Madoc Microbial Source Tracking Survey
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INTRODUCTION

The Village of Madoc, Ontario, has a municipal well supply that is believed to be influenced by surface waters from a local stream (Groundwater Under the Direct Influence (GUDI) of surface water). Madoc is located just north of the town of Belleville that is situated on the Bay of Quinte, Lake Ontario. Quinte Conservation has indicated that Deer Creek is thought to be the surface water of influence for the Village of Madoc. Madoc Creek flows from the north-east into Deer Creek at a point just south of HWY 7, and then Deer Creek flows south past the location of two municipal wells (see Figure 1). A survey was conducted in the fall of 2010 to provide a preliminary characterization of the microbial water quality at several surface water and municipal groundwater sampling locations in the Madoc study area. Weekly water sampling was carried out by Quinte Conservation, and Environment Canada analyzed water samples to enumerate *E. coli* and determine whether the DNA from a strain of *Bacteroidales* bacteria that is unique to the human gut, could be detected in the water samples. The objective was to understand the extent of fecal pollution at the surface water and groundwater sampling locations, and to determine if there was evidence for human sewage contamination as a contributor to any detectable fecal pollution. Human sewage is generally regarded to present the highest concern among fecal pollution sources for potential human health risks from the occurrence of waterborne pathogens.

This study applied *E. coli* enumeration and a microbial source tracking approach to investigate the potential sources of fecal contamination in the Madoc study area. Microbial source tracking techniques compare the similarity of microorganisms from fecal pollution sources and water samples in order to make inferences about the source of water contamination (U.S. EPA, 2005; Edge and Schaefer, 2006). There are two general approaches to microbial source tracking: library-dependent methods and library-independent methods. Library-dependent methods select an indicator microorganism like *E. coli*, and collect hundreds to thousands of isolates from fecal sources and nearby water samples of interest. The similarity of fecal and water *E. coli* isolates is measured by DNA fingerprinting or other forensic-like techniques to infer the likely source of the water isolates. In this sense, the similarity of DNA fingerprints of *E. coli* from water samples are compared to the "library" of DNA fingerprints of *E. coli* from known fecal pollution sources. While these methods can provide very useful information for beach managers (Edge and Hill, 2007; Edge et al. 2007; Edge et al. 2010), they are time consuming and very labour-intensive to perform. For this reason, a library-independent method was selected for preliminary investigations in the Madoc area.

Library-independent source tracking methods are based on searching for host-specific microorganisms in water samples rather than building large libraries based on fecal indicator bacteria. These host-specific microorganisms are adapted to specific gastrointestinal tracts, and have a restricted distribution, occurring only in the gut of their host such as humans or ruminant animals. If the DNA sequence of such a microorganism is detected in a water sample, it is an indication of fecal contamination from that human or animal host. Some of the most promising library-independent methods are based on detecting host-specific strains of anaerobic bacteria in the *Bacteroidales*. This group of

bacteria is generally found in much greater numbers in mammalian gastrointestinal tracts than *E. coli*. In addition, human-specific strains of *Bacteroidales* are increasingly under investigation as indicators of the presence of fecal contamination from sources like municipal sewage (Bernard and Field, 2000; Bower et al. 2005; Field and Samadpour, 2007; Gawler et al. 2007; Ahmed et al. 2009). The present study investigated the occurrence of a DNA sequence uniquely found in human strains of *Bacteroidales* in water samples from the Madoc study area to assess impacts from human sewage contamination.

