

Measuring the Amount of Bacteria in Calvin's Water: Is it Really Safe to Throw R.A.'s in the Seminary Pond?

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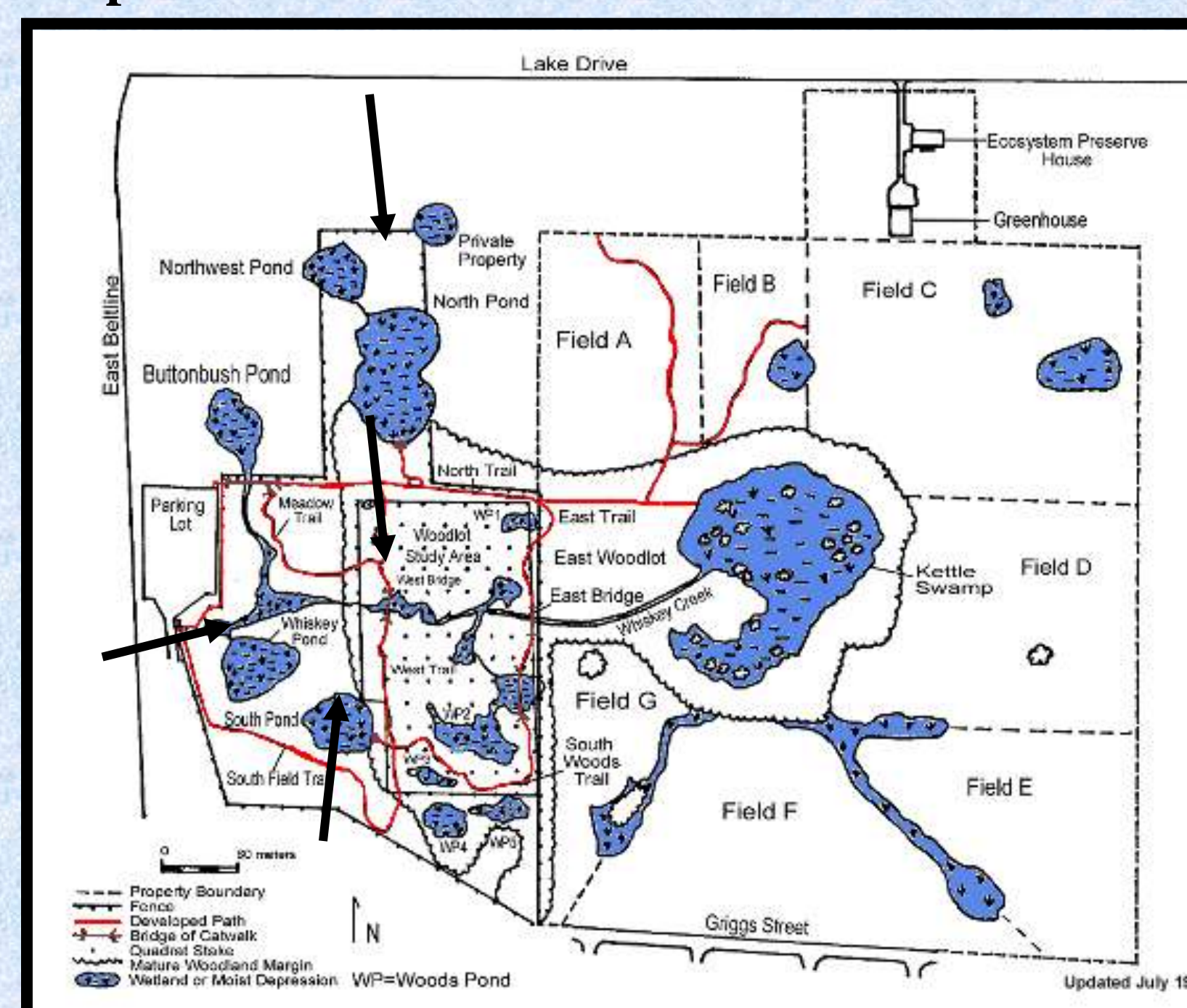
INTRODUCTION

The purpose of this study was to determine the number of coliform and *E. coli* bacteria in the ponds and creeks of Calvin's campus. The Environmental Protection Agency (EPA) uses *E. coli* bacteria counts to determine if a body of water is safe for human contact; this is a widely accepted method for determining the amount of fecal matter contamination in water. While many coliforms are commonly found in decaying matter in soil, *E. coli* is only found in fecal matter from warm-blooded animals. The overall goal is to see if the composting toilet, soon to be installed, will have any effect on the nearby water sheds. If the composting toilet works as it should then there should be little to no change seen in the amount of coliform bacteria in the bodies of water found in the Calvin Ecosystem Preserve. In addition, new Resident Assistants are routinely thrown into the Seminary Pond, and we wish to determine if the fecal content of the water is safe for this practice.



