NJ Department of Environmental Protection
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WATER MONITORING MANAGEMENT
James Mumman, Administrator

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SEDIMENT TOXICITY TEST USING THE AMPHIPOD *Hyalella azteca* (Burrs Mill Brook) October/November 1996

Assay Number(s): 96H007a, 96H007d, 96H007e

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EXECUTIVE SUMMARY

A toxicity test using the amphipod *Hyalella azteca* was performed on sediments collected from Burrs Mill Brook (AN0153) and a reference site on Burrs Mill Brook (AN0154) in the Delaware Basin. The reference was selected on Burrs Mill Brook (AN0154) due to a "non-impaired" biological assessment by the Ambient Biomonitoring Network (AMNET). The Burrs Mill Brook (AN0153) test site was chosen because it was suspected of toxicity due to a "severely impaired" assessment by the AMNET program. The Burrs Mill Brook (AN0153) test site did not exhibit acute toxicity, in survival results, when statistically compared to the reference station. Burrs Mill Brook (AN0153) test site did exhibit acute toxicity, in growth results, when statistically compared to the reference station. It is suspected that the numerous cranberry bogs directly upstream from the test site may be contributing to the toxicity and biological impairments observed. Further routine AMNET bioassessments will determine if additional testing in necessary.

INTRODUCTION

The Ambient Biomonitoring Network (AMNET) program is designed to establish biologically impaired stream segments throughout the state using EPA's Rapid Bioassessment Protocol (RBP). The RBP assesses impairment through the collection, identification, and classification of macroinvertebrates. Although the RBP is an excellent way in which to assess impairment, it may sometimes be difficult to distinguish if impairment is due to water quality or habitat destruction. Sediment Toxicity Testing is an additional tool to narrow down the cause of impairment to an acute toxicity problem before resorting to costly chemical monitoring.

Hyalella azteca is an epibenthic detritovore reported to also digest bacteria and algae from ingested sediment particles (Hargrave, 1970). This amphipod burrows into the sediment surface and inhabits lakes, ponds, and streams throughout North and South America (de March, 1981; Pennak, 1989). *H. azteca* is a sensitive benchmark species that can be cultured in the laboratory with relative ease.

METHODS

Sample sites were selected based on available AMNET data (see appendix a) and proximity to NJPDES facilities.
The sites selected are as follows (see map):

AMNET STATION#	BIOLOGICAL ASSESSMENT	LOCATION(see map)
AN0153	severely impaired	Burrs Mill Brook @ Hedgerhouse Rd., Woodland Twp.
AN0154	non-impaired	Burrs Mill Brook @ Sooey Place Rd., Tabernacle

Sediment samples were collected from sites AN0154 and AN0153 on October 16, 1996 at 9:40 and 10:00 hours respectively. At each station the sediment was collected in the stream channel using a stainless steel scoop sampler and placed into one liter amber glass bottles and stored at less than 4EC until the start of the test (NJDEP, 1992).

Prior to test initiation the sample sites were assigned assay numbers as follows:

96H007a = control
96H007d = AN0153
96H007e = AN0154

Testing methodology followed the Bureau of Water Monitoring Standard Operating Procedures (NJDEP, SM001.0795, 1995). 24 hours prior to the start of the test, the sediment from each station was mixed to provide a homogeneous sample and hand picked of any visible indigenous organism. For each site, 100 ml of sediment was added to each of the five 300 ml replicate test vessels and topped with laboratory grade freshwater to the 250 ml mark. The test vessels were then held at the test temperature (23EC) for 24 hours to allow the sediment to settle(NJDEP, SM001.0795,1995). After this time period, the overlying water was syphoned, and fresh water was added. A control set of replicates was also set up using 250 ml of overlying water only.

1 - 7 day *H. azteca* juveniles were collected and held for one week prior to the start of the test (NJDEP, 1995).

The test was initiated on October 22, 1996 at 10:45 hours, by adding ten 7 - 14 day old organisms from the holding chamber to each test series replicate. Each day the overlying water was exchanged, and each test replicate was fed 1.5 ml of YCT and 1.5 ml of the green algae *Selenastrum capricornutum* at a concentration of 35 X 10^6 cells/ml. Mortalities were noted if visible. pH, dissolved oxygen, and conductivity were measured from aliquots of each test series; measurements were made at the start of the test and after each 24 hour period (see table 3).