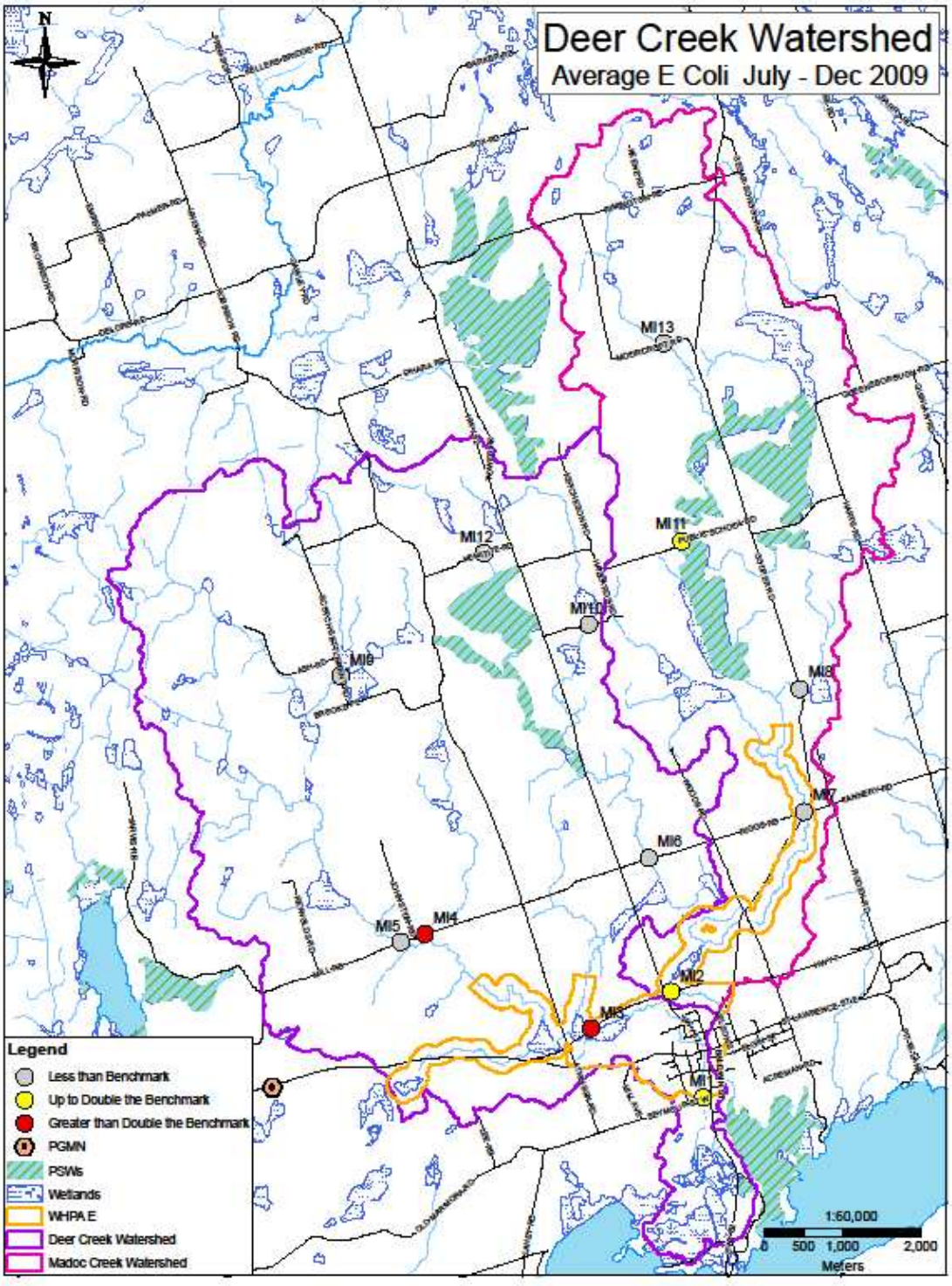

Figure 1. The Madoc study area showing Deer and Madoc Creek watersheds, the Wellhead Protection Area (WHPA), surface water sampling locations (map supplied by Quinte Conservation).

METHODS

Study area

Study sites were selected by Quinte Conservation (Figure 1) and were as follows:

Surface water site MI 1 is a Provincial Water Quality Monitoring Network station with several years of water quality data but no data on bacteria or organic nitrogen. It is located on Deer Creek on Seymour Street in Madoc, close to municipal wells and it is a downstream location representing the majority of the upper watershed;

Surface water site MI 3 is located on Deer Creek upstream from sampling location MI 1;

Surface water site MI 2 is located on Madoc Creek as a downstream site representing the upper watershed;

Municipal well sites - Rollins well and Whytock well were selected as municipal groundwater sampling sites in Madoc. Only raw water samples that are untreated were tested; and

Madoc sewage lagoon site - Initial 8 samples were taken from the final treated effluent as a human sewage reference location. However, subsequently 2 raw samples were taken at the Alum Shack before alum was added which were more appropriate as reference samples for human sewage.

