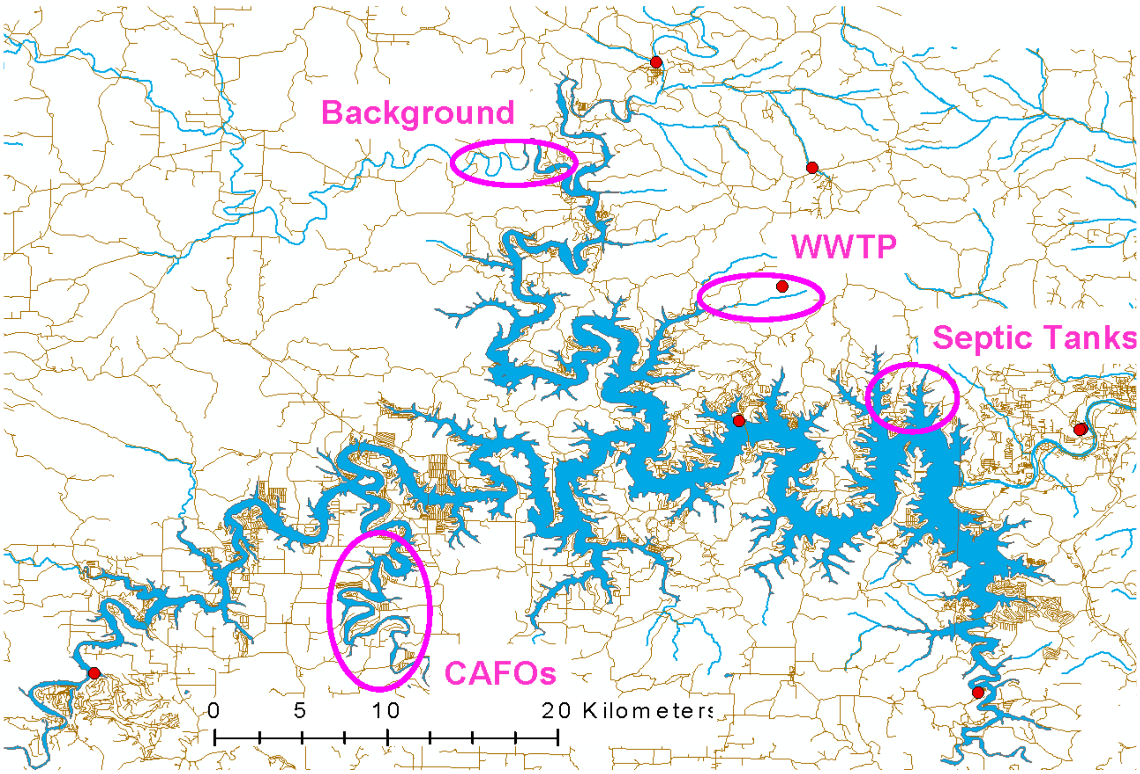
National Decentralized Water Resources
Capacity Development Project

Executive Summary



Evaluation of Chemical and Biological Indicators for Source Apportionment of Phosphorus in Table Rock Lake, on the Missouri-Arkansas Border

Submitted by Washington University
St. Louis, Missouri

February 2006

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EXECUTIVE SUMMARY

Phosphorus Pollution of Surface Waters and Table Rock Lake

Phosphorus contamination of surface waters from point and nonpoint sources remains an environmental problem of great concern. Excess loading of phosphorus to surface waters can cause eutrophication. The identification of sources and determination of their relative contributions to the total pollutant load are essential to achieving water quality goals. Source identification and apportionment are challenging due to the large number of potential sources and the significance of nonpoint sources.

This project evaluated chemical and biological species as potential indicators of specific phosphorus source types. Evaluation of a suite of chemical and biological species was performed through field sampling and laboratory analysis. Samples were collected from potential sources and at locations in the near-field of sources to determine whether the source profiles were apparent in the receiving water.

