A Brief Comparison of Hardness, Ammonia, and *E. coli* levels Pre- and Post-Filter Water Sourced from Margarita Creek

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Introduction

On a tributary stream for the Sibun River known as Margarita Creek, located in the Cayo District of Belize, is the Sleeping Giant Lodge. For the lodge to have suitable water, a part of the stream has been diverged to a collection cistern to pool water, for which the lodge to pull from. This water is then treated and sent to every faucet in the complex. To determine the effect of the filtration system, I am going to sample the water from the stream before the divergence to the intake cistern, and then I am going to sample the water after the water has been treated. The hypothesis is that there will be more hardness to the water, as well as more ammonia concentration and higher levels of *E.coli*. Belize is located in a karst environment, meaning that the majority of bedrock and general geologic features are composed of limestone. Limestone is the common name of Calcium Carbonate. When water percolates through calcium, the water gains an amount of hardness, which is the parts per million(ppm) of calcium in the water. Hardness can also be expressed as Milligrams per Litre(mg/L), which is the same measurement as ppm. While water hardness does not pose any adverse health effects on humans, it can wreak havoc on machinery, appliances, and other items with which it is in constant contact. This is

because of the limescale and other buildup as well as the resistance to soap lather. Ammonia can be present in untreated water due to it being a waste product excreted by fish through their skin and gills. Ammonia can also be present due to decaying food or a buildup of waste. It is also used as a fertilizer. Ammonia is not toxic in low doses to humans, but it is toxic to fish. If ammonia solutions are ingested by humans in excess amounts, it can cause corrosive damage to the mouth, throat, and stomach. *Escherichia coli (E. coli)* is a rod shaped bacterium that is present in the lower intestines of humans as well as most other endotherms. As a general rule, most strains of *E. coli* are harmless, but there are a few serotypes that can cause food poisoning. *E. coli* is normally spread through contact with fecal material. While the first two variables of the three variables to be tested are generally harmless to humans, resulting data from all three tests will indicate effectiveness of the filtration system as well as general health of the stream.

Methods and Materials

Due to the close proximity of the cistern to the stream source, as well as the uniformity of the plumbing around the lodge post-filtration, I have determined to draw samples from six sites: Three sites before the water is filtered, and three sites of post filtration water. From these sites, I will draw three samples for each of my tests. See appendix for site details. For testing water hardness, I have selected to use the KH Carbonate Hardness Test Kit for Freshwater and Saltwater, made by Aquarium Pharmaceuticals Inc. This kit comes with a liquid indicator as well as a small test tube, along with a conversion chart from drops of indicator to ppm. Directions on operating this test are included with the kit. For testing ammonia, I will use the Quick Dip Ammonia Test Kit Aquarium Test, made by Jungle Laboratories Corporation. This kit contains indicator test strips as well as a vial for testing. There is also a color indication chart for identifying ammonia levels in ppm(mg/L). Directions on operating this test are included with the kit. For testing for *E. coli*, I will use Eosin-Methylene Blue agar plates, more commonly referred to as EMB Plates. These agar plates will be used to identify presence of *E. coli*, however, determining amount may be a bit difficult in the field. To use the EMB Plates, one must use

