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A Comparison Study of Sediment Microbial Enzyme Activities to Biochemical Oxygen Demand, Nitrate Concentration and Phosphate Concentration, in the Sediments of a Fecally-contaminated Stream in Northeast Tennessee Relative to Season and Land Use

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Q-365

ABSTRACT

Microbial metabolism reacts quickly to environmental conditions. These reactions are dependent on the need for nutrients and respiration and can be measured using an assay of individual microbial enzyme activities (MEA's). In this study, we measured MEA's in the sediments of a stream in northeast Tennessee that had an approved fecal coliform Total Maximum Daily Load (TMDL). These values were compared to biochemical oxygen demand (BOD), phosphate concentration and nitrate concentration in the water column of this stream. Comparisons were grouped by season and land use. Stream sediments and water were collected monthly for one year and then quarterly for an additional two years at 14 sites located in agricultural, urban and forest regions. Dehydrogenase (DHA), a measure of microbial respiration, along with acid phosphatase (AcidPA), alkaline phosphatase (AlkPA), galactosidase (GalA) and glucosidase (GluA) activities were measured using colorimetric assays. BOD was determined using the standard 5-day BOD test (BOD_5). Nitrate and phosphate concentrations were measured using colorimetric procedures. There were significant positive and negative correlations ($p<.05$ Pearson) by season. In the cooler months of fall, we found positive correlations between DHA vs. BOD_5, DHA vs. nitrate concentration, and DHA vs. phosphate concentration. Also in the fall months there were significant negative correlations between GalA and GluA vs. BOD_5, and concentrations of nitrate and phosphate. There was also a negative correlation between AcidPA and BOD_5. In the warmer months of spring and summer, there were positive correlations between AcidPA, AlkPA, GalA and GluA vs. the BOD_5's, and the concentrations of nitrate and phosphate. The only negative correlation in a warmer season was in the summer between AlkPA vs. BOD_5 and phosphate concentration. No significant correlations were found by land use type. Results indicate that significant relationships may exist between MEA's and other water quality measures (e.g. BOD_5, nitrate concentration, and phosphate concentration) that could make it possible to use MEA's as another tool for water quality assessment.

INTRODUCTION

Microbial metabolism reacts quickly to environmental conditions. These reactions are dependent on the need for nutrients and respiration and can be measured using an assay associated with microbial enzyme activities (MEA's) (Frankenberger, et al. 1983). In a previous study, positive correlations between MEA's and total and fecal coliforms were noted (Evanshen et al. 2005). Other water quality parameters might also show a relationship. The specific five day measured biochemical oxygen demand (BOD_5) assists in evaluation of water systems by determining the need for oxygen by microbial populations. A higher BOD_5 signifies more oxygen depletion that can often lead to anaerobic conditions (Burton, et al. 1987). The activity of dehydrogenase (DHA) is a measure of microbial respiration and might be directly proportional to the BOD_5. Another measure of water health is seen with the concentrations of the simple nutrients, nitrates and phosphates. Higher values are often affiliated with biologically stressed streams affiliated with higher amounts of organic loadings from fecal matter and fertilizers. Acid phosphatase activity (AcidPA) and alkaline phosphatase activity (AlkPA) are necessary for the conversion of organic phosphate to inorganic ortho-phosphates. Higher organic pollutant loadings will result in higher activities. Galactosidase activity (GalA) and glucosidase activity (GluA) produce energy from conversions of maltose and lactose. Higher values will be seen in nutrient-rich systems, often as a result of organic discharges.
In this study we compared the activities of these enzymes with BOD_5, nitrates and phosphates from 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 2 classifies each region. Pictures representative of each region are shown in Figure 2.

OBJECTIVES

The objectives of this study were to:
•Compare the concentrations of MEA's in stream sediments to BOD_5 and the simple nutrient concentrations of nitrates and phosphates relative to the seasons and/or different watershed regions of a moderate flowing stream in Northeast Tennessee to determine if significant positive and/or negative correlations exist.
•Note the direct comparisons of the relationships between the quotient of enzyme activities over each concentration of BOD_5, nitrates and phosphates, based on season and region. This can help determine if the specific enzyme:parameter ratios show a significant trend for each season and for each region.