Water and fecal reference sampling

Water samples were collected weekly by Quinte Conservation from September 7 to November 8, 2010 at Madoc sampling locations for this study. Water samples were collected in sterile polypropylene 500mL bottles, placed on ice in a cooler, and shipped overnight by courier to Environment Canada in Burlington for next day laboratory analysis.

Reference samples from fecal pollution sources were collected by Quinte Conservation from the Madoc sewage lagoon. Initial samples were collected from the final treated effluent, but raw untreated sewage from the lagoon was later used to test the host-specificity of the human *Bacteroidales* DNA marker assay. Municipal wastewater samples (raw untreated influent and final treated effluent) have also been collected from the Ashbridges Bay and Humber Sewage Treatment Plants in Toronto, as well as final effluents from Sewage Treatment Plants in Ottawa, Hamilton, and the Niagara Region. Other fecal samples from dog and cat droppings have been collected previously from Toronto and Ottawa animal shelters. Fecal samples from bird droppings (e.g. gull, geese, duck) have also been collected previously from the Toronto, Hamilton, Ottawa, and Niagara Region. Livestock and poultry fecal samples have been collected from farms in the Niagara Region.

E. coli enumeration

Water and lagoon wastewater samples were analyzed by membrane filtration and *E. coli* enumeration was expressed as colony forming units per 100 mL of water (CFU/100mL). Serial dilutions of water samples were performed and membrane filters were placed on the chromogenic differential coliform (DC) agar media supplemented with cefsulodin (Oxoid Inc.) for 18 hour incubation at 44.5°C. Sterile lab water samples were routinely filtered as negative controls to test potential for inadvertent sample contamination.

Bacteroidales DNA marker analysis

Water samples were assessed for the presence of strains of the anaerobic bacterium *Bacteroidales* that are associated with human fecal pollution (Bernhard and Field, 2000; Bower et al. 2005). This assay involved filtering as much water as the sample permitted, generally up to 300 mL for water samples. After filtration, the 0.45 µm membrane filters were frozen at -80C before subsequent DNA extraction steps. Each water sample was analyzed for the presence of human-specific strains of *Bacteroidales* bacteria (human *Bacteroidales* DNA marker HF183), as well as for the presence of a broader range of *Bacteroidales* bacteria (generic *Bacteroidales* DNA marker BAC32). Since the *Bacteroidales* group consists of a broad range of bacteria (beyond human-specific strains) that can be commonly found in the environment, analysis for the generic *Bacteroidales* BAC32 marker serves as a form of positive reference to confirm the assay is capable of detecting and amplifying DNA targets in an environmental sample.

Membrane filters with total genomic DNA from water or wastewater samples were removed from -80C, and then homogenized in a Mini-Beadbeater (BioSpec Products Inc.) for 2 min. DNA was purified using a Powersoil DNA isolation kit (Mo BIO Laboratories, Inc.). A 1 µl extract was used as template in a polymerase chain reaction (PCR) assay using primer HF183F to amplify the human *Bacteroidales* DNA sequences and BAC32 to amplify generic *Bacteroidales* sequences if they were present in the sample. Primer BAC708R was the reverse primer for both reactions. For the PCR reaction, the following concentrations were used: 0.05 U/µl Hotmaster Taq and 1 x buffer (Intermedico), 0.8 mM dNTP mixture, 0.06% BSA, 1.56 pmol/ µl each primer and water to 25 µl. The PCR cycling conditions were: 2 min at 94°C followed by 35 cycles of 20 sec at 94°C, 10 sec anneal at 53°C for BAC32 or 63°C for HF183 primers, 50 sec at 65°C and a final single step at 65 °C for 7 min. A human fecal DNA extract was run as a positive control for each set of reactions, along with sterile water as a negative control. A 5 µl amount of dye DNA mix was loaded into wells of a 1.25% agarose gel, and run at 170 V for approximately 1 hr to resolve the bands which were visualized by staining with ethidium bromide and imaging under UV light.

