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SEDIMENT TOXICITY TEST USING THE AMPHIPOD *Hyalella azteca* Watershed Management Area # 19 (Lower Delaware River Basin)



New Jersey Department of Environmental Protection ROBERT C. SHINN, JR. COMMISSIONER

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New Jersey Department of Environmental Protection Division of Watershed Management P.O. Box 427, Trenton, NJ 08625-0427

WATER MONITORING MANAGEMENT James Mumman, Administrator

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Bureau of Freshwater and Biological Monitoring Biomonitoring Section

Assay Number(s): 99H002e, 99H002f

Report Prepared By: Thomas Miller

> Analysts: Thomas Miller Dean Bryson Victor Poretti

> > Samplers:

Thomas Miller Dean Bryson Victor Poretti

Supervisor: Paul Olsen

Chief Bureau of Freshwater & Biological Monitoring Alfred Korndoerfer, Jr.

EXECUTIVE SUMMARY

Toxicity tests using the amphipod *Hyalella azteca* were performed on sediments collected from two sites in the lower Delaware River system. This initiative was undertaken by the Bureau of Freshwater and Biological Monitoring in conjunction with the Office of Watershed Management, both administered under the New Jersey Department of Environmental Protection's Division of Watershed Management. The sites tested were situated on Mt. Misery Brook and North Branch Rancocas Creek. Both sites are within Watershed Management Area # 19 in the lower Delaware "Water Region". Suspected toxicity at one of the sites (North Branch Rancocas Creek) was based on its "moderately impaired" biological assessments (i.e. degraded quality of benthic macroinvertebrate communities) as compared to its "non-impaired" assessment found in previous survey(s) of New Jersey statewide Ambient Biomonitoring Network (AMNET). A reference site was selected at Mt. Misery Brook because of its "non-impaired" AMNET assessment. The sediment toxicity tests were conducted to evaluate whether or not toxic materials in the streambed sediments could account for the observed macroinvertebrate impairment. Tests were conducted in accordance with the Bureau Standard Operating Procedures, which incorporate protocols recognized by the U.S. Environmental Protection Agency. When test results were statistically compared to that of the reference, the test site did exhibit acute toxicity, as measured by survival of test organisms.

INTRODUCTION

The Ambient Biomonitoring Network (AMNET) program of the New Jersey Department of Environmental Protection (NJDEP), Bureau of Freshwater and Biological Monitoring (BFBM), is designed to establish a biological database for use in gauging stream quality throughout the state. This database, in turn, can be an invaluable aid to New Jersey's water quality and watershed planning and management efforts. Levels of impairment are shown through the use of Rapid Bioassessment Protocols (RBP) advised by the U.S. Environmental Protection Agency (EPA)(1). The RBP assesses impairment through the collection, identification, categorizing, and quantification of instream macroinvertebrate communities. Although the RBP is an excellent way in which to assess impairment, it may sometimes be difficult to distinguish whether impairment is due to water quality degradation or habitat destruction.

Sediment toxicity testing is an additional tool used to determine whether toxicity is the cause of impairment, before resorting to costly chemical monitoring. The test organism, *Hyalella azteca* is an epibenthic detritivore, reported to also digest bacteria and algae from ingested sediment particles (2). This amphipod crustacean inhabits lakes, ponds, and streams throughout North and South America, typically burrowing into the sediment surface (3,4). *H. azteca* is a sensitive "benchmark" species, which can be cultured in the laboratory with relative ease.

In March of 1999, the Bureau of Freshwater and Biological Monitoring conducted sediment toxicity tests on two stream sites, within an area of southwestern New Jersey, which had exhibited varying degrees of impairment in previous AMNET sampling (5). The new initiative was designed to support management efforts in Watershed Management Area (WMA) #19. Administratively, this includes the lower Delaware river drainage, in the lower Delaware "Water Region".