MATERIALS AND METHODS

Water samples were collected in pre-rinsed 2 liter PPE sample bottles and processed immediately upon arrival at the lab.
Nitrates and phosphates were analyzed by their specific Hach procedures (Hach,1999). Nitrates were determined by adding a Nitra-Ver 5 Reagent Powder Pillow® to a 10 mL sample in a specific sample cell designed for the analysis. The sample cell was repeatedly inverted for one minute and then allowed to sit undisturbed for 5 minutes. The same was done for the sample blank without the NitraVer-5. This blank cell was then placed in the Hach-designed spectrophotometer and zeroed. It was imperative that the cover for the vial was tightly inserted to prevent any entry of light. The blank was removed and the sample cell was put into the unit and read. The values were reported as mg/L nitrate as nitrogen.
Phosphates were determined using PhosVer Powder Pillows that were added to the specific test cell containing a 10 mL water sample. The mixture was repeatedly inverted then allowed to sit for a minimum of 2 minutes but not more then 10 minutes. As noted above, a blank was prepared and zeroed on the spectrophotometer. The sample was then placed in the spectrophotometer cell and the concentration in mg/L was determined.
The BOD_5 was determined per the specific procedure in Standard Methods for the Examination of Water and Wastewater (2005). Specific 300 mL BOD bottles were either filled completely with sample (100%) or with 225 mL of sample diluted to 300 mL with a specific dilution buffer to give a 75% BOD sample. The dissolved oxygen (D.O.) in each sample was immediately measured (Day0) using a specific D.O. meter. The sample was allowed to incubate at 25°C for 5 days and the D.O. was again read. The difference in dissolved oxygen concentrations between Day 0 and Day 5 was noted as BOD_5.
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.