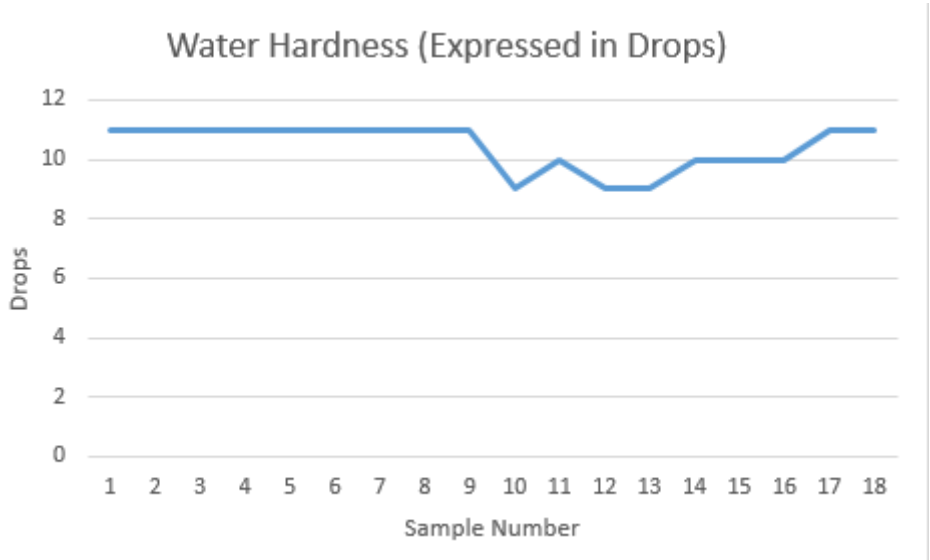
a pipette to place a drop of sample water on the plate, and then use a sterile cotton swab to spread the water around the plate. Then, the plates must incubate and bacterial growth will appear on the plate in various forms if it is present in the water sample. If *E. Coli* is present in the sample, the plate will show dark blue/black colonies with a bright metallic green sheen. Example included in Appendix. For additional supplies needed, I will also need the 18 containers for obtaining my samples and transporting them to a lab area. I can utilize the same pipette from my EMB plates for my hardness and ammonia tests. It would also be ideal to have a few spare pipettes in the event that a pipette were to malfunction. I also need a couple containers for my hardness test waste water. I will need 25 EMB plates and a like amount of sterile cotton swabs. A compound microscope would be handy to have in conjunction with the EMB plates as well. A notebook and half a dozen pens and pencils are necessary for data recording, as well as a pack of 100 note cards for labels and a roll of masking tape for sealing the plates. A camera for collecting imagery would also be of use. Any additional materials can be acquired at the lodge if need-be.

Results

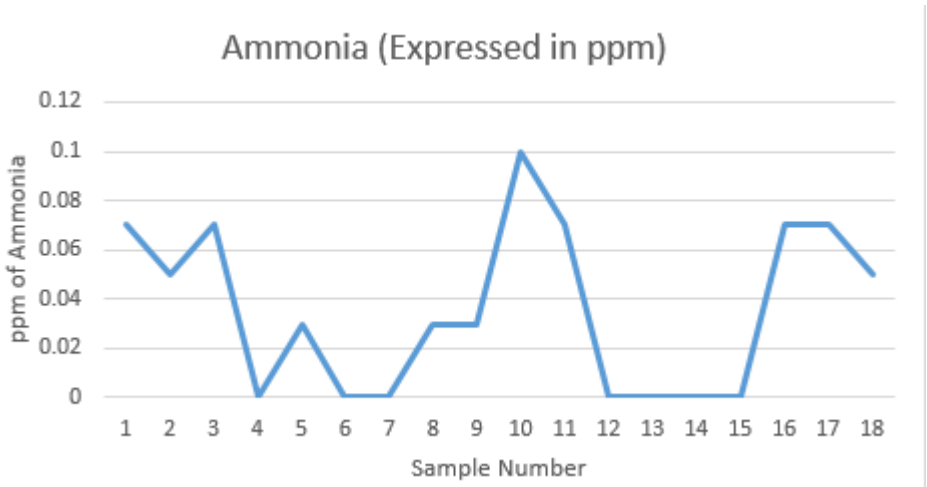
Hardness			Ammonia		
Sample	Site	Result	Sample	Site	Results
1	1	11	1	1	0.07
2	1	11	2	1	0.05
3	1	11	3	1	0.07
1	2	11	1	2	0
2	2	11	2	2	0.03
3	2	11	3	2	0
1	3	11	1	3	0
2	3	11	2	3	0.03
3	3	11	3	3	0.03
1	4	9	1	4	0.1
2	4	10	2	4	0.07
3	4	9	3	4	0
1	5	9	1	5	0
2	5	10	2	5	0
3	5	10	3	5	0
1	6	10	1	6	0.07
2	6	11	2	6	0.07
3	6	11	3	6	0.05