METHODS

Sample sites were selected based on previous AMNET results (5) (see appendix A), proximity to urban and/or agricultural areas, and proximity of point source discharges (i.e. effluents from facilities with New Jersey Pollutant Discharge Elimination System (NJPDES) permits). The sites selected are as follows (see map):

AMNET	BIOLOGICAL	
STATION#	ASSESSMENT	LOCATION(see map)
AN0145	non-impaired	Mt. Misery Brook @ Rt.602, Hardwick Twp.
AN0143	moderately impaired	North Branch Rancocas Creek @ outlet of
		Hanover Lake, Pemberton Twp.

Sediment samples were collected from both sites on March 11, 1999. 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, then stored at # 4EC until the start of the test (6).

Prior to test initiation the sample sites were assigned assay numbers, in accordance with our ongoing series of toxicity tests, as follows:

99H002e = AN0145 (reference, nonimpaired site) 99H002f = AN0143

Testing methodology followed the BFBM Standard Operating Procedures (7). 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 organisms. 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 (7). After this time period, the overlying water was siphoned, and fresh water was added. A control set of replicates was also set up using 250 ml of laboratory grade freshwater only.

One to seven-day old *H. azteca* juveniles were collected, from our cultures, and held for one week prior to the start of the test (7).

Testing was initiated on March 16, 1999 at 10:40 hours, by adding ten 7 to 14- day old organisms from the holding chamber to each test replicate series. Each day the overlying water was exchanged, and each test replicate was fed 1.5 ml of yeast, CEROPHYLL7, Trout chow (YCT)(8), and 1.5 ml of the green algae *Selenastrum capricornutum* at a concentration of 35×10^6 cells ml⁻¹ (after centrifugation). 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 1).

The test was concluded after ten days (March 26, 1999). As a measure of acute toxicity, live organisms were counted against those dead or missing (see Table 2). As an indication of chronic toxicity, dry weights of surviving organisms were measured (see Table 3). Statistical analysis was performed following EPA guidelines (8). Results of the reference test were compared against the control, and, providing the reference and the control were statistically similar, the remaining tests were compared to the reference.

RESULTS

The tests were valid by virtue of meeting the acceptability requirements of \$ 80% survival (see Table 2) in the control test series (7). The survival data was not distributed normally as 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 in survival results between the reference test (99H002e) and the control. The test sample was then compared to the reference. The survival data was not distributed normally as analyzed by the Shapiro-Wilks test for normality, and therefore, the Wilcoxan Rank Sum Test was used when comparing test survival results. The result for tests 99H002f showed significant difference in mortality from that of the reference sample. A dry weight analysis was performed on the blank control and the reference site (99H002e) and showed no significant difference (see Table 3). The dry weight data was distributed normally as analyzed by the Shapiro-Wilks test, therefore, an F-Test and T-Test were used when comparing test dry

weight results (see appendix B for statistical printout). Since there were no surviving organisms in sample 99H002f, dry weights comparison was not be performed.

Although all indigenous organisms observed in the samples before the start of the test were removed, some did remain; however, their presence did not invalidate test results. Test chamber 99H002e contained a dragonfly larva.

DISCUSSION

The test site (North Branch Rancocas Creek) in WMA 19 was chosen based on the results of previous macroinvertebrate studies and the presence of likely sources of impairment; these included the proximity of NJPDES facilities, and of urbanization, or agriculture. The reference site at Mt. Misery Brook, was chosen because of its prior "non-impaired" bioassessment in the AMNET survey (5), and because it is within the same Water Region (Lower Delaware River Basin) as the test sites. Also considered in choosing the reference site, were similarities in stream morphology and position within the New Jersey Ecomap or ecoregion scheme (1,9).

