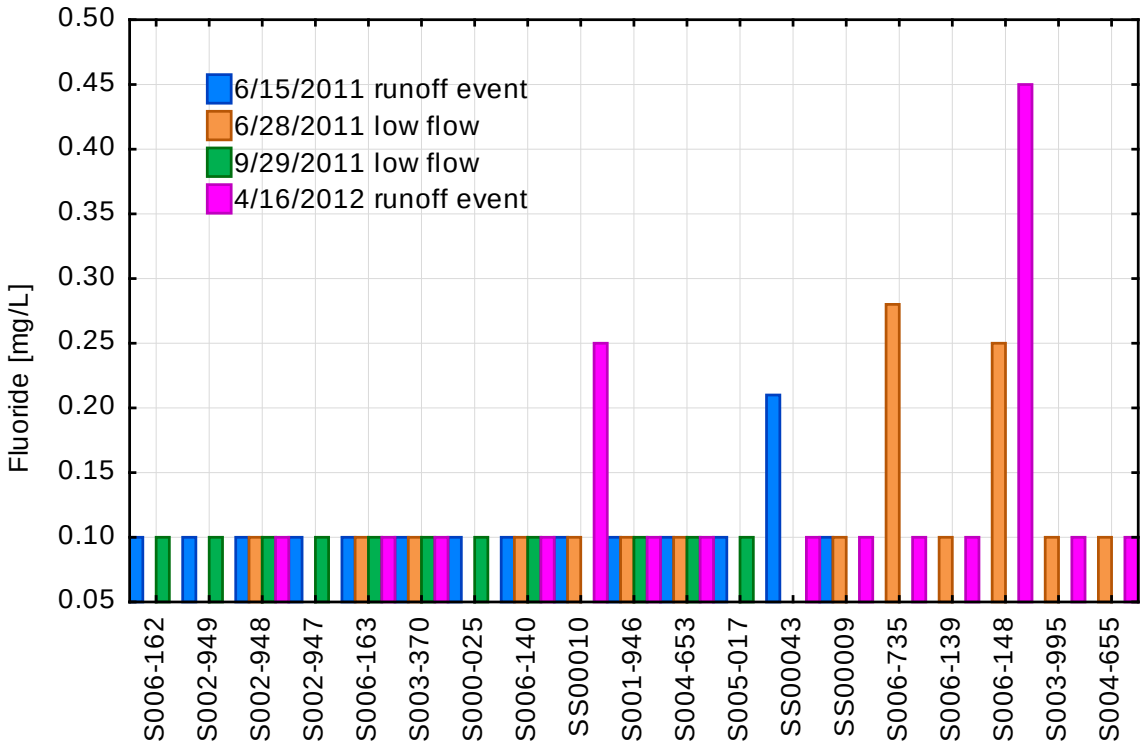

Figure 7. Fluoride concentrations from each sampling event at microbial source tracking monitoring sites.
The majority of the fluoride samples were found to have concentrations below the detection limit of the laboratory analytical method; the detection limit was 0.2 mg/L and, per standard protocol, these samples are shown here assumed to be at a value of half the detection limit (at 0.1 mg/L).

