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SEDIMENT TOXICITY TEST
USING THE AMPHIPOD
Hyalella azteca
(Parsonage Run, Cohansey River)

New Jersey Department of Environmental Protection
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COMMISSIONER

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Assay Number(s): 97H002e, 97H002f

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EXECUTIVE SUMMARY

A toxicity test using the amphipod *Hyalella azteca* was performed on sediments collected from Parsonage Run (AN0711) and a reference site on Cohansey River (AN0709) in the Delaware Basin. The reference was selected on Cohansey River because of its "non-impaired" biological assessment as per the Ambient Biomonitoring Network (AMNET). The Parsonage Run site was chosen because of suspected toxicity due to a "severely impaired" assessment as per the AMNET program. Sediment toxicity testing provides further data which can be related to previous evaluations. When statistically compared to the reference the test site did not exhibit acute toxicity, as measured by survival of test organisms, however it did exhibit acute toxicity for growth results.

INTRODUCTION

The Ambient Biomonitoring Network (AMNET) program of the New Jersey Department of Environmental Protection (NJDEP) is designed to establish biologically impaired stream segments throughout the state using the U.S. Environmental Protection Agency (EPA) Rapid Bioassessment Protocol (RBP)(10). The RBP assesses impairment through the collection, identification, catagorizing, and quantification 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 (2). This amphipod burrows into the sediment surface and inhabits lakes, ponds, and streams throughout North and South America (1)(8). *H. azteca* is a sensitive benchmark, i.e. established, test species that can be cultured in the laboratory with relative ease.

METHODS

Sample sites were selected based on available AMNET data(5) (see appendix A) and proximity to New Jersey Polutant Discharge Elimination System (NJPDES) facilities.
The sites selected are as follows (see map):

AMNET STATION#	BIOLOGICAL ASSESSMENT	LOCATION(see map)
AN0711	severely impaired	Parsonage Run @ Finley Rd., Upper Deerfield Twp.
AN0709	non-impaired	Cohansey River @ Beal Rd., Alloway Twp.

Sediment samples were collected from sites AN0709 and AN0711 on March 5, 1997 at 12:45 and 13:20 hours respectively. At each station the sediment was collected in the stream channel using a stainless steel scoop sampler,placed into one liter amber glass bottles and stored at less than 4EC until the start of the test (4).

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

97H002a = control
97H002e = AN0709
97H002f = AN0711

Testing methodology followed the Bureau of Water Monitoring Standard Operating Procedures(6). 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 at the bottom(6). 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 overlying water only.

One to seven day old *H. azteca* juveniles were collected and held for one week prior to the start of the test (6).

The test was initiated on March 11, 1997 at 10:40 hours, by adding ten 7 - 14 day old organisms from the holding chamber to each test series replicates. Each day the overlying water was exchanged, and each test replicate was fed 1.5 ml of Yeast, CEROPHYLL7, Trout chow(YCT)(11), and 1.5 ml of the green algae *Selenastrum capricornutum* at a concentration of 35×10^6 cells/ml (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 21, 1997). Live organisms were counted (see table 2) and the dry weights measured (see table 3). Statistical analysis was performed following EPA guidelines (11). The reference test was compared against the control, and the remaining tests compared to the reference, providing the reference and the control were statistically similar.

RESULTS

The test was valid by virtue of meeting the acceptability requirements of $ 80% survival (see table 2) in the control test series (6). 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 between the reference test, 97H002e, survival results and the control survival results. Test 97H002f 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. 97H002f and showed no significant difference from the reference for mortality. Test 97H002f was then compared to the reference for dry weight. The dry weight data (see table 3) was distributed normally by the Shapiro-Wilks test, and therefore an F-Test and T-Test were used when comparing test dry weight results. 97H002f showed a significant difference from the reference for dry weight.(see appendix B for statistical printout)

On day 2 the dissolved oxygen in 97H002f fell below the test criterion of 40% saturation (to 33%), thus continuous aeration was maintained in all test vessels.

Although all visible indigenous macroinvertebrate organisms had apparently been removed from the sediment samples,tubifex worms and harpacticoid copepods were observed in the 97H002f test vessels.

DISCUSSION

The sample site on Parsonage Run was chosen based on the results of macroinvertebrate studies and the proximity of NJPDES facilities and urbanization. Site AN0711 had a Aseverely impaired@ bioassessment rating as analyzed by AMNET. The reference site at Cohansey River, AN0709, was chosen because it had a Anonimpaired@bioassessment based on results from the AMNET program and was within the same major drainage basin as the test sites. Similar stream morphology and similar New Jersey Ecomap(7) designation (based on geology, soil, and natural vegetation) was also considered in choosing the reference site.

