MICROBIOLOGICAL QUALITY OF SAGINAW BAY CITY PARK

Draft Report

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Joan B. Rose, Ph.D
Homer Nowlin Chair in Water Research

Rebecca L. Ives
and
Lauren Bellows

Department of Fisheries and Wildlife
13 Natural Resources Building
Michigan State University
East Lansing, MI 48824

Phone: (517) 432-4412
Fax: (517) 432-1699
rosejo@msu.edu

Introduction

The purpose of this study was to evaluate the microbiological quality of the algal debris and muck at the shoreline in Saginaw Bay for fecal indicators as well as to evaluate and develop protocols for the testing of DNA from the samples. Analyses to date have included conventional water quality indicators including *Escherichia coli*, as well as the alternative indicators *Enterococci*, coliphage and *Clostridium perfringens*.

Table 1 below describes the indicators and their applications. All of these indicators are found in the feces of humans and animals and are referred to as fecal indicators and are used to indicate fecal or wastewater contamination of water and potential for waterborne disease and presence of pathogens. They are also all found in sewage.

- *E.coli* and Enterococci may regrow in the environment (e.g. on algae and in soil and sand).
- *Clostridium* bacterium does not regrow but does survive a long time, and survives chlorination, it is not found in as high of concentrations in wastewater as the first bacteria mentioned above, but survives longer.
- The coliphage (virus indicator) does not survive in the environment as long as the others and indicates more recent pollution.
- Finding all of these particular indicators shows greater impact than finding just one.

Table 1. Indicators and their Applications

Indicator	Definition	Drinking Water Standards (limits/100mL)	Recreational Use Standards* (limits/100mL)	Advantages of Use as an Indicator	Disadvantages of Use as an Indicator
Total Coliforms	Members of the family Enterobacteriaceae (includes E. coli) Make up 10% of intestinal organisms in humans and animals Facultatively anaerobic, gram-negative, nonsporing, rod-shaped bacteria (14) Ferments lactose	Not more than 1 positive sample found per month (applies to Public Water Systems serving 25-33,000 people) Not more than 5.0% of samples can be total coliform-positive in a month (applies to Public Water Systems serving >33,000 people)		Used as an indicator of bacteriological quality in drinking water assessment according to the Total Coliform Rule. (USEPA 816-F-01-035) (15) Lose viability in fresh water at a slower rate than most other intestinal bacterial pathogens Will usually be detected in waters impacted by animal feces or sewage	"Coliforms" represent a large class of microbes, including bacteria that don't come from the intestinal tract. Therefore, it is critical to use more specific indicators, such as fecal coliforms, as indicators of potential risk Waters containing total coliforms may not have been impacted by fecal contamination due to the wide variety of potential sources of total coliform contamination. Coliform bacteria can re-grow Not directly related to human health risk

Table 1. Indicators and their Applications (cont)

Indicator	Definition	Drinking Water Standards (limits/100mL)	Recreational Use Standards* (limits/100mL)	Advantages of Use as an Indicator	Disadvantages of Use as an Indicator
E. Coli	A type of coliform bacteria that naturally occurs in the human intestinal tract. Many strains exist – only a few of these are pathogenic (14)	ZERO (water must be boiled before consumption if E. coli is present)	**FRESHWATER ONLY** PRIMARY CONTACT RECREATION – GENERAL (3): < 126cfu/100mL(based on geometric mean) PRIMARY CONTACT RECREATION - MICHIGAN (8): < 130cfu/100mL (based on a geometric mean) < 300cfu/100mL (for a single sample from a designated beach area)	Used as an indicator of bacteriological quality in both drinking and recreational waters. Found to have a high correlation with gastroenteritis associated with bathing in fresh water (6) Source tracking methods have been developed (7)	May grow in the soil in tropical locations. Found to be poorly correlated with gastroenteritis in marine waters (5). E. coli presence does not correlate with the presence of enteric viruses and parasites.

Table 1. Indicators and their Applications (cont)

Indicator	Definition	Drinking Water Standards (limits/100mL)	Recreational Use Standards* (limits/100mL)	Advantages of Use as an Indicator	Disadvantages of Use as an Indicator
Enterococci	A gram-positive non-spore forming member of the Streptococci bacteria Commonly found in the feces of humans and other warm-blooded animals Many strains are not harmful, however the presence of enterococci is an indication of the possible presence of enteric pathogens.	NONE	<u>FRESHWATER</u> PRIMARY CONTACT RECREATION – GENERAL (1): < 33cfu/100mL (based on a geometric mean) < 61cfu/100mL (based on a single sample) <u>MARINE WATER</u> PRIMARY CONTACT RECREATION - GENERAL (1): < 35cfu/100mL (based on a geometric mean) < 104cfu/100mL (for a single sample at a designated beach area)	Used as an indicator of bacteriological quality of recreational waters. Enterococci may die at a slower rate than fecal coliforms in water and sediments, providing more reliable indications of possible recent pollution (7). Multi-site epidemiological studies have shown that enterococci have a higher correlation with gastroenteric disease related to swimming in both fresh and marine waters than fecal coliforms (5).	Can regrow in the environment Less data is available