Hardness result recorded as drops(conversion to follow)

Ammonia results recorded as ppm



Note that Samples 1-9 were pre-filter and Samples 10-18 were post-filter



Note that Samples 1-9 were pre-filter and Samples 10-18 were post-filter

CONVERSION CHART

# of Drops	°dKH	ppm GH/KH
1	1	17.9
2	2	35.8
3	3	53.7
4	4	71.6
5	5	89.5
6	6	107.4
7	7	125.3
8	8	143.2
9	9	161.1
10	10	179
11	11	196.9
12	12	214.8

Conversion from Drops to General Hardness

*There were no colonies of *E. coli* detected in any sample. For the sake of space, the pictures of the 18 EMB plates has been omitted.*

Data source: Hardness

Normality Test (Shapiro-Wilk) Failed (P < 0.050)

Test execution ended by user request, Signed Rank Test begun

Wilcoxon Signed Rank Test

Data source: Hardness

Group N Missing Median 25% 75%

BEFORE 9 0 11.000 10.500 11.000

AFTER 9 0 10.000 9.000 10.500

W= -31.000 T+ = 7.000 T-= -38.000

Z-Statistic (based on positive ranks) = -1.903

P(est.)= 0.066 P(exact)= 0.074

The change that occurred with the treatment is not great enough to exclude the possibility that it is due to chance (P = 0.074).

Data source: Ammonia

Normality Test (Shapiro-Wilk) Passed (P = 0.800)

Treatment Name N Missing Mean Std Dev SEM

BEFORE 9 0 0.0311 0.0280 0.00935

AFTER 9 0 0.0400 0.0400 0.0133

Difference 9 0 -0.00889 0.0408 0.0136

t = -0.654 with 8 degrees of freedom. (P = 0.531)

95 percent confidence interval for difference of means: -0.0402 to 0.0224

The change that occurred with the treatment is not great enough to exclude the possibility that the difference is due to chance (P = 0.531)

Power of performed test with alpha = 0.050: 0.050

The power of the performed test (0.050) is below the desired power of 0.800.

Less than desired power indicates you are less likely to detect a difference when one actually exists. Negative results should be interpreted cautiously.

Statistical Analysis of Hardness and Ammonia Data

Analysis performed by Prof. Anthony Tate, Lincoln Land CC

Conclusion

To make a blanket statement concerning my data and my hypothesis, I would have to say that the hypothesis "…that there will be more hardness to the water, as well as more ammonia concentration and higher levels of *E.coli*." was not supported. I say that because my hypothesis was phrased as an all inclusive manner, meaning that I needed all three aspects to be supported. However, this was not the case.

When looking at hardness, all nine pre-filtration samples produced the same result, that result being 11 drops, or 196.9ppm. This in itself confirmed my theory of the water being of relatively high amounts of hardness due to the karst topography. Furthermore, when samples were drawn from the post-filter sites, I received varying results (albeit by only one or two drops), and the trend was that they required fewer indicator drops, meaning that the water was not as hard. When the hardness data was put through a statistical analysis, it was determined that there was an uneven distribution(which would be correct, being that the first deviation was at a post-filter site), and that there was a statistically significant difference in pre- and post-filtration samples. This proves the first aspect of my hypothesis: There was "more hardness to the water before it went through the filtration system." For additional details concerning the statistical analysis, refer to the analysis

itself on Page 8.

When looking at ammonia levels, my levels were of a very small amount. I received generally varying results through all samples from all sites, and without an apparent trend. When statistically analyzed, my data was indeed in a normal distribution, meaning that the data would fit into a normal bell shaped curve. In addition to its normality, it was also proven to be not statistically significantly different in levels when pre- and post-filtration samples were compared. When observing the ammonia level chart on Page 7, it is understandably visible that there is not a trend, neither an increase nor decrease in levels pre- and post-filtration. This does not support my hypothesis in stating that there would be “more ammonia concentration.” Furthermore, I indicated very low amounts of ammonia overall. The highest reading I received was .1 ppm, while .25 is still “safe” for fish and Danger level is 6. I can conclude from this that ammonia does not pose an immediate threat to life involved with Margarita Creek, both aqueous and terrestrial, at this time.

