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ESCHERICHIA COLI COLIFORM ANALYSIS USING MICROBIAL SOURCE TRACKING IN EMIGRATION CREEK

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ABSTRACT

The presence of *Escherichia coli* within a water source can be an indicator of the presence of pathogens that are harmful to human health. Previous studies have shown that Emigration creek has exhibited high levels of *E. coli* in the past. However, these studies are several years to decades old and did not identify the potential source of *E. coli* and/or fecal contamination. In this study, samples were taken from four sites throughout the canyon over 8 sampling events from September 15, 2021 to January 22, 2022, were cultured for *E. coli,* and were compared with EPA water quality standards. Additionally, microbial source tracking using quantitative polymerase chain reaction (qPCR) was conducted to determine a biological source for *E. coli* contamination. *E. coli* was enumerated in water samples using the culture plate method. these results showed the presence of *E. coli* at all sample sites throughout the canyon. Generally, the presence of *E. coli* decreased the further up the canyon that was sampled. However, sample sites located close to residential areas showed a higher amount than other sites. All collected samples from the study showed that *E. coli* levels within Emigration Creek are within the EPAs water quality standards for recreational waters. The qPCR analysis showed the presence of human host associated *Bacteriodales* HF-183 16s rRNA at all sample sites, but a higher amount of HF-183 was measurable in close proximity to residential areas. The dog associated *Bacteriodales* BacCan 16S rRNA was also measurable in the canyon, typically higher in residential areas, but was detected less frequently than the human fecal markers. This would suggest that further investigation of human fecal sources within Emigration Creek should be conducted.

INTRODUCTION

Emigration Canyon is located near Salt Lake City, Utah. It has a small township with a population of 1,466 people. [6] A small creek runs through this canyon and most of the human development in the canyon is built around this creek. Human interaction with this creek is common, especially around Rotary Glen Park. Interaction with this creek is expected to increase as the population of Emigration Canyon and the surrounding area increases. Previous studies have shown elevated levels of *Escherichia Coli* within Emigration Creek. A 2006 study by a University of Utah student showed that *E. coli* levels exceeded state criteria of 409 MPN/100 ml [11] in the months of July and August, with values ranging from 200-500 MPN/100 ml depending on the sample site. [14] Additionally, a 2016 study by the Salt Lake County showed elevated *E. coli* values during summer and winter months. These values ranged from a geometric mean of 129.1-386.6 MPN/100 ml below Rotary Glenn Park. [2] This was attributed to a number of causes including decreased flow rate, increased human activity in the canyon, wildlife within the canyon, increased runoff, and possible contamination from septic systems. [16] Both studies showed that *E. coli* values trended upwards further downstream. Knowing the amount of *E. coli*

present in a water body is important as it acts as in indicator organism for other, more dangerous, organisms. This is because *E. coli* is commonly found within the intestinal tract of warm-blooded mammals and other species. [17] So, the presence of certain types of *E. coli* in water systems suggests that fecal contamination may have occurred.

Fecal contamination of a water body can mean possible exposure to various diseases, such as cryptosporidium, cholera, bacterial transmitted hepatitis, and other infections that can cause diarrhea, vomiting, and other symptoms. [13] Contraction of these diseases can occur through the fecal-oral route [13]. In order for disease to occur, direct contact with the stream is needed and the water would need to come in contact with the mucus membranes of a susceptible individual. Human interaction with this creek is most likely to occur in public parks such as Rotary Glenn Park or where the creek flows through residential yards.