The test was concluded after ten days (November 1, 1996). Live organisms were counted (see table 1) and the dry weights measured (see table 2). Statistical analysis was performed, following EPA guidelines (U.S.E.P.A., 1991). The reference test was compared against the control and the remaining tests compared to the reference, providing the reference and the control were statistically the same.

RESULTS

The test was valid by meeting the acceptability requirements of $ 80% survival (see table 1) in the control test series (NJDEP, SM001.0795,1995). The survival data was not normally distributed when analyzed by the Shapiro-Wilks test for normality, and therefore the Wilcoxan Rank Sum Test was used when comparing test survival results. There was no significant difference between the reference test, 96H007e, survival results and the control survival results. Test 96H007d was then compared to the reference test. 96H006d showed no significant difference from the reference test for mortality.

Growth data (see table 2) was normally distributed when comparing the reference test with 96H007d. Normality was analyzed by the Shapiro-Wilks test for normality, and an F-Test and T-Test was performed when comparing test results. 96H007d exhibited a significant difference from the reference for growth.(see appendix b for statistical printout)

All visible indigenous organisms were removed from the sediment samples.

DISCUSSION

The sample sites on Burrs Mill Brook were chosen based on the results of macroinvertebrate studies and the proximity of NJPDES facilities and urbanization. Site AN0153 had severely impaired bioassessment results as analyzed in AMNET. The reference site, AN0154, was chosen because it had a nonimpaired bioassessment, based on results from the AMNET program, and was within the same major drainage basin as the test site. Similar stream morphology and similar ecological region designation to the sample site suspected of toxicity also factored into choosing the reference site. Survival results showed no significant differences between the control treatment and reference test. Survival results showed no significant differences between the reference test, or between the reference test and 96H007d, Burrs Mill Brook (AN0153). Growth results for the test site however did show toxicity, as a significant difference, when compared to the reference site. Since toxicity was not exhibited in the survival results, the test site may be in the process of undergoing biological recovery. More probable, the severe impairment, demonstrated by the AMNET program, may be due to a chronic toxicity problem which is more likely to be shown in growth data. Burrs Mill Brook (AN0153) is downstream of numerous cranberry bogs which may be contributing to the impairments shown in both the AMNET program and this sediment toxicity test. The reference site does not show toxicity or biological impairment and is actually downstream from the test site, however, it is not directly downstream from cranberry bogs. Future macroinvertebrate bioassessments will show if biological impairment still exists in the stream and additional testing will be considered at that time. A more comprehensive study on the effects of the cranberry bogs located on Burrs Mill Brook should be conducted

