

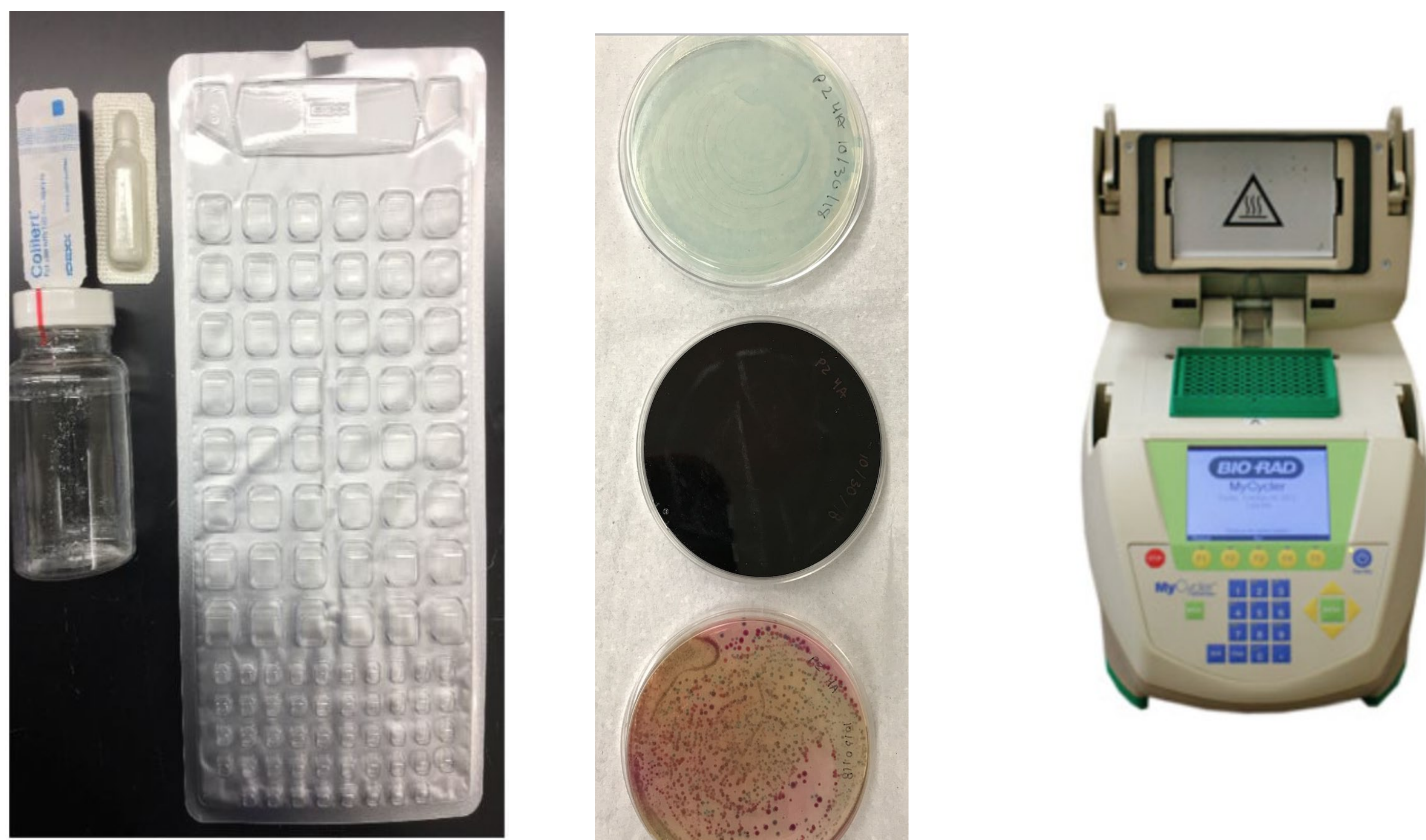
Abstract

In 2015, the city of Ontario constructed the Mill Creek Wetlands, a natural treatment wetland facility to improve local water quality management, improve water quality for users downstream, and also serve as a recreational area for the community's habitants. Bacteria (*Escherichia coli*, *Streptococcus*, and *Enterococcus* spp.) in the water has been testing higher in the last stop of filtration than at the start which means one of the filtering pools has stopped working. Our job was to collect water from each filtering pond (9 ponds total) each month and test it to see what types of bacteria are present in the water. We tested these samples for eighteen months. Our tests were positive for both *E. coli* and *Enterococcus* spp. Additional testing is being done to further identify the types of *E. coli*, such as STEC, and types of *Enterococcus* spp., such as *E. faecalis* and *E. faecium*.

