

# *Alternative Wastewater Demonstration Project*

## **Left Fork Watershed of the Mud River**



## **Lincoln County Commission ~ US EPA Cooperative Project**

### ***Bacterial Source Tracking***

**BACKGROUND.** Bacterial Source Tracking is a relatively new technique which compares DNA e. coli “fingerprints” to evaluate the presence of certain species.

This project has collaborated with Marshall University’s Forensic Science Center’s Bacterial Source Tracking Laboratory to determine the presence of human e. coli in tributaries in the Left Fork of the Mud River Watershed. Though the project has a series of historic watershed tributary samplings dating from the late 1990’s, until the work with Marshall there had been no attempt to separate out human from non-human e. coli.

Clearly there is serious contamination in the watershed. During November 2005 and July 2006, twenty-three sites in the watershed were sampled between seven to ten times. At seventeen of these sites samples were over the acceptable limits for e. coli (200 parts per 100 milliliter) 50% or more of the times they were sampled. Viewed another way, of the 195 different water samples taken, 119 or 61% were over the acceptable e. coli limit.

**PROCEDURE.** Marshall has been willing to donate lab analysis for 50 water samples to the project. Bio-Chem Testing analyzes the project’s water samples for e. coli. They save the spent filters from these tests. Marshall, then, uses the e. coli colonies grown on these filters for their analysis. For each site analyzed, the spent filter which had the greatest dilution is used. Ten different pure isolates (clones of 10 separate bacteria colonies) from each site’s water sample are analyzed. The Left Fork isolate’s “fingerprint” is compared to Marshall’s library of 14,000 isolates to attempt both a 3-way (wildlife, domestic animal, and human) and a 2-way (nonhuman and human) match.

**PROJECT SAMPLING AND RESULTS.** To date, the project has asked Marshall to analyze e. coli from seven different sites throughout the watershed from two different dates (September 2006 and March 2007). With only seven sites and two samplings, results can not be assumed to be definitive, but they do confirm what can be assessed from visual observation and from home dye tests: current septic systems are failing. **Marshall’s BST results show that five of the seven sites (71%) have a human e. coli isolate in one of the two analyses. Three of the seven sites showed a human e. coli isolate in both samplings.**

**APPLICABILITY.** The project would like to do at least one bacterial source tracking sampling during the 2007 summer, but drought conditions have made it impossible to gather appropriate samples. The project will continue tributary sampling as new systems are put in. However, it looks as if systems will be installed broadly throughout the watershed, rather than concentrating on one tributary section. This will make it difficult to determine using BST technique whether without question those new systems have reduced human e. coli in the watershed. The BST results will only give a limited snapshot of a moment in the health of a tributary. Instead BST will be used as part of a broader investigation of tributary general e. coli results, field analysis, and analysis of effluent from new septic systems.

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