

Quantifying and Identifying *E. coli* in Buffalo Creek
499B/C Research Report
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Author Note

This research Report was prepared as a 499B/C under the guidance of Dr. Michael Whitney, as the summation of an Undergraduate Research Project at Milligan College.

Background:

Escherichia coli (*E. coli*) represents a potential danger to students and locals who use Buffalo Creek. It is known that portions of Buffalo creek do not meet water quality standards for permitted levels of *E. coli* (TDEC, 2014, p. 52). This research seeks to identify whether the portion of Buffalo creek running through Milligan College's campus contains levels of *E. coli* that exceed safe levels as deemed by the Tennessee Department of Environment and Conservation. To accomplish this, water samples were collected from Buffalo Creek at three locations along its length through Milligan College's campus. Each sample was evaluated to enumerate colonies of *E. coli* by the membrane filter technique using m-ColiBlue24 Broth as a medium. The m-ColiBlue24 Broth grows both *E. coli* and *fecal coliform* bacteria and differentiates the bacteria based on what colors the bacteria colonies turn. After 24 hours of incubation, *E. coli* turn blue and can be counted with a simple magnifying glass. The Tennessee Department of Environment and Conservation deems that for purposes of "recreation" the average number of colonies per 100ml water sample should not exceed 127, and that for any one sample there should be no more than 941 (Tennessee Water Quality, 2011, p. 10). *E. coli* levels have been found to meet standards set by the Tennessee Department of Environment and Conservation for water uses of "recreation." The safe levels of *E. coli* in Buffalo Creek found thus far should be taken with some caution because consistent and relatively high levels of *E. coli* have been shown to be present in Buffalo Creek as it runs through Milligan College's campus. In addition, levels of *E. coli* could fluctuate in other seasons of the year not tested during this experiment, namely during the summer months.

Buffalo creek originates near the peak of Pinnacle trail in Unicoi County and runs northeast along Unicoi Drive, to South Roan Street, to Okolona Road, to Milligan Highway, through Milligan College's campus, and ends as it crosses highway 91 and intersects the Watauga River. Previous testing at Milligan College has suggested that levels of *E. coli* exceed TDEC standards for recreational water use in the portion of Buffalo creek running through Milligan College's campus. It is likely then that the 6.08 miles of Buffalo creek impaired by fecal contamination includes the portion of Buffalo creek running through Milligan College's campus. According to TDEC, the pollutant source of *E. coli* in Buffalo creek is "pasture grazing" (TDEC, 2014, p. 52). *E. coli* lives in the digestive tract of animals (Tortora, 2010, p. 71). When animals defecate, the *E. coli* living in the intestines is transmitted to the environment and, subsequently, to any sources of water that the fecal matter washes into (Tortora, 2010, p. 705).

While prior research has indicated the presence of excessive levels of *E. coli* in Buffalo creek and identified grazing animals as its source, there is no data showing the extent of fecal contamination in the portion of Buffalo creek running through Milligan College's campus. The extent of fecal contamination in Buffalo creek should be determined, because students and local residents interacting with Buffalo creek are potentially subject to any pathogens present in its water. To determine the extent of fecal contamination in Buffalo creek, I measured levels of *fecal coliform* in its water. *Coliform bacteria* are "aerobic or facultatively anaerobic, gram-negative, non-endospore-forming, rod-shaped bacteria that ferment lactose to form gas within 48 hours of being placed in lactose broth at 35°C" (Tortora, 2010, p. 781). *Fecal coliform* designates *coliform bacteria* that are solely enteric, living only in the intestines of animals (p. 781). Measuring the level of *fecal coliform* in Buffalo creek indicates the extent of fecal

contamination in the creek. *E. coli* is a species of *fecal coliform* and can be enumerated and identified through further testing (p. 781). Because the EPA designates levels of *E. coli* as the most accurate indicator of fecal contamination in creeks, the results of this study will focus on numbers of *E. coli* colonies found in the creek.