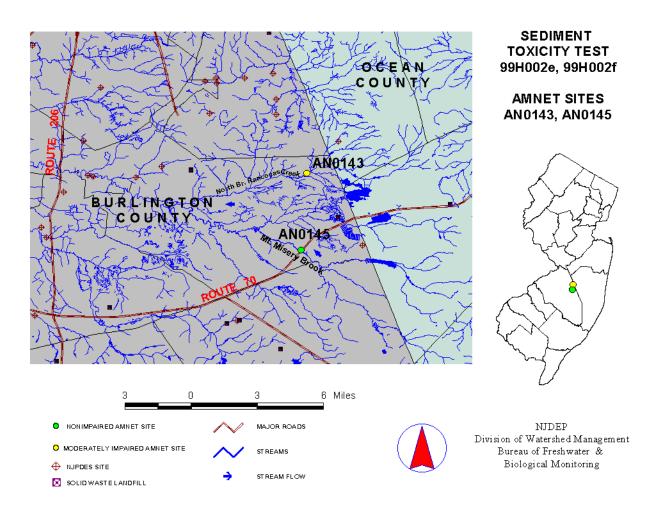
Site AN0143 (North Branch Rancocas Creek), was chosen for testing based on its degraded bioassessment results from "non-impaired" to "moderately impaired", and to the upstream presence of NJPDES facilities (Jackson Estates), and the high agricultural land use in the area. The latter two features may have influenced the bioassessment results. The test site is located downstream of the Fort Dix Military Base.

Survival and dry weight results showed significant differences between the reference and the test site treatments. The test site results indicate acute toxicity probably due to a high concentration of heavy metals such as arsenic, copper, lead, and zinc (Table 4). Impairment may also have been caused by the presence of other toxic substances at chronically, but not acutely, toxic levels, which could be introduced episodically, rather than continuously, into the stream. Therefore, it is advisable by these study results, that supplemental sampling be performed for target analytes, such as excessive nutrients (usually forms of nitrogen or phosphorus), and pesticides or other known toxic compounds.

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MAPS



Bureau of Freshwater and Biological Monitoring Sediment Toxicity Tests Watershed Management Area # 19 Lower Delaware River Basin

Control	HIGH	LOW	AVG.	STD. DEV.	% CV
pН	7.9	7.3	7.5	0.17	2.25
cond. Fmhos	150	141	146	3.21	2.20
D.O. mg/L	7.9	7.0	7.6	0.35	4.56
99H002e	HIGH	LOW	AVG.	STD. DEV.	% CV
pН	7.7	7.0	7.5	0.18	2.47
cond. Fmhos	161	116	147.2	12.64	8.59
D.O. mg/L	8.2	6.9	7.8	0.35	4.51
99H002f	HIGH	LOW	AVG.	STD. DEV.	% CV
рН	7.9	7.2	7.5	0.22	2.94
cond. Fmhos	170	126	147.3	13.10	8.89
D.O. mg/L	8.2	6.9	7.7	0.40	5.18

Table 1 Test Chamber Chemical/Physical Parameters

Bureau of Freshwater and Biological Monitoring

Sediment Toxicity Tests Watershed Management Area # 19 Lower Delaware River Basin

TABLE 2

MORTALITY DATA (number surviving)

ASSAY #	REP. A	REP. B	REP. C	REP. D	REP. E	%survival
Control	10	10	10	10	10	100
99H002e	10	8	9	8	10	90
99H002f	0	0	0	0	0	0

Statistical Analysis

Test Endpoint: Su	urvival	
Test Used:	Wilcoxan Ra	ank Sum Test
Results:	99H002e:	no significant difference from control
	99H002f:	significant difference from reference station

*see appendix B for statistical printout

Bureau of Freshwater and Biological Monitoring Sediment Toxicity Tests Watershed Management Area # 19 Lower Delaware River Basin