Microbial source tracking (MST) is a method which can use qPCR to determine biological sources of fecal contamination. [21] qPCR works by using *Bacteroidales*, a bacterium which is found in the intestines of mammals. [20] These bacteria contain 16S rRNA genetic markers which can be associated with a specific host. The marker HF-183 can be used to determine an association with human markers. [5] Another marker is BacCan, which can be used to determine an association with canines. [7] While these markers are overall specific and precise, they can only be associated with the targeted species as it is possible another species contains the same *bacteroidales.*

Despite a large amount of existing data relating to the presence of *E. coli*, the previous studies mentioned are many years old and may not accurately reflect current conditions within Emigration Creek. Additionally, no prior study has used qPCR to determine a source of contamination. This study aims to determine current *E. coli* levels within Emigration Creek as well as use microbial source tracking methods to pinpoint specific sources of contamination.

METHODS

2.1 Sampling Sites

Four water sample collection sites shown in figure 1 were identified in upper Emigration Creek running from the canyon mouth to Burr Fork 5.6 miles upstream on repeated sampling events. Sites were selected such that areas of human development existed between each site. This would theoretically show how increased human development upstream from sampling sites effected results. Sample site 1 was located in Rotary Glenn Park. This site was the furthest downstream and below all developments in Emigration Canyon. Sample site 2 was located where Mayfield Drive crosses Emigration Creek. This site was located upstream of a development cluster and an equestrian camp. Site 3 was located near Red Hill Lane approximately two thirds of the way up the canyon. This site was in close proximity to a residential area and has easy access from the road. Site 4 was located off of Pinecrest Canyon Road near the Burr Fork. This site was the furthest upstream and in theory would have the least amount of human influence.

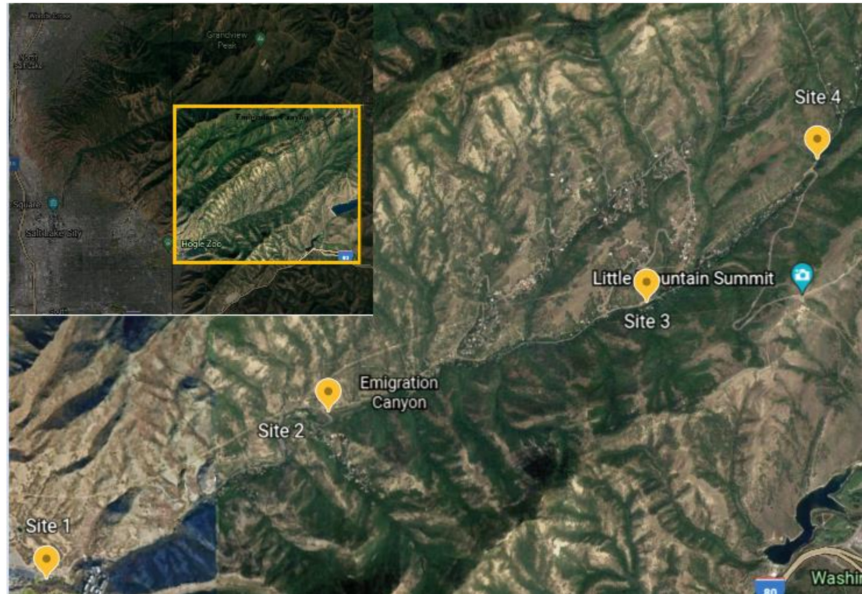

Figure 1. Sample Site map

2.2 Sample Collection Methods

Water samples were collected from each sampling site in 1-L, autoclaved PS bottles. Grab samples consisting of 1-L of stream water were collected by hand in the creek. Samples were collected mid-day and weather conditions were recorded. Several sample runs were collected within 24 hours of a precipitation event. Samples were collected on September 15th 2021, October 2nd , October 10th, October 16th, November 6th, December 1st, January 8th 2022, and January 22nd. Sampling on October 10th and October 16th occurred within 24 hours of a precipitation event. Rainfall preceding a sample event could theoretically allow for overland runoff to flow into the creek, potentially carrying additional sources of contamination. Samples were not collected during periods of heavy precipitation as this resulted in increased turbidity which heavily influenced *E. coli* results.