Materials and Methods:

Materials:

Equipment:

Sampling:

24 100ml sterile sampling containers

pH paper (able to read 6-8 pH)

Thermometer

Turbidity Tester

Laboratory Testing:

Sterile Membrane Filter Funnels

Sterile Membrane Filters (.45 μm pore size)

Petri Dishes (48x8.5 mm)

Pipets

Forceps

Sterile flasks and beakers

Vacuum source- Erlenmeyer flask with attachment

Bunsen Burner

Inoculation Loops

Autoclave

Incubator- set to 37.5 °C

Magnifying Glass (to count colonies)

Media:

Presence Absence (P-A) broth

m-ColiBlue24 broth

MacConkey agar

Methods:

Duration of Testing:

Testing was conducted over the course of 34 days. The requirements for submitting data to TDEC require testing to be within 30 days, but due to classes and unforeseen events such as weather, testing was extended. Throughout these 34 days, testing occurred approximately twice every week. Prior to the experiment, the source (Whistle-Stop Deli) and base (Watauga River) of Buffalo creek were tested once each.

Locations of Testing:

Samples from Buffalo creek were collected at the entry of Buffalo Creek into Milligan College's campus, in front of the "Gazebo" (a popular swimming place on campus), and as Buffalo Creek exists Milligan College's campus behind the bridge moving away from campus .

Experimental Controls:

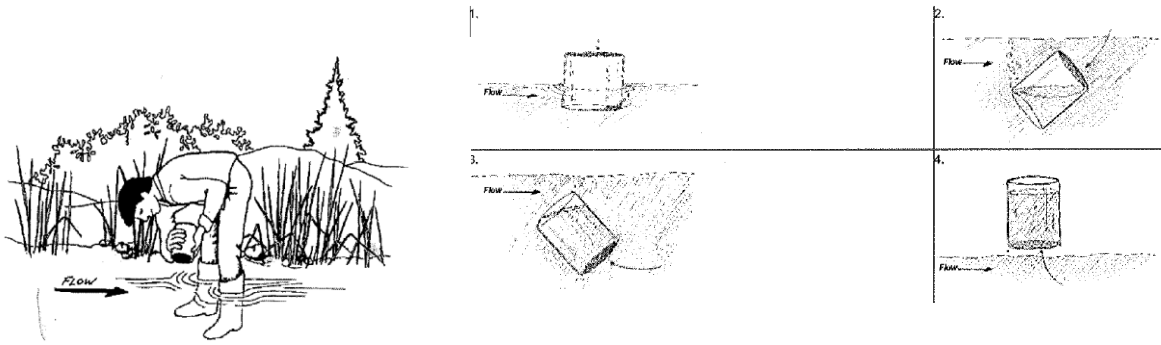
For each sampling, a sample of tap water was transported to the sampling site to ensure no experimental errors sampling. These controls follow considerations by the EPA in "Water: Monitoring and Assessment" (EPA 2002).

Recording Environmental Conditions:

For each collection, water temperature, turbidity, and pH were recorded in order to determine any trends between *E. coli* levels and environmental conditions.

Collection and Transportation of Samples:

To collect and transport samples to the lab, the EPA's considerations for "Water: Monitoring and Assessment" (EPA 2002) were followed, as well as the quality control guidelines the EPA recommends in "Water: Monitoring and Assessment" (EPA 2002).



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Testing Methods:

The Presence Absence (P-A) [Total] Coliform Test was used initially in the experiment to make sure qualitatively that *E. coli* were present in Buffalo Creek. This procedure followed the methods found in the twenty-first edition of "Standard Methods for the Examination of Water and Wastewater" (APHA 2005).

To enumerate *E. coli* colonies as well as *fecal coliform* colonies, the Membrane Filter Procedure for *Escherichia coli* was followed as found in "Test Methods for Escherichia coli and Enterococci in Water by the Membrane Filter Procedure" (EPA 1985). However, commercially bought m-ColiBlue24 Broth was used in place of self-made broth.

Results:

Data from this experiment has been organized into 5 graphs that represent the most significant results of the experiment. First, *E. coli*/100ml represents the number of colonies counted per 100ml of water sample collected at each sampling site. Data from each sampling site was originally collected per 50ml water sample, but was doubled to obtain the shown results (Figure A). Total coliform counts were also measured via the membrane filtration method (Figure B). *Fecal coliform* represents all the enteric bacteria that were present but that were not *E. coli*. *Total coliform* is the sum of *fecal coliform* colonies and *E. coli* colonies per 100ml. Temperature (Figure C), turbidity (D), and pH (E) are most beneficial when compared directly to "*E. coli*/100ml" or "*Fecal & Total Coliform*/100ml."