RESULTS AND DISCUSSION

E. coli surveillance

Whytock well is the backup well to the municipal drinking water supply for the Village of Madoc while Rollins well is the main well supply for the village. *E. coli* was not detected from any Whytock well raw water samples over the study period (n=10). However, *E. coli* was detected on two occasions from Rollins well raw water samples during the study period (n=10). On both September 20 and 27, 2010, 1 *E. coli* CFU / 100 mL were detected from this well location in the raw water. While this is a very low concentration of *E. coli*, it is nonetheless suggestive of fecal contamination at this well at times. As will be discussed later, the human *Bacteroidales* DNA marker was not detected in these two well water samples, so there is no evidence of human sewage as a source of this fecal contamination. This fecal pollution source could be animal as suggested later, or it is still possible that it could have been from a human sewage or septic contamination source that was below our level of detection. Additional investigation of the Rollins well would be advisable, possibly using total coliforms that could be a more sensitive indicator than *E. coli* of surface water impacts. It should be recognized that while total coliforms or *E. coli* can be practical indicators of surface and fecal contamination of well waters, they remain indicators and are only imperfect surrogates for predicting the potential presence of protozoa or viruses that may persist in groundwater longer than fecal indicator bacteria.

E. coli concentration data are found in Appendx 1 and a summary of the results for the surface water sampling locations in the Madoc study area is as follows:

MI 1 (Deer Creek downstream)
- mean *E. coli* = 54 CFU / 100 mL, (n=10);
- *E. coli* range = 15 – 103 CFU / 100 mL;
- higher *E. coli* concentrations in September (always above 60 CFU / 100 mL).

MI 3 (Deer Creek upstream)
- mean *E. coli* = 275 CFU / 100 mL, (n=10);
- *E. coli* range = 21 – 940 CFU / 100 mL ;
- higher *E. coli* concentrations in September (always above 300 CFU / 100 mL).

MI 2- (Madoc Creek)
- mean *E. coli* = 84 CFU / 100 mL, (n=10);
- *E. coli* range = 13 – 300 CFU / 100 mL ;
- higher *E. coli* concentrations in September (always above 70 CFU / 100 mL).

The only time *E. coli* was detected in the Rollins well was in the September period roughly corresponding to when *E. coli* concentrations were highest in nearby Creek

waters. On September 20, site MI 1 had 103 *E. coli* CFU / 100 mL; site MI 3 had 450 *E. coli* CFU / 100 mL, and site MI 2 had 119 *E. coli* CFU / 100 mL. On September 27, site MI 1 had 64 *E. coli* CFU / 100 mL, site MI 3 had 300 *E. coli* CFU / 100 mL, and site MI 2 had 77 *E. coli* CFU / 100 mL. It was notable that 96 hrs preceding the September 20 sampling date there was a considerable rain event (precipitation = 31 mm measured at Skootamatta River rain gauge station, Figure 2). While the *E. coli* concentrations in Deer Creek were fairly high in September (particularly at MI 3), these concentrations are still below what might be expected from gross sewage contamination of the Creek or stormwater outfalls (e.g. > 10,000 *E. coli* CFU / 100 mL).