TABLE 3

WEIGHT DETERMINATION

Drying Oven Temperature: <u>105EC</u>

Duration: 17.5 hours

Analyst:T. Miller

REPLICATE.		WGT. OF BOAT (mg)	DRY WGT: BOAT + ORGANISMS (mg)	TOTAL WGT. OF ORGANISMS (mg)	NUMBER OF ORGANISMS	ORGANISM AVG. DRY WGT. (mg)	GROUP AVG. (mg)
CONTROL	А	431.66	432.64	0.98	10	0.098	
	В	435.95	437.01	1.06	10	0.108	
	С	434.68	435.98	1.30	10	0.130	0.108
	D	432.53	433.62	1.09	10	0.109	
	Е	433.66	434.65	0.99	10	0.099	
99H002e	А	436.42	437.71	1.29	10	0.129	
	В	433.03	434.07	1.04	8	0.130	
	С	434.51	435.78	1.27	9	0.141	0.160
	D	434.24	435.87	1.63	8	0.204	
	Е	434.32	436.32	2.00	10	0.200	
99H002f	А	432.09	432.09	0	0	0	
	В	435.11	435.11	0	0	0	
	С	433.76	433.76	0	0	0	0
	D	433.58	433.58	0	0	0	
	Е	433.09	433.09	0	0	0	

Statistical Analysis Test Endpoint: Growth Results:

F-test and T-test99H002e:Wilcoxan Rank Sum Test99H002f:

no significant difference from control significant difference from reference station

*see appendix B for statistical printout

Bureau of Freshwater and Biological Monitoring Sediment Toxicity Tests Watershed Management Area # 19 Lower Delaware River Basin

TABLE 4 Sediment Sample Heavy Metal Analysis Results

Sediment samples were collected on 11/10/98 and assayed for heavy metal contamination.

Analyte	105704*	105705*	105706*
Arsenic	4	31.5	4
Boron	1.27	2.49	16.9
Chromium	2.47	6.52	25.5
Copper	192	8.69	1
Iron	3810	20.8	22.7
Lead	6970	110	57
Manganese	6.94	34.4	12.8
Zinc	23.8	41.9	52.5

Results are in mg/Kg (PPM) based on a dry weight basis

Results shown in shaded boxes represent a concentration above which adverse effects occurred frequently (10)

* Sediment Sampling sites:

105704	N. Branch Rancocas Creek @ Outlet of Hanover Lake (AN0143)
105705	N. Branch Rancocas Creek @ Main Street (Hanover St)
105706	N. Branch Rancocas Creek @ Pine Street

APPENDIX A

AMNET DATA(5)

Sediment Toxicity Tests Watershed Management Area # 19 Lower Delaware River Basin Lower Delaware Basin - Browns Mills USGS Quadrangle Station: AN0145 Mt Misery Bk, Rt 70, Pemberton Twp, Burlington County Date Sampled: 02/03/98

Family	Family Tolerance Value (FTV)	Number of Individuals	
Chironomidae	6	29	
Limnephilidae	4	13	
Capniidae	1	10	
Asellidae	8	7	
Lepidostomatidae	1	6	
Leptophlebiidae	2	6	
Philopotamidae	3	5	
Ceratopogonidae	6	4	
Enchytraeidae	10	3	
Polycentropodidae	6	3	
Heptageniidae	4	3	
Taeniopterygidae	2	3	
Hydropsychidae	4	2	
Tipulidae	3	1	
Perlodidae	2	1	
Phryganeidae	4	1	
Sialidae	4	1	
Metretopodidae	2	1	
Tabanidae	6	1	

Statistical Analysis

Number of Taxa: 19 Total Number of Individuals: 100 % Contribution of Dominant Family: 29.00 % (Chironomidae) Family Biotic Index: 4.44 Scraper/Filterer Collector Ratio: 0.30 Shredder/Total Ratio: 0.40 E+P+T (Ephemeroptera, Plecoptera, Trichoptera): 12 % EPT: 54.00 EPT/C: 1.86 NJIS Rating: 30 Biological Condition: Nonimpaired

Observations

Streamwater: Clear....Flow: Moderate....Width/Depth (ft): 11/1 Substrate: Gravel,sand....StreamBank Vegetation/Stability: Trees,shrubs/Stable

Appendix A

Lower Delaware Basin - Browns Mills USGS Quadrangle Station: AN0143 Rancocas Ck N Br, Blw Hanover Lk, Hanover Furnace, Burlington County Date Sampled: 02/10/98