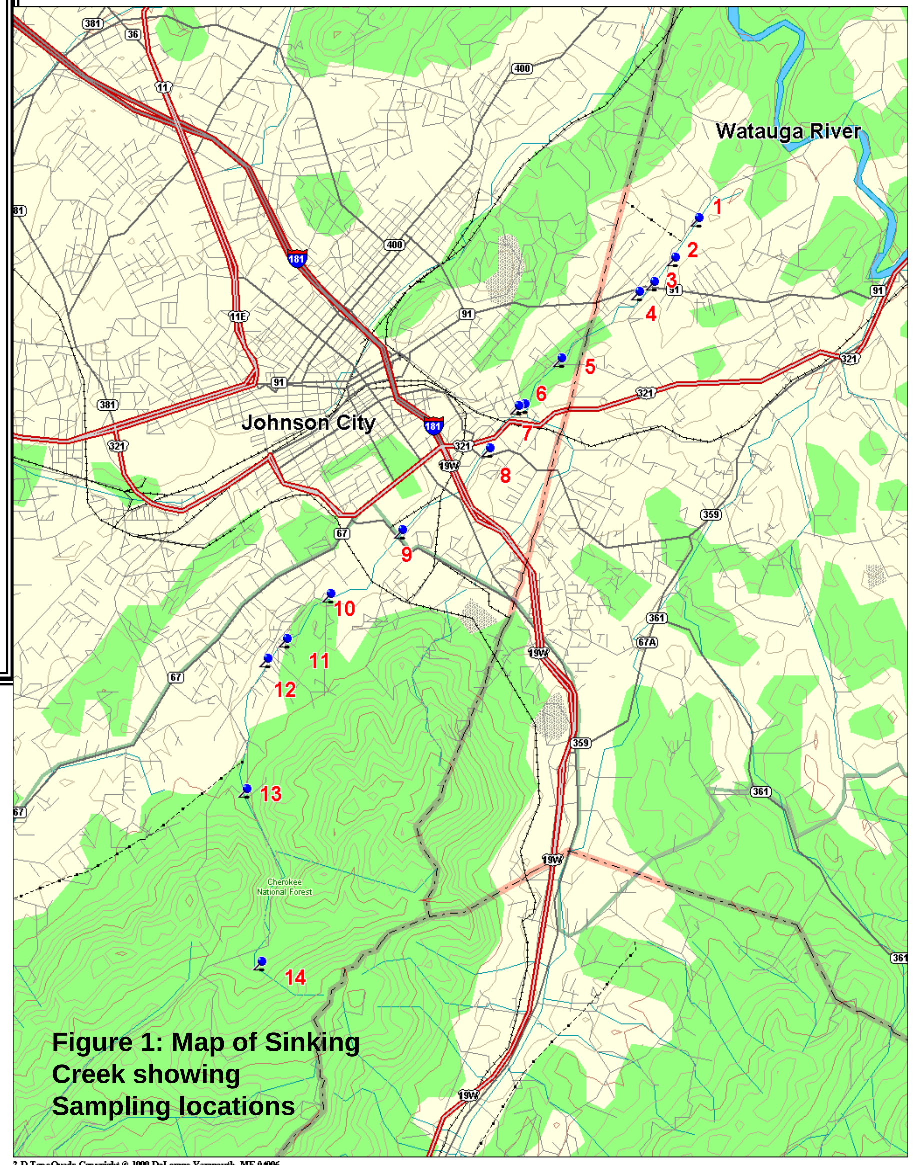

Figure 1: Map of Sinking Creek showing Sampling locations

SEASON

Pearson Correlations p<.05

BOD_5	DHA				0.592
	AcidP			0.321	-0.322
	AlkP	-0.445	0.619	-0.729	0.8
	Gal				-0.696
	Glu				-0.535
Nitrates	DHA				0.498
	AcidP		0.74		
	AlkP				
	Gal		0.565		-0.469
Phosphates	DHA				0.652
	AcidP		0.789		
	AlkP			-0.544	
	Gal			0.49	-0.381
	Glu				-0.311

REGION

Pearson Correlations p<.05

BOD_5	DHA			
	AcidP			
	AlkP			
	Gal		-0.321	
	Glu			
Nitrates	DHA			
	AcidP		-0.398	
	AlkP	0.45		
	Gal			
Phosphates	DHA			-0.627
	AcidP	0.433	0.388	
	AlkP			
	Gal			
	Glu			

Table 1: Pearson Correlation by Season and Region versus MEA concentration based on BOD_5, nitrates and phosphates.

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

Table 2: Region Classification

Figure 2: Typical sampling sites from the Agricultural, Urban and Forest regions.

RESULTS

Correlations of Enzyme Activities versus Parameter

There were both positive and negative correlations ($p<.05$ Pearson) during winter, spring, summer and fall that involved all five enzymes versus the nitrate and phosphate nutrients, and BOD_5 (Figure 1).
•Two significant trends were seen in the Fall: There were positive correlations with DHA versus BOD5, nitrate concentration and phosphate concentration.
Also, significant negative correlations were noted with GalA and GluA versus BOD_5, nitrate concentration, and phosphate concentration.
•In the warmer months of spring and summer, nitrates and phosphates had positive correlations with AcidPA, GalA and GluA.
•In the winter, only BOD_5 had a negative correlation with AlkPA.
•The only negative correlation in the warmer months of summer was between AlkPA versus BOD_5 and the phosphates.
•In regions, GalA showed positive correlations with phosphates in agriculture and urban regions. It showed a negative correlation with BOD_5 in the urban region.

Trends of Quotient of Enzyme Activities to Parameters

The quotient of enzyme activities over the concentrations of nitrates, phosphates and BOD_5 demonstrated noticeable trends based on season and region (Figure 2). All concentration ratios were normalized to percentiles for relative comparisons within each parameter.
•During the seasons, the AcidPA/nitrate ratio exhibited a strong positive trend from the winter through the fall seasons. Data showed the nitrate concentration progressively decreasing through the seasons while the AcidPA increased.
BOD_5 showed the greatest ratio for all enzyme activities during the summer months. This was primarily a result of decreased BOD_5 concentrations in the summer.
•In regard to the regions, a number of enzyme activities with the nitrates, phosphates and BOD_5 were evident.
•AcidPA and AlkPA clearly exhibited a greater ratio ranging from agriculture through urban to forest regions for all three parameters. As AcidPA and AlkPA uniformly increased, all three parameters decreased.
•The urban region exhibited the greatest ratio among all three parameters for GalA, GluA and DHA. Agriculture and forest regions were very similar at a lower ratio.