2.3 Analytical Methods

Suspended solids were measured using a turbidity meter- (LaMotte, Chestertown, Maryland) in units of NTU. Collected samples were allowed to settle for a period of 20 minutes before measuring. This reduces the amount of solids that can clog the filter paper used for *E. coli* culturing. However, this can result in under reporting of turbidity.

Ion concentration was measured using a pH probe- (Avantor-VWR, Radnor Township, Pennsylvania). Measurements were taken from raw sample as well as diluted samples ready for filtering.

2.4 Culture Plate Methods

Two types of culture plates were used during experimentation. The first method used a modified mTEC agar. This agar was prepared using the standard method [15] and aseptically poured into sterile 50mm petri dishes. The second method used *E. coli* specific agar prepared using standard methods [18] and was also poured aseptically into sterile 50mm petri dishes. Samples to be cultured were prepared using the membrane filtration method [18]. Samples were prepared in various concentrations using a 1x phosphate buffer solution (PBS). Two 100 ml replicates of a 1X dilution, 0.1X dilution, and no dilution were prepared and filtered through a sterile 0.45 um nitrocellulose filter paper- (Advantec, Dublin, California) using a vacuum

filtration apparatus. The filter papers were then placed onto pre-prepared agar culture-plates and labeled. The culture plates were incubated for 2 hours at 35 °C, then at 44.5 °C for 22-24 hours. *E. coli* cultures on plates containing mTEC agar were identified by a strong purple coloration. On plates using standard agar filter plates were transferred to empty culture plates. Then, 5 ml of a urea substrate-phenol red solution was added to the plates and allowed to sit for 20 minutes. *E. coli* cultures using this method were identified by a yellow-green to yellow-brown coloration. After culturing, colony forming units were counted. An ideal range of 20-200 CFUs were desired for use in calculations. For calculations, plates with the highest CFU count within the 20-200 range were used. After calculating the CFU count with the dilution, a final value of CFU/100 ml was determined. The geometric mean of one sample site over all sampling dates was also determined to compare average values between sampling sites.

2.5 DNA extraction and qPCR methods

In addition to the diluted filtered samples, 400 ml of stream sample was filtered onto a sterile 0.45 um filter paper- (Advantec, Dublin, California). These filter papers were stored in a 50 ml test tube and frozen at -80 °C. Once the filters were completely frozen, the papers were broken up in the test tube, added to a 2ml lysing matrix tube, and processed according to previously published methods [19]. Samples were eluted in 25 uL of 10 uM Tris-HCl (pH 7.4) after DNA concentration. Quantity and purity of DNA was determined by placing 2uL of sample on a plate reader and analyzed using a BioWin UV spectroscopy analyzer at 260 and 280 nm. The nucleic acid concentration was logged and saved for use in qPCR calculations.

Concentrated samples were analyzed using probe-based qPCR for *E. Coli* and SYBR Green qPCR for HF-83 and BacCan. The following table outlines the primers and probes used for each assay.

Table 1. qPCR assay and primer list [5,7,9]

ASSAY	**GENE**	**F PRIMER**	**R PRIMER**	**PROBE**
E. coli	uidA	GTC CAA AGC GGC GAT TTG	CAG GCC AGA AGT TCT TTT TCC A	ACG GCA GAG AAG GTA
HF183 (human)	16S	ATC ATG AGT TCA CAT GTC CG	TAC CCC GCC TAC TAT CTA ATG	TTA AAG GTA TTT TCC GGT AGA CGA TGG
BacCan (canine)	16S	GGA GCG CAG ACG GGT TTT	CAA TCG GAG TTC TTC GTG ATA TCT A	6-FAM-TGGTGTAGCGGTGAAA-TAMRA-MGB

These assays were selected based on their specificity and precision. After making the master mix for *E. coli*, HF-183, and BacCan, the samples were placed in the thermocycler. *E. coli*, HF-183, and BacCan were run at 50 °C for 2 minutes, followed by 95 °C for 10 minutes, then cycled 45

times at 95 °C for 15 seconds followed by 60 °C for 60 seconds. Data was then collected from the thermocycler and cells/100 ml recorded.