When looking at the EMB plates, I took note of a few key points. After four days allowing the cultures to grow, there was no indication of *E. coli* on any plate. There were cultures forming on the majority of the plates, however, due to the circumstances of the testing, I was not able to

identify them. I was not in the possession of a gram test, and did not have the background information or additional microbiological resources available to further investigate and analyze these cultures. It would have been ideal to transport them back to the Microbiology Lab at Lincoln Land Community College where I would have information at my disposal to identify these cultures, but that is not allowed through Customs. Furthermore, the validity and integrity of the EMB tests was compromised by the travel and the environment. Several plates had cracked lids and the vast majority had condensation on the inside of the lids as well. Before any testing was done, I wiped away, to my best extent, the moisture inside the plates with a cotton cloth. I made sure not to tough the plate itself and contaminate it in the process. However, some plates had accumulated enough condensation that the moisture had already gathered on the agar itself, rendering it unusable. There were three plates that were not used. When these three unused plates were left to incubate with the other plates, they too grew cultures, even though they were isolated from the sample waters. Concerning the actual testing done, there was a general trend of being smaller/fewer cultures on the plates inoculated with post-filtration water. There was one exception to this statement. On the second night of incubation, there was a distinct pink culture growing on a

plate that was being tested with water from the Garden Room 3 bathroom sink. This caused a some slight alarm with myself and the others who saw it, and I took two more tests with the Garden Room 3 bathroom sink water, using EMB plates that I had indicated were in good order. These two plates did not produce any cultures by the time of departure, over two days later. I took it as plate B5-B was an isolated incident and not a need for immediate concern. Images of Plate B5-B as well as the unused plates growing cultures will be in the appendix. Being that there were no *E. coli* colonies detected in any samples, my hypothesis that there would be "…higher levels of *E. coli.*" was not supported.

While the first element of my hypothesis concerning water hardness was proven accurate, the other two aspects did not support it. Therefore, since I stated that "…there will be more hardness to the water, as well as more ammonia concentration and higher levels of *E.coli*," I must say that my hypothesis as a whole was not supported. However, this one hypothesis can spawn off different hypotheses and one could take different routes and angles along the same ideas presented by myself and my data. If I were to return, I would probably so some more analysis of Margarita Creek, and do additional analyses comparing the jungle water with the manipulated water. A composite comparison report of the jungle stream as compared to the manipulated water would be

a handy piece of information. Handy not only for the owners, staff, and patrons of the Sleeping Giant Lodge, but for similar locations in the area, and the data could be extrapolated to different environments globally. It would not be of pertinence just to the Eco-Tourism sector, but to all who derive their daily water in a similar fashion. I do recommend that for future students an/or educators considering using EMB plates in this environment that they take the raw materials with them and make them on site. The accuracy would be much improved and the margin of error would be significantly reduced.

Works Cited

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"The Facts About Ammonia." New York State Department of Health. https://www.health.ny.gov/environmental/emergency/chemical_terrorism/ammonia_tech.htm (accessed April 16, 2014).