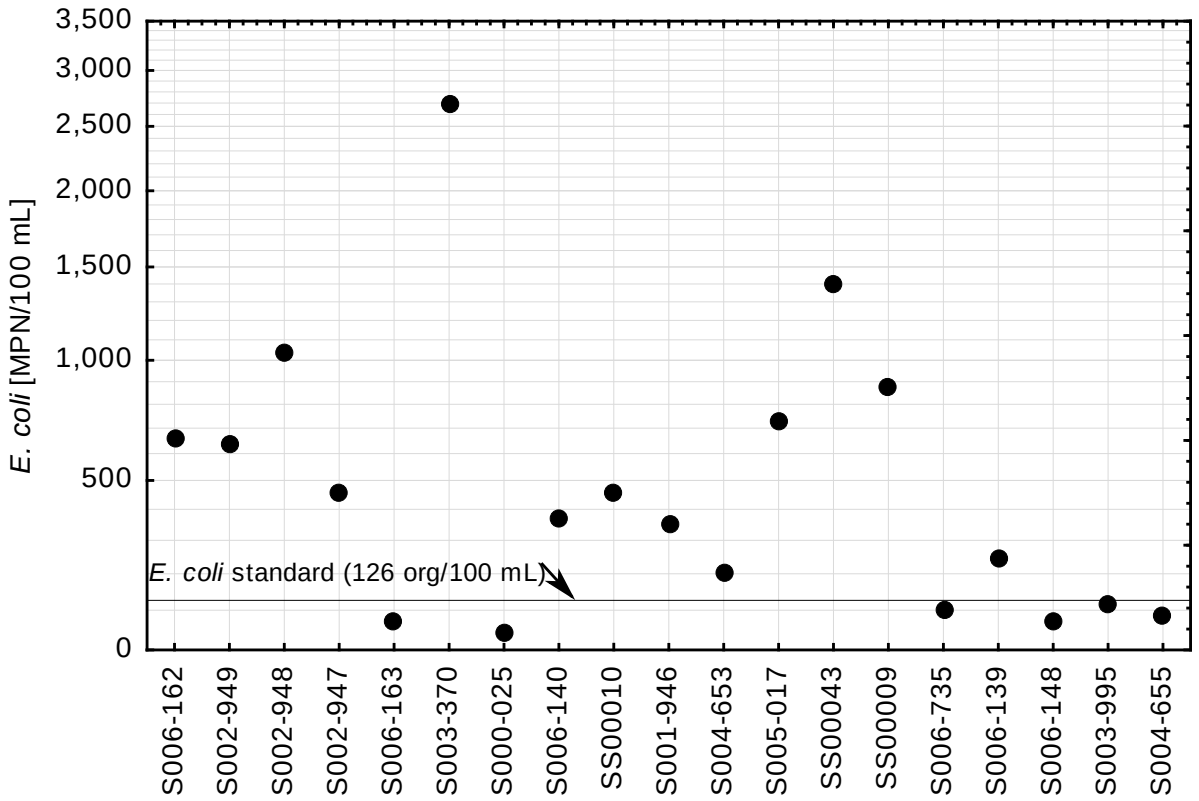

Figure 8. Geometric mean *E. coli* concentrations from all sampling events at microbial source tracking monitoring sites.
Note y-axis logarithmic scale and scale break. The sampling regime of this study does not meet MPCA assessment protocol; comparing *E. coli* data from this project to the standard (126 org/100 mL) provides merely a reference point.