Introduction

Bacteria in the water is not a good sign, which is why the United States Environmental Protection Agency (USEPA) considers all presence of enterococci in water as polluted. The enterococci we will be focusing on is *Enterococcus faecalis* and *Enterococcus faecium* because both are found in feces. Gross! The other bacteria we are focusing on is *E. coli*. Some strains of *E. coli* may be harmless, while other strains, such as STEC O157:H7, are one of the causes of foodborne disease. A foodborne disease is caused by consuming a food product that is contaminated with the bad type of bacteria! The bad bacteria, STEC O157:H7, can cause severe sickness if consumed such as, bloody diarrhea and cramps. STEC can be found in the intestinal tract of animals, food, water, and soil. For this study, we will be testing the pond water to see if it contains STEC because the inflow of water passes through dairy farms before entering the wetland. The first step towards the prevention of water and foodborne disease outbreaks is early detection and monitoring the bacteria in water sources prior to human consumption.

Materials and Methods



- Collecting:** Use the water samples from the ponds
- Growing:** Put the pond water and bacteria food in these trays (top left) to help us count and grow more bacteria
- Isolating:** Take water from the trays and put them on these different plates to isolate the different bacteria.
- Amplifying:** Use a PCR machine to grow thousands more bacteria so the bacteria can be identified as *Enterococcus* spp. and *E. coli* spp.
- Identifying:** Put the PCR samples on a jelly tray with buffer liquid and watch the samples flow across the jelly plate by using electric waves!