	Family Tolerance	Number of	
Family	Value (FTV)	Individuals	
Chironomidae	6	78	
Protoneuridae	9	6	
Polycentropodidae	6	3	
BloodRed Chironomidae	8	2	
Hydropsychidae	4	2	
Tubificidae	10	2	
Tipulidae	3	2	
Asellidae	8	1	
Gomphidae	1	1	
Molannidae	6	1	
Limnephilidae	4	1	
Elmidae	4	1	

Statistical Analysis

Number of Taxa: 12 Total Number of Individuals: 100 % Contribution of Dominant Family: 78.00 % (Chironomidae) Family Biotic Index: 6.13 Scraper/Filterer Collector Ratio: 0.40 Shredder/Total Ratio: 0.82 E+P+T (Ephemeroptera, Plecoptera, Trichoptera): 4 % EPT: 7.00 EPT/C: 0.09 NJIS Rating: 12 Biological Condition: Moderately Impaired Habitat Analysis: 178

Observations

Streamwater: Clear....Flow: Moderate....Width/Depth (ft): 17/2 Substrate: Gravel,sand....StreamBank Vegetation/Stability: Shrubs,trees/Stable Canopy: Mostly Open....Other: Forested/Rural/Lake upstream; Water temp.4.4 /pH 4.3 /DO 11.9 /Cond.45 Bureau of Freshwater and Biological Monitoring

APPENDIX B STATISTICAL DATA

Sediment Toxicity Tests Watershed Management Area # 19 Lower Delaware River Basin

Sediment Toxicity Tests Watershed Management Area # 19

SURVIVAL RESULTS

CONTROL VS. AN0145 (reference)

Survival Proportions with Arc Sine Square Root Transformation

Blank	AN0145	Blank Trans	AN0145 Trans
1.0	1.0	1.4127	1.4127
1.0	0.8	1.4127	1.1071
1.0	0.9	1.4127	1.249
1.0	0.8	1.4127	1.1071
1.0	1.0	1.4127	1.4127

Shapiro-Wilks Test for Normality

Blank Trans	AN0145 Trans	Pooled	Mean	Centered	Ordered	D-value	W-value	Critical-W (0.05)	Result
1.4127	1.4127	1.4127		0.0775	-0.2281				
1.4127	1.1071	1.4127		0.0775	-0.2281				
1.4127	1.249	1.4127	1.3352	0.0775	-0.0862	0.1535	0.6301	0.842	Not Normal
1.4127	1.1071	1.4127		0.0775	0.0775				
1.4127	1.4127	1.4127		0.0775	0.0775				
		1.4127		0.0775	0.0775				
Mean	Mean	1.1071		-0.2281	0.0775				
1.4127	1.2577	1.249		-0.0862	0.0775				
		1.1071		-0.2281	0.0775				
		1.4127		0.0775	0.0775				

Sediment Toxicity Tests Watershed Management Area # 19

SURVIVAL RESULTS continued...

CONTROL VS. AN0145 (reference)

wncoxa	Vilcoxan Rank Sum Test									
Pooled	Sorted	Point	Wilcoxan Rank	Blank	AN0145	Critical(from Table K=1)	Result			
1.4127	1.1071	9	1.5	0	1.5	19	No Significant Difference			
1.4127	1.1071	7	1.5	0	1.5					
1.4127	1.249	8	3	0	3					
1.4127	1.4127	10	7	0	7					
1.4127	1.4127	6	7	0	7					
1.4127	1.4127	5	7	7	0					
1.1071	1.4127	4	7	7	0					
1.249	1.4127	3	7	7	0					
1.1071	1.4127	2	7	7	0					
1.4127	1.4127	1	7	7	0					
				Sum	Sum					
				35	20					