Table 1. Indicators and their Applications (cont)

Indicator	Definition	Drinking Water Standards (limits/100mL)	Recreational Use Standards* (limits/100mL)	Advantages of Use as an Indicator	Disadvantages of Use as an Indicator
Clostridium perfringens	Obligate anaerobic gram-positive bacteria that forms endospores and does not carry out dissimilatory sulfate reduction (14) Found in sewage and highly impacted waters (7). An opportunistic pathogen that produces enterotoxin.		**FRESHWATER (standards for the state of Hawaii)** < 50cfu/100mL <u>MARINE WATER (standards for the state of Hawaii)</u> < 5cfu/100mL (guidelines used by the state of Hawaii based on research by Dr. Roger Fujioka et al. at the University of Hawaii)	C. perfringens spores could be an index parameter for the occurrence of persistent intestinal pathogens like viruses and (oo)cysts of protozoa (7). Useful in such specific situations as the examination of chlorinated waters or industrial waters that may contain compounds lethal to non-spore forming bacterial indicators, samples that cannot be processed within 12 hours and the detection of recent as well as long term inputs of fecal pollution (4)	May be too conservative an indicator (7) Found in low concentrations
Coliphage	Viruses (also known as Bacteriophages) whose hosts are strains of the bacteria E. coli (13). Found wherever fecal contamination occurs.	ZERO (suggested by the ground water rule)	< 100 pfu/100mL (based on previous studies by Dr. Joan Rose, USF)	A good indicator of Enteroviruses due to similar seasonal variation, propensity for removal and resistance to environmental stress (7).	Coliphage is not specific to human sewage

* The indicators used and standards enforced differ from state to state for recreational waters. For information about a particular state refer to reference #2.

Methods

Five water samples were collected directly at the shoreline where the waves were breaking onto the sand, containing large amounts of algae and debri. No samples were collected from waters at depths out from the shore used for swimming. Two samples were collected from Muck debri itself up on the shoreline.

Samples were analyzed using the Colilert® kits by IDEXX. *Enterococci* were analyzed using membrane filtration according to USEPA Method 1600 (*Enterococci*) (9). Coliphage were analyzed via a modification of USEPA Methods 1601 and 1602 (10,11). (Table 2 includes the media and conditions for monitoring these bacteria)

Table 2. Media and Methods used for Indicator Testing

Test	**Media**	**Incubation**	**Reference**
E.coli	Colilert®	24-28 hours at 37°C	APHA Standard Method 9223B (2)
Enterococci	mEI agar	24 hours at 41°C	USEPA Method 1600 (9)
Clostridium perfringens	mCP agar	24 hours at 45°C in anaerobic chamber	Bisson *et al* (1979)
Coliphage	Tryptic Soy Agar	16 – 24 hours at 37°C	USEPA Method 1601/1602 (10, 11)

Sample Collection:

Grab samples were collected into sterile sampling containers, and placed on ice for transport to the laboratory. Samples were processed within 24 hours of collection.

Bacterial indicators: Membrane Filtration

Volumes for bacteriological analysis via membrane filtration (*Clostridium perfringens,* and *Enterococci*) ranged from one millilter of a 10^{-1}dilutions to 100ml. Samples collected on 6/5/07 and were analyzed on that same day. Serial dilutions were made from log phase cultures of *Enterococcus faecalis* and *E. coli* C3000 for use as positive controls. One millilter from each of the 10^{-6}, 10^{-7}, and 10^{-8} dilutions was filtered in duplicate.

Plates were incubated for 24 hours at 41°C for *Enterococci* analysis,. Plates for *Clostridium perfringens* analysis were incubated for 24 hours at 45°C in anaerobic chamber. Solid samples were diluted 10 g into 100ml and assayed similarly.

Bacterial indicators: IDEXX

In order to measure *E.coli* 100 mL samples were assayed in a Quanti-Tray/2000 (WQT-2K) using the IDEXX (Westbrook, Maine) Colilert (98-21375-00) Bacterial counts exceeded the detection limits of the systems, samples were diluted 1/100 with a sterile phosphate buffered water solution, and the dilution reprocessed. Assays were

conducted by adding sample to a powdered reagent (Colilert for *E.coli*) in a sterile container, mixing until reagent was dissolved, and then sealing in a Quanti-Tray/2000 plate. Colilert samples were placed in a 36.5°C incubator for 24 hours.

