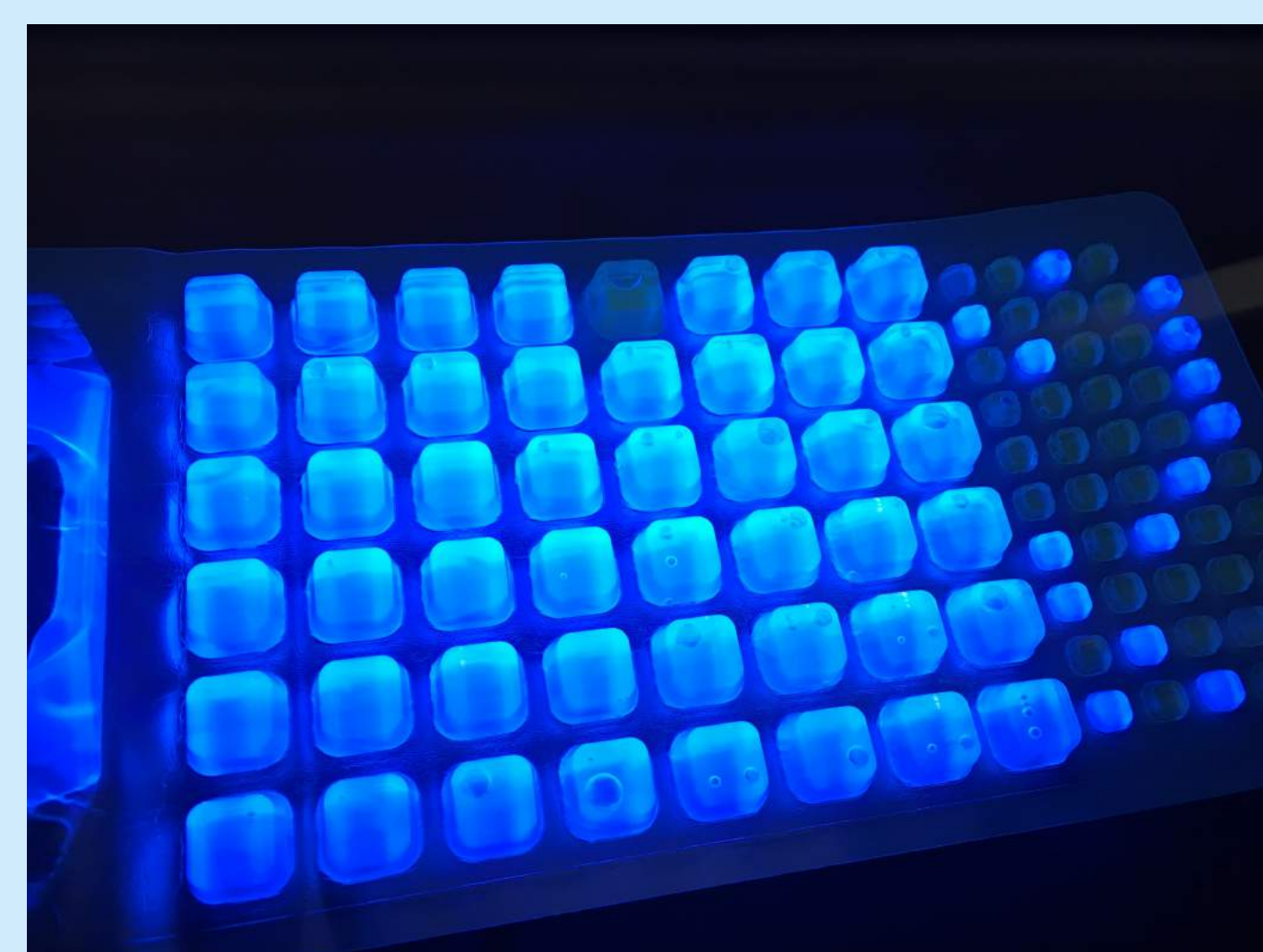


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Abstract

Bacteria and pathogen contamination has been ranked as the leading cause for impaired and threatened waters nationwide by the United States' Environmental Protection Agency (EPA). However, most current available data has relied on the collection and analysis of single grab samples at discrete times for various locations. Effective restoration and watershed managements require a comprehensive understanding of the origin, transport, and dynamics of these bacterial contaminants. In this study, we monitored fecal indicator bacteria (FIB) including total coliform, *E. coli* and *Enterococcus* at 46 sites across White Clay Creek, Red Clay Creek, Brandywine Creek, and Schuylkill sub-watersheds. The data indicated an increasing occurrence of high FIB in the Delaware River watershed. The concentrations of total coliform, *E. coli* and *Enterococcus* were significantly higher than the EPA standards, suggesting a rising public health threat, a potential risk for surface-fed drinking water suppliers, and a challenge for watershed managers. In addition, molecular source tracking methods were used to identify the possible sources of FIB contamination, and our results indicated that the bacterial contaminants were likely related to local land uses—including agriculture, urbanization, mushroom operations, and wildlife.



