

## ABSTRACT

The rapid growth and urbanization that is occurring in many locations is increasing the already significant deterioration of surface water quality. To better understand the chemical and biological factors influencing water quality and to predict water quality changes as development occurs, monthly sampling at 13 sites on Little Limestone Creek was conducted from November 2008 through June 2010. Little Limestone Creek (HUC 060101080206) is a tributary of the Nolichucky River located in Washington County in Northeast Tennessee. The data suggest that upstream urban nonpoint source inputs and significant input from a wastewater treatment plant contribute to water quality deterioration, as the chemical and biological parameters typically associated with wastewater (biochemical oxygen demand, phosphate concentration and *E. coli*) all increase just downstream of the wastewater treatment plant. Rainfall during the late summer seems to increase the influence of non-point source runoff upstream of the wastewater treatment plant. Water quality downstream from the wastewater treatment plant appears to be influenced by the wastewater outfall during the late fall, winter and early spring, and is more heavily influenced by nonpoint source inputs during the summer. Multivariate statistical analyses also demonstrate spatial and temporal variability among the water quality variables, suggesting the influence of multiple sources that are contributing to nutrient and bacteria concentrations in Little Limestone Creek.

Keywords: water quality, fecal pollution, multivariate statistics

## INTRODUCTION

The rapid growth and urbanization that is occurring in many locations is increasing the already significant deterioration of surface water quality. Increased listing of surface water bodies on impaired waters (303d) lists for pathogen impairment and the need to address these through the Total Maximum Daily Load (TMDL) process as required under the Clean Water Act of 1972 has resulted in increased research to find methods that effectively and universally identify sources of fecal pollution. To better understand the chemical and biological factors influencing water quality and to predict water quality changes as development occurs, monthly sampling at 13 sites on Little Limestone Creek was conducted from November 2008 through June 2010. Little Limestone Creek was listed as impaired on the State of Tennessee's 303d list in 2008 for failure to meet surface water quality standards and impairment of its beneficial uses due to excessive nitrate, phosphate, ammonia and *E. coli* concentrations, and habitat alteration (1). A TMDL for *E. coli* was approved by the U.S. Environmental Protection Agency (USEPA) for Little Limestone Creek in 2007 (2). TMDL development is currently based on a limited 30-day geometric mean, but this method does not take into consideration seasonal effects, variability in land use patterns, or the influence of runoff events on water quality.

In addition, the interactions between chemical and microbial processes in the water add to the complexity of understanding pathogen loading and transport within the watershed. To account for these sources of variability, alternative methods of water quality monitoring and data analysis may be necessary to remove impaired waters from 303d lists. Examining these relationships using multivariate statistical tools such as canonical correlation and canonical discriminant analysis can help quantify nonpoint sources of pollution and improve our understanding of the influences of chemical and microbial processes on water quality to help identify sources of fecal pollution. Canonical correlation analysis examines the linear combinations of the variables in two or more data sets and then determines the largest correlation between the data sets to provide a measure of the strength of association between data sets and help explain how chemical parameters influence microbial fate and transport and how these interactions influence fecal coliform loading in the watershed (3). Discriminant analysis produces linear combinations of independent variables that are used to evaluate separation between groups (e.g., season, site). A plot of the first two canonical variables will display the degree of discrimination between each group. In addition to a targeted sampling program, the identification of pollution sources using such data analysis tools can prove to be a cost-effective method for water quality monitoring and assessment to aid in effective TMDL development and the implementation of best management practices (BMPs).

## OBJECTIVES

1. Assess the overall chemical and microbial factors that influence the water quality of Little Limestone Creek using a targeted sampling approach.
2. Apply multivariate statistical methodology to the collected data to better understand how chemical and biological factors are influencing quality, and
3. Using Little Limestone Creek as a model, determine the usefulness of this approach to identify common patterns associating these monitored water quality parameters to sources of fecal pollution.

## MATERIALS AND METHODS

**Site Description** – Little Limestone Creek (HUC 060101080206) is a tributary of the Nolichucky River located in Washington County in Northeast Tennessee, and is partially located within the downtown section of Jonesborough, Tennessee. Land use along Little Limestone Creek transitions from pasture land areas at the headwaters through the downtown section of Jonesborough, into agricultural areas at the downstream locations. Little Limestone Creek enters the Nolichucky River with headwaters located at the southeastern town limits next to the intersection of Spring Street and the railroad tracks for Southern Pacific Railroad. We selected 13 sites in the Little Limestone Creek watershed these included 11 sites in Little Limestone Creek and two sites in tributaries to Little Limestone Creek (Figure 1).

**Sample Collection** – Water and sediment samples were collected monthly from Little Limestone Creek from November 2008 to January 2010. Water samples for chemical analyses were collected in triplicate in 2L Nalgene™ bottles, and water and sediment samples for microbial analyses were collected in 100ml sterile Whirl-pak bags (Nasco, Fort Atkinson, WI). Water samples for ColiTrak® Quanti-Tray method were collected in sterile 100ml plastic bottles. All samples were transported to the laboratory on ice and analyzed within 6 hours of arrival. **Microbial Analyses** – Analyses for the determination of total fecal coliform concentrations, standard plate count (SPC) concentrations, and the ColiTrak® Quanti-Tray method were performed according to Standard Methods for Examination of Water and Wastewater (4). Microbial Enzyme Activity (MEAs) assays for acid phosphatase, alkaline phosphatase, dehydrogenase, galactosidase and glucosidase activities were also performed (5, 6). **Chemical Analyses** – Nitrate, phosphate, alkalinity and hardness analyses were performed in triplicate using colorimetric HACH™ methods as described by the manufacturer (7). All HACH™ analyses were performed in triplicate. Biochemical oxygen demand (BOD<sub>5</sub>) analyses were analyzed in triplicate using the YSI Model 5000 dissolved oxygen meter (YSI Inc., Yellow Springs, OH).