Brian Konynenbelt demonstrates water sampling on a clear day. Water sampling actually occurred during a downpour of cold rain.

Map of Nature Preserve Collection Sites



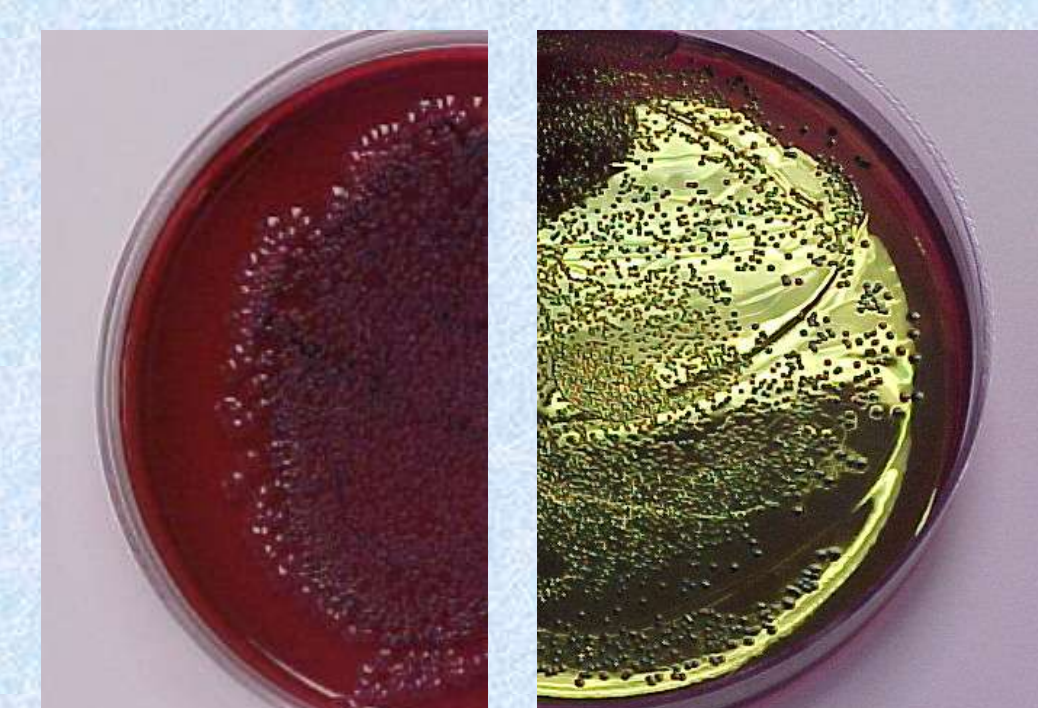
Arrows indicate ponds sampled on Nature Preserve. The Seminary Pond, President's Pond, and Ravenswood Pond of the West Campus are not pictured.

METHODS

Four sites in the nature preserve, the seminary pond, and two ponds near the athletic fields were tested for fecal bacteria. Sterile tubes were secured on the end of a water-sampling pole. At each site a surface sample (A) and a subsurface sample (B) were taken. The temperature of each sampling site was recorded. The labeled tubes were transported to the lab on ice.

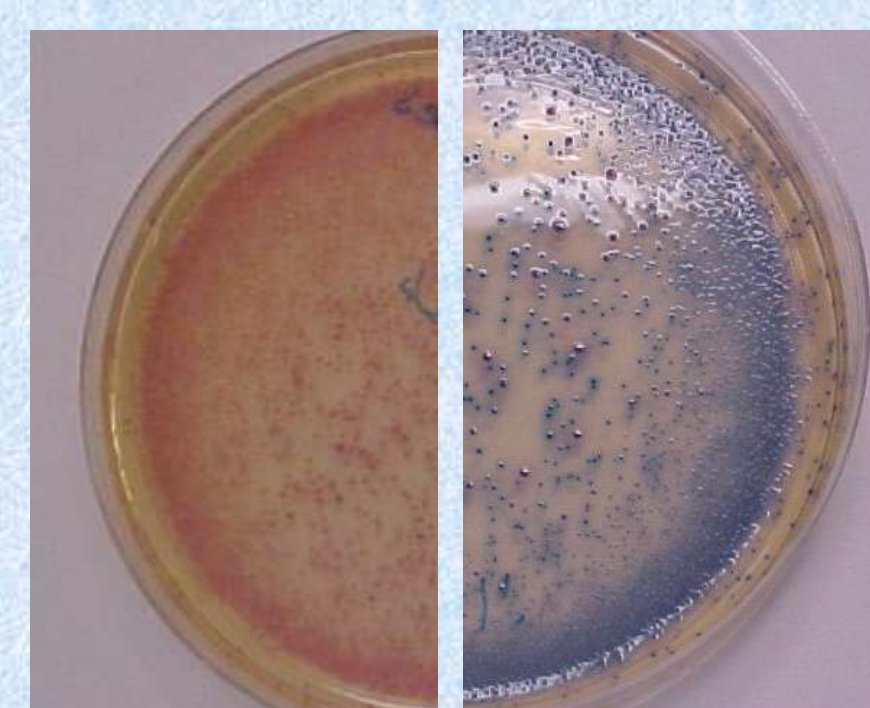
For each sample, two Eosin-Methylene Blue (EMB) agar plates were prepared. One was cultured with 100 μ L of the water sample while the second was cultured with 500 μ L of the water sample. Additionally, for each sample an Easygel® plate was prepared. 2 mL of the water sample was mixed with the Easygel®, poured into a plate containing divalent cations, and allowed to harden. All plates were incubated at 37 C for 48 hours. After the incubation period, the plates were photographed and the colonies counted to assess the presence of fecal bacteria.