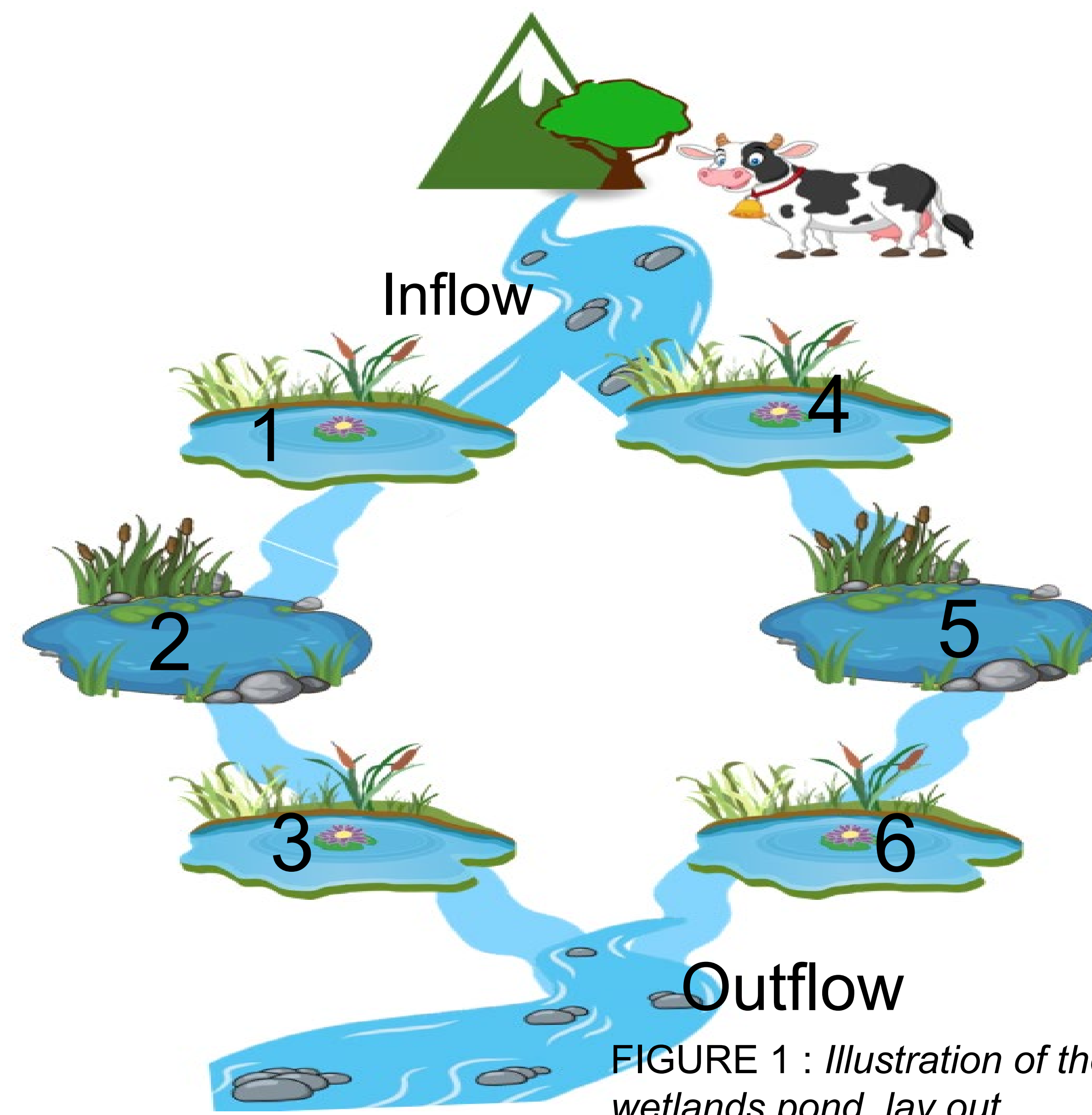


FIGURE 1 : Illustration of the wetlands pond lay out.