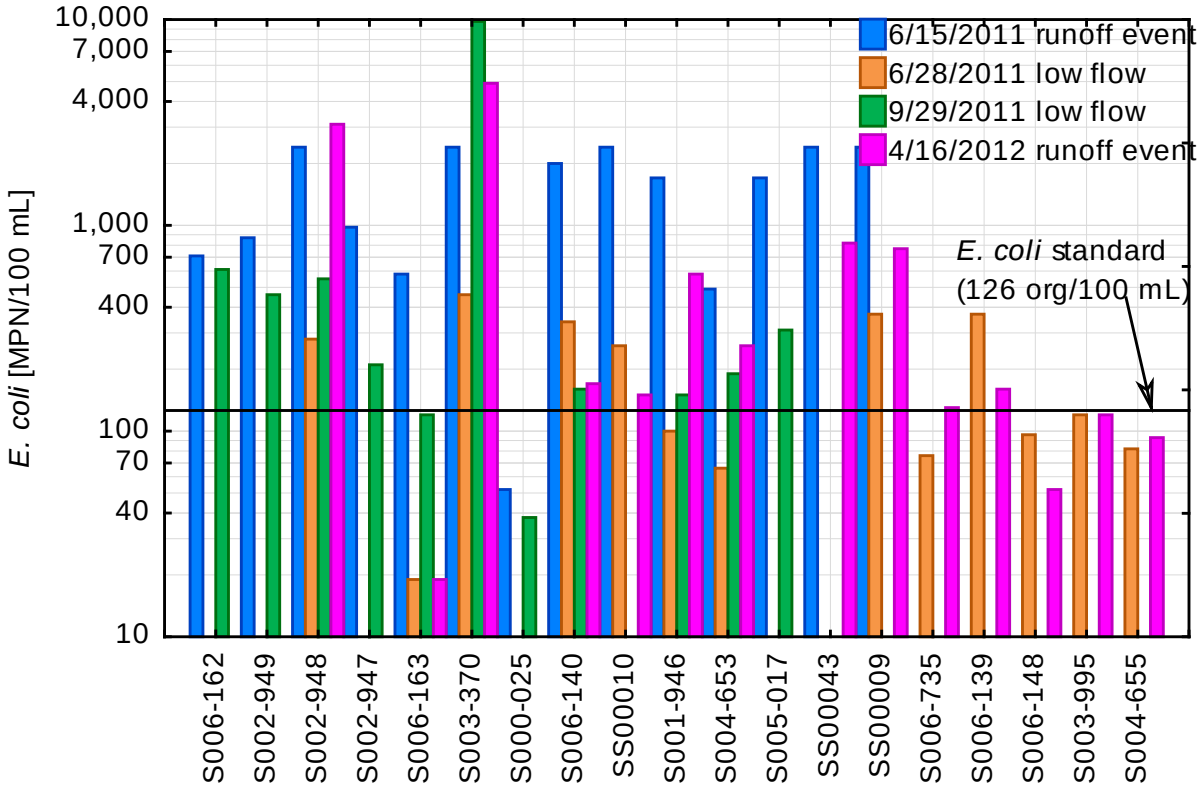

Figure 9. *E. coli* concentrations from all sampling events at microbial source tracking monitoring sites.
Note y-axis logarithmic scale and scale break. The sampling regime of this study does not meet MPCA assessment protocol; comparing *E. coli* data from this project to the standard (126 org/100 mL) provides merely a reference point.

Conclusions

The following conclusions are based on the observations from this monitoring:

- Bovine and human (and cat/dog) sources of fecal contamination are present throughout the watershed.
- Unexpected results suggest areas of future investigation: are there bovine sources in the watersheds of County Ditch 17, 65th Avenue outfall in Brooklyn Center, and Shingle Creek?
- Stormsewers show a consistent presence of the human (and cat/dog) marker; are there sources of human fecal contamination such as leaking sanitary sewers?

Appendix: Laboratory Reports

The following two reports are the laboratory reports from the Research Analytical Laboratory at the University of Minnesota.

The site numbers in the reports may be different from the site numbers reported in Table 2. Site #5001-303 in the U of MN report is SS00043 in Table 2. The site numbers in Table 2 that begin with either “S” or “SS” are indicated in the U of MN report as beginning with “5” or “55.”

ANALYTICAL DATA REPORT

Research Analytical Laboratory
University of Minnesota
1902 Dudley Ave.
Saint Paul, MN 55108
Telephone: 612.625.3101
E-mail: ral@umn.edu

Report to: Minnesota Pollution Control Agency
Date received: 6/15/11 & 6/28/11
Date reported: 7/27/11
Study name: Upper Mississippi Bacteria TMDL
Sample type: Water
Analyses for: *Bacteroides* by qPCR