Materials & Methods

- Sampling: weekly samples were collected from White Clay, Red Clay, Brandywine, and Schuylkill sub-watersheds
- *E. coli*/*Enterococcus* detection: Colilert and Enterolert water testing kit from IDEXX Laboratories, Westbrook, ME
- Environmental DNA collection and extraction: membrane filtration (0.22 µm) and genomic DNA were extracted by using Power Soil DNA Isolation Kit (MoBio Laboratories, Carlsbad, CA)
- Source tracking: environmental DNA were amplified by PCR using group-specific primers (*Bacteroides*), and compared to potential sources

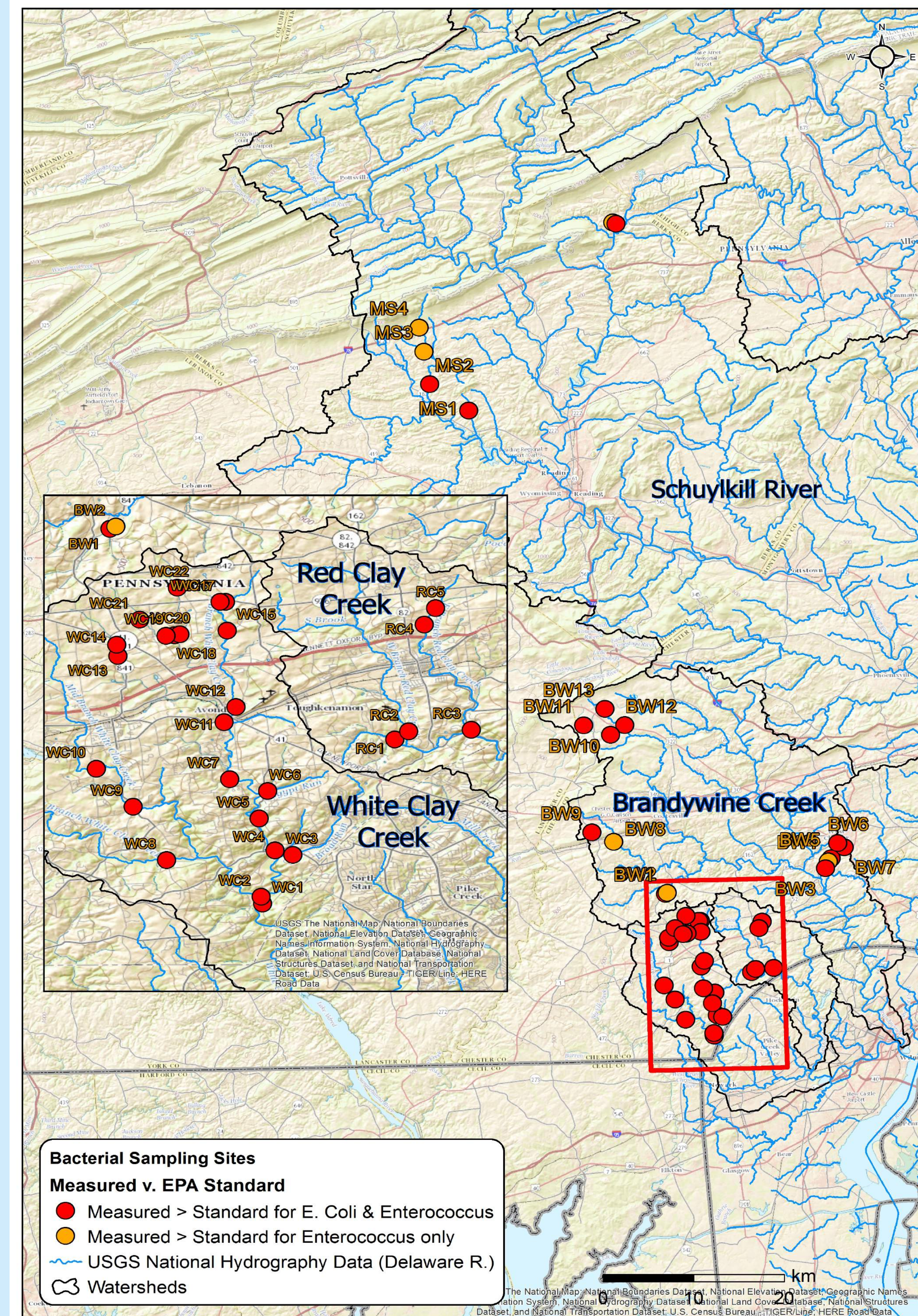


Figure 1. Map of site locations throughout the four sub-watersheds. Red dots indicate sites that measured greater than EPA standard for *E. coli* and *Enterococcus* concentrations. Orange dots indicate sites that measured greater than EPA standard for *Enterococcus* concentrations only.

