Q-333

A COMPARISON STUDY OF MICROBIAL ENZYME ACTIVITIES AND COLIFORMS IN THE SEDIMENTS OF A FECALLY-CONTAMINATED TENNESSEE STREAM RELATIVE TO SEASON AND LAND USE

B. G. Evanshen, K. J. Maier, P. R. Scheuerman

Department of Environmental Health, East Tennessee State University, Johnson City, Tennessee

College of Public and Allied Health, East Tennessee State University

ETSU

ABSTRACT

Enzymes react quickly to environmental stress and can serve as sensitive indicators of environmental change. Microbial enzyme activities (MEA's) can be a useful tool to evaluate the health of an aquatic ecosystem. In this study we compared the trends of MEA's (µg/g) to total and fecal coliform concentrations (CFU/g) in sediments from a stream in Northeast Tennessee that had an approved fecal coliform Total Maximum Daily Load (TMDL). The comparisons were based on season and land use through which the stream flowed. Triplicate grab samples of stream sediments were collected monthly for 29 months at 14 sites located in agricultural, urban, and forest regions. Dehydrogenase, acid phosphatase, alkaline phosphatase, galactosidase and glucosidase activities were determined using specific colorimetric analyses. Total coliforms and fecal coliforms were determined using the membrane filtration method. There was significant positive correlation ($p<0.05$ Pearson) between the total coliform concentrations and all five enzyme activities in the winter (January, February and March). A positive correlation was also seen with alkaline phosphatase in the summer. Fecal coliform concentration was positively correlated with dehydrogenase activity in the winter and spring (April, May and June), and with galactosidase activity in the winter, spring and summer (July, August and September). Fecal coliforms were also positively correlated with acid phosphatase in the summer. Only those sediments located in the urban region showed a positive correlation between total coliforms and dehydrogenase, acid phosphatase, alkaline phosphatase and glucosidase. DHA also showed a positive correlation between total coliforms and the forest region. The only correlation between fecal coliforms and region was with acid phosphatase in the urban region. A strong inverse relationship existed with the ratio of each specific MEA over the fecal coliform concentration versus both the seasons and regions. These correlations show that elevated activities of these five microbial enzymes can serve as another indicator of stream impairment.

INTRODUCTION

Enzymes can serve as indicators of microbial activity and environmental stress and stimulation in waters and sediments. Microbial enzyme activities (MEA's) can measure these activities in natural waters and sediments to help determine the health of an aquatic ecosystem. Microbial activity reflects how microorganisms react to changes in the habitat and the amount of availability of nutrients. The activities are dependent on needs for nutrient cycling and respiration. Since microbial metabolism reacts sensitively and quickly to environmental conditions, MEA's can serve both as indicators of microbial growth and activity (Frankenberger, et al. 1983) and as an indicator of environmental toxicity (Burton, et al. 1987).

In this study we compared these activities from Sinking Creek, a stream in Northeast Tennessee that was classified as polluted with high levels of fecal coliforms. MEA comparisons were based on the seasons and the regions through which the stream flowed. Regions were defined as agricultural, urban, and forest. The land use characteristics of Sinking Creek transitions from forested at the upstream locations through urban areas then into agricultural areas at the downstream locations. Fourteen sites were identified based on land use patterns, population demographics, and bracketing of tributaries (Figure 1). Table 1 classifies each region. Pictures representative of each region are shown in Figure 2. We employed five different MEA analyses for both stream water and sediment. MEA's are reported as µg of the specific enzyme product per gram of sediment.

Dehydrogenase (DHA), a measure of microbial respiration, is directly proportional to microbial population and may indicate the potential for oxygen depletion of the stream. Acid phosphatase and alkaline phosphatase are necessary for the production of inorganic phosphate. Those streams with excess phosphate, a sign of pollution, will generally show lower values for the concentrations of phosphatases. Galactosidase and glucosidase are necessary for the production of their sugars, galactose and glucose. A higher microbial population will cause more enzyme activity.

OBJECTIVES

The objectives of this study were to:

1. Determine if microbial enzyme activity (MEA) in sediment bacteria exhibit an activity pattern associated with the seasons and/or regions of a moderate flowing stream traveling through different regions of a watershed.
2. Compare the trends of MEA's to the concentrations of total and fecal coliforms in the stream sediments to determine if significant correlations exist.
3. Determine the ratio of MEA's over coliform concentrations versus the season or region to determine if a significant positive or negative correlation existed.

MATERIALS AND METHODS

Sediment samples were collected in two sterile Whirl-Pak bags designated for MEA's and for total and fecal coliforms.