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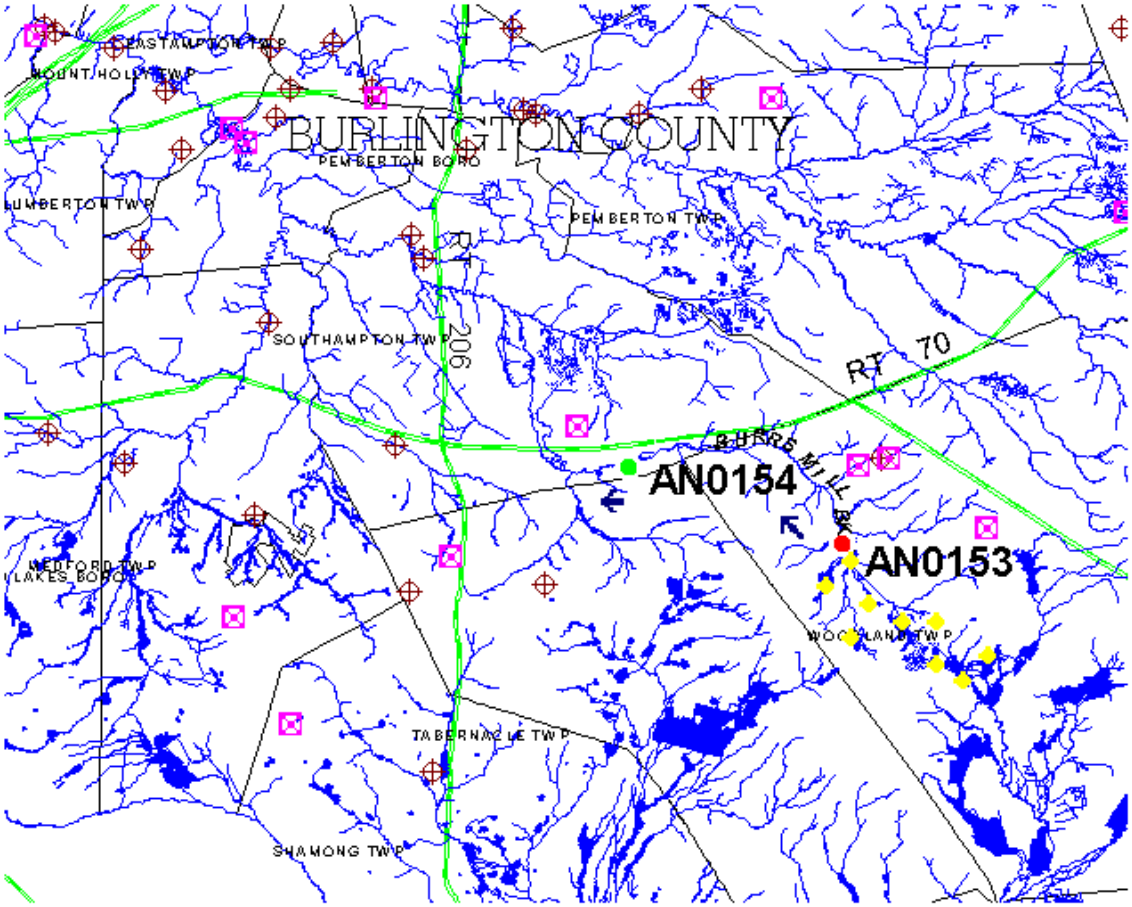
BURLINGTON COUNTY
PEMBERTON BORO
PEMBERTON TWP
LUMBERTON TWP
SOUTHAMPTON TWP
206
RT 70
BURRS MILL RD
AN0154
AN0153
MEDFORD TWP
LAKES BORO
WOODLAND TWP
TABERNACLE TWP
SHAMONG TWP

TABLE 1

MORTALITY DATA
(number surviving)

ASSAY #	REP. A	REP. B	REP. C	REP. D	REP. E	%survival
Control	10	10	10	10	10	100
96H007d	9	10	9	9	10	94
96H007e	10	10	10	9	10	98

Statistical Analysis

Test Endpoint: Survival
Test Used: Wilcoxan Rank Sum Test
Results: 96H007e - no significant difference from control
96H007d - no significant difference from reference station

Test Endpoint: Growth
Test Used: F-Test, T-Test
Results: 96H007e- no significant difference from control
96H007d - significant difference from reference station

*see appendix b for statistical printout

TABLE 2

WEIGHT DETERMINATION

Drying Oven Temperature: 105EC Time/Date Start Drying: 1420 /11-1-96
Time/Date End Drying: 1620 /11-1-96

Analyst: T. Miller

REPLICATE.	WGT. OF BOAT (mg)	DRY WGT: BOAT + LARVAE (mg)	TOTAL WGT. OF LARVAE (mg)	NUMBER OF LARVAE	LARVAE AVG. DRY WGT. (mg)	GROUP AVG. (mg)
CONTROL A	20.61	22.00	1.39	10	0.139	
B	15.49	16.43	0.94	10	0.094	
C	11.71	12.93	1.22	10	0.122	0.119
D	15.59	16.66	1.07	10	0.107	
E	12.10	13.44	1.34	10	1.134	
95H007d A	16.20	18.16	1.96	9	0.218	
B	14.50	17.11	2.61	10	0.261	
C	14.71	16.82	2.11	9	0.234	0.233
D	14.51	16.66	2.15	9	0.239	
E	10.84	12.96	2.12	10	0.212	
95H007e A	16.72	19.47	2.75	10	0.275	
B	13.49	16.19	2.70	10	0.270	
C	15.61	18.14	2.53	10	0.253	0.261
D	14.76	16.90	2.14	9	0.238	
E	12.69	15.38	2.69	10	0.269	