This project was conducted at the Table Rock Lake watershed on the Missouri-Arkansas border. Table Rock Lake is a reservoir that was created by a dam built by the US Army Corps of Engineers in 1958. The lake has a surface area of approximately 43,100 acres and the watershed upstream of the dam encompasses 4,020 square miles. Phosphorus concentrations in Table Rock Lake have increased over the past two decades, and previous studies reveal that phosphorus is the limiting nutrient in the lake. The lake is impacted by point sources (for example, municipal wastewater treatment plants) and nonpoint sources including decentralized wastewater treatment systems (for example, septic systems) and animal feeding operations.

Project Objectives

The project objective was to evaluate chemical and biological species that could potentially be used as indicators of specific types of phosphorus sources so they could be used in source apportionment methods. Due to the duration and size of the project, the objective was limited to evaluating potential indicators. This project did not encompass actual source apportionment. The hypothesis was that certain chemical and/or biological species would be found that met requirements of useful indicators. It was not known at the start of the project whether such species would be found.

Three specific objectives were pursued:

- Apply a multicriteria geospatial information systems (GIS) analysis to identify suitable sampling locations in Table Rock Lake impacted by single source types
- Evaluate a suite of chemical species (anions, major and trace elements, and synthetic organic compounds) to see if they were useful indicators for phosphorus source apportionment
- Evaluate selected bacteriophage species as biological indicators, as their presence—or absence—could act as an indicator of human versus nonhuman fecal contamination, which could correlate with sources of phosphorus

These three objectives were pursued in an integrated project involving geospatial data analysis, aquatic chemistry, and environmental microbiology. The GIS approach is described in Chapter 2. Chapters 3 and 4 are dedicated to the evaluation of chemical and biological indicators, respectively.

Multicriteria Approach to Selecting Sampling Sites

To ensure the development of representative source profiles, it was important to sample at locations impacted by a single source type. A GIS-based multicriteria decision analysis was applied to identify suitable sampling locations in Table Rock Lake to capture the influence of discharges from wastewater treatment plants (WWTPs) and septic systems, and runoff from animal feeding operations (AFOs). Sampling locations were also determined for background sites.

The GIS used for site selection was developed using data gathered from Missouri and Arkansas spatial data clearinghouses and environmental protection offices. GIS-multicriteria suitability analysis was determined based on characteristics of datasets (for example, soil depth in a soil type dataset) and distances from attributes (for example WWTP location). Data used in this analysis included:

- Topography
- Water bodies
- Land cover
- Soil type
- Roads
- Water discharge permit facility locations

The suitability analysis resulted in four site selection (suitability surface) maps, one for each of the siting criteria—WWTP, septic system, AFO, and background conditions. Creating the multicriteria model provided a systematic approach to site selection and a flexible and dynamic tool for future variations in the site selection process. The most suitable background sampling sites were identified on the James River arm in the northern branch of the lake.

The most suitable locations for isolating wastewater treatment plant effluent were in the central regions of the lake. The Kings River arm in the southwestern section of the lake stood out as most suitable for sampling animal feeding operation runoff. Indian Point in the eastern portion of the lake was identified as the most suitable area for sampling septic system effluent. The suitability maps were provided as guidance to the water quality sampling team who ultimately—based on personal lake experience and logistical considerations—chose the final sampling locations:

- The James River arm (background)
- The Branson West WWTP (centralized wastewater treatment)
- Water surrounding Indian Point (septic system influence)
- The Kings River arm (AFOs)

Evaluation of Chemical Indicators

A suite of chemical species was selected for evaluation for potential indicators. These species included:

- Anions (Br^-, Cl^-, F^-, NO_2^-, NO_3^-, PO_4^{3-}, SO_4^{2-})
- Major elements (Ca, Mg, K, Na)
- Dissolved and total trace elements (As, Ba, Cd, Co, Cr, Cu, Hg, Mo, Ni, Pb, Sb, Sr, U, V, Zn)
- Synthetic organic compounds (SOCs) (acetaminophen, caffeine, sulfamethoxazole, trimethoprim)

The utility of the selected species was evaluated for the following requirements of useful indicators:

- Presence in the receiving waters at detectable concentrations
- Uniqueness of source signatures
- Consistent concentration ratios of potential indicators to phosphorus

The potential indicators were not evaluated for requirements regarding transport and degradation, which are also important and can be evaluated in subsequent work focused on those species that meet the first three requirements.