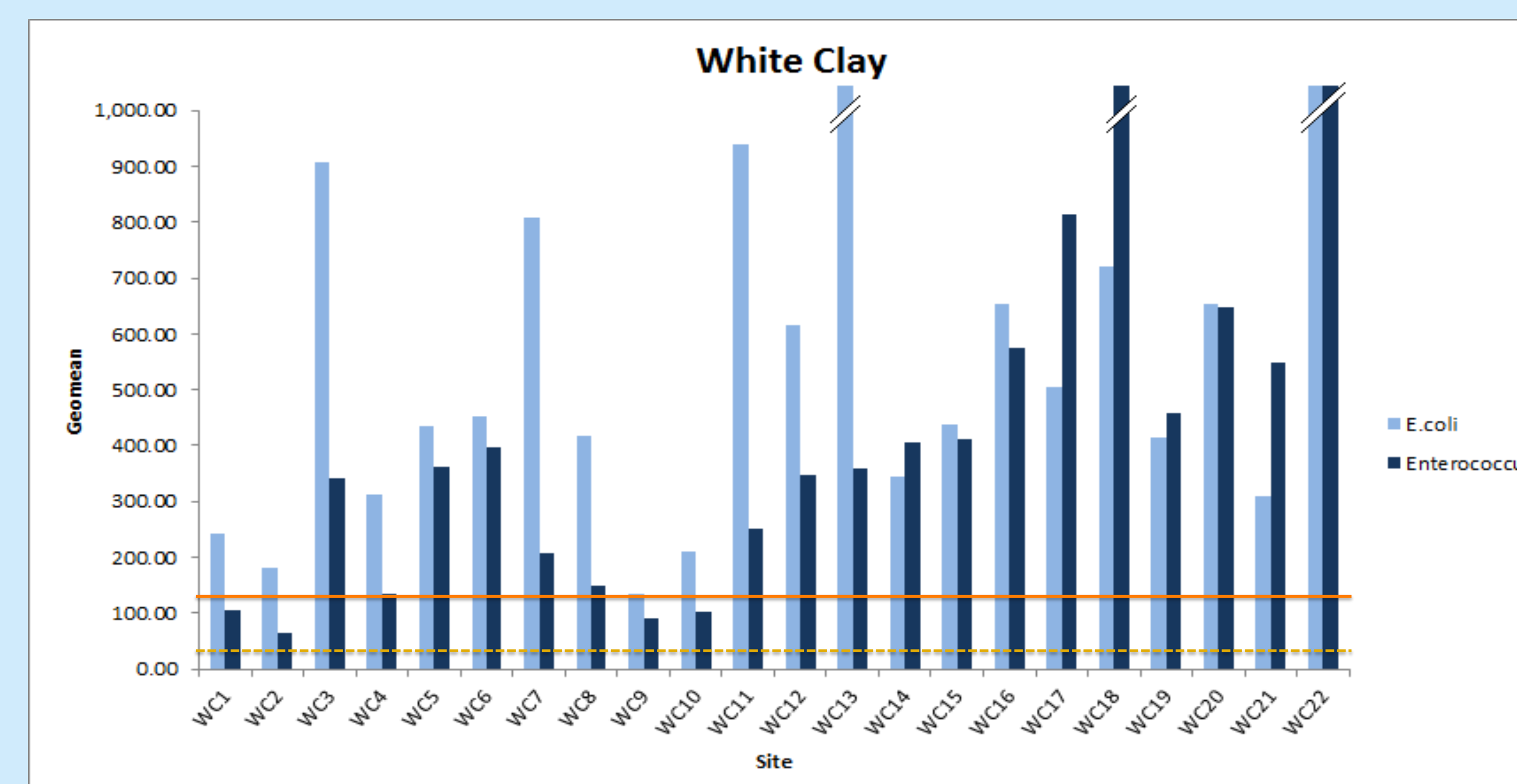