RESULTS

3.1 E. coli Enumeration Results

The recording of sampling data includes the date of collection, sample number, pH, dilution, volume filtered, and *E. coli* colonies counted. The raw sampling data can be found in Appendix A. For an accurate representation of *E. coli* presence, agar plates should contain 20-200 colonies of *E. coli*. The following table (Table 2) shows the maximum detected levels of *E. coli* using the ideal plate method.

Table 2. Sample *E. Coli* site maximum using the ideal plate method in CFU/100 ml

E. COLI (CFU/100 ML)

SITE #	DATE							
	9.15.21	10.2.21	10.10.21	10.16.21	11.6.21	12.1.21	1.8.22	1.22.22
SITE 1	118	86	78	27	8	19	7	76
SITE 2	46	5	240	92	7	13	83	42
SITE 3	*	37	210	35	22	8	28	72
SITE 4	9	9	46	15	200	25	11	57

*Site location not Sampled.

Table 3. Geometric mean in CFU/100 ml by site for all sample dates

SITE GEOMETRIC MEAN (CFU/100 ML)

SITE #	Geometric Mean
site 1	33.86
site 2	33.57
site 3	37.09
site 4	25.44

The site geometric mean indicates that, with the exception of site 3, the level of culturable *E. coli* increases the further downstream a sample site is located.

3.2 Turbidity and Flow Rate Results

Turbidity measurements were generally low during sampling except during periods immediately after precipitation events as seen on October 10th and 16th. Attempts were made to collect samples where stream flow was deep and slow flowing, as shallow or fast flowing areas would increase turbidity levels.

Table 4. Site Turbidity measured in NTU

TURBIDITY (NTU)

SITE #	DATE							
	9.15.21	10.2.21	10.10.21	10.16.21	11.6.21	12.1.21	1.8.22	1.22.22
SITE 1	*	0.72	0.55	0.6	0.59	1.55	0.56	0.79
SITE 2	*	1.84	33.9	6.25	3.35	0.95	2.18	0.56
SITE 3	*	2.03	11	13.1	3.4	2.92	5.07	5.78
SITE 4	*	0.75	3.43	2.65	1.4	1.17	5.21	0.49

*Turbidity readings not tested on 9.15.21

Emigration creek flow measurements were obtained from a flow meter located in Rotary Glenn Park and managed by the Salt Lake County. A flow rate for January 8th was not obtained due to a problem with the meter.

Table 5. Emigration Creek flow rate in cubic feet per second

Emigration Creek Flow Rate (CFS)

	9.15.21	10.2.21	10.10.21	10.16.21	11.6.21	12.1.21	1.8.22	1.22.22
Flow (CFS)	1	0.8	1.32	1.08	0.87	0.87	*	0.45

3.3 qPCR Results

After determining the amount of culturable *E. coli* present in Emigration Creek, qPCR was used to determine the presence of pathogenic *E. coli* cells per 100 ml. This measurement represents the total amount of *E. coli* present in the creek, including non-infective strains of the pathogenic *E. coli* and dead cells. Initially, all sampling dates were to be analyzed using qPCR. However, samples from 11.6.21 and 12.1.21 were accidentally disposed of during a lab clean out. The following results (Table 6) show the qPCR results for *E. coli* for the available dates.

Table 6. *E. coli* gene copies (GC) per 100 ml estimated via qPCR

***E. COLI* GENE COPIES (GC)/100 ML**

SITE #	DATE					
	9.15.21	**10.2.21**	**10.10.21**	**10.16.21**	**1.8.22**	**1.22.22**
SITE 1	4507	384	203	ND	ND	ND
SITE 2	155	595	1779	228	153	1752
SITE 3	*	108	256	478	70	1503
SITE 4	357	236	ND	ND	632	2089

*site location not sampled

Data points on October 10th, 16th, January 8th and 22nd for *E. coli* cell analysis produced no data. Comparing culturable *E. coli* to *E. coli* gene copies shows that the values are not correlated. For instance, October 10th and 16th had a high presence of culturable *E. coli* after a rain event but a lower presence of *E. coli* gene copies compared to other days.

