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Comparison Study of the Averaged Sediment Microbial Enzyme Activities in Four Fecally-Contaminated Streams in the Same Watershed in Northeast Tennessee, to Biochemical Oxygen Demand, Nitrate Concentration and Phosphate Concentration Relative to Season

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ABSTRACT

Microbial enzyme activities (MEA's) are measurements of microbial metabolism. These activities are dependent on the need for nutrients and respiration. This extended study evaluated four streams in the same watershed that had an approved fecal coliform Total Maximum Daily Load. Sediment and water samples were collected monthly for the first year of each specific stream study, and then quarterly to the end of 2006. Dehydrogenase, a measure of microbial respiration, along with acid phosphatase, alkaline phosphatase, galactosidase and glucosidase activities were measured using colorimetric assays. Biochemical oxygen demand (BOD) was determined using the standard 5-day test (BOD_5). Nitrate and phosphate concentrations were measured using colorimetric procedures. Sediment MEA values were compared to the BOD, nitrate concentration and phosphate concentration in the overlying water. Seasonal means of each parameter were not significantly different (p<.05) between the four streams. For this reason the results were combined from the four streams by season to allow a larger sample size. Positive correlations were found between phosphates and nitrates versus the phosphatases in the winter and spring. Phosphates and nitrates were also correlated with galactosidase and glucosidase during this time. During the summer months, positive correlations were noted between BOD and most enzyme activities. Ratios of MEA/phosphates were highest for all MEA's during summer months. This was probably due to the lower phosphate concentrations during the summer. BOD exhibited similar results. Significant relationships exist between MEA's and other water quality measures (e.g., BOD_5, nitrate concentration, and phosphate concentration). This suggests to us that MEA's may be an alternative tool for water quality assessments.

INTRODUCTION

Biological recovery from adverse effects in aquatic systems must be monitored to evaluate the significance of alterations to the environment caused by human activity (Lanza and Dougherty, 1991). Microbial activity reflects how microorganisms react to these changes by measuring their dependence on nutrients and respiration. An important method that can help measure these needs is measurement of microbial enzyme activities (MEA's) (Burton and Lanza, 1987). In a previous study, positive correlations were noted between the presence of higher populations of total and fecal coliforms and MEA's (Evanshen et al. 2005). Large inverse relationships between fecal coliform concentrations and enzyme activities were found. Measurements of the simple nutrients, phosphate and nitrate, as well the specific five day measured biochemical oxygen demand (BOD_5), were also associated with MEA concentrations (Evanshen et al. 2006). Higher nutrient concentrations are often associated with biologically stressed streams affiliated with higher amounts of organic loadings from fecal matter and fertilizers. Acid phosphatase (AcidP) activity and alkaline phosphatase (AlkP) activity are necessary for the conversion of organic phosphate to inorganic ortho-phosphates. Higher organic pollutant loadings will result in higher activities. Galactosidase (Gal) activity and glucosidase (Glu) activity produce energy from conversions of maltose and lactose. Higher values will be seen in nutrient-rich systems, often as a result of organic discharges. 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.
In this study we compared the concentrations of these enzymes with BOD_5, nitrates and phosphates from four streams in the same watershed in Northeast Tennessee. All of these streams were classified as polluted with high levels of fecal coliforms. MEA comparisons were based on the seasons when the samples were collected. Pictures of each stream are shown in Figure 2.

OBJECTIVES

The objectives of this study were to:
•Compare the seasonal concentrations of MEA's in the stream sediments to BOD_5 nitrates and phosphates of four streams in Northeast Tennessee.
•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.

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 (1995). 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.

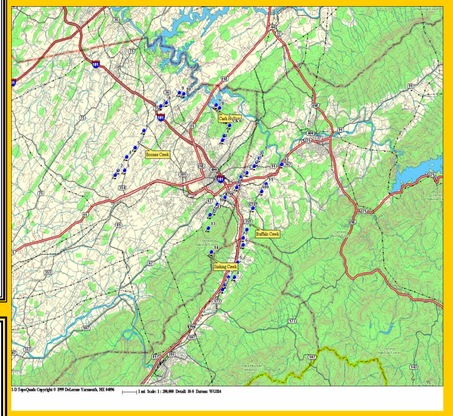

Figure 1: Map of Watauga Watershed in Northeast Tennessee With Location of Sampling Sites on Boones Creek, Buffalo Creek, Cash Hollow, and Sinking Creek

