Title: Microbial Source Tracking in the Plum Creek Watershed, Nebraska

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Introduction and Problem

Excessive levels of fecal bacteria are the most prevalent causes of beneficial use impairment in Nebraska streams and account for 65 of 67 stream segments on the 2002 Nebraska section 303(d) list. Control techniques (i.e., best-management practices (BMPs) and/or effluent limits) must be considered for both point and nonpoint sources to bring impaired water bodies to water-quality objectives (e.g., achieving total maximum daily loads (TMDLs)). In order to successfully attain these water quality objectives, it is important that the contributing sources of bacteria be identified. Microbial source tracking may prove to be a useful approach to identifying contributing sources of bacteria. The Ohio District Microbiology Lab is involved as one of the few USGS facilities capable of providing analytical support for source tracking.

Goals and Objectives

The goal of this project will be to evaluate, develop, and if appropriate, apply microbial source tracking procedures in the source identification, implementation, and planning phase of the Total Maximum Daily Load (TMDL) process for waters identified as being impaired by fecal contamination, either because of fecal coliform bacteria concentrations or concentrations of other indicator organisms. Initially, the MST procedures will be tested in a small watershed, and then, if successful, they will be applied on a regional or statewide basis. In order to achieve this goal, the objectives of the project are to:

➢ Evaluate the applicability of microbial source tracking for use in fecal-indicator bacteria source identification
➢ Initiate the establishment of a state and/or regional database for possible use during future MST endeavors in Nebraska.

Approach

The study approach centers on upstream/downstream monitoring to examine point sources and to subdivide Plum Creek watershed into several monitored subwatersheds. Bacterial source tracking is proposed to discriminate the relative contributions of human versus nonhuman sources to the fecal contamination. Fixed-station monitoring will document the overall level of fecal contamination from the watershed during the study period. Site selection will be aided by a field reconnaissance of water-quality indicator constituents.

Initially, spatial analyses of digital map data will be used to characterize the drainages of all tributaries to Plum Creek that are 6 mi^2 (15 km^2) or larger in size. Land

use characteristics, human populations, livestock inventories, and densities of point sources will be summarized by tributary watershed to the extent that available data permit. Field reconnaissance samples will be collected near the mouth of each such tributary (or a subset if they are too numerous) and analyzed on-site for selected nutrient species and turbidity. Turbidity has been correlated with fecal contamination in previous studies. At sites where turbidity is relatively high (defined as >85 NTU), a sample also will be collected for fecal bacteria density analysis.

Sampling stations will be selected from among the reconnaissance sites. Laboratory analyses of water samples will include concentrations of total coliform and *Escherichia coli* bacteria densities, and suspended-sediment concentration. Also, *E. coli* isolates will be analyzed using microbial source-tracking (MST) methods from 16 samples from one sampling station indicated by field reconnaissance as representing a suspected principal source of fecal contamination. MST analysis will be performed by the USGS research laboratory in Columbus, Ohio, by repetitive element-PCR (specifically, BOX-PCR), to determine the originating host, whether from human sources, cattle, swine, or other sources. This technique requires considerable effort to develop a reference database of genetic "fingerprints" of known-source isolates, to which unknown isolates are compared. Existing molecular methods for bacterial source tracking are considered experimental and may classify many isolates as originating from unknown sources, i.e., insufficient confidence in pattern match.

Given the experimental status of MST methods, a second analytical approach is warranted as a check for corroboration of evidence. Resource limitations constrain the choice of a library-independent method for this purpose. Coliphage typing is suitable as a second line of evidence. The 16 samples collected for rep-PCR analysis also will be analyzed by coliphage typing to indicate whether the coliphage was human or animal in origin. Ten coliphage isolates per sample will be typed. The USGS research laboratory in Columbus, Ohio will also conduct this analysis.