Results

1. March 2018 Data

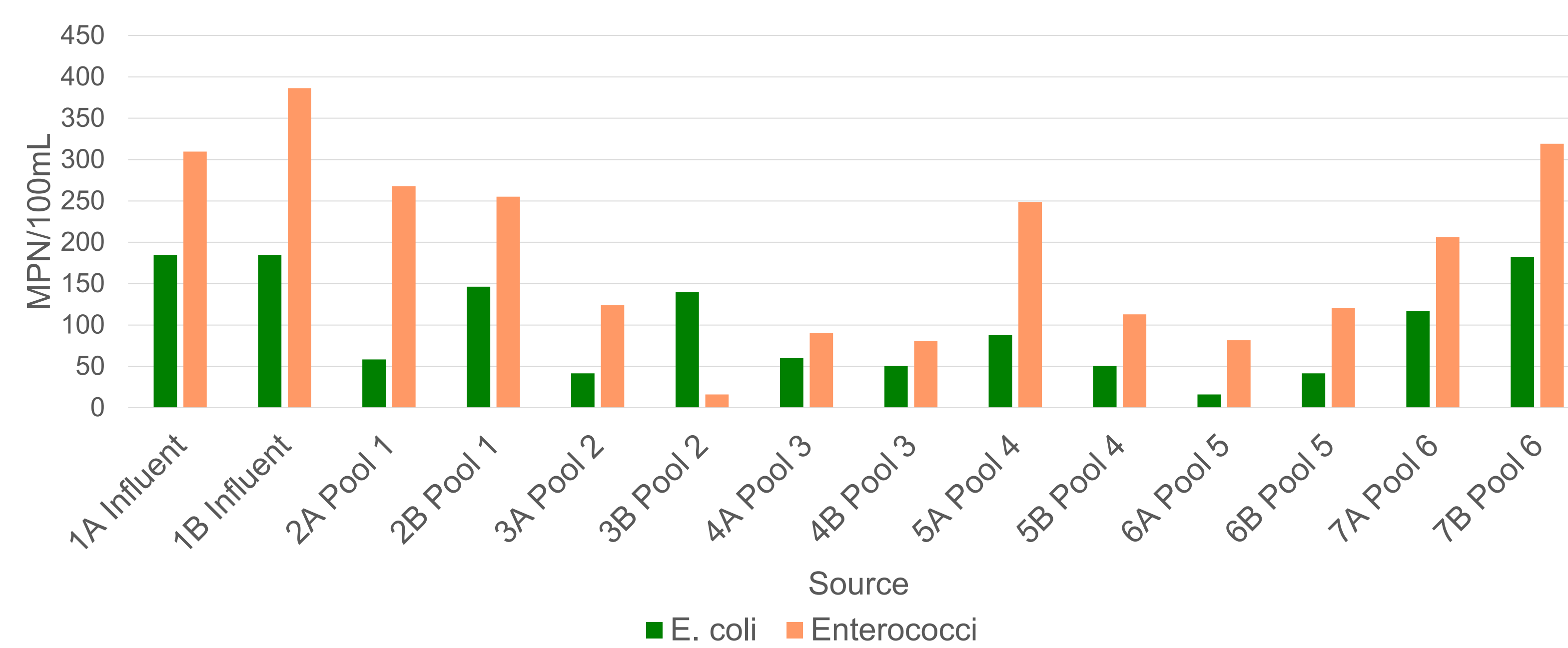


FIGURE 2. Bacteria count from the start of the project

3. September 2018 Data

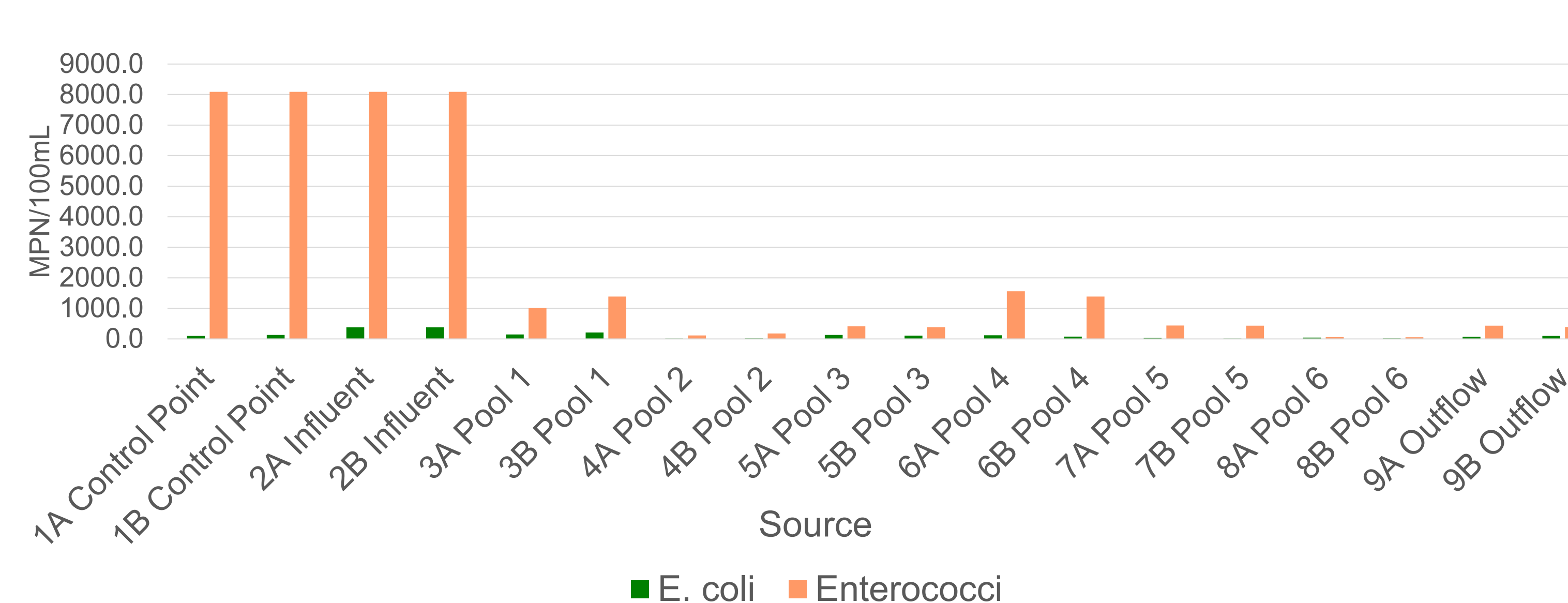


FIGURE 3. Bacteria count near the end of the project

