The Analysis for Coliforms in Calvin's Ponds

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Introduction

We drink in it. We bathe in it. We cook with it. We even play in it. What is it? It is water! We know that water has a profound impact on our lives since we use it each and every day. One might wonder, just what is in that water? Well, there are many microbes that call water their home. Some of these microbes that live in the water are human pathogens, meaning they cause disease. Many pathogenic microbes are transmitted via the fecal-oral route. In this instance, the pathogen leaves its host in feces and enters the new host by mouth. Because of the fact that these microbes are pathogenic, several ways have been devised to test for the presence of these microbes. One test that is used is the coliform test. Coliform bacteria are gram-negative rods that are non-spore-forming and ferment lactose. They are commonly found in soil and water. Not all coliforms indicate the presence of fecal-oral contamination. For example, *Enterobacter aerogenes* is a coliform that is often found at sites of decaying vegetation. However, *E. coli* is a specific coliform that can be used to indicate fecal contamination because it a bacterium found in the intestinal tracts of both humans and warm-blooded animals. As *E. coli* itself is not usually pathogenic, the presence of high levels of *E. coli* is not a direct measure of pathogens, but it does indicate that pathogenic fecal organisms may be present. Coliscan® Easygel® is one medium that can be used to identify both total coliforms and *E. coli*. This medium can differentiate between total coliforms and *E. coli* based on differences in enzymes secreted by the bacteria. Other classical media include MacConkey agar, m-Endo agar, and Eosin-methylene blue (EMB) agar.

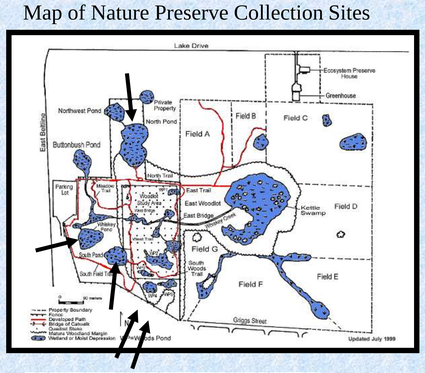
Several ponds on Calvin's campus were surveyed for fecal contamination, which would indicate increased human and animal influence. The purpose of this experiment was to test for the presence of coliforms in these bodies of water. It is hypothesized that the ponds by the residential areas will have an increased coliform count because of human and animal presence in those areas. Also, ponds in the construction sites will have more contamination due to increased human presence in the construction zone.

Methods and Materials

Water Sampling: To begin, water samples were obtained from seven different ponds on Calvin's campus. (The ponds sampled are as follows: North Pond, South Pond, the middle drainage pond, the east drainage pond, and Whiskey Pond on the Nature Preserve. President's Pond and Ravenswood Pond near the athletic fields.) Two samples were taken at each location (except the drainage ponds), each sample in a slightly different spot. For each sample, a sterile collecting tube was filled with pond water. This was accomplished by one of two methods: either the tube was directly submersed or a bottle on the end of a telescopic pole was filled, and then water was transferred to the collecting tube. Samples were all obtained from the top eight inches of water. The water temperature of each lake was also recorded. All the water samples were taken back to the lab where various media were used to determine the presence of coliform bacteria.

Sample Plating: The first medium used was Coliscan® Easygel® agar. Three mL of each pond sample was added to each bottle of Easygel. The Easygel was then poured onto pre-treated petri dishes and allowed to harden. The plates were inverted and incubated at 35ºC for 48 hours. Next, a series of dilutions were carried out for one sample from each site. After the dilutions were prepared, 1mL and 0.1 mL of the original sample, and dilutions that resulted in the equivalent volume of 0.01 mL, 0.001 mL, and 0.0001 mL of the original sample were plated onto m-Endo agar plates. Micropipettes were used to transfer the samples to the plates and a glass spreader (soaked in ethanol and then flamed) was used to spread the sample evenly on the agar. This procedure was repeated with EMB and MacConkey agar. All of the plates were then incubated at 35ºC for 48 hours. After the 48 hour incubation the plates were examined for growth characteristics of coliform or *E. coli* colonies. The colonies on appropriate plates were counted and expressed as the number of bacteria per 100 mL water sample. Appropriate known bacteria were also plated on each of the four media, and water from a drinking fountain in the Science Building (outside SB210) was plated on Coliscan® Easygel®.