In order to determine a potential biological source (human or dog) for *E. coli* contamination, qPCR analysis was done to test for human and dog associated *Bacteroidales* marker genes in the water samples. The following table (Table 7) denotes the quantities of HF-183 16s rRNA in the stream water.

Table 7. HF-183 gene copies (GC) per 100 ml estimated via qPCR

HF-183 GENE COPIES (GC)/100 ml

SITE #	DATE					
	9.15.21	**10.2.21**	**10.10.21**	**10.16.21**	**1.8.22**	**1.22.22**
SITE 1	36	7	192	190	46	66
SITE 2	402	18	46	113	494	247
SITE 3	*	14	ND	399	373	184
SITE 4	170	258	34	35	46	450

*Site location not sampled

The test for HF-183 human indicator produced few sample dates with no data, only occurring on October 10th. Additionally, the control samples showed that HF-183 was not detected in negative samples. This would suggest that the qPCR data for human indicators is accurate.
The following table (Table 8) shows the detection of the canine marker for individual site samples. Generally, the dog marker was detected less frequently in the water than the *E. coli* and HF-183 marker genes.

Table 8. Presence or absence of the dog marker BacCan in each sample.

BacCan Cell Presence

SITE #	DATE					
	9.15.21	10.2.21	10.10.21	10.16.21	1.8.22	1.22.22
SITE 1	ND	ND	ND	X	X	X
SITE 2	ND	ND	ND	ND	ND	X
SITE 3	*	X	X	X	X	ND
SITE 4	X	ND	ND	ND	X	X

ND denotes No Data, X denotes BacCan presence, * indicates site location not sampled.

While a quantifiable number of cells in each sample could not be determined, the relative concentration of detected sample would indicate that the overall presence of Canine cells in individual samples is relatively low when compared to the concentration of HF-183 cells in each sample.

DISCUSSION

Visual observations of the sampling sites showed many factors that could affect human contact with the stream. The location of sampling site 1 is easily accessible from the Rotary Glenn Park parking area. The ground leading to the site is heavily trampled from people walking along the creek. The creek itself has clear water but is easily disturbed from the soft bottom. A large amount of plant life can be seen in the water during the summer months. Sampling site 2 has no common foot traffic as it is located under heavy vegetation near a bridge. The creek is relatively fast moving at this site and there are many rocks in the creek where samples were collected. There is visible trash caught on rocks and vegetation in the creek. Sampling site 3 is easily accessible from the road of a residential neighborhood. An old picnic table is located near the collection site. The creek is very shallow at this location. Sampling site 4 is hard to access from the road and requires climbing down the side of a hill. The creek flows very fast at this site; however, the water appears the cleanest of all the collection sites. Small fish were visible in the creek at this location.