Figure A

E. coli/100ml

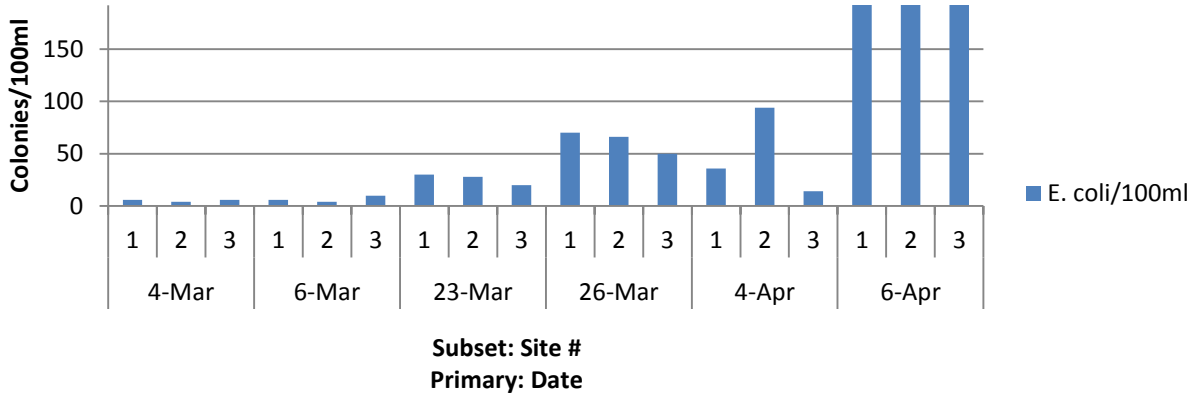


Figure B

Fecal & Total Coliform/100ml

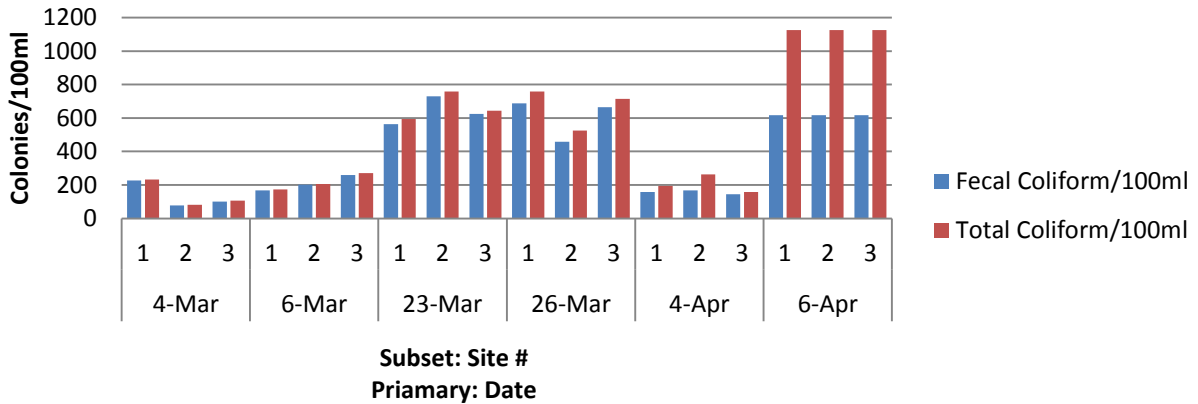