Coliphage Analysis

Agar overlays were utilized to detect coliphage present in the samples. Filtered volumes of the water sample were used to enumerate coliphage. Two types of overlays were conducted for samples collected on 5/23/06 and 6/28/06, one using *E.coli* F^+amp as a host, the other using *E.coli* C3000. Overlays using *E.coli* C3000 as a host were conducted for samples collected on 7/19/06

Overlays: For each sample, 20 mls were syringe-filtered through a 0.45 micron filter. 0.5mls of host and 2 ml of sample were added to melted top agar before mixing and pouring onto a tryptic soy agar plate (TSA). Two negative control plates were made, one with each host, by adding 1.5mls host to the top agar, mixing and pouring onto a TSA plate. A positive control was run for each host type by adding 1.5mls host to the top agar, mixing and pouring onto a TSA plate. Stock MS-2 phage was spotted onto the hardening agar layer. For each sample, 5 overlays of each host type were performed. Overlays were incubated at 37C for 24 hours, and then assessed for plaque formation.

Muck moisture analysis

Muck samples were dried to assess the moisture content of the material. Table 3 shows the % moisture of the muck samples.

Table 3. Moisture content of muck samples

Sample	**Collection Date**	**% Moisture**
Muck M6	6/5/07	55 %
Muck M7	6/5/07	64 %

Results: Bacterial and Phage indicators

Table 4 shows the average bacterial and coliphage indicator results to date.

Table 4a. Bacterial and coliphage indicator results from water samples

Sample	**Collection Date**	***E.coli* CFU/100ml (MPN)***	**Enterococci CFU/100ml**	***Clostridium Perfringens* CFU/100ml**	**F specific Coliphage PFU/100ml**	**SomaticColiphage PFU/100ml****
Water M1	6/5/07	$3.6x10^3$	$6.9x10^3$	67	40	70
Water M2	6/5/07	$3.8x10^3$	$8.3x10^3$	120	150	40
Water M3	6/5/07	$2.7x10^3$	$6.4x10^4$	93	130	70
Water M4	6/5/07	$2.4x10^3$	$6.7x10^3$	60	170	90
Water M5	6/5/07	$3.4x10^3$	$7.8x10^3$	82	80	30

Table 4b. Bacterial and coliphage indicator results from muck samples

Sample	**Collection Date**	***E.coli* CFU/g wet wt (MPN)****	**Enterococci CFU/ g wet wt**	***Clostridium Perfringens* CFU/ g wet wt**	**F specific Coliphage PFU/ g wet wt**	**SomaticColiphage PFU/ g wet wt**
Muck M6	6/5/07	$9.2x10^3$	$9.7x10^4$	87	$<10^a$	$< 10^a$
Muck M7	6/5/07	$3.0x10^2$	$3.6x10^2$	$1.1x10^3$	$<10^a$	$< 10^a$

*MPN =most probable number calculated from colilert
**MPN =most probable number calculated from colilert, converted to gram of wet weight

[a] Value shown is the limit of detection, phage concentrations were below the limit of detection in these samples

***esp* marker analysis**

DNA was extracted from enterococci colonies that grew on the membrane filters. The polymerase chain reaction was used to amplify the DNA *esp* marker (Scott et al., 2005). Table 4 displays the results of the *esp* marker analysis.

Table 4. *esp* marker results

Enterococci CFU/100mL	Sample ID	# of membranes analyzed for *esp* marker	# of membranes testing positive for *esp* marker
$6.9x10^3$	Water M1	1	0
$19.6x10^3$	Water M2	1	1
$8.3x10^3$	Water M3	1	1
$6.3x10^3$	Water M4	1	1
$9.0x10^3$	Water M5	1	1
$7.6x10^4$	Muck M6	1	1
$3.7x10^3$	Muck M7	0*	0*

*The number of colonies on the membrane were too low to warrant *esp* marker analysis

Four of the 5 water samples contained the human sewage marker and 1 of the 2 muck samples. The esp marker has been found in septic tank effluent and in CSO effluents as well as untreated sewage, Poorly treated secondary sewage which has not received proper disinfection was also positive (Kumar, 2007). We have never detected the esp in non-human wastes (including cows, sheep, pigs and birds) and ran a blind study with samples sent from USGS, only human wastes were positive. However, recently Whitman et al. (2007) has reported that they have detected this marker on occasion in dog feces and bird feces .

In conclusion this sampling shows high levels of fecal contamination in waters containing suspended algae (muck) and in the solids material on the shore. There is strong evidence that at least one of the sources is human (whether septic tanks, CSOs or poorly treated sewage is not known). Attention to the public health messages and good hygienic procedures is needed.

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