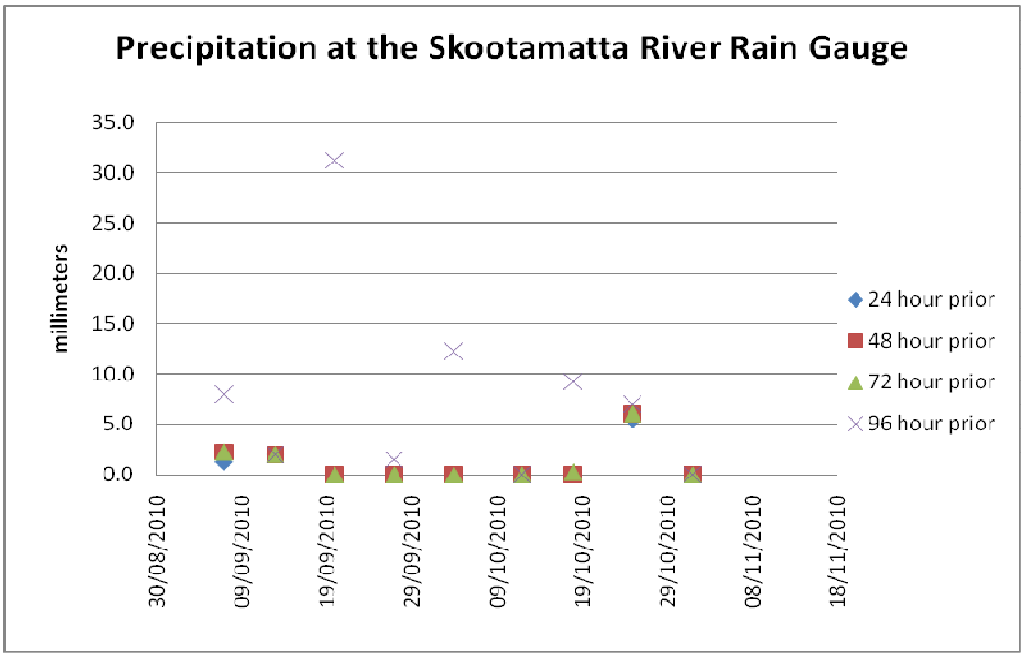

Figure 2: Precipitation (mm) recorded at the Skootamatta River Rain Gauge managed by Quinte Conservation (graph supplied by Quinte Conservation)

<u>*Bacteroidales* DNA marker analyses</u>

The host-specificity of the human *Bacteroidales* DNA marker has been examined using negative and positive control samples in our laboratory, as well as testing it against a variety of fecal samples collected from fecal pollution sources around southern Ontario. The human *Bacteroidales* DNA marker has been regularly amplified from human fecal samples run in our laboratory as positive control samples. It has not been detected in sterile water samples regularly run in our laboratory as negative control samples. The human *Bacteroidales* DNA marker was not detected in 8 final effluent samples from the Madoc lagoon, suggesting a pretty clean final effluent where this DNA marker was below our level of detection (Appendix 2). It is uncertain why some wastewater effluents seem to have low frequencies of human *Bacteroidales* DNA marker, although it could be related to whether rainwater or other inputs into a wastewater system might dilute the human signature at times. *E. coli* concentrations in this final effluent were usually very

low and ranged from 0 to 105 CFU / 100 mL. However, both raw sewage samples collected upstream from the Alum shack were positive for the human DNA marker as expected. These two sewage samples had *E. coli* concentrations of 4.7×10^7 and 1.1×10^7 CFU/100 mL.

The human *Bacteroidales* DNA marker has been detected in 87 % of final effluent samples from Toronto's Ashbridge Bay sewage treatment plant (n=52). This might suggest the DNA marker is a conservative one, as it probably occurs below our level of detection in the final effluent from sewage treatment plants at times, similar to our experience with the Madoc sewage lagoon. A consequence is that our results represent more of a relative comparison of the occurrence of the human *Bacteroidales* DNA marker across water samples. Where the marker was not detected, the water sample could be truly negative for the DNA marker, or the DNA marker could be present, but only at a relatively low concentration below our limit of detection. In this sense, our % positive results for the human *Bacteroidales* DNA marker at a site are probably minimum values.

Host-specificity assessment of the human *Bacteroidales* DNA marker indicated false positive results in our lab to date are extremely rare. To date, the results of our host specificity testing of the human *Bacteroidales* DNA marker has not detected this marker in the following fresh fecal samples: dog (n=16), cat (n=17), gull (n=85), Canada geese (n=58), mallard duck (n=10), cow (n=31), pig (n=8), and chicken (n=14). In addition, several presumptive human *Bacteroidales* PCR amplicons obtained from Toronto water samples were subjected to DNA sequencing for confirmatory analyses. These polymerase chain reaction (PCR) amplicons were the amplified fragments of DNA obtained from our assay that are assumed to be the same DNA sequence as the human *Bacteroidales* DNA marker we are looking for. The DNA sequences of these PCR amplicons were found to be most similar to *Bacteriodales* strains of *B. thetaiotaomicron* and *B. vulgatis* which have been associated with human fecal sources. However, host-specificity testing of the human DNA marker has found cross-amplification with two fresh fecal samples showing false positive results. One fecal sample was from a Toronto dog (1 of 17 samples tested) and one was from a Niagara chicken (1 of 15 samples tested). While the human *Bacteroidales* DNA marker is not expected to be a perfect host-specific marker for human fecal pollution (Kildare et al. 2007), our host-specificity testing to date, and results from other studies (Gawler et al. 2007; Ahmed et al. 2009) indicate there is little evidence for concern about significant effects from false positive results in our study around Madoc area.