4. Detection of *Enterococcus*, *E. coli*, & STEC

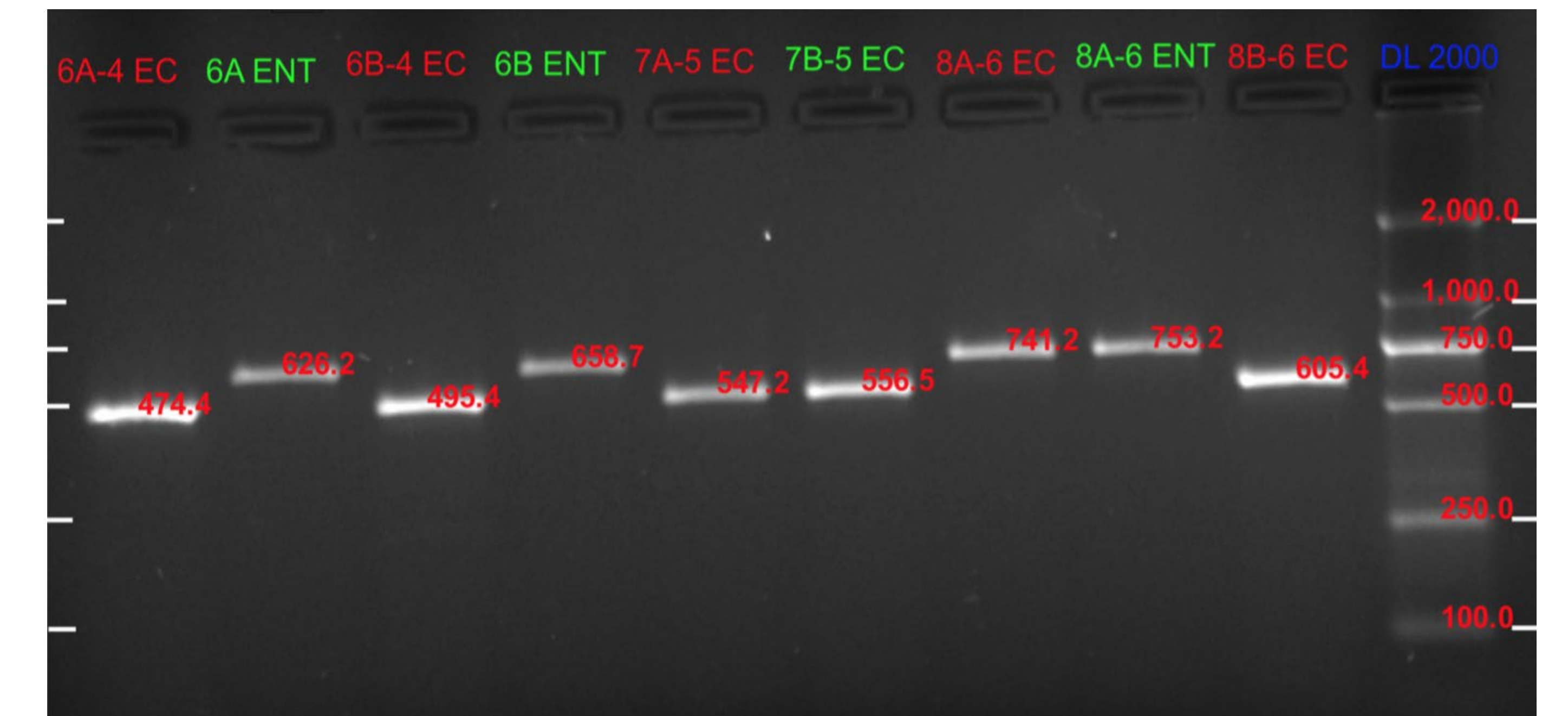


FIGURE 4: Detection of *E. coli* and *Enterococcus* spp. utilizing primers targeting the 16S and *uidA* gene sequence.