**Statistical Analysis** – Statistical analyses were performed using SAS/STAT software (SAS Institute, Cary, NC). Fecal coliform data were analyzed by ANOVA to compare concentrations by site and season. Canonical correlations were performed using the CANCORR procedure and discriminant analysis was performed using the CANDISC procedure. Canonical correlations and canonical discriminant analyses were performed at the season and site levels.

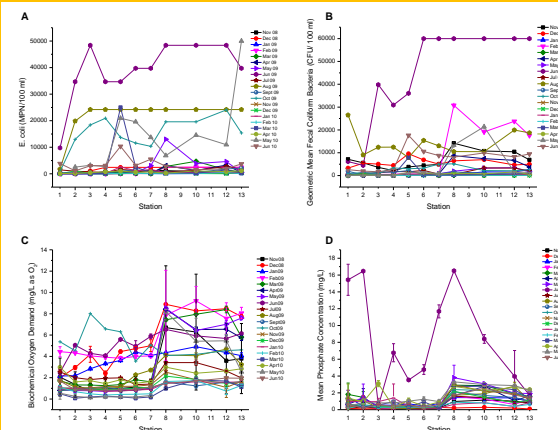


Figure 2. *E. coli* (A), fecal coliform (B), biochemical oxygen demand (C) and phosphate (D) concentrations by site and month (Data not included for sites 9 and 11 which are tributaries).

## RESULTS

- Fecal coliform concentrations display spatial and seasonal trends. Fecal coliform concentrations are significantly higher at downstream sites compared to upstream sites (Figure 3). Significantly higher fecal coliform concentrations are observed during the summer and fall months (Figure 4). Mean values for each site and season are above the regulatory limit of 200 colony forming units (CFUs) per 100ml.
- *E. coli*, fecal coliform and phosphate concentrations, and BOD<sub>5</sub> levels are elevated at site 8 and at all downstream sites (Figure 2).
- Canonical correlation analysis of water quality data for the entire stream indicates that soil erosion is most often associated with fecal pollution during all seasons. The parameters associated with fecal pollution also show spatial variation. When analyzed by site, the data indicate that fecal pollution is influenced by a combination of soil erosion and urban runoff.
- Canonical discriminant analysis by site demonstrates a grouping effect at both levels (Figure 5). A plot of the first two canonical variables displays the degree of discrimination between each group. The canonical variables show strong separation between sites, demonstrating the strong influence of spatial specific conditions on water quality in Little Limestone Creek.

## CONCLUSIONS

- Fecal coliform concentrations vary temporally and spatially and temporally. Upstream sources are contributing large fecal coliform bacteria and *E. coli* loads which are most likely associated with runoff and the high rainfall amounts experienced during the late summer and fall months.
- In addition to nonpoint sources, the introduction of wastewater at site 8 is influencing Little Limestone Creek. The chemical and biological parameters typically associated with wastewater (*E. coli*, fecal coliform bacteria, phosphates and BOD<sub>5</sub>) increase at site 8 and remain high downstream of the WWTP outfall compared to upstream sites.
- Canonical correlation analysis demonstrates that sources of impairment vary spatially. Nonpoint sources are predominant contributors of fecal pollution upstream while the WWTP influences fecal pollution downstream of the outfall point at site 8.
- Canonical discriminant analysis further illustrates the influence of the WWTP on water quality. The first canonical variable is influenced by phosphate and alkalinity concentrations and BOD<sub>5</sub> levels. The second canonical variable is influenced by phosphate, dehydrogenase and glucosidase concentrations. Downstream sites are clustered based on these variables, further indicating the influence of the WWTP outfall on Little Limestone Creek. Site 3 is discriminated by heterotrophic activity and high fecal coliform concentrations in the sediments, while site 9 is a tributary and is discriminated by the influence of urban runoff.
- The application of multivariate statistical methods to water quality data has helped to identify common patterns associating monitored water quality parameters to various pollution sources. Combined with a targeted water quality monitoring program, this data analysis approach is a useful method to identify sources of impairment and to identify BMPs that can prevent and remediate the effects of rapid urbanization.

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Figure 1. Map of Little Limestone Creek with sampling sites marked

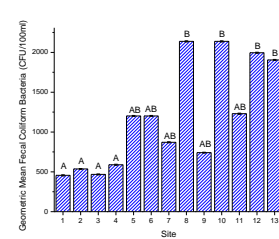


Figure 3. Mean fecal coliform concentrations by site

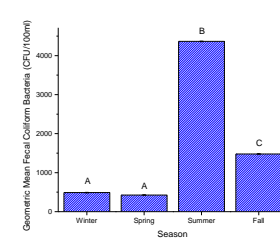


Figure 4. Mean fecal coliform concentrations by season

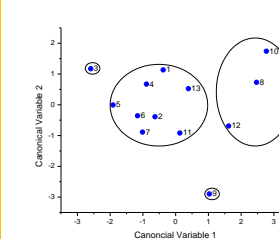


Figure 5. Class means on canonical variables by site

Table 1. Description of Canonical Structure

Canonical Variable	Water Quality Variables Describing Canonical Structure
Canonical Variable 1	PO <sub>4</sub> (0.6726) BOD <sub>5</sub> (0.5689) Alkalinity (-0.4992)
Canonical Variable 2	Dehydrogenase (0.5252) PO <sub>4</sub> (0.4626) Glucosidase (0.3121)