Analyses for *Bacteroidales* DNA markers in water samples found that the generic BAC32 marker was detected in 100 % of surface water samples (n=40), suggesting little concern about inhibition of PCR assays for detecting the human DNA marker in these samples (Appendix 2). However, only 21% (n=19) of the well water samples had a detectable BAC32 presence. This is likely a reflection of the generally lower level of bacteria contamination in groundwater, although it is possible it could suggest inhibition of the PCR assay.

The human *Bacteroidales* DNA marker was not detected in any of the surface water samples (n=40) or the well water samples (n=19) collected during this study in the Madoc area (Appendix 2). It was not detected in 8 Madoc sewage lagoon final effluent samples, but it was detected in both Madoc raw untreated sewage lagoon samples. These results provide no evidence of the presence of human sewage contamination at these surface water and well water sampling locations over our 2010 study period. This is consistent with generally low *E. coli* concentrations observed over much of the study period. Based on these preliminary data, it would appear more likely that fecal contamination at surface water sampling locations and the Rollins well during this study period was from a non-human fecal source such as livestock or wildlife. However, it should be recognized that this does not preclude human fecal contamination from septic or other sources to be present in the study area at other times during the year outside our sampling period.

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APPENDIX 1. *E. coli* data from Madoc 2010 water sampling. MI1… = Madoc Issues surface water sites, MSL = Madoc Sewage Lagoon.

Sublocation	Date	E. coli cfu/100ML	Unique sample id
ROLLINS WELL	9/7/2010	0	QUINTEROLLINS WELL9/7/2010W.
MSL	9/7/2010	70	QUINTEMSL9/7/2010W.
MI2	9/7/2010	300	QUINTEMI29/7/2010W.
MI3	9/7/2010	710	QUINTEMI39/7/2010W.
WHYTOCK WELL	9/7/2010	0	QUINTEWHYTOCK WELL9/7/2010W.
MI1	9/7/2010	70	QUINTEMI19/7/2010W.
ROLLINS WELL	9/13/2010	0	QUINTEROLLINS WELL9/13/2010W.
WHYTOCK WELL	9/13/2010	0	QUINTEWHYTOCK WELL9/13/2010W.
MSL	9/13/2010	91.25	QUINTEMSL9/13/2010W.
MI3	9/13/2010	940	QUINTEMI39/13/2010W.
MI2	9/13/2010	147	QUINTEMI29/13/2010W.
MI1	9/13/2010	90	QUINTEMI19/13/2010W.
ROLLINS WELL	9/20/2010	1	QUINTEROLLINS WELL9/20/2010W.
MI3	9/20/2010	450	QUINTEMI39/20/2010W.
MI1	9/20/2010	103	QUINTEMI19/20/2010W.
MSL	9/20/2010	105	QUINTEMSL9/20/2010W.
WHYTOCK WELL	9/20/2010	0	QUINTEWHYTOCK WELL9/20/2010W.
MI2	9/20/2010	119	QUINTEMI29/20/2010W.
MSL	9/27/2010	54	QUINTEMSL9/27/2010W.
WHYTOCK WELL	9/27/2010	0	QUINTEWHYTOCK WELL9/27/2010W.
MI2	9/27/2010	77	QUINTEMI29/27/2010W.
MI3	9/27/2010	300	QUINTEMI39/27/2010W.
ROLLINS WELL	9/27/2010	1	QUINTEROLLINS WELL9/27/2010W.
MI1	9/27/2010	64	QUINTEMI19/27/2010W.
MI3	10/4/2010	83	QUINTEMI310/4/2010W.
MI2	10/4/2010	78	QUINTEMI210/4/2010W.
WHYTOCK WELL	10/4/2010	0	QUINTEWHYTOCK WELL10/4/2010W.
MI1	10/4/2010	61	QUINTEMI110/4/2010W.
ROLLINS WELL	10/4/2010	0	QUINTEROLLINS WELL10/4/2010W.
MSL	10/4/2010	2	QUINTEMSL10/4/2010W.
ROLLINS WELL	10/12/2010	0	QUINTEROLLINS WELL10/12/2010W.
MI2	10/12/2010	43	QUINTEMI210/12/2010W.
WHYTOCK WELL	10/12/2010	0	QUINTEWHYTOCK WELL10/12/2010W.
MI1	10/12/2010	47	QUINTEMI110/12/2010W.
MI3	10/12/2010	116	QUINTEMI310/12/2010W.
MSL	10/12/2010	0	QUINTEMSL10/12/2010W.
MSL	10/18/2010	1	QUINTEMSL10/18/2010W.
ROLLINS WELL	10/18/2010	0	QUINTEROLLINS WELL10/18/2010W.
MI1	10/18/2010	59	QUINTEMI110/18/2010W.
MI3	10/18/2010	48	QUINTEMI310/18/2010W.
MI2	10/18/2010	18	QUINTEMI210/18/2010W.