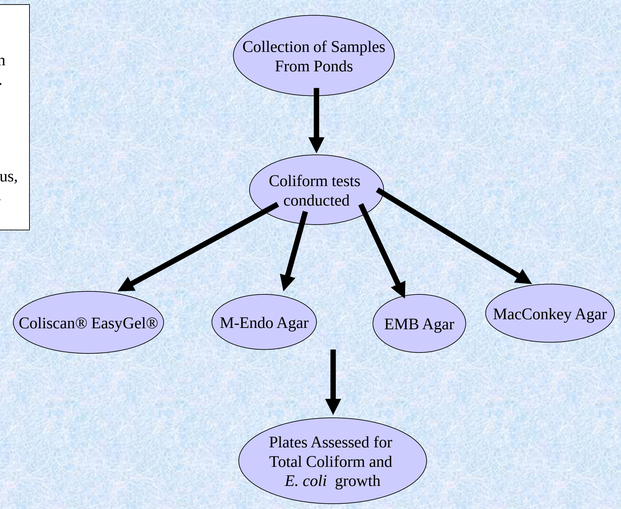
Map of Nature Preserve Collection Sites



Arrows indicate Ponds sampled on Nature Preserve. Two other sites, President's and Ravenswood, located on the North side of campus, are not pictured.

Approximate locations of Middle and West Drainage Ponds (not on map)

Summary of Methods



Results

Numerous bacteria were present on all plates after 48 hours incubation at 35 C. The known bacteria that were plated on each of the different media resulted in the expected characteristic growth, with the exception of the m-Endo agar. The number of total coliforms determined by the distinguishing characteristic growth on Easygel®, EMB Agar, and MacConkey Agar is shown in Table 1. The ponds with the greatest numbers of coliforms include the South Pond and the Middle Drainage Pond within the Calvin College Ecosystem Preserve, while the North Pond and Whiskey Pond had the lowest coliform counts. The concentration of *E. coli* in each of the ponds is shown in Table 2. The highest values were observed in South Pond and Middle Drainage pond, where the number of *E. coli* per 100 ml was greater than 2000. The ponds with lower E. coli numbers include Whiskey Pond and the two ponds adjacent to the athletic fields (Ravenswood and Presidents Ponds). The water obtained from the drinking fountain did not contain either *E. coli* or other coliforms (See Figure 1).

Table 1: Total Coliform Counts

Pond	Easygel®	EMB Agar	MacConkey Agar
	(Bacteria/100 mL)		
North Pond A	14,500	49,000	10,100
North Pond B	8,100	*N.D.*	*N.D.*
South Pond A	107,000	24,000	40,000
South Pond B	85,500	*N.D.*	*N.D.*
Whiskey Pond A	10,800	40,000	8,500
Whiskey Pond B	5,000	*N.D.*	*N.D.*
West Drainage Pond	34,000	38,000	6,000
Middle Drainage Pond	150,000	*N.D.*	*N.D.*
Ravenswood Pond A	17,200	36,000	16,700
Ravenswood Pond B	10,200	*N.D.*	*N.D.*
Presidents Pond A	26,700	42,000	41,000
Presidents Pond B	13,000	*N.D.*	*N.D.*
Drinking Fountain	0	*N.D.*	*N.D.*

N.D. indicates that total coliform counts were not determined by this method for the sample.

Table 2: *E. coli* Counts

Pond	Easygel®	EMB Agar
	(Bacteria/100 mL)	
North Pond A	830	400
North Pond B	400	*N.D.*
South Pond A	2,000	3,000
South Pond B	2,400	*N.D.*
Whiskey Pond A	100	900
Whiskey Pond B	370	*N.D.*
West Drainage Pond	100	1,000
Middle Drainage Pond	2,700	*N.D.*
Ravenswood Pond A	260	700
Ravenswood Pond B	230	*N.D.*
Presidents Pond A	70	500
Presidents Pond B	300	*N.D.*
Drinking Fountain	0	*N.D.*

N.D. indicates that E. coli counts were not determined by this method for the sample.