- The total and fecal coliform procedures were carried out within 6 hours of collection. One gram of sediment was mixed with 50 ml of a 1% Tween 80 and vortexed for 1 minute. A 1 ml aliquot was filtered through a 47mm Millipore MF (Millipore Bedford, MA) type mixed cellulose ester filter with a 0.45 µm pore size. The filters were placed in sterile petri dishes containing adsorbent pads soaked in either m-Endo or m-FC broth, for total coliforms and fecal coliforms, respectively. All petri dishes were inverted and incubated at 35±0.2°C for total coliforms and 44.5±0.2°C for fecal coliforms for 24±2 hours. A default value of 300 was entered for plates containing colonies above this upper limit of the countable colonies. A default value of 0.5 Colony Forming Units (CFU) was entered for plates with no growth of fecal or total coliforms. This value represents ½ of the detection limit, (1 colony). Results were reported as CFU per 100 ml.
- The assays for MEA's required one gram of sediment added to a test tube containing a specific buffer per analysis. Dehydrogenase (DHA) employed 0.1M phosphate buffer, pH7.6. Acid phosphatase and alkaline phosphatase used a TRIS buffer, pH 4.8 and 8.6, respectively (Salyer, et al. 1979) Both galactosidase and glucosidase utilized a phosphate buffer, pH9.0 (Morrison, et al. 1977). The specific substrate for each enzyme was then added and thoroughly mixed. DHA was determined using the tetrazolium salt (INT). Acid and alkaline phosphatase measurements employed TRIS buffer with phosphatase substrate, pH 7.6. Galactosidase and glucosidase measurements required phosphate buffer with glucopyranoside and galactopyranoside, respectively. All mixtures were measured on a spectrophotometer 18-24 hours later. The wavelength for DHA was 460nm. All other reactions were measured at 418nm. Results were calculated on a standard curve and reported as ug/g of sediment.

Figure 1: Map of Sinking Creek showing sampling locations.

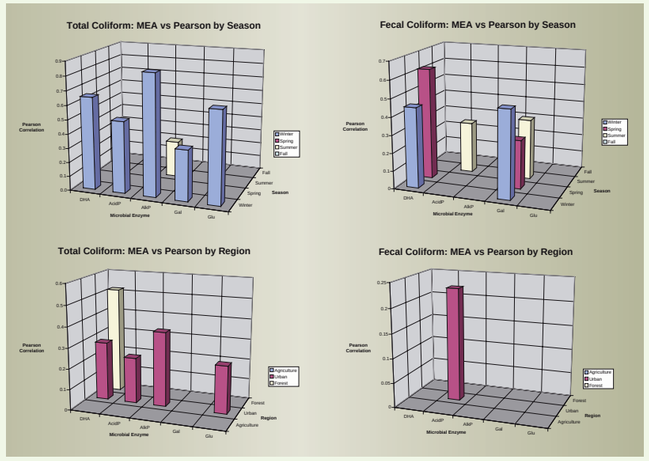


Figure 3: Pearson coefficients by season and by region versus MEA concentrations based on total and fecal coliform concentrations.

Table 1: Region Classification

Classification	Sample Sites	Stream Section	Concentration of livestock/ wildlife	Population Density
Agricultural	1-4	Downstream, below city limits	High	Low
Urban	5-12	Median, within city limits	Low	High
Forest	13-14	Headwaters, above city limits	High	Low

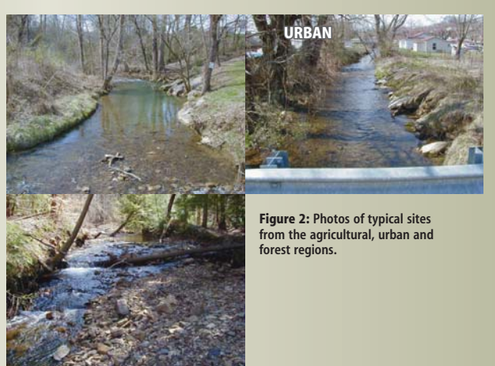
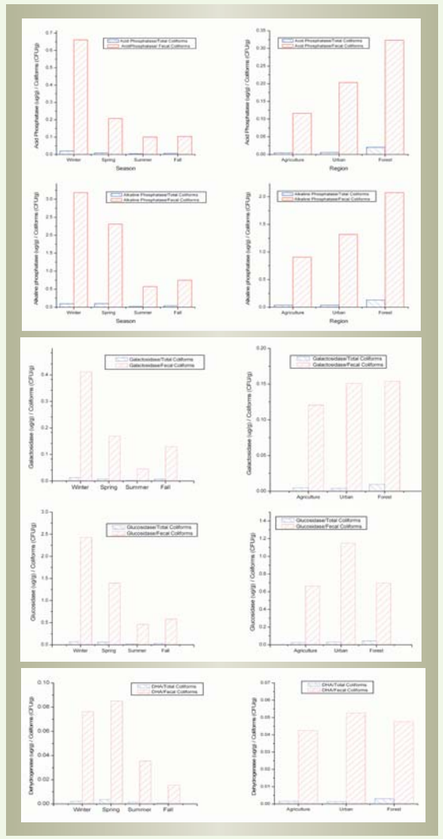


Figure 2: Photos of typical sites from the agricultural, urban and forest regions.