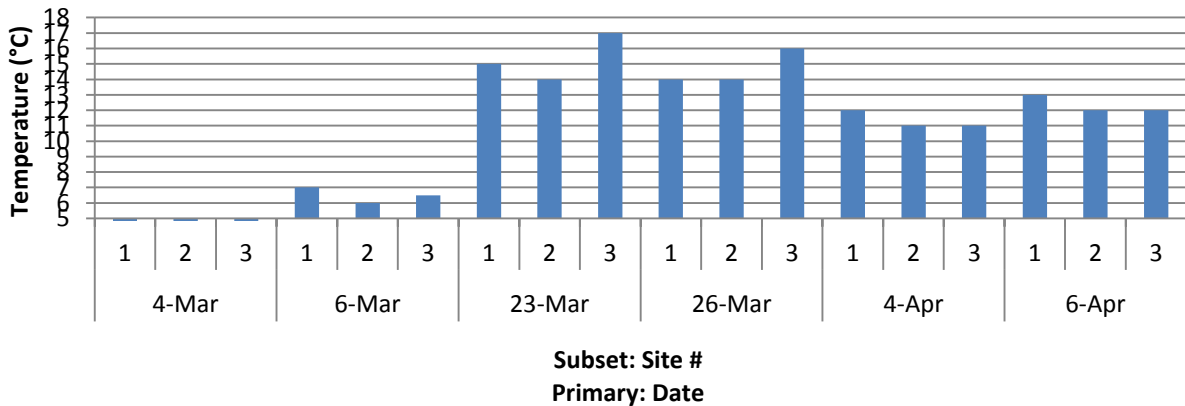
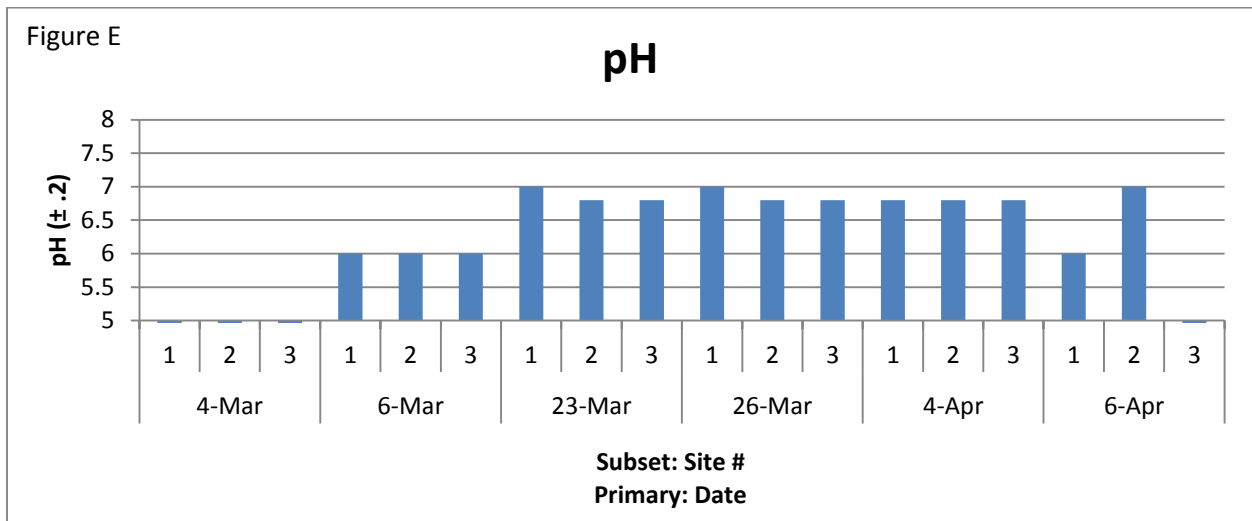
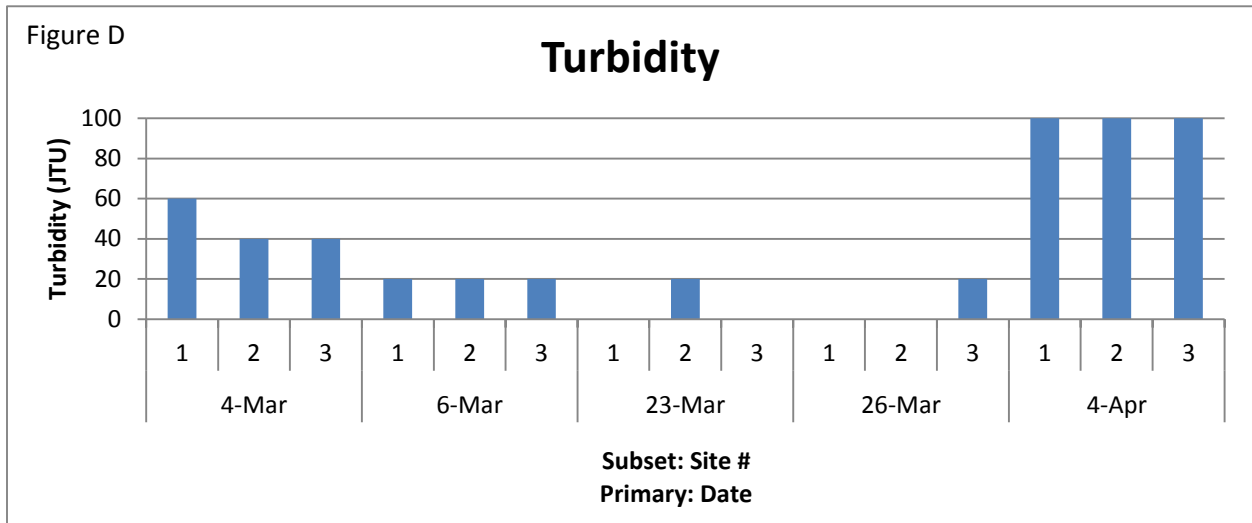