Wilcoxan Rank Sum Test

Sediment Toxicity Tests Watershed Management Area # 19

SURVIVAL RESULTS

AN0145 (reference) VS. AN0143

Survival Proportions with Arc Sine Square Root Transformation

AN0145	AN0143	AN0145 Trans	AN0143 Trans
1.0	0.0	1.4127	0.1581
0.8	0.0	1.1071	0.1581
0.9	0.0	1.249	0.1581
0.8	0.0	1.1071	0.1581
1.0	0.0	1.4127	0.1581

Shapiro-Wilks Test for Normality

AN0145 Trans	AN0143 Trans	Pooled	Mean	Centered	Ordered	D-value	W-value	Critical-W (0.05)	Result
1.4127	0.1581	1.4127		0.7048	-0.5498				
1.1071	0.1581	1.1071		0.3992	-0.5498				
1.249	0.1581	1.249	0.7079	0.5411	-0.5498	3.1164	0.7418	0.842	Not Normal
1.1071	0.1581	1.1071		0.3992	-0.5498				
1.4127	0.1581	1.4127		0.7048	-0.5498				
		0.1581		-0.5498	0.3992				
Mean	Mean	0.1581		-0.5498	0.3992				
1.2577	0.1581	0.1581		-0.5498	0.5411				
		0.1581		-0.5498	0.7048				
		0.1581		-0.5498	0.7048				

Sediment Toxicity Tests Watershed Management Area # 19

SURVIVAL RESULTS continued...

AN0145 (reference) VS. AN0143

Wilcoxan Rank Sum Test

Pooled	Sorted	Point	Wilcoxan Rank	AN0145	AN0143	Critical(from Table K=1)	Result
1.4127	0.1581	10	3	0	3	19	Significantly Different
1.1071	0.1581	9	3	0	3		
1.249	0.1581	8	3	0	3		
1.1071	0.1581	7	3	0	3		
1.4127	0.1581	6	3	0	3		
0.1581	1.1071	4	6.5	6.5	0		
0.1581	1.1071	2	6.5	6.5	0		
0.1581	1.249	3	8	8	0		
0.1581	1.4127	5	9.5	9.5	0		
0.1581	1.4127	1	9.5	9.5	0		
				Sum	Sum		
				40	15		

Sediment Toxicity Tests Watershed Management Area # 19

GROWTH

CONTROL VS. AN0145 (reference)

Average Dry Weights (in mg)

Blank	AN0145
0.098	0.129
0.106	0.130
0.130	0.141
0.109	0.204
0.099	0.200

Shapiro-Wilks Test for Normality

Blank	AN0145	Pooled	Mean	Centered	Ordered	D-value	W-value	Critical-W (0.05)	Result
0.098	0.129	0.098		-0.0366	-0.0366				
0.106	0.130	0.106		-0.0286	-0.0356				
0.130	0.141	0.130	0.1346	-0.0046	-0.0286	0.0133	0.816	0.842	Not Normal
0.109	0.204	0.109		-0.0256	-0.0256				
0.099	0.200	0.099		-0.0356	-0.0056				
		0.129		-0.0056	-0.0046				
Mean	Mean	0.130		-0.0046	-0.0046				
0.108	0.161	0.141		0.0064	0.0064				
		0.204		0.0694	0.0654				
		0.200		0.0654	0.0694				

Sediment Toxicity Tests Watershed Management Area # 19

GROWTH continued...

CONTROL VS. AN0145 (reference)

Wilcoxan Rank Sum Test

Pooled	Sorted	Point	Wilcoxan Rank	Blank	AN0145	Critical(from Table K=1)	Result
0.098	0.098	1	1	1	0	19	No Significant Difference
0.106	0.099	5	2	2	0		
0.130	0.106	2	3	3	0		
0.109	0.109	4	4	4	0		
0.099	0.129	6	5	0	5		
0.129	0.130	7	6.5	0	6.5		
0.130	0.130	3	6.5	6.5	0		
0.141	0.141	8	8	0	8		
0.204	0.200	10	9	0	9		
0.200	0.204	9	10	0	10		
				Sum	Sum		
				16.5	38.5		