Table 3

Test Chamber Chemical/Physical Parameters

Control	HIGH	LOW	AVG.	STD. DEV.	% CV
pH	7.2	6.8	7.0	0.170	2.426
cond. Fmhos	141	130	136	3.557	2.609
D.O. mg/L	8.1	6.4	7.2	0.427	5.964

96H007d	HIGH	LOW	AVG.	STD. DEV.	% CV
pH	6.6	6.2	6.4	0.166	2.605
cond. Fmhos	137	98	125	13.141	10.467
D.O. mg/L	6.6	4.4	5.5	0.606	11.949

96H007e	HIGH	LOW	AVG.	STD. DEV.	% CV
pH	6.8	6.4	6.5	0.130	2.003
cond. Fmhos	141	100	128	13.556	10.576
D.O. mg/L	6.6	4.6	5.4	0.606	11.191

APPENDIX A
AMNET DATA

Delaware Basin - Chatsworth USGS Quadrangle
Station AN0153
Burrs Mill Brook, Hedgerhouse Road, Woodland Township
March 2, 1993

Family	Number of Individuals	Family Tolerance Value (FTV)
Tubificidae	32	10
Dytiscidae	3	4
Asellidae	1	8
Sialidae	1	4
Chironomidae	10	6
Nematoda	2	6
Leptoceridae	1	4
Simuliidae	1	6

Statistical Analysis

Number of Taxa = 8
Total Number of Individuals = 51
% Contribution of Dominant Family = 62.75
Family Biotic Index = 8.35
Scraper/Filterer Collector Ratio = 0.00
Shredder/Total Ratio = 0.02
E+P+T* = 1 *(Ephemeroptera, Plecoptera and Trichoptera)
%EPT = 1.96
EPT/C* = 0.10 *(Chironomidae)
NJIS Rating = 3
Biological Condition = severely impaired
Deficiency(s) noted: paucity of clean water organisms
Tubificidae overwhelmingly dominant
significant organic pollution

Observations

Streamwater: clear/cedar...Flow: slow...Width/Depth(ft): 20/1.5...
Substrate: sand/gravel...Streambank Vegetation/Stability: poor/
poor...Canopy: open...Other: wooded; twigs and leaves on substrate

Delaware Basin - Pemberton USGS Quadrangle
Station AN0154
Burrs Mill Brook, Sooey Place Road (off Route 70)
March 2, 1993

Family	Number of Individuals	Family Tolerance Value (FTV)
Simuliidae	37	6
Hydropsychidae	33	4
Leptophlebiidae	7	2
Philopotamidae	3	3
Asellidae	6	8
Heptageniidae	1	4
Chironomidae	3	6
Ceratopogonidae	1	6
Limnephilidae	3	4
Lumbriculidae	1	8
Nematoda	2	6
Molannidae	1	6
Leptoceridae	1	4
Aeshnidae	1	3

Statistical Analysis

Number of Taxa = 14
Total Number of Individuals = 100
% Contribution of Dominant Family = 37.00
Family Biotic Index = 4.98
Scraper/Filterer Collector Ratio = 0.00
Shredder/Total Ratio = 0.11
E+P+T* = 7 *(Ephemeroptera, Plecoptera and Trichoptera)
%EPT = 49.00
EPT/C* = 16.33 *(Chironomidae)
NJIS Rating = 30
Biological Condition = non-impaired
Deficiency(s) noted: none

Observations

Streamwater: clear/cedar...Flow: slow...Width/Depth(ft): 20/1... Substrate: sand/gravel...Streambank Vegetation/Stability: poor/ poor...Canopy: open...Other: wooded

APPENDIX B

STATISTICAL DATA

Survival Proportions with Arc-Sine Square Root Transformation

Blank	AN0154	Blank Trans	AN0154 Trans
1	1	1.4127	1.4127
1	1	1.4127	1.4127
1	1	1.4127	1.4127
1	0.9	1.4127	1.249
1	1	1.4127	1.4127

Shapiro-Wilks Test for Normality

Blank Trans	AN0154 Trans	Pooled	Mean	Centered	Ordered	D-value	W-value	Critical-W (0.05)	Result
1.4127	1.4127	1.4127		0.0164	-0.1473				
1.4127	1.4127	1.4127		0.0164	0.0164				
1.4127	1.4127	1.4127	1.3963	0.0164	0.0164	0.0241	0.3662	0.842	Not Normal
1.4127	1.249	1.4127		0.0164	0.0164				
1.4127	1.4127	1.4127		0.0164	0.0164				
		1.4127		0.0164	0.0164				
Mean	Mean	1.4127		0.0164	0.0164				
1.4127	1.38	1.4127		0.0164	0.0164				
		1.249		-0.1473	0.0164				
		1.4127		0.0164	0.0164				