Survival results showed no significant differences between the reference and the test site treatments. Growth results showed significant difference between the reference and the test site treatments. Prior to the test all visible indigenous organisms were removed from the sediment samples; tubifex worms and harpacticoid copepods were later observed in the 97H002f test vessels. *H. azteca* and copepods have some similarities in their feeding habits, i.e. they both ingest algae. This competition for food sources may have resulted in the lower weights of *H. azteca* in the test sample as compared to the reference. On day 2, the dissolved oxygen fell below 40% saturation in 97H002f (to 33%), and continuous aeration was maintained in all test vessels thereafter. Since the D.O. problem was resolved immediately, the single day drop probably did not effect the test results. If a Aseverely impaired@ bioassessment continues at site AN0711, further intensive studies should be initiated.

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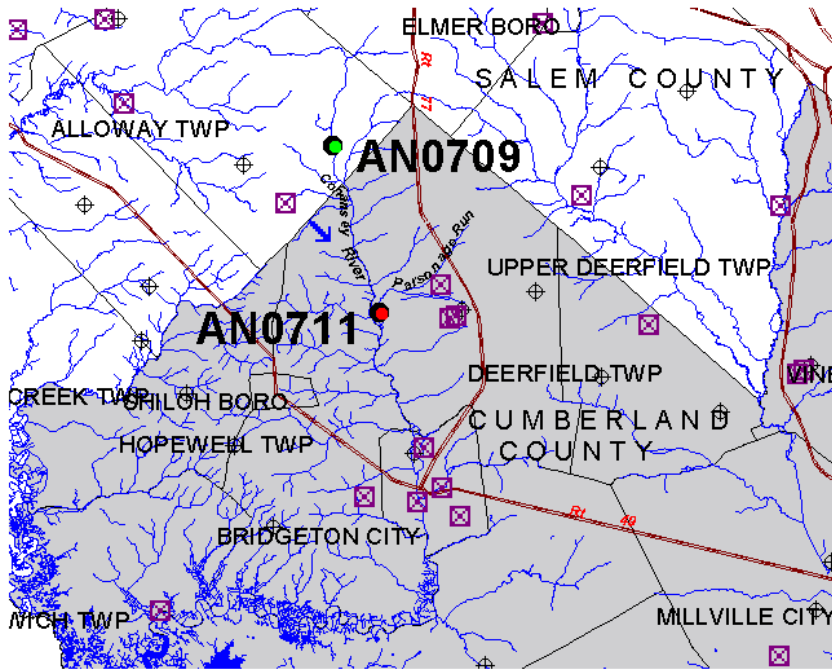
SEDIMENT TOXICITY TEST 97H002e, 97H002f

NJDEP
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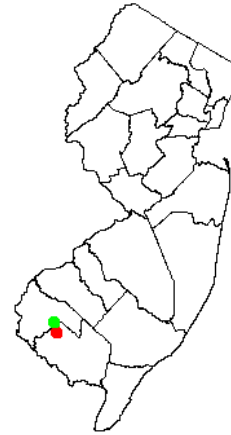
Legend:
- NONIMPAIRED AMNET SITE
- SEVERELY IMPAIRED AMNET SITE
- NJPDES SITE
- SOLID WASTE LANDFILL
- MAJOR ROADS
- STREAMS
- STREAM FLOW

Scale: 3.2 0 3.2 6.4 Miles

Table 1

Test Chamber Chemical/Physical Parameters

Control	HIGH	LOW	AVG.	STD. DEV.	% CV
pH	7.8	7.2	7.5	0.23	3.04
cond. Ϝmhos	148	126	140	7.16	5.11
D.O. mg/L	8.1	6.0	7.3	0.81	11.15

97H002e	HIGH	LOW	AVG.	STD. DEV.	% CV
pH	7.5	6.6	7.1	0.33	4.70
cond. Ϝmhos	134	105	123	9.89	8.04
D.O. mg/L	7.9	5.4	6.9	0.92	13.46

97H002f	HIGH	LOW	AVG.	STD. DEV.	% CV
pH	7.2	6.2	6.9	0.34	4.90
cond. Ϝmhos	184	119	134	18.56	13.84
D.O. mg/L	8.0	2.8*	5.9	2.10	35.58

* The D.O. fell below 40% saturation on day 2 of the test.