Methods

A 300 ml subsample of the provided water sample was filtered through a 0.45μm nitrocellulose filter (Millipore) and extracted using a Power Water DNA Isolation Kit (MoBio). The isolated DNA was eluted into 50 μl of Tris buffer. The DNA was analyzed for source-specific *Bacteroides* marker genes ses by using a Quantitative PCR assay and the following primers:

bovine primers CowM3F (5'- CCTCTAATGGAAAATGGATGGTATCT-3') and CowM3R (5'-CCATACTTCGCCT GCTAATACCTT-3') and probe (5'-(FAM)TTATGCATTGAGCATCGAGGCC(TAMRA)-3'),

porcine primers Pig-2-Bac41F (5'-GCATGAATTTAGCTTGCTAAATTTGAT-3') and Pig-2-Bac163Rm (5'-ACCTCATACGGTATTAATCCGC-3') and probe Pig-2Bac113MGB (5'-(VIC)TCCACGGGATAGCC(NFQ-MGB)-3')

human primers HF183f (5'-ATCATGAGTTCACATGTCCG-3') and HF183r (5'-CCATCGGAGTTCTTCGTG-3').

Plasmid standards were created by cloning the target gene from PCR product of animal fecal DNAs using the StrataClone PCR kit (Stratagene). Purified plasmid DNA was quantified by the Qubit 1.0 flourometer (Invitrogen) before preparation of 10-fold dilutions for qPCR standards. The qPCR reaction mixture consisted of 12.5 μl of iTaq SYBR Green Supermix with ROX (BioRad) for the human assay or iTaq Supermix with ROX (BioRad) for bovine and porcine assays, primers and probe, and 5 μl of a ten-fold dilution of template DNA in 25 uL reaction volume. Concentration of primers and probe were optimized at 200 nM primer and probe for the bovine assay, 300 nM primer and 100 nM probe for the porcine assay, and 300 nM primer for the human assay. Reaction conditions for bovine and porcine qPCR consisted of an initial denaturation at 95°C for 5 min and 40 cycles of 95°C for 15 s, and 60°C annealing/extension for 1 min. Reaction conditions for human-specific qPCR consisted of an initial denaturation at 95°C for 5 min and 40 cycles of 95°C for 15 s, 56°C annealing for 30 s, and 60°C extension for 45 s. For the SYBR

green assay, melt curves were used to verify specific product amplification. Each reaction plate contained three non-transcript controls, six plasmid standards run in triplicate, and test samples run in triplicate.

qPCR reactions were carried out in the ABI Prism7000 Sequence Detection System (Applied Biosystems), and threshold cycle number was determined automatically using SDS software (Applied Biosystems). The qPCR reaction efficiency ranged from 95 to 102% and the standard curve R^2 ranged from 0.994 to 0.998. No amplification was detected in non-transcript controls.

Water Sample			*Bacteroides* marker gene copies per ml Mean ± SE			*Bacteroides* marker gene copies per 100 ml Mean ± SE		
Code	**Date**	**Time**	**Bovine**	**Porcine**	**Human**	**Bovine**	**Porcine**	**Human**
5001-946	6/15/2011	5:30	BDL*	BDL	3.1±0.6	BDL	BDL	311±57
5006-162	6/15/2011	10:03	BDL	BDL	5.2±0.3	BDL	BDL	520±33
5002-949	6/15/2011	10:15	1.9±0.1	BDL	BDL	193±9.3	BDL	BDL
5002-948	6/15/2011	10:35	17±1.0	BDL	BDL	1,700±96	BDL	BDL
5002-947	6/15/2011	11:05	BDL	BDL	BDL	BDL	BDL	BDL
5006-163	6/15/2011	11:32	1.8±0.4	BDL	BDL	175±40	BDL	BDL
5003-370	6/15/2011	12:15	23±3.3	2.6±0.7	BDL	2,272±329	261±66	BDL
5000-025	6/15/2011	13:32	BDL	BDL	3.1±0.7	BDL	BDL	306±66
5006-140	6/15/2011	14:17	BDL	BDL	1.6±0.2	BDL	BDL	162±23
65th Ave Outfall	6/15/2011	14:40	5.0±1.1	BDL	2.1±0.4	503±107	BDL	213±43
5004-653	6/15/2011	15:20	BDL	BDL	1.9.±0.4	BDL	BDL	190±39
MCES-002	6/15/2011	16:30	BDL	BDL	4.9±0.8	BDL	BDL	485±82
6 UMN	6/15/2011	16:38	BDL	BDL	1,957±57	BDL	BDL	195,676±5,687
St. Anthony Park	6/15/2011	17:25	116±2.7	BDL	52±2.6	11,564±270	BDL	5,227±265
MCES-113	6/28/2011	6:05	6.5±0.3	BDL	BDL	653±31	BDL	BDL
5006-139	6/28/2011	6:35	BDL	BDL	BDL	BDL	BDL	BDL
5002-948	6/28/2011	8:50	BDL	BDL	BDL	BDL	BDL	BDL
5006-163	6/28/2011	9:23	BDL	BDL	BDL	BDL	BDL	BDL
5003-370	6/28/2011	10:06	25±4.0	BDL	BDL	2,536±400	BDL	BDL
5006-148	6/28/2011	10:55	3.0±0.6	BDL	BDL	296±61	BDL	BDL
5003-995	6/28/2011	11:40	BDL	BDL	5.7±0.4	BDL	BDL	574±38
5006-140	6/28/2011	12:00	19±1.6	BDL	BDL	1,868±163	BDL	BDL
5001-946	6/28/2011	12:37	BDL	BDL	BDL	BDL	BDL	BDL
5004-653	6/28/2011	12:58	2.9±0.7	BDL	BDL	285±66	BDL	BDL
5004-655	6/28/2011	13:36	2.8±0.7	BDL	BDL	278±68	BDL	BDL