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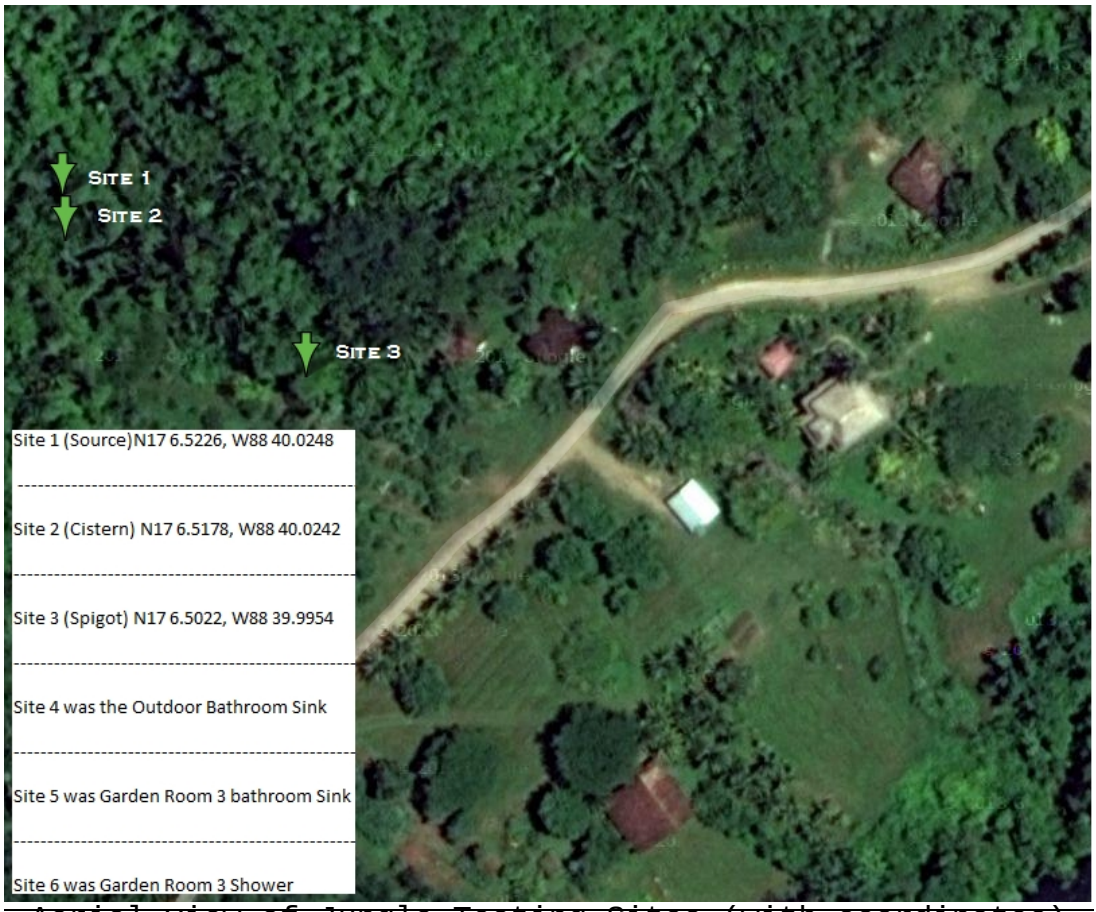
Lal, Archana, & Cheeptham, Naowarat. "Eosin-Methylene Blue Agar Plates Protocol." American Society for Microbiology. http://www.microbelibrary.org/component/resource/laboratory-test/2869-eosin-methylene-blue-agar-plates-protocol (accessed April 16, 2014).

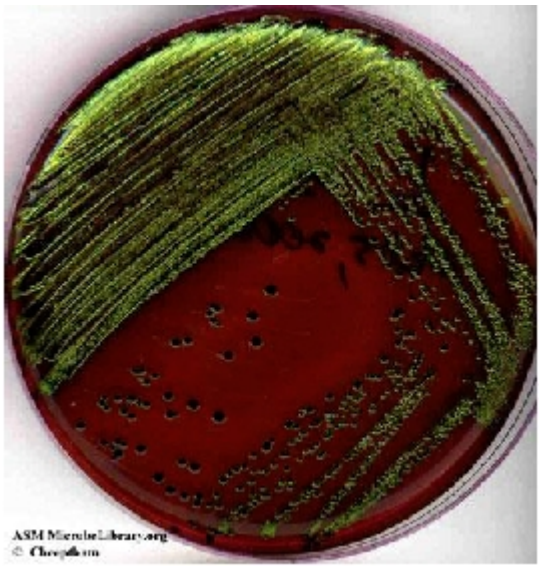
"Eosin-Methylene Blue." American Society for Microbiology. http://www.microbelibrary.org/library/laboratory-test/2871-eosin-methylene-blue (accessed April 16, 2014).