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
97H002e	8	10	10	9	lab accident	92.5
97H002f	5	10	9	9	10	86

Statistical Analysis

Test Endpoint: Survival
Test Used: Wilcoxan Rank Sum Test
Results: 97H002e - no significant difference from control
                         97H002f - no significant difference from reference station

TABLE 3

WEIGHT DETERMINATION

Drying Oven Temperature: <u>105EC</u>　　　　　Time/Date Start Drying: <u> 1400 /3-21-97</u>
　　　　　　　　　　　　　　　　　　　　　Time/Date End Drying: <u> 1600 /3-21-97</u>

Analyst:<u>T. Miller</u>

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	12.05	13.48	1.43	10	0.143	
B	10.87	11.51	0.64	10	0.064	
C	9.74	10.99	1.25	10	0.125	0.109
D	8.63	9.48	0.85	10	0.085	
E	9.73	11.01	1.28	10	0.128	
97H002e A	6.58	7.75	1.17	8	0.146	
B	7.04	8.29	1.25	10	0.125	
C	16.15	18.21	2.06	10	0.206	0.150
D	13.27	14.38	1.11	9	0.1.23	
E	---	---	---	---	---	
97H002f A	9.62	10.08	0.46	5	0.092	
B	8.48	9.83	1.35	10	0.135	
C	7.60	8.25	0.65	9	0.072	0.099
D	10.30	11.12	0.82	9	0.091	
E	9.87	10.93	1.06	10	0.106	

Statistical Analysis*

Test Endpoint:　Growth
Test Used:　　　　　F-test and T-test
Results:　　　　　　97H002e - no significant difference from control
　　　　　　　　　　97H002f - significant difference from reference station

*see appendix b for statistical printout

APPENDIX A

AMNET DATA(5)

AN0709 # Cohansey R, Beal Rd, Alloway Twp, Salem Co, Alloway Quad

October 19, 1995

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Taxon (Family Level)	FTV	NOI
ENCHYTRAEIDAE	10	1
TUBIFICIDAE	10	4
ANCYLIDAE	7	2
SPHAERIIDAE	8	2
HEPTAGENIIDAE	4	6
EPHEMERELLIDAE	1	4
CORDULEGASTRIDAE	3	2
COENAGRIONIDAE	9	1
TAENIOPTERYGIDAE	2	3
VELIIDAE	9	1
HALIPLIDAE	5	1
DYTISCIDAE	5	1
ELMIDAE	5	1
CORYDALIDAE	0	5
HYDROPSYCHIDAE	4	50
PHRYGANEIDAE	4	1
MOLANNIDAE	6	1
CHIRONOMIDAE	6	14

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Number of Taxa +18 # Individuals per subsample +   100

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Dominant Family(s) +HYDROPSYCHIDAE   50.00%
Family Biotic Index + 4.47
Scraper/Filterer Collector Ratio + 0.04
Shredder/Total Ratio + 0.23
E(phemeroptera)+P(lecoptera)+T(richoptera) +6
%EPT +65.00
EPT/Chironomids + 4.64

NJIS/Rating +27/non-impaired

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OBSERVATIONS
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Clarity +clear
Flow +slow
Width/Depth(ft) +4-5/1-2
Substrate +sand/gravel
Streambank Vegetation/Stability +good/good
Canopy +mostly closed
Other +woods/rural; frog

FTV = Family Tolerance Value,   NOI = Number Of Individuals(per 100 organism subsample)

AN0711 # Parsonage Run, Finley Rd, U Deerfield Twp, Cumberland Co,
    Shiloh Quad

October 19, 1995

Taxon (Family Level)	FTV	NOI
PROSTOMATIDAE	7	1
TUBIFICIDAE	10	79
SPHAERIIDAE	8	6
GAMMARIDAE	4	11
COENAGRIONIDAE	9	1
ELMIDAE	5	1
CHIRONOMIDAE	6	1

Number of Taxa +7 # Individuals per subsample +   100

Dominant Family(s) +TUBIFICIDAE   79.00%
Family Biotic Index + 9.09
Scraper/Filterer Collector Ratio + 0.00
Shredder/Total Ratio + 0.01
E(phemeroptera)+P(lecoptera)+T(richoptera) +0
%EPT + 0.00
EPT/Chironomids + 0.00

NJIS/Rating +3/severely impaired

Deficiency(s) noted +TUBIFICIDAE overwhelmingly dominant
            paucity of clean water organisms
            significant organic pollution

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OBSERVATIONS
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Clarity +clear
Flow +slow-moderate
Width/Depth(ft) +8-10/1-2
Substrate +sand/mud
Streambank Vegetation/Stability +good/good
Canopy +mostly open
Other +woods/agricultural; macrophytes; fish (minnows)

FTV = Family Tolerance Value,   NOI = Number Of Individuals(per 100 organism subsample)