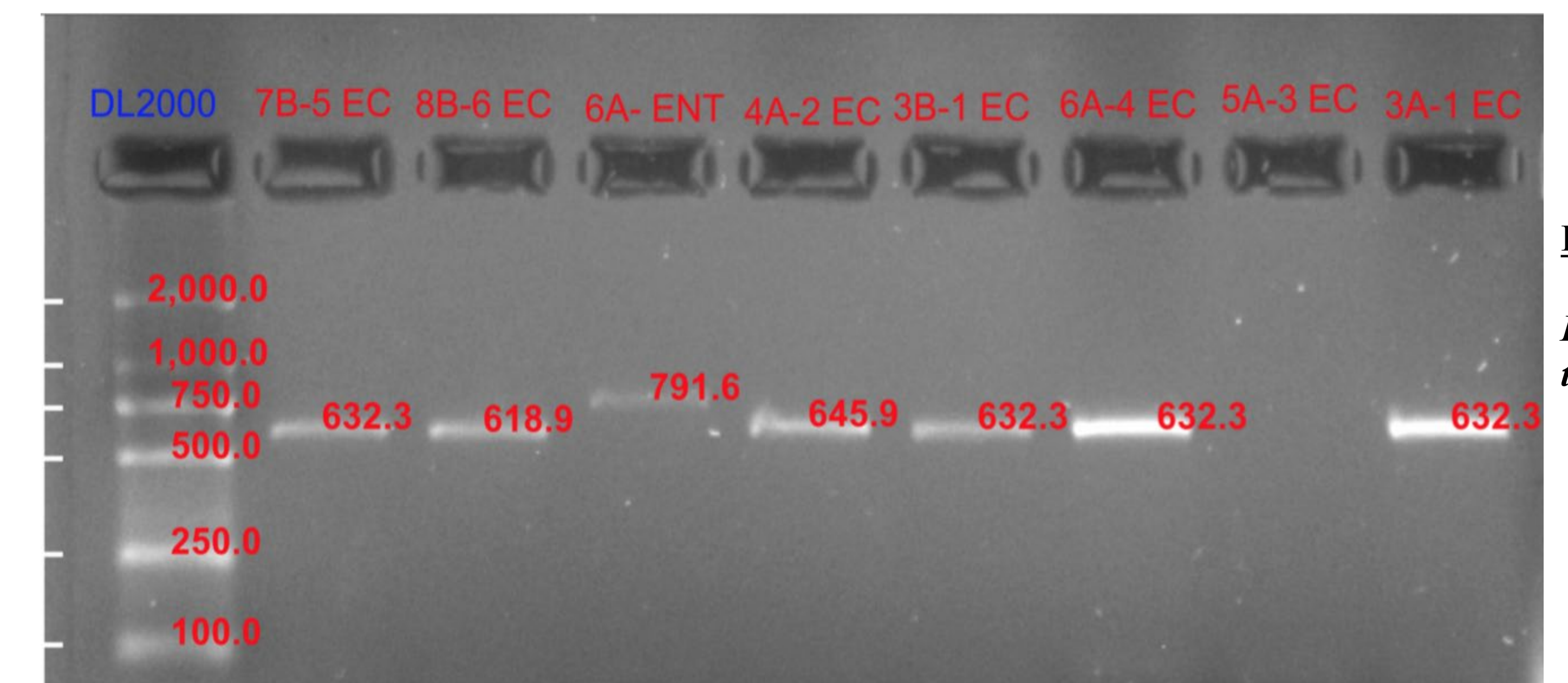


FIGURE 6: Detection of STEC targeting *stx* genes

Conclusion

- Microbial MPN counts continued to decrease with each monthly sample
- Achieved successful identification of *E. coli* and *Enterococcus* spp. utilizing 16SE1, 16SE2 (r), *uid298F*, *uid884R*, and STEC using *stx1-F* and *stx1-R* primers
- Igem and Glantz's colony PCR method yielded less reliable results compared to the original PCR method used for DNA extraction

Future Studies

- Continue monitoring and identifying microbe levels by:
 - Collecting monthly samples from the wetlands.
 - Tracking MPN using the IDEXX system
 - Identifying microbes using PCR
- Refine our current PCR protocol by:
 - Establishing a more effective DNA extraction method
 - Implementing more control samples
 - Run more PCR trials utilizing primers mdF (F1), mdR (F2), sdF (E1), sdR (E2), to test for presence of *E. faecium* and *E. faecalis*

References

Science & Technology, 42(13), 4676-4682.
 Maheux, Bissonnette, Boissinot, Bernier, Huppé, Béroubé, Bergeron. (2011). Method for rapid and sensitive detection of *Enterococcus* sp. and *Enterococcus faecalis/faecium* cells in potable water samples. *Water Research*, 45(6), 2342-2354.
 Igem, P., & Glantz, S. (2013). <http://journal.ru/wp-content/uploads/2017/03/a-2017-023.pdf>. *Colony PCR*. doi:10.18411/a-2017-023