Source profiles of various phosphorus sources within the Table Rock Lake watershed were examined through quarterly field sampling for one year. Samples were collected from potential sources, at locations in the near-field of sources, to determine whether the source profiles were apparent in the receiving water, at a background site not expected to be impacted by anthropogenic sources, and in public water supplies that are the influents to the wastewater treatment systems.

The concentrations of chemical species were determined using laboratory analysis following standard methods. The interpretation of the large dataset was supported by principal component analysis (PCA), a multivariate statistical method that examines the relationships within a large set of variables. PCA was used in an effort to establish source signatures—a unique combination of several species that are characteristics of a certain source—based on concentrations measured at source-rich receiving water locations.

Almost all of the chemical species met the requirement of detectable concentrations, with the exception of SOCs, as analyzed by high-performance liquid chromatography (HPLC) with ultraviolet detection. To measure trace-level SOCs present in environmental water samples, a mass spectrometric detector is required. Efforts were made to have SOCs analyzed by an outside laboratory with expertise in this area, but these efforts were ultimately unsuccessful because the receiving lab lost the samples.

Bromide (Br^-) is a unique indicator for the large WWTP, which was confirmed by PCA. No other chemical species were observed that could be used as unique indicators of other sources. However, nickel and copper can potentially be used as indicators of the septic system effluents, and sulfate as an indicator of WWTPs for receiving waters with larger proportions of water from these source types. SOCs might be more unique indicators than the other chemical species because human inputs are the main sources of SOCs, while the other species also have natural sources. Because of the large volume of the lake, smaller discharges from septic systems can be rapidly diluted with water from other locations. Consequently, the imprint of the source profiles on the receiving water is difficult to observe. In contrast, the effect of the larger WWTP on downstream river and lake sites can be observed by current analytical methods.

No chemical species were observed to have consistent concentration ratios to phosphorus for all sources and seasons, due to the high variability of phosphorus concentrations for the three septic systems and the smaller WWTP. However, some chemical species had consistent concentration ratios to phosphorus in a single source type (larger WWTP), and some species' concentrations (not ratios) were relatively constant throughout the whole year and may be used to apportion the contribution of water (not phosphorus) from various sources.

Evaluation of Coliphages as Biological Indicators

A suite of three RT-PCR primers specific for F^+ RNA coliphages was designed to discriminate between human and nonhuman fecal pollution. This method was tested with samples collected from Table Rock Lake. Sampling and onsite analyses were performed once per season to assess the effects of seasonal variation in source loadings and lake dynamics. Sampling locations and events for July 2004 through January 2005 were the same as those for chemical species. In addition, a subset of samples was also collected during May and August 2005. The RT-PCR technique was used to identify bacteriophages, and single agar layer (SAL), double agar layer (DAL), and traditional most probable number (MPN) assays were used to quantify the bacteriophages.

The study shows that F^+ RNA phages can be used as biological indicators for fecal pollution; however, the phages cannot be used to distinguish between human and nonhuman sources, nor for phosphorus source apportionment. Environmental samples from locations in the watershed that were most impacted by fecal pollution, as determined by the GIS approach, gave higher levels of F^+ RNA coliphages than the least impacted locations. However, results with genotyping did not show a correlation between the presence of human coliphages at expected human impacted locations and the presence of nonhuman coliphages at nonhuman impacted locations. Genotyping was not successful in determining the source of pollution, primarily because nonhuman bacteriophages were present in sources of human fecal pollution. There was no statistically significant correlation between phage numbers and total phosphorus concentrations.