Distinguishing Coliform Colonies on Various Media



EMB Agar:
E. Coli are blue-black with a metallic sheen
Other Coliform are red-pink
Non-Coliform are uncolored or transparent

E. aerogenes (other coliform) *E. Coli*



Coliscan EasyGel®:
E. Coli purple or blue-purple
Other Coliform are pink
Non-Coliform are uncolored or teal-green

E. aerogenes (other coliform) *E. Coli*

RESULTS

The greatest number of colony forming units (CFU) of coliforms on EMB agar was found in the Whiskey Pond B subsurface sampling site. The greatest number of CFUs of *E. coli* on EMB agar was found from the Seminary Pond A sampling site. Easygel® and EMB agar displayed varying degrees of colony forming units. The least number of CFUs plated on EMB agar of coliforms was found from the North Pond B sampling site. The least number of CFUs of *E. coli* on EMB agar was also found from the South Pond A as well as the Whiskey Creek A sampling site. The positive control, *E. coli* and *E. aerogenes*, showed metallic sheen colony formation on the EMB agar and blue-purple coloration on Easygel® as expected. The negative controls, drinking fountain water and *S. aureus*, exhibited no growth on either the EMB agar or the Easygel®.

Table 1: Total Coliform Counts

Pond	Easygel®	EMB Agar (Bacteria/100 mL)
North Pond A	2,500	16,600
North Pond B	2,700	6,800
South Pond A	297,400	316,800
South Pond B	398,800	408,000
Whiskey Pond A	6,000	250,000
Whiskey Pond B	9,900	532,000
Whiskey Creek A	7,400	55,600
Whiskey Creek B	9,000	142,000
Seminary Pond A	16,600	51,200
Seminary Pond B	21,000	40,000
Ravenswood Pond A	21,200	48,200
Presidents Pond A	8,800	25,300
Drinking Fountain	0	0

Table 2: *E. coli* Counts

Pond	Easygel®	EMB Agar (Bacteria/100 mL)
North Pond A	100	2,400
North Pond B	50	1,600
South Pond A	400	0
South Pond B	3,600	100
Whiskey Pond A	250	14,000
Whiskey Pond B	150	15,000
Whiskey Creek A	900	0
Whiskey Creek B	100	600
Seminary Pond A	2,400	48,400
Seminary Pond B	2,050	36,400
Ravenswood Pond A	2,800	2,200
Presidents Pond A	2,500	1,600
Drinking Fountain	0	0