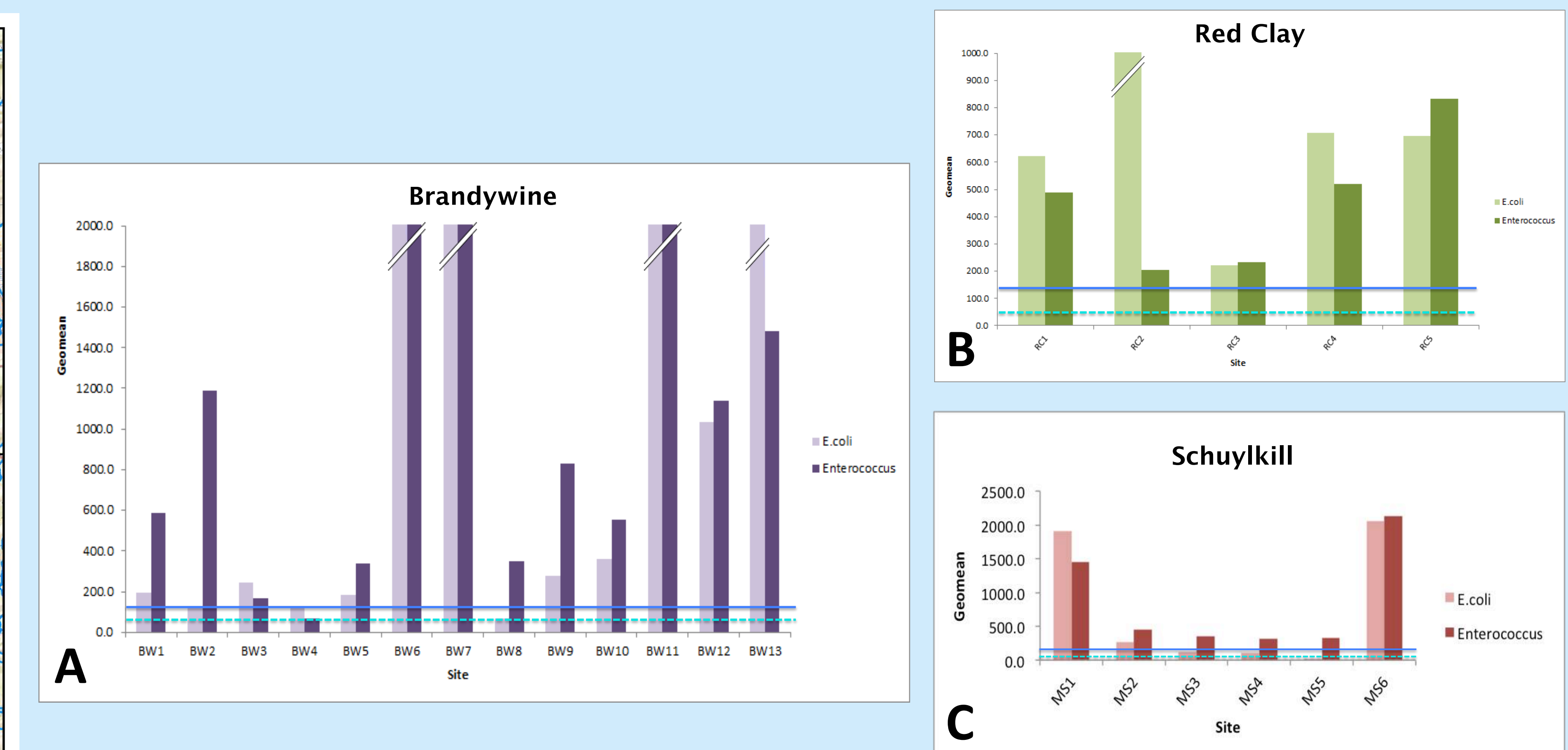