*BDL = Below Detection Limit - 1 DNA copy per ml .*BDL = Below Detection Limit - 100 DNA copies per ml.

ANALYTICAL DATA REPORT

Research Analytical Laboratory
University of Minnesota
1902 Dudley Ave.
Saint Paul, MN 55108
Telephone: 612.625.3101
E-mail: ral@umn.edu

Report to: Minnesota Pollution Control Agency
Date received: 9/29/11 & 4/16/12
Date reported: 4/19/12
Study name: Upper Mississippi Bacteria TMDL
Sample type: Water
Analyses for: *Bacteroides* by qPCR

Methods

A 300 ml subsample of the provided water sample was filtered through a 0.45μm nitrocellulose filter (Millipore) and extracted using a Power Water DNA Isolation Kit (MoBio). The isolated DNA was eluted into 50 μl of Tris buffer. The DNA was analyzed for source-specific *Bacteroides* marker genes ses by using a Quantitative PCR assay and the following primers:

Bovine primers: CowM3F (5'- CCTCTAATGGAAAATGGATGGTATCT-3') and CowM3R (5'-CCATACTTCGCCT GCTAATACCTT-3') and probe (5'-(FAM)TTATGCATTGAGCATCGAGGCC(TAMRA)-3'),

Porcine primers: Pig-2-Bac41F (5'-GCATGAATTTAGCTTGCTAAATTTGAT-3') and Pig-2-Bac163Rm (5'-ACCTCATACGGTATTAATCCGC-3') and probe Pig-2Bac113MGB (5'-(VIC)TCCACGGGATAGCC(NFQ-MGB)-3')

Human primers: HF183f (5'-ATCATGAGTTCACATGTCCG-3') and HF183r (5'-CCATCGGAGTTCTTCGTG-3').

Plasmid standards were created by cloning the target gene from PCR product of animal fecal DNAs using the StrataClone PCR kit (Stratagene). Purified plasmid DNA was quantified by the Qubit 1.0 flourometer (Invitrogen) before preparation of 10-fold dilutions for qPCR standards. The qPCR reaction mixture consisted of 12.5 μl of iTaq SYBR Green Supermix with ROX (BioRad) for the human assay or iTaq Supermix with ROX (BioRad) for bovine and porcine assays, primers and probe, and 5 μl of a ten-fold dilution of template DNA in 25 uL reaction volume. Concentration of primers and probe were optimized at 200 nM primer and probe for the bovine assay, 300 nM primer and 100 nM probe for the porcine assay, and 300 nM primer for the human assay. Reaction conditions for bovine and porcine qPCR consisted of an initial denaturation at 95°C for 5 min and 40 cycles of 95°C for 15 s, and 60°C annealing/extension for 1 min. Reaction conditions for human-specific qPCR consisted of an initial denaturation at 95°C for 5 min and 40 cycles of 95°C for 15 s, 56°C annealing for 30 s, and 72°C extension for 1 min. For the SYBR