The original experimental plan was designed to identify sources of human fecal pollution by enumeration and detection of phages specifically infecting *B. fragilis* HSP40. This target phage was chosen because in European studies, primers that amplified human-specific bacteriophages for *B. fragilis* showed them to be present in water that was impacted by human fecal pollution. However, the primers that targeted *B. fragilis* HSP40 phages in Europe were unable to detect *B. fragilis* HSP40 phages in the USA.

Seasonal effects on bacteriophage presence were found in this study. Winter samples contained the highest concentration of coliphages, while fall and spring samples contained the lowest. Samples from the summer showed higher F^+ coliphage concentrations than the spring and fall, but not as high as the winter. The propagated coliphages during the summer of 2005 were not amplified with the primers used in this study and remain an unknown strain(s). In the summer, a more resilient and unknown type of coliphage may have been present. Other factors besides seasonal fluctuations may have contributed to different coliphage numbers and types. For example, the hydraulics of the lake may have also contributed to the different types and concentrations of F^+ RNA coliphages over the seasons.

Summary of Recommendations for Future Work

After completing this 18-month project, insights were gained that can be useful in planning future studies. Several lessons from this project are summarized below, with respect to GIS-based multicriteria analysis, chemical indicator evaluation, and bacteriophage indicator evaluation.

Sampling site selection is ultimately determined by human knowledge and experience. Suitability analysis is a valuable tool in systematically providing information for multicriteria decision making. When combined with "local" or onsite information, it guides the final determination of sampling sites. The development of a GIS-multicriteria tool allows the site selection process to include feedback and adjust to new information as it becomes available in improving site selection. Analyzed samples can provide data on whether samples collected were actually influenced primarily by a single source type or if the location on the lake is impacted by other sources. This information can be assimilated into the suitability analysis to provide revised sampling sites for future sampling campaigns.

In the evaluation of chemical species, the inability of any species to meet all of the requirements of useful indicators was strongly affected by

- Variation of phosphorus concentrations in the sources
- Lack of species that were completely unique to a source type (except for bromide)
- Large size of the main body of the lake, which obscured the imprint of source profiles on the receiving lake waters

The four sampling campaigns of the current project were carried out in four different seasons in one year. The phosphorus concentrations of investigated source sites varied considerably, which made finding suitable indicators with constant indicator ratios impossible. Alternatively, if multiple sampling campaigns (and more sampling campaigns) for the source sites and receiving waters could be performed in a short period, the concentrations of phosphorus and other chemical species are less likely to vary as much in one year. This practice could help identify suitable indicators more easily because the distribution pattern of chemical species among sites would be more stable. However, these indicators may only be useful for short periods of time, and different seasons may have different indicators due to seasonal variation in the composition of sources. In addition, the more frequently the sites are sampled, the more accurate the PCA interpretation is as a statistical tool.

SOCs might be better indicators with respect to uniqueness because human inputs are the main sources of certain SOCs. Collections of septic systems may have discharge volumes that are too small compared to the large lake water body and their effects on the water quality of receiving waters are difficult to observe. Several indicators would have been evaluated more favorably for a smaller lake with similar source magnitudes.

Additional studies are needed to elucidate the diversity of the coliphage population in the Table Rock Lake watershed. These studies could shed light on coliphages that could be used as an indicator of phosphorus pollution. To ascertain which coliphages are present in the watershed, all 18 known F^+ RNA coliphages in the four subgroups must be targeted instead of just the three coliphages used in this study. Thus, an additional 15 primer sets must be developed and tested.

In terms of methodology, development of a direct quantitative polymerase chain reaction (qPCR) assay of filtered environmental samples should have high priority. Such an assay would both identify and quantify the phages present, while eliminating the propagation step. This is advantageous because propagation adds time and effort, introduces the potential for contamination, and may have a lower sensitivity compared to qPCR.

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