Figure 2. Geometric mean of *E. coli* and *Enterococcus* concentrations at White Clay. Solid line represents EPA standard of 126 cells/100 mL for *E. coli* and dashed line represents EPA standard of 35 cells/100 mL for *Enterococcus*



Figures 3. Geometric mean of *E. coli* and *Enterococcus* concentrations at Brandywine (A), Red Clay (B), and Schuylkill (C). Solid line represents EPA standard of 126 cells/100 mL for *E. coli* and dashed line represents EPA standard of 35 cells/100 mL for *Enterococcus*.

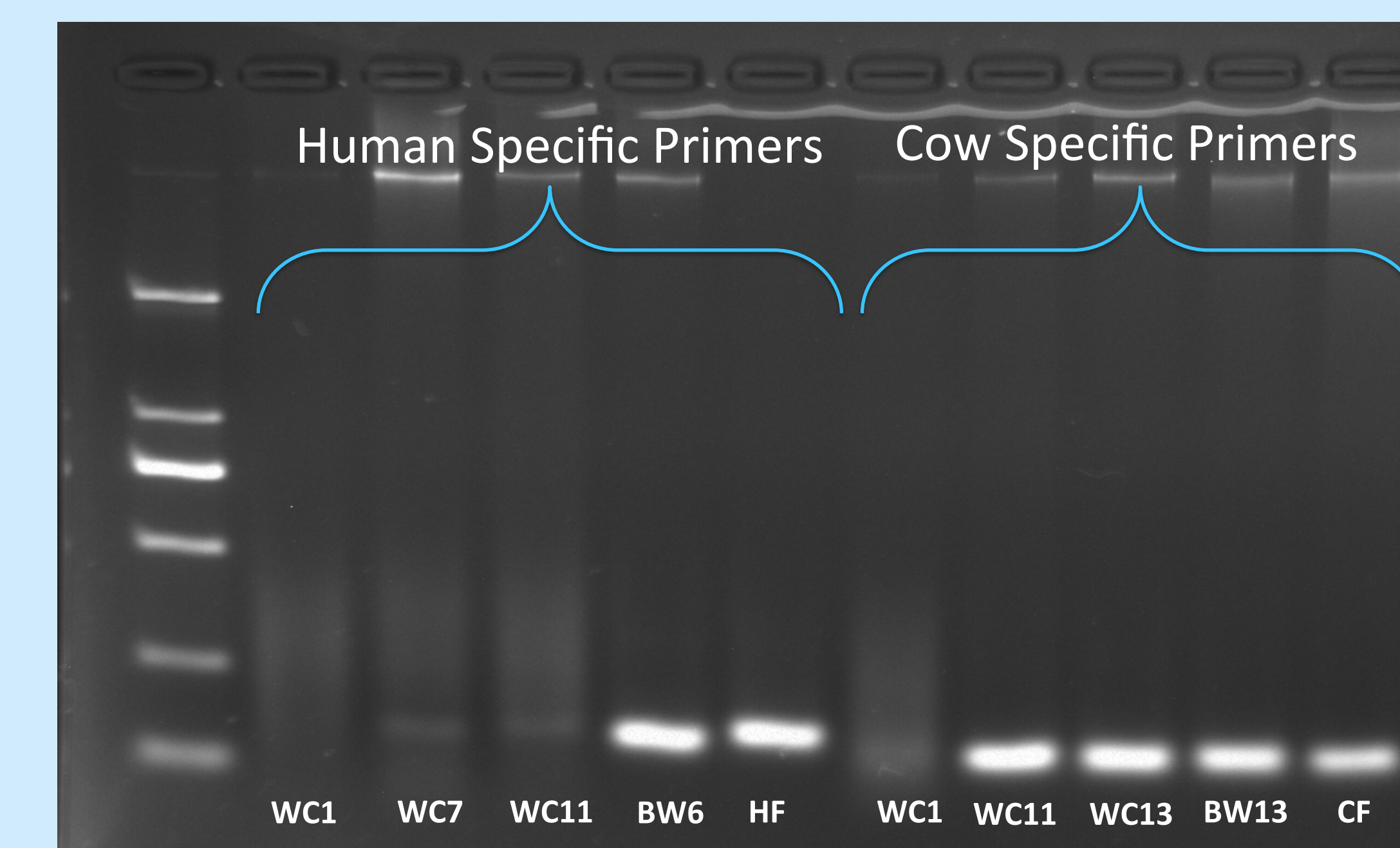


Figure 4. PCR detection of host-specific *Bacteroides*. Illuminated bands indicate the presence of bacteroides in the water at the sample site. HF stands for human fecal sample and CF stands for cow fecal sample. Fecal samples are included for comparisons.

Results/Discussion

- Every sampling site exceeded EPA standard for *Enterococcus* and only a few sites did not exceed EPA standard for *E. coli*.
- Sites with some of the highest concentrations of both *E. coli* and *Enterococcus* were often surrounded by farm fields where animals were present, and in some cases had direct access to the stream.
- For example, sites WC13, BW11, and BW13 were located in cow pastures, site WC18 was downstream of a dairy farm, and site WC22 was located on a horse farm where geese and chickens were also present. However, sites BW6 and BW7 were located in urbanized areas, indicating that farmland is not the only cause of bacterial contamination in water systems.
- PCR results demonstrated the capability of identifying bacterial contamination sources by comparing environmental DNA to known host sources, which was likely relevant to local land uses.

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