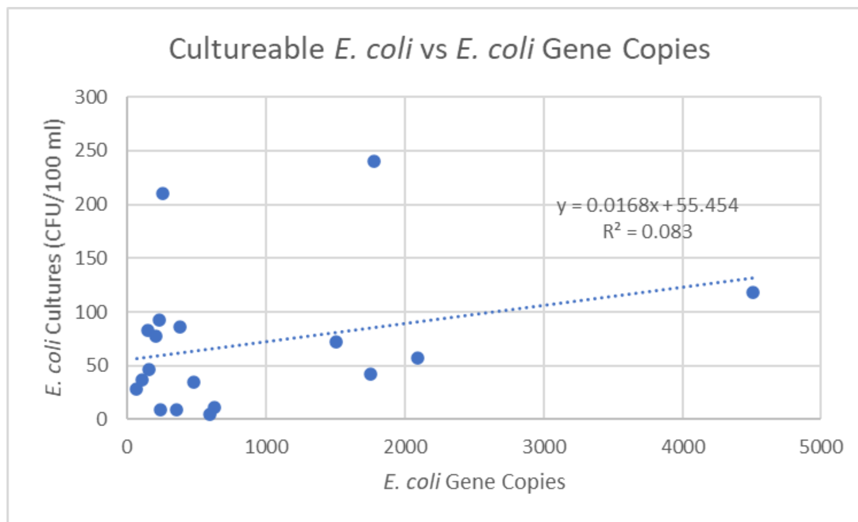

Fig. 2, culturable *E. coli* vs *E. coli* Gene Copies

Overall, the amount of culturable *E. coli* was lower than *E. coli* detected using qPCR. A comparison of culturable *E. coli* to *E. coli* detected using qPCR seen in figure 2 shows that there is not a strong correlation between the two sets of data. Table 3 shows the maximum culturable *E. coli* using the ideal plate method. This data represents a grab sample rather than a composite sample. As a result this sample may be dependent on the time of day the sample was taken. Site 1 has the highest average of culturable *E. coli* compared to other sites. This site is the furthest downstream from other sites. Although site 1 has the highest geometric mean of culturable *E. coli*, qPCR tests for human cells have a lower geometric mean compared to other sites. This would suggest that *E. coli* contamination at site 1 comes from multiple biological sources. BacCan qPCR analysis did detect the presence of Canine indicators at site 1. Although site 4 has the lowest geometric mean of culturable *E. coli* detected, qPCR values for HF-183 cells are relatively high when compared to other sites. One possible reason for this is that there is possible contamination from a human source further upstream. However, there is not enough human presence to cause a large amount of *E. coli* contamination. While site 1, 2, and 4 show *E. coli* decrease the further up the canyon the sample site is located, site 3 is an exception to this, having the highest geometric mean of any of the sites. This could be caused either by site characteristics unique to site 3, such as ease of access and proximity to structures. Site 3 is located in a residential neighborhood with houses directly across from the sample site. At no other sample site are residential dwellings as close to the sampling location as site 3. qPCR data for human cells would support this. Site 3 has the highest geometric mean for HF-183 cells than any other sample site. Another interesting factor for site 3 is despite having the largest geometric mean of culturable *E. coli* it has the lowest geometric mean of *E. coli* cells from qPCR analysis. The high geometric mean for HF-183 and low geometric mean for *E. coli* cells at site 3 could suggest that the majority of *E. coli* contamination is from an human sewage source. This could be the result of leaking sewage pipes, a faulty septic system, or a septic system that is located too close to the creek.

During visual analysis of cultured samples, several samples of *E. coli* counts would dramatically increase at higher concentrations to the point where it was impossible to count individual colonies. This often occurred when samples were collected within 24 hours of a precipitation event in the canyon, as seen on October 10th and 16th. This is likely due to precipitation causing runoff from the surrounding area carrying additional *E. coli* contamination sources into the creek. This extra runoff would likely influence flow rate within the creek as well

as turbidity at the sample site location. A linear regression model was used to compare culturable *E. coli* colonies to creek turbidity and flow.