green assay, melt curves were used to verify specific product amplification. Each reaction plate contained three non-transcript controls, six plasmid standards run in triplicate, and test samples run in triplicate.

qPCR reactions were carried out in the ABI Prism7000 Sequence Detection System (Applied Biosystems), and threshold cycle number was determined automatically using SDS software (Applied Biosystems). The qPCR reaction efficiency ranged from 90 to 105% and the standard curve R^2 ranged from 0.995 to 0.998. No amplification was detected in non-transcript controls.

Water Sample			*Bacteroides* marker gene copies per ml Mean ± SD			*Bacteroides* marker gene copies per 100 ml Mean ± SD		
Code	**Date**	**Time**	**Bovine**	**Porcine**	**Human**	**Bovine**	**Porcine**	**Human**
5002-948	9/29/11	8:32	BDL*	BDL	BDL	BDL*	BDL	BDL
5006-162	9/29/11	6:00	BDL	BDL	BDL	BDL	BDL	BDL
5006-163	9/29/11	9:25	BDL	BDL	BDL	BDL	BDL	BDL
5002-949	9/29/11	8:17	BDL	BDL	BDL	BDL	BDL	BDL
5005-017	9/29/11	5:52	BDL	BDL	BDL	BDL	BDL	BDL
5002-947	9/29/11	9:01	BDL	BDL	BDL	BDL	BDL	BDL
5000-025	9/29/11	11:15	BDL	BDL	BDL	BDL	BDL	BDL
5006-140	9/29/11	11:57	BDL	BDL	BDL	BDL	BDL	BDL
5003-370	9/29/11	10:05	39±1.5	BDL	BDL	3,854±147	BDL	BDL
5004-653	9/29/11	12:45	BDL	BDL	BDL	BDL	BDL	BDL
5001-946	9/29/11	--	1.9±0.4	BDL	BDL	189±41	BDL	BDL
5006-735	4/16/12	5:40	BDL	BDL	BDL	BDL	BDL	BDL
5500009	4/16/12	6:45	BDL	BDL	1.6±0.3	BDL	BDL	163±34
5006-139	4/16/12	6:15	BDL	BDL	BDL	BDL	BDL	BDL
5004-655	4/16/12	7:10	BDL	BDL	BDL	BDL	BDL	BDL
5004-653	4/16/12	7:45	BDL	BDL	BDL	BDL	BDL	BDL
5001-946	4/16/12	8:00	BDL	BDL	BDL	BDL	BDL	BDL
5500010	4/16/12	8:20	BDL	BDL	3.6±0.6	BDL	BDL	357±59
5002-948	4/16/12	10:00	8.0±1.5	BDL	BDL	802±149	BDL	BDL
5006-163	4/16/12	10:43	BDL	BDL	BDL	BDL	BDL	BDL
5003-370	4/16/12	11:30	172±16	BDL	BDL	17,166±1,590	BDL	BDL
5006-148	4/16/12	12:16	BDL	BDL	BDL	BDL	BDL	BDL
5003-995	4/16/12	13:06	BDL	BDL	BDL	BDL	BDL	BDL
5006-140	4/16/12	13:30	BDL	BDL	BDL	BDL	BDL	BDL
5001-303	4/16/12	14:40	BDL	BDL	10±1.1	BDL	BDL	998±106

*BDL = Below Detection Limit = 1 DNA copy per ml.

*BDL = Below Detection Limit = 100 DNA copies per ml.