Figure C

Temperature





Conclusions:

E. coli is present in Buffalo creek in the region running through Milligan College's campus in consistent and significant amounts. These amounts are about 1/5 the amount of colonies that would be deemed to be unsafe for "recreation" (Tennessee Water Quality, 2011, p. 10). Buffalo creek is then safe to swim in for purposes of "recreation" but anyone interacting with the creek should take precautions to avoid ingesting the water.

Discussion:

Extension of Results:

Data from the experiment will be submitted to the Tennessee Department of Environment and Conservation for consideration to be included in data for the annual 303(d) list publication.

Further Considerations:

Because levels of *E. coli* were found to be both present and consistent in the portion of Buffalo creek running through Milligan College's campus it is important that further research be conducted on levels of *E. coli* present in Buffalo creek. Two potentially beneficial studies that could be conducted in the future would be to determine the levels of *E. coli* in seasons of the year other than early spring and to identify the specific strains of *E. coli* that are present in Buffalo creek. Information on levels of *E. coli* present in Buffalo creek throughout the year can be found online at <http://watersgeo.epa.gov/mywaterway>. STEC and EPEC are two pathotypes of *E. coli* that are potentially present in Buffalo creek. Both live in the digestive tract of animals, particularly cattle, so both of these pathotypes could be present in the portion of Buffalo creek running through the campus of Milligan College (p. 2, 10). Further information on pathotypes and strains of *E. coli* can be found under "References" in "*Microbiology: an introduction* (10th ed.)" by J.G. Tortora.

References

- American Public Health Association, American Water Works Association, Water Environment Federation. (2005). *Standard Methods for the Examination of Water & Wastewater* (21st ed.) (A. D. Eaton, L. S. Clesceri, E. W. Rice, & A. E. Greenberg, Eds.). Baltimore, MD: American Public Health Association.
- Centers for Disease Control and Prevention. (2013, January 24). Global Diarrhea Burden. In *Global Water, Sanitation, & Hygiene (WASH): Policy and Recommendations*. Retrieved from <http://www.cdc.gov/healthywater/global/policy.html>
- Morabito, S. (Eds.). (2014). *Pathogenic Escherichia Coli: Molecular and Cellular Biology*. Norfolk, UK: Caister Academic Press.
- Nickel, C. J. (2007, Spring). Urinary Tract Infections and Resistant Bacteria. *Reviews in Urology*, 9(2), 78-80. Retrieved from <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1892623/#!po=12.5000>
- Tennessee Department of Environment and Conservation. (2014, July). *Draft: Year 2014 303(d) List*. Nashville, TN: Planning and Standards Unit Division of Water Resources.
- Tennessee Water Quality Control Board, Division of Water Pollution Control. (2011). *Rules of Tennessee Department of Environment and Conservation*. Nashville, TN: Tennessee Water Quality Control Board.
- Tortora, J. G., Funke, B. R., Case, C. L. (2010). *Microbiology: an introduction* (10th ed.). San Francisco, CA: Pearson Benjamin Cummings.
- United States Environmental Protection Agency. (1993). *Preventing Waterborne Disease: A Focus on EPA's Research*. Retrieved from United States Environmental Protection Agency website: <http://www.epa.gov/nerlcwww/documents/h2odis.pdf>
- United States Environmental Protection Agency. (1985). *Test Methods for Escherichia coli and Enterococci in Water by the Membrane Filter Procedure*. Cincinnati, OH: Environmental Monitoring and Support Laboratory.
- United States Environmental Protection Agency. (2002, March 6). Water: Monitoring and Assessment. *5.11 Fecal Bacteria*. Retrieved from <http://water.epa.gov/type/rsl/monitoring/vms511.cfm>
- United States Environmental Protection Agency. (2002, March 6). Water: Monitoring and Assessment. *Chapter 5 Water Quality Conditions*. Retrieved from <http://water.epa.gov/type/rsl/monitoring/vms50.cfm>