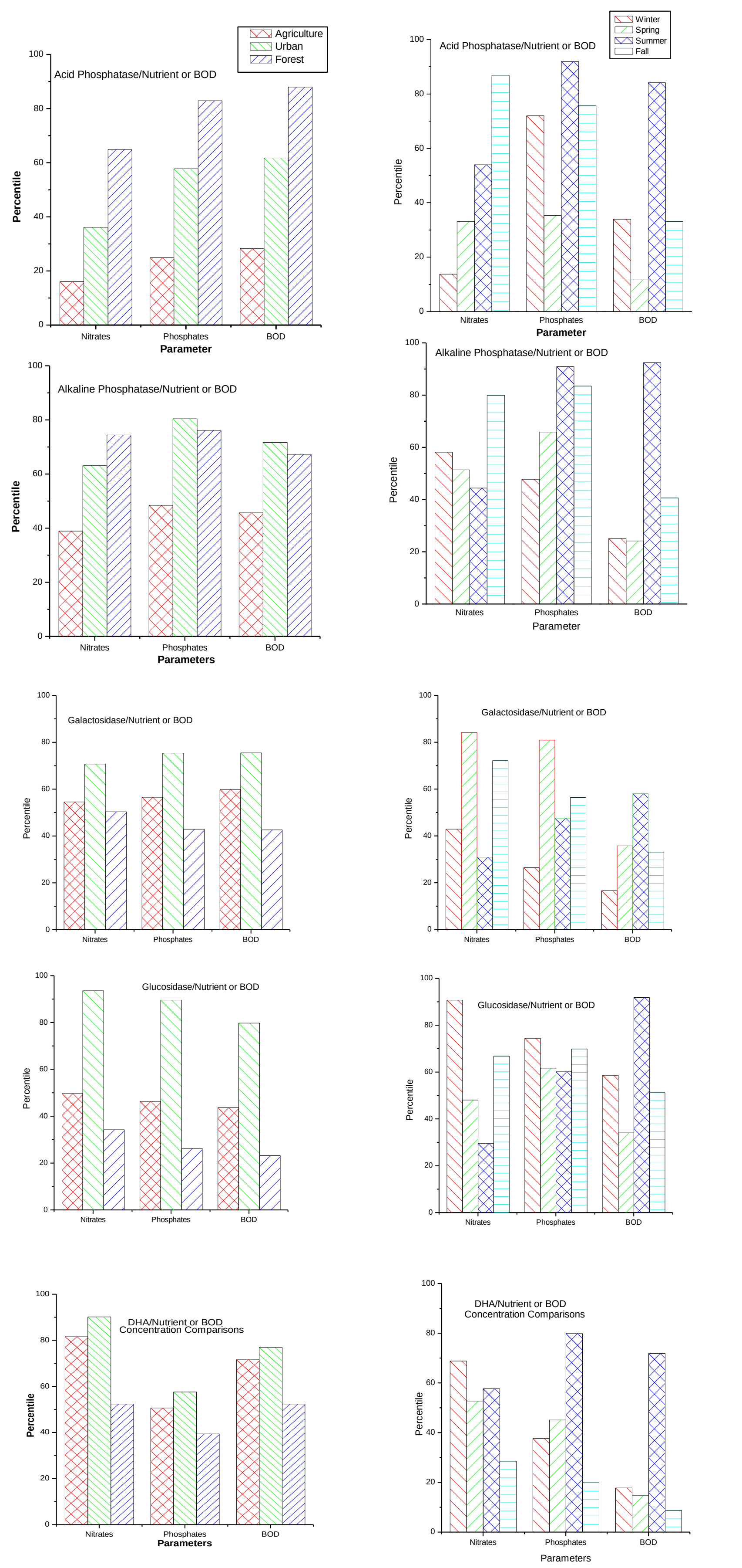

Figure 3: Relative Concentration Comparisons based on quotient of enzyme activities to parameters

CONCLUSIONS

•In the cooler fall season, lower DHA values reflected less microbial respiration. This correlated (Pearson $p<.05$) with the comparatively lower concentrations of nitrates, phosphates, and BOD_5 (Table 1). This trend was also seen in the low ratios from the concentration comparisons (Figure 3).
•Also noted in the fall, the significant negative correlation with the GalA and GluA versus the concentrations of the nitrates, phosphates and BOD_5, do not fall in line with the expected high ratios. Lower parameter values of the nutrients and BOD_5 may be a factor for this negative correlation.
•Review of the quotient of enzyme activities over parameter values helped illustrate the trends of each specific parameter based on season and region. Of immediate note is the uniformly high ratio between each enzyme activity and BOD_5 during the summer months.
•Review of the concentration comparisons for the regions shows a uniform trend for each enzyme activity. Both acid and alkaline phosphatase activities for nitrates, phosphates, and BOD_5, show the lowest ratio for agriculture, and then to urban and forest.
•The information determined by the ratios between enzyme activities versus BOD_5, and the simple nutrients, nitrates and phosphates, show that relationships might exist with MEA's that can further characterize a stream.

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