Appendix of

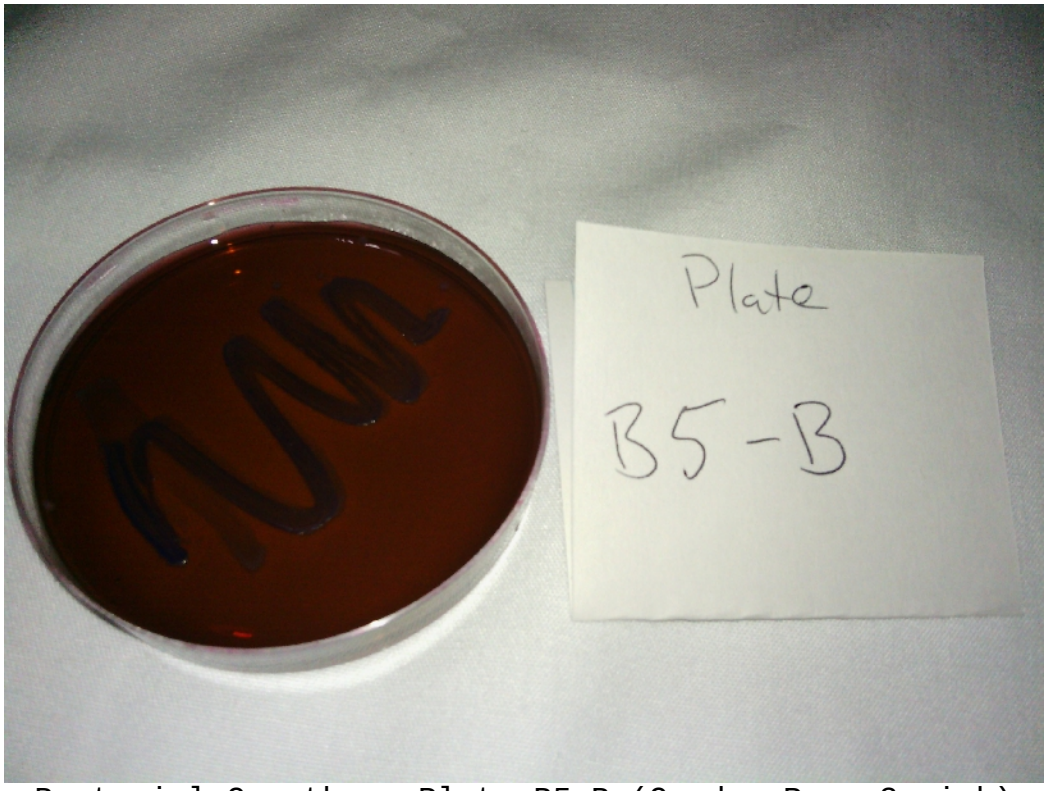
Images



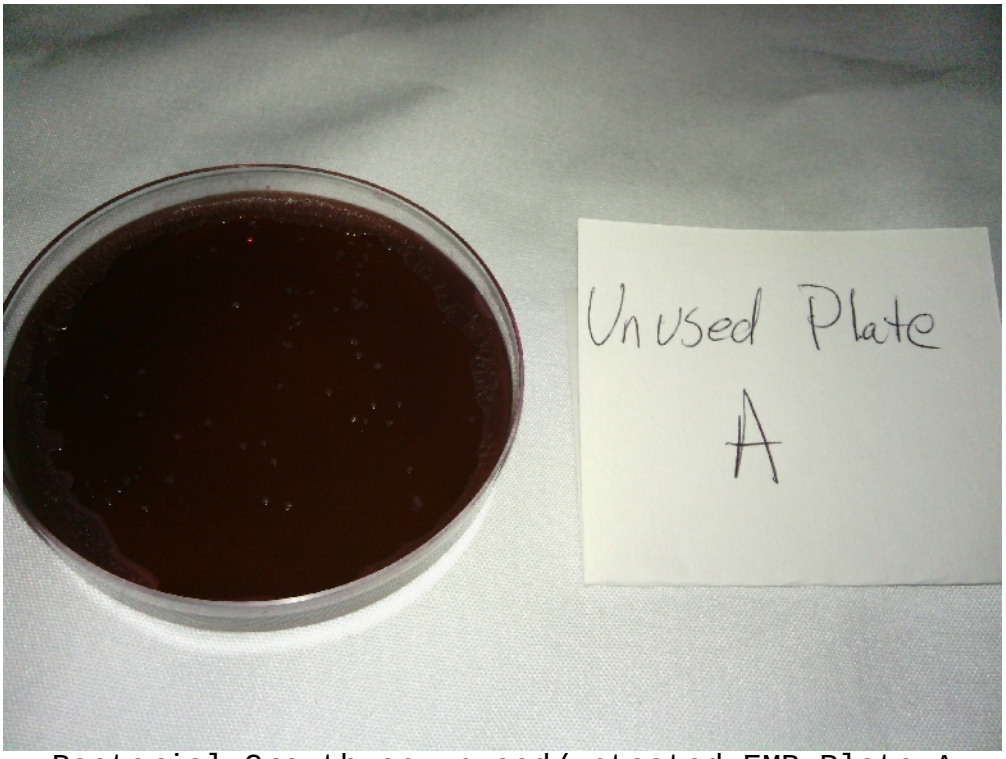
Aerial View of Jungle Testing Sites (with coordinates)