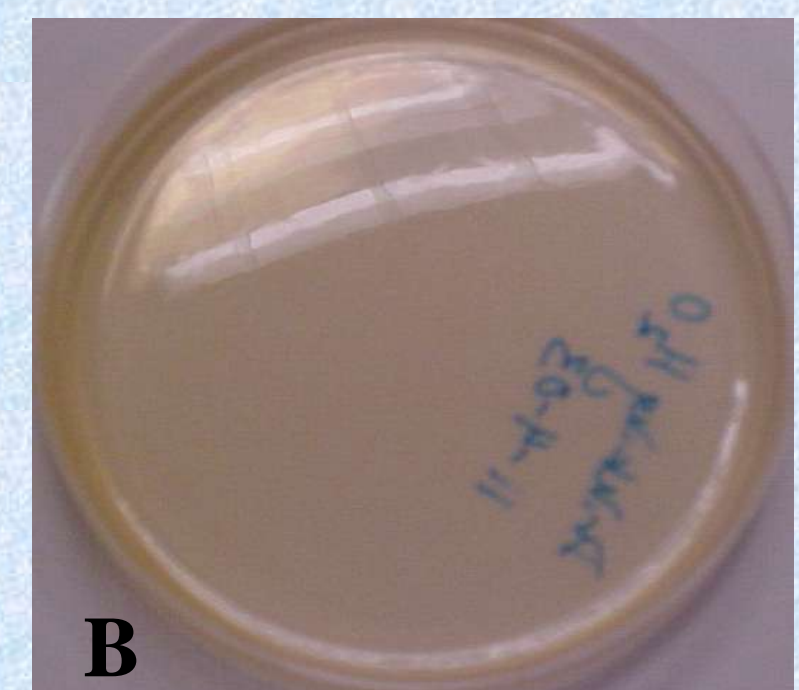
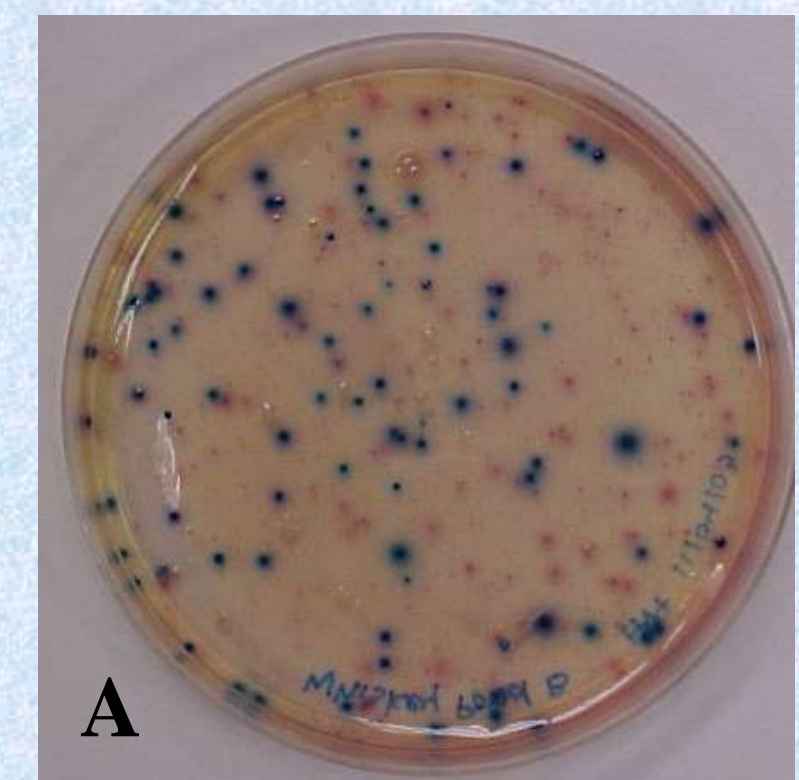


Figure 1. Example of Easygel® plates after incubation using water from Whiskey Creek (A) or from a campus drinking fountain (B).

DISCUSSION

For each data selection site there were tests done to measure total coliform counts as well as *E. coli* counts. These tests consisted of culturing water samples on Easygel® and EMB agar and counting colonies. Although both methods should have given similar results, it was found that the data was different using the two methods. Typically counts taken from the Easygel® were considerably lower than those taken on EMB. In comparing the total coliform counts from this year's data, taken on November 2, 2003, to the data taken on October 30, of 2001, the data showed that for North Pond counts using both Easygel® and EMB were three-fold smaller this year. The decrease in counts also occurred in South Pond and President's pond. In Ravenswood and Whiskey Ponds, total coliform counts increased between the two years. This year data was also collected from Seminary pond and from Whiskey Creek. The data from Whiskey Pond, Whiskey Creek and Seminary Pond had the highest total coliform counts of the sampled sites.

The ponds were also analyzed for safety for full or partial body contact. This is determined by using *E. coli* counts. Rule 62 of the Michigan Water Quality Standards states that waters safe for total body contact should contain no more than 130 *E. coli* per 100 mL of water, and for partial body contact, no more than 1000 *E. coli* per 100 mL. Data collected from the Easygel® was used, since it is EPA-approved and is often used in the field for beach testing. North Pond and Whiskey Pond were found to be safe for full body contact. Seminary Pond, South Pond, President's Pond, and Ravenswood Pond were found not to be safe for even partial body contact. This suggests that college students should stop throwing people in the Seminary Pond.

As a note, the law requires that for data to be considered a reliable indication of water quality, a minimum of five sampling events (consisting of three samples per event) must be collected within a thirty-day period. This was not done, data was only collected on one day and that day had heavy rainfall. This rainfall could have skewed our data due to the ability of the rain to wash full fecal matter into the water body.

REFERENCES

Michigan Water Quality Standards, Rule 62 (Part 4 of Act 451). Website for the Department of Environmental Quality. <http://www.deq.state.mi.us/beach/about.asp>