RESULTS

- There was significant positive correlation ($p<.05$ Pearson) between the total coliform concentrations and all five enzyme activities in the winter months (January, February and March) (Figure 3). A positive correlation was also seen with alkaline phosphatase in the summer.
- Fecal coliform concentration was positively correlated with dehydrogenase activity in the winter and spring (April, May and June), and with galactosidase activity in the winter, spring and summer (July, August and September) (Figure 3). There was also a positive correlation with acid phosphatase in the summer.
- There was significant positive correlation ($p<.05$ Pearson) between the total coliform concentrations and all five enzyme activities in the winter months (January, February and March) (Figure 3). A positive correlation was also seen with alkaline phosphatase in the summer.



Figures 4: Ratios of enzyme concentrations (µg/g) over coliform concentrations (CFU/g) versus season and region.

- Fecal coliform concentration was positively correlated with dehydrogenase activity in the winter and spring (April, May and June), and with galactosidase activity in the winter, spring and summer (July, August and September) (Figure 3). There was also a positive correlation with acid phosphatase in the summer.
- Sediments located in the urban region showed a positive correlation between total coliforms and dehydrogenase, acid phosphatase, alkaline phosphatase and glucosidase (Figure 3).
- DHA also showed a positive correlation between total coliforms and the forest region. The only correlation between fecal coliforms and region was with acid phosphatase in the urban region (Figure 3).
- Based on ratio of enzyme activities over total coliform concentrations versus all seasons and regions, we see very low values indicating a strong positive correlation (Figure 4). When one parameter is lower or higher, the other follows the same trend.
- During the seasons, there is a very strong inverse correlation between all enzyme activities and fecal coliforms (Figure 4). The greatest ratio is seen in the winter months when enzyme activity is highest and fecal coliform concentrations are lowest. The ratio steadily decreases through the warmer season when enzyme activity decreases and fecal coliform populations increase.
- An inverse relationship was also seen in the regions (Figure 4). The highest fecal coliform values were noted in the lower agricultural region and the lowest fecal concentrations were in the upper forest region. Because the MEA's remained relatively constant throughout the regions, the enzyme activity/fecal coliform ratio increased from agricultural to forest.

CONCLUSIONS

1. The higher activities for all microbial enzymes during the winter months for total coliforms may be a response to stress factors. This could be due to lower nutrient sources during the colder months resulting in greater activity to produce the needed nutrients.
2. A very strong inverse relationship was noted with the ratios of microbial enzyme activities over fecal coliform concentrations versus temperatures associated with seasons. This might show a possible trend for greater MEA's required in the cooler months, when populations of fecal coliforms are less.
3. A very strong inverse correlation also existed between the ratios of microbial enzyme activities over fecal coliform concentrations versus region locations. This relationship was primarily due to greater numbers of fecal coliforms found in the lower agricultural parts of the watershed.
4. The information determined by the large inverse ratios between enzyme activities and fecal coliform concentrations show that relationships might exist with MEA's and fecal coliform concentrations.

REFERENCES

APHA, AWWA, and WPCF. 1992. *Standard Methods for the Examination of Water and Wastewater, 18th Edition*. A.E. Greenberg, L.S. Clesceri, and A.D. Eaton, Eds. American Public Health Association, Washington, D.C.

Burton,Jr.,G.A. and G.R. Lanza. 1987. *Aquatic microbial activity and macrofaunal profiles of an Oklahoma stream*. Wat.Res. 21:1173-1182.

Frankenburger, Jr.,W.T., and W.A. Dick. 1983. *Relationships between enzyme activities and microbial growth and indices in soil*. Soil Sci. Soc. Am. J. 47:945-951.

Morrison, S.J., King, F.D., Bobbie R.J., Beechtold, R.E., and D.C. White. 1977. *Evidence for microfloral succession on allochthonous plant litter in Apalachicola Bay, Florida, USA*. Marine Bio. 41:229-240.

Salyer, G.S., Puziss, M., and M. Silver. 1979. *Alkaline phosphatase assay for freshwater sediments: Application to perturbed sediment systems*. Amer. Soc. Microbiol. 38:922-927.