Wilcoxan Rank Sum Test

Pooled	Sorted	Point	Wilcoxan Rank	Blank	AN0154	Critical (from Table K=1)	Result
1.4127	1.249	9	1	0	1	19	No Significant Difference
1.4127	1.4127	10	6	0	6		
1.4127	1.4127	8	6	0	6		
1.4127	1.4127	7	6	0	6		
1.4127	1.4127	6	6	0	6		
1.4127	1.4127	5	6	6	0		
1.4127	1.4127	4	6	6	0		
1.4127	1.4127	3	6	6	0		
1.249	1.4127	2	6	6	0		
1.4127	1.4127	1	6	6	0		
				Sum	Sum		
				30	25		

Survival Proportions with Arc-Sine Square Root Transformation

AN0154	AN0153	AN0154 Trans	AN0153 Trans
1	0.9	1.4127	1.249
1	1	1.4127	1.4127
1	0.9	1.4127	1.249
0.9	0.9	1.249	1.249
1	1	1.4127	1.4127

Shapiro-Wilks Test for Normality

AN0154 Trans	AN0153 Trans	Pooled	Mean	Centered	Ordered	D-value	W-value	Critical-W (0.05)	Result
1.4127	1.249	1.4127		0.0655	-0.0982				
1.4127	1.4127	1.4127		0.0655	-0.0982				
1.4127	1.249	1.4127	1.3472	0.0655	-0.0982	0.0643	0.6403	0.842	Not Normal
1.249	1.249	1.249		-0.0982	-0.0982				
1.4127	1.4127	1.4127		0.0655	0.0655				
		1.249		-0.0982	0.0655				
Mean	Mean	1.4127		0.0655	0.0655				
1.38	1.3145	1.249		-0.0982	0.0655				
		1.249		-0.0982	0.0655				
		1.4127		0.0655	0.0655				

Wilcoxan Rank Sum Test

Pooled	Sorted	Point	Wilcoxan Rank	AN0154	AN0153	Critical (from Table K=1)	Result
1.4127	1.249	9	2.5	0	2.5	19	No Significant Difference
1.4127	1.249	8	2.5	0	2.5		
1.4127	1.249	6	2.5	0	2.5		
1.249	1.249	4	2.5	2.5	0		
1.4127	1.4127	10	7.5	0	7.5		
1.249	1.4127	7	7.5	0	7.5		
1.4127	1.4127	5	7.5	7.5	0		
1.249	1.4127	3	7.5	7.5	0		
1.249	1.4127	2	7.5	7.5	0		
1.4127	1.4127	1	7.5	7.5	0		
				Sum	Sum		
				32.5	22.5		

Average Dry Weight per Replicate (in mg)

AN0154	AN0153
0.275	0.218
0.27	0.261
0.253	0.234
0.238	0.239
0.269	0.212

Shapiro-Wilks Test for Normality

AN0154	AN0153	Pooled	Mean	Centered	Ordered	D-value	W-value	Critical-W (0.05)	Result
0.275	0.218	0.275		0.0281	-0.0349				
0.27	0.261	0.27		0.0231	-0.0289				
0.253	0.234	0.253	0.2469	0.0061	-0.0129	0.0044	0.9349	0.842	Normal
0.238	0.239	0.238		-0.0089	-0.0089				
0.269	0.212	0.269		0.0221	-0.0079				
		0.218		-0.0289	0.0061				
Mean	Mean	0.261		0.0141	0.0141				
0.261	0.2328	0.234		-0.0129	0.0221				
		0.239		-0.0079	0.0231				
		0.212		-0.0349	0.0281				

F-test and T-Test

AN0154 Var	AN0153 Var	F-Value	Critical-F (Two-Tailed 0.05)	Variances	T-value	Deg. of Freedom	Critical-T (One-Tailed 0.05)	Result
0.0002	0.0004	2	6.3882	Equal	2.5743	7	1.8946	Significantly Different