		Pearson Correlations p<.05			
		SEASON			
		Winter	Spring	Summer	Fall
BOD_5	DHA	-0.249	0.3	0.31	
	AcidP			0.283	
	AlkP		-0.265	0.314	0.372
	Gal				-0.422
	Glu	0.189		0.261	
NITRATES	DHA			0.46	0.234
	AcidP	0.397	0.454		
	AlkP	0.549		0.215	
	Gal				-0.304
	Glu	0.355	0.438		-0.255
PHOSPHATES	DHA	0.39		0.394	
	AcidP	0.493	0.296		0.421
	AlkP	0.492			
	Gal	0.328			
	Glu				

Table 1: Pearson Correlation by season versus average MEA concentrations from four streams based on BOD_5, nitrates and phosphates.

Figure 2: Pictures of the four streams studied.

RESULTS

Correlations of Enzyme Activities versus Parameter
•No significant seasonal differences in BOD_5, nitrates and phosphates (p<.05 Pearson) in the four streams studied.
•Both positive and negative correlations (p<.05 Pearson) noted during winter, spring, summer and fall between all five enzymes and BOD_5, nitrate and phosphate concentrations, (Table 1).
•In the colder winter months, found positive correlations between AcidP and AlkP and nitrates and phosphates.
•Also in colder months, Gal and Glu positively correlated with nitrates and phosphates.
•In the spring months, AcidP also positively correlated with nitrates and phosphates.
•In warmer summer, DHA, AcidP, AlkP and Glu positively correlated with BOD.
•DHA also positively correlated with BOD, nitrates and phosphates.
•Negative correlations were noted in the fall between Gal and Glu versus BOD and nitrates.

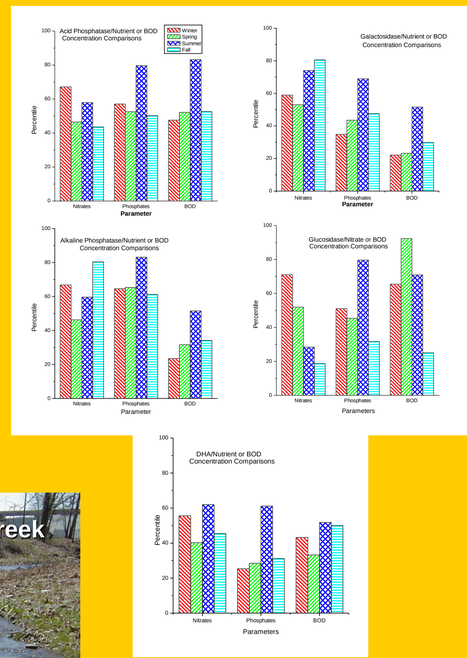

Figure 3: Relative Concentration Comparisons based on quotient of enzyme activities to parameters

Trends of Quotient of Enzyme Activities to Parameters
•Quotient of enzyme activities to nitrates, phosphates and BOD_5 varied seasonally (Figure 3).
•All concentration ratios were normalized to percentiles for relative comparisons within each parameter.
•All enzymes exhibited a greater ratio for phosphates during the summer. Might be due to higher enzyme activity during this season
•AcidP and AlkP also showed a slightly higher ratio during these warmer months.

CONCLUSIONS

•Higher DHA values in the warmer summer months reflected greater microbial respiration. This correlated (Pearson p<.05) with the nitrates, phosphates, and BOD_5 (Table 1).
•Also noted in summer, the BOD_5 was positively correlated with AcidP, AlkP and Glu. Higher concentrations of these enzymes indicate organic pollution.
•In winter months, significant positive correlation noted between AcidP, AlkP, Gal and Glu versus the nitrates and phosphates. Concentration of these enzymes in less biologically active colder environments might be an indicator of lower amounts of these nutrients.
•Review of the quotient of enzyme concentrations over parameter values (Figure 3) helped illustrate trends of each specific parameter. Graphs illustrate greater or lesser activities of the different enzymes based on season.
•Information determined by the ratios between enzyme activities versus BOD_5, nitrates and phosphates, show that relationships might exist with MEA's that can further characterize a stream.

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