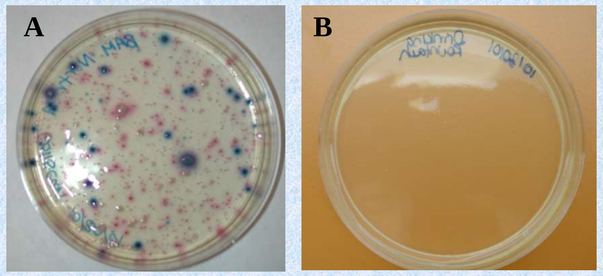


Figure 1. Example of the appearance of Coliscan® Easygel® plates after 48 h incubation at 35 C. A. The media was prepared with 3 mL of water obtained from the North Pond. Note the presence of purple colonies (E. coli), pink colonies (other coliforms), and teal and tan colonies (non-coliforms. **B.** The media was prepared with 3 mL of water from a campus drinking fountain.

Discussion

Overall, the EMB agar plates showed a much higher total coliform and *E. coli* count when compared with the other media that were used. It is possible that these higher readings are false, and that the error could be due to inconsistencies in the agar itself or due to a misinterpretation of the types of colonies on the plates. Perhaps it is possible that some environmental bacteria that have not yet been cultured and identified show growth characteristics similar to *E. coli*, contributing to a false high reading. Also, it should be noted that the known bacteria did not show characteristic growth on m-Endo agar plates, so the results from this type of media were disregarded.

Looking at the results of the two different types of agar that were used (MacConkey and EMB agar) and the results from the Coliscan®Easygel® it is fairly obvious that *E. coli* and other coliforms are present in the waters on Calvin College's campus. However, it is important to note that, with the exception of the South Pond and the middle Drainage Pond at Calvin, all other ponds that were tested had *E. coli* counts well under the accepted legal limits set my the Michigan Department of Environmental Quality, Surface Water Quality Division. The limits that were set by the DEQ were for total body contact, 300 *E. coli*/100ml and for partial body contact, 1000 *E. coli*/100ml.

The DEQ does suggest to do tests over a 30-day period possibly to decrease the error in the data collected. Perhaps, if samples were taken from different locations of the pond over a longer period of time, the average coliform count would be different. Several possible explanations exist for why the coliform counts were higher in South Pond and in the middle Drainage Pond when compared to the other ponds. These include run-off from rain in the days before, a high number of animals which inhabit the area surrounding these ponds, and that the samples could have been taken too close to shore where the soil may have contained higher amounts of fecal coliforms from the animal droppings.

We suggest that further tests be run before a conclusion is drawn as to the exact amount of fecal and non-fecal coliforms that are found in the ponds located on Calvin College's campus.

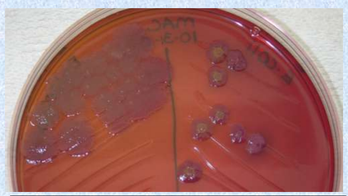
Distinguishing Coliform Colonies on Various Media

MacConkey Agar:
Coliform are red
Non-Coliform are uncolored or transparent

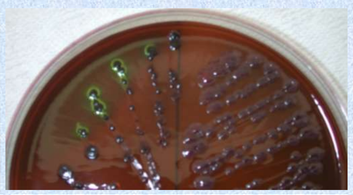
EMB Agar:
E.Coli are blue-black with a metallic sheen
Other Coliform are red-pink
Non-Coliform are uncolored or transparent

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

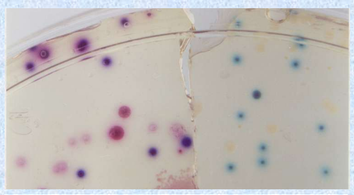
M-Endo Agar:
Coliform are red with a metallic sheen
Non-Coliform are red with no metallic sheen



Enterobacter aerogenes *E. coli*



E. coli *Enterobacter aerogenes*



Mixed Coliforms Non-Coliforms

No Photo Available. Streaks with known bacteria did not occur as expected on this agar.

References

Hudson, B.K. and Sherwood, L. *Explorations in Microbiology.* Prentice Hall: New Jersey, 1997.