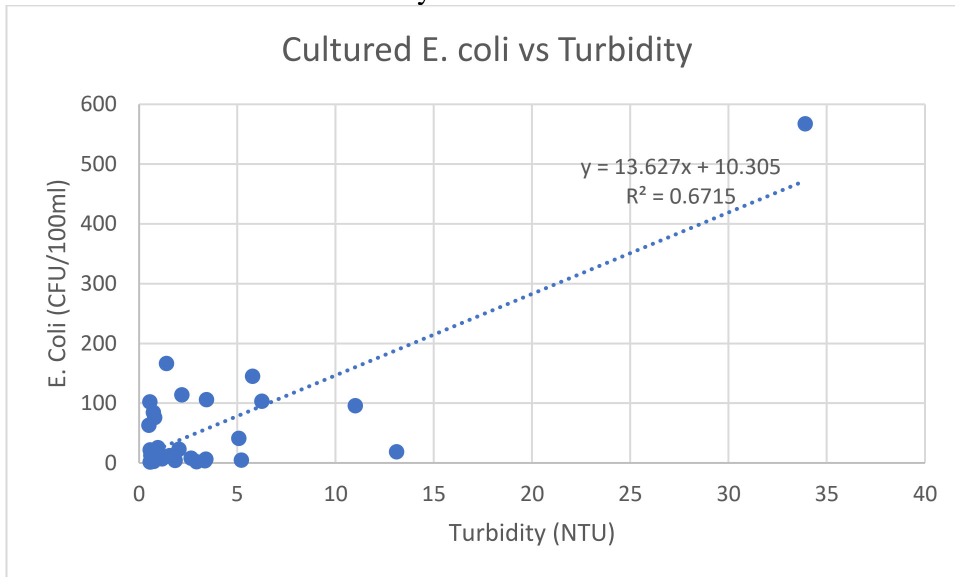

Figure 3. cultured *E. coli* vs turbidity linear regression

The linear regression model comparing *E. coli* cultures and turbidity produced an R^2 value of 0.67 This would suggest that there is a strong correlation between turbidity and culturable *E. coli*.

Observed flow rates in Emigration Creek were relatively low, with a geometric mean flow rate of 0.87 CFS. Flow rate within the creek increased in the time after a precipitation event. Table 6 shows that for October 10th the flow rate was greater than 1 standard deviation from the mean. A precipitation event had occurred within 24 hours of sampling on October 10th. The comparison between flow rate and culturable *E. coli* is shown below.

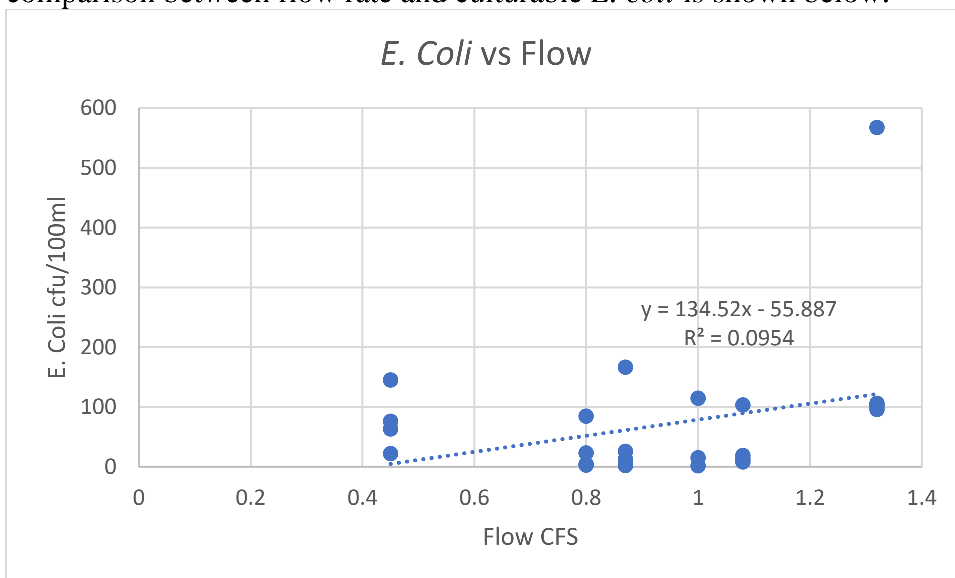

Figure 4. Culturable *E. coli* vs Flow linear regression model.