WHYTOCK			QUINTEWHYTOCK
WELL	10/18/2010	0	WELL10/18/2010W.
MI1	10/25/2010	18	QUINTEMI110/25/2010W.
MI3	10/25/2010	56	QUINTEMI310/25/2010W.
MSL	10/25/2010	0	QUINTEMSL10/25/2010W.
ROLLINS WELL	10/25/2010	0	QUINTEROLLINS WELL10/25/2010W.
WHYTOCK			QUINTEWHYTOCK
WELL	10/25/2010	0	WELL10/25/2010W.
MI2	10/25/2010	19	QUINTEMI210/25/2010W.
MSL-2	11/1/2010	47200000	QUINTEMSL-211/1/2010W.
WHYTOCK			QUINTEWHYTOCK
WELL	11/1/2010	0	WELL11/1/2010W.
MI3	11/1/2010	21	QUINTEMI311/1/2010W.
MI1	11/1/2010	15	QUINTEMI111/1/2010W.
ROLLINS WELL	11/1/2010	0	QUINTEROLLINS WELL11/1/2010W.
MI2	11/1/2010	13	QUINTEMI211/1/2010W.
MI1	11/8/2010	15	QUINTEMI111/8/2010W.
WHYTOCK			QUINTEWHYTOCK
WELL	11/8/2010	0	WELL11/8/2010W.
ROLLINS WELL	11/8/2010	0	QUINTEROLLINS WELL11/8/2010W.
MSL-2	11/8/2010	11000000	QUINTEMSL-211/8/2010W.
MI2	11/8/2010	21	QUINTEMI211/8/2010W.
MI3	11/8/2010	23	QUINTEMI311/8/2010W.

APPENDIX 2. Presence (1) or absence (0) of human (HF183) and universal (BAC32) *Bacteroidales* DNA markers in Madoc 2010 water samples. MI1… = Madoc Issues surface water sites, MSL = Madoc Sewage Lagoon.

Sublocation	Date	Sample	Rack	Well	HF183	BAC32	Tracker
MI1	9/7/2010	A2778	Q001	A09	0	1	1048338689
MI2	9/7/2010	A1978	Q001	B05	0	1	1048338700
MI3	9/7/2010	A1974	Q001	B01	0	1	1048338704
MSL	9/7/2010	A2781	Q001	A12	0	1	1048338692
ROLLINS WELL	9/7/2010	A1979	Q001	A11	0	0	1048338691
WHYTOCK							
WELL	9/7/2010	A2779	Q001	A10	0	0	1048338690
MI1	9/13/2010	A2782	Q001	A01	0	1	1048338681
MI2	9/13/2010	A2783	Q001	B04	0	1	1048338701
MI3	9/13/2010	A1976	Q001	B02	0	1	1048338703
MSL	9/13/2010	A1977	Q001	A02	0	1	1048338682
ROLLINS WELL	9/13/2010	A2780	Q001	A05	0	0	1048338685
WHYTOCK							
WELL	9/13/2010	A1975	Q001	B03	0	1	1048338702
MI1	9/20/2010	A1985	Q001	A03	0	1	1048338683
MI2	9/20/2010	Z0037	Q001	A06	0	1	1048338686
MI3	9/20/2010	Z0035	Q001	A07	0	1	1048338687
MSL	9/20/2010	Z0038	Q001	A04	0	1	1048338684
ROLLINS WELL	9/20/2010	Z0036	Q001	A08	0	1	1048338688
MI1	9/27/2010	A1980	Q001	D05	0	1	1048338724

