**Abstract**

Sources of fecal water pollution were assessed in the Sloan Creek sub-watershed (located in the Red Cedar river watershed), which was newly listed as impaired according to MDEQ 2014 integrated report. The current work aims to determine whether microbial water quality standards are being met, and to identify the probable source of microbial contamination in the sub-watershed by using: a fecal indicator (E. coli), host-specific (human and bovine) Bacteroidales genetic markers, and microbial diversity identification using Illumina sequencing and metagenomics analysis. These methods were used in conjunction with environmental information such as land use, precipitation, and water flow rate in the creek. Water samples were collected from three sites in the sub-watershed twice a week and following rain events during spring and summer of 2015. E. coli was routinely detected in the three sampled sites. 75% of samples (n=192) exceed the recreational water quality guidelines by several orders of magnitude. High concentrations of human and bovine-associated Bacteroides were detected in the three sites indicating influence of multiple sources of contamination. Results suggest that the probable sources of contamination are: leakage from septic system and runoff from a concentrated animal feeding operation nearby Sloan Creek.

**Site description and sampling locations**

**Sample collection:** Three sites were selected and tested during spring and summer 2015 (March to August). Samples were collected twice per week and following every rain event. A total of 192 samples were analyzed for E. coli and Bacteroides markers, and 3 samples were analyzed by Illumina.

**Sampling processing and sequencing**

- **Culture Method**
  - E. coli enumeration using Colilert-18TM IDEXX
- **Molecular Method**
  - Sample concentration (membrane filtration and centrifugation)
  - DNA extraction
  - Bacteroides quantification with qPCR
  - Illumina sequencing (HiSeq)
  - Sequence Reads Quality Control: Trimming Trimmomatic
  - DeNovo Assembly IDBA – UD, Annotation, Taxonomic Classification
  - Genome Alignment with MgRust

**Results**

**Microbial Indicator**

- Fig4: E. coli concentration in 3 sampling sites
- Fig5: Water Flowrate in Sloan creek

**E. coli** trend is consistent with the flowrate in the creek and the precipitation has a significant effect in the E. coli concentration.

**High concentrations of Bacteroides** have been observed in Sloan creek following spring rainfall events. The peak concentrations of Bacteroides were associated with the water discharge peaks at the creek.

**Microbial Source Tracking Markers**

- Fig6: Bovine-associated Bacteroides
- Fig7: Human-associated Bacteroides

**Metagenomic Analysis**

- Fig8: Metagenome summary for three samples with the highest E. coli concentration
- Fig9: Breakdown of affiliated bacterial sequences shows Bacteroides are the dominant species. Among Proteobacteria

**Conclusions**

The preliminary microbial source tracking suggests that septic systems and runoff from agricultural areas were a probable microbial pollution source as indicated by elevated human and bovine-associated Bacteroides in Sloan creek and tributaries water samples.