Figure 4 shows the linear regression for all data points related to flow. This linear regression produced an R^2 value of 0.09. This would suggest that the relation between culturable *E. coli* and stream flow rate is low. However, when comparing flow rates and *E. coli* between individual

sites, the R^2 value was much more varied. The highest R^2 value between flow and culturable *E. coli* was at site 2 at 0.51 and the lowest at site 4 at 0.05. Based off the differing R^2 values between flow rate and turbidity, turbidity is a much better indicator for the presence of *E. coli.*

The Environmental Protection Agency has set criteria for the presence of *E. coli* in fresh water. [1] The recreational water quality criteria states that the geometric mean should not exceed 126 CFU/100 ml and the statistical threshold value should be no greater than 410 CFU/100 ml. All collected samples using both the ideal plate method, the daily geometric mean, and the overall geometric mean fall under these values. Therefore current *E. coli* contamination is within acceptable standards for water quality.

CONCLUSION

After conducting sampling, results for culturable *E. coli* show the presence of *E. coli* at all sample sites, generally decreasing further up the canyon with the exception of one site located in a residential neighborhood. The results show that *E. coli* is likely to increase in the creek as turbidity increases. Sampling results show that *E. coli* contamination within Emigration Creek is within the EPA's acceptable standards of 410 CFU/100ml. qPCR results show the presence of *E. coli* and HF-183 cells at all sample sites. Comparative results would indicate that *E. coli* contamination within Emigration Creek is the produced of a human source, especially at site 3. This could include leaking sewer lines, faulty septic systems, or septic system drainage fields placed too close to Emigration Creek. Further studies can be done to provide additional evidence that *E. coli* contamination in the creek is human caused. Mass spectrometry can be used to search for particulates that are routinely disposed of in sewage such as pharmaceuticals or microparticles specific to toothpaste and other toiletries.

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Fig. 5, Office of Undergraduate Research at the University of Utah

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APENDEX A

Table of Results

Site ID	Sample Date	*E. coli* plate count, CFU/100 mL	*E. coli* uidA GC/100 mL	Turbidity NTU	HF-183 16S rRNA GC/100 ml	BacCan present (P)/Absent (A)	pH
1	9.15.21	118	4507	ND	36	A	8.55
2	9.15.21	46	155	ND	402	A	8.34
4	9.15.21	9	357	ND	170	P	8.63
1	10.2.21	86	384	0.72	7	A	8.49
2	10.2.21	5	595	1.84	18	A	8.24
3	10.2.21	37	108	2.03	14	P	7.90
4	10.2.21	9	236	0.75	258	A	8.34
1	10.10.21	78	203	0.55	192	A	8.54
2	10.10.21	240	1779	33.9	46	A	8.28
3	10.10.21	210	256	11	ND	P	8.07
4	10.10.21	46	ND	3.43	34	A	8.66
1	10.16.21	27	ND	0.6	190	P	8.46
2	10.16.21	92	228	6.25	113	A	8.48
3	10.16.21	35	478	13.1	399	P	8.12
4	10.16.21	15	ND	2.65	35	A	8.78
1	11.6.21	8	ND	0.59	ND	ND	8.69
2	11.6.21	7	ND	3.35	ND	ND	8.67
3	11.6.21	22	ND	3.4	ND	ND	8.19
4	11.6.21	200	ND	1.4	ND	ND	8.81
1	12.1.21	19	ND	1.55	ND	ND	8.35
2	12.1.21	13	ND	0.95	ND	ND	8.23
3	12.1.21	8	ND	2.92	ND	ND	8.25
4	12.1.21	25	ND	1.17	ND	ND	8.32
1	1.8.22	7	ND	0.56	46	P	8.52
2	1.8.22	83	153	2.18	494	A	8.44
3	1.8.22	28	70	5.07	373	P	8.59
4	1.8.22	11	632	5.21	46	P	8.33
1	1.22.22	76	ND	0.79	66	P	8.66
2	1.22.22	42	1752	0.56	247	P	7.75
3	1.22.22	72	1503	5.78	184	A	8.92
4	1.22.22	57	2089	0.49	450	P	8.47

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