MI2	9/27/2010	Z0031	Q001	D06	0	1	1048338723
MI3	9/27/2010	A1984	Q001	D09	0	1	1048338720
MSL	9/27/2010	A1983	Q001	D10	0	1	1048338719
ROLLINS WELL	9/27/2010	A1987	Q001	D07	0	0	1048338722
WHYTOCK WELL	9/27/2010	A1982	Q001	D08	0	0	1048338721
MI1	10/4/2010	Z0034	Q003	A06	0	1	1048337054
MI2	10/4/2010	Z0033	Q003	A05	0	1	1048337053
MI3	10/4/2010	A1986	Q003	A02	0	1	1048337050
MSL	10/4/2010	Z0032	Q003	A04	0	1	1048337052
ROLLINS WELL	10/4/2010	A1981	Q003	A01	0	1	1048337049
WHYTOCK WELL	10/4/2010	A1988	Q003	A03	0	0	1048337051
MI1	10/12/2010	A2846	Q003	A11	0	1	1048337059
MI2	10/12/2010	A2844	Q003	A09	0	1	1048337057
MI3	10/12/2010	A2845	Q003	A10	0	1	1048337058
MSL	10/12/2010	A2840	Q003	A08	0	1	1048337056
ROLLINS WELL	10/12/2010	A2848	Q003	A12	0	0	1048337060
WHYTOCK WELL	10/12/2010	A2839	Q003	A07	0	0	1048337055
MI1	10/18/2010	A2843	Q003	B05	0	1	1048337068
MI2	10/18/2010	A2838	Q003	B04	0	1	1048337069
MI3	10/18/2010	A2849	Q003	B06	0	1	1048337067
MSL	10/18/2010	A2837	Q003	B03	0	1	1048337070
ROLLINS WELL	10/18/2010	A2834	Q003	B02	0	0	1048337071
WHYTOCK WELL	10/18/2010	A2841	Q003	B01	0	0	1048337072
MI1	10/25/2010	A2830	Q005	C10	0	1	1048340346
MI2	10/25/2010	A2831	Q005	C08	0	1	1048340344
MI3	10/25/2010	A2835	Q005	C09	0	1	1048340345
MSL	10/25/2010	A2842	Q005	C07	0	1	1048340343
ROLLINS WELL	10/25/2010	A2833	Q005	C06	0	0	1048340342
WHYTOCK WELL	10/25/2010	A2836	Q005	C05	0	0	1048340341
MI1	11/1/2010	A2832	Q005	D05	0	1	1048340356
MI2	11/1/2010	A2850	Q005	D06	0	1	1048340355
MI3	11/1/2010	A2827	Q005	D04	0	1	1048340357
MSL-2	11/1/2010	A2828	Q005	D03	1	1	1048340358
ROLLINS WELL	11/1/2010	A2829	Q005	D02	0	0	1048340359
WHYTOCK WELL	11/1/2010	A2826	Q005	C11	0	0	1048340347
MI1	11/8/2010	A2824	Q005	E10	0	1	1048340370
MI2	11/8/2010	A2825	Q005	F01	0	1	1048340384
MI3	11/8/2010	A2823	Q005	E12	0	1	1048340372
MSL-2	11/8/2010	A2822	Q005	E11	1	1	1048340371
ROLLINS WELL	11/8/2010	A2821	Q005	E09	0	1	1048340369
WHYTOCK WELL	11/8/2010	A2847	Q005	E08	0	0	1048340368