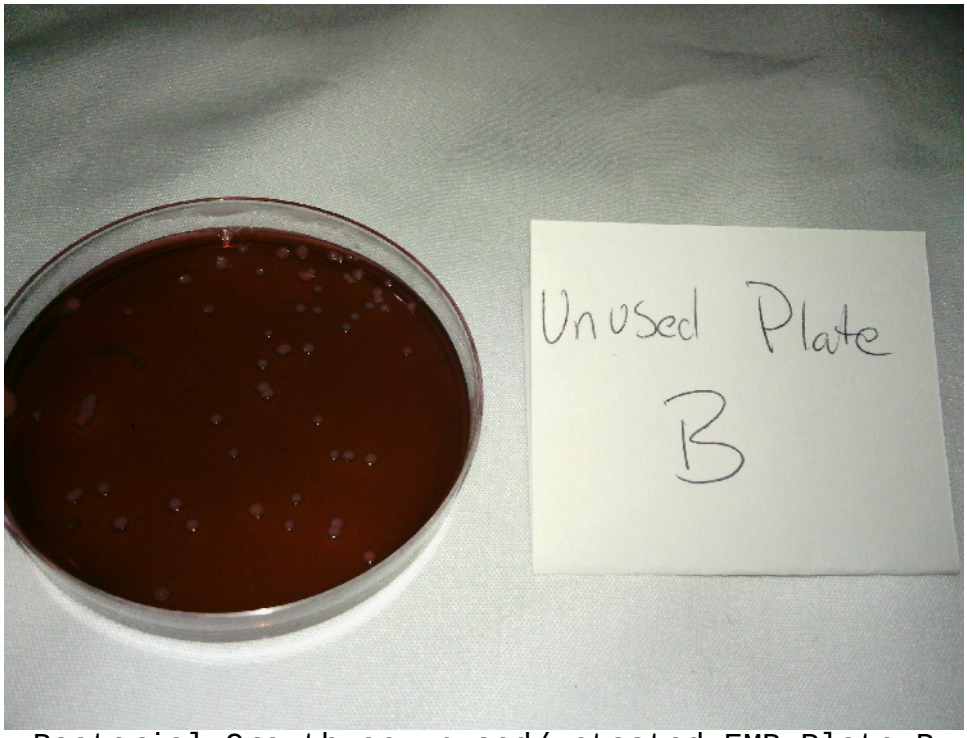
EMB Plate inoculated with *E. coli* (example)



Bacterial Growth on Plate B5-B (Garden Room 3 sink)



Bacterial Growth on unused/untested EMB Plate A



Bacterial Growth on unused/untested EMB Plate B

Bacterial Growth on unused/untested EMB Plate C

Photos of remaining EMB Plates available upon request

*Additional photos placed on Display Board, see for details”