APPENDIX B

STATISTICAL DATA

SURVIVAL RESULTS

Survival Proportions with Arc-Sine Square Root Transformation

BLANK	AN0709	BLANK Trans	AN0709 Trans
1	0.8	1.4127	1.1071
1	1	1.4127	1.4127
1	1	1.4127	1.4127
1	0.9	1.4127	1.249
1		1.4127	

Shapiro-Wilks Test for Normality

BLANK Trans	AN0709 Trans	Pooled	Mean	Centered	Ordered	D-value	W-value	Critical-W (0.05)	Result
1.4127	1.1071	1.4127		0.0521	-0.2535				
1.4127	1.4127	1.4127		0.0521	-0.1116				
1.4127	1.4127	1.4127	1.3606	0.0521	0.0521	0.0957	0.5675	0.829	Not Normal
1.4127	1.249	1.4127		0.0521	0.0521				
1.4127		1.4127		0.0521	0.0521				
		1.1071		-0.2535	0.0521				
Mean	Mean	1.4127		0.0521	0.0521				
1.4127	1.2954	1.4127		0.0521	0.0521				
		1.249		-0.1116	0.0521				

Wilcoxan Rank Sum Test

Pooled	Sorted	Point	Wilcoxan Rank	BLANK	AN0709	Critical(from Table K=1)	Result
1.4127	1.1071	6	1	0	1	12	No Significant Difference
1.4127	1.249	9	2	0	2		
1.4127	1.4127	1	6	6	0		
1.4127	1.4127	2	6	6	0		
1.4127	1.4127	3	6	6	0		
1.1071	1.4127	4	6	6	0		
1.4127	1.4127	5	6	6	0		
1.4127	1.4127	7	6	0	6		
1.249	1.4127	8	6	0	6		
				____	____		
				Sum	Sum		
				30	15		

Survival Proportions with Arc-Sine Square Root Transformation

AN0709	AN0711	AN0709 Trans	AN0711 Trans
0.8	0.5	1.1071	0.7854
1	1	1.4127	1.4127
1	0.9	1.4127	1.249
0.9	0.9	1.249	1.249
	1		1.4127

GROWTH RESULTS

Shapiro-Wilks Test for Normality

AN0709 Trans	AN0711 Trans	Pooled	Mean	Centered	Ordered	D-value	W-value	Critical-W (0.05)	Result
1.1071	0.7854	1.1071		-0.1474	-0.4691				
1.4127	1.4127	1.4127		0.1582	-0.1474				
1.4127	1.249	1.4127	1.2545	0.1582	-0.0055	0.342	0.7796	0.829	Not Normal
1.249	1.249	1.249		-0.0055	-0.0055				
	1.4127	0.7854		-0.4691	-0.0055				
		1.4127		0.1582	0.1582				
Mean	Mean	1.249		-0.0055	0.1582				
1.2954	1.2218	1.249		-0.0055	0.1582				
		1.4127		0.1582	0.1582				
				0	0				

Wilcoxan Rank Sum Test

Pooled	Sorted	Point	Wilcoxan Rank	AN0709	AN0711	Critical(from Table K=1)	Result
1.1071	0.7854	5	1	0	1	17	No Significant Difference
1.4127	1.1071	1	2	2	0		
1.4127	1.249	4	4	4	0		
1.249	1.249	7	4	0	4		
0.7854	1.249	8	4	0	4		
1.4127	1.4127	2	7.5	7.5	0		
1.249	1.4127	3	7.5	7.5	0		
1.249	1.4127	6	7.5	0	7.5		
1.4127	1.4127	9	7.5	0	7.5		
				___	___		
				Sum	Sum		
				21	24		

Average Dry Weight per Replicate (in mg)

BLANK	AN0709
0.143	0.146
0.064	0.125
0.125	0.206
0.085	0.123
0.128	

Shapiro-Wilks Test for Normality

BLANK	AN0709	Pooled	Mean	Centered	Ordered	D-value	W-value	Critical-W (0.05)	Result
0.143	0.146	0.143		0.0158	-0.0632				
0.064	0.125	0.064		-0.0632	-0.0422				
0.125	0.206	0.125	0.1272	-0.0022	-0.0042	0.0126	0.9195	0.829	Normal
0.085	0.123	0.085		-0.0422	-0.0022				
0.128		0.128		0.0008	-0.0022				
		0.146		0.0188	0.0008				
Mean	Mean	0.125		-0.0022	0.0158				
0.109	0.15	0.206		0.0788	0.0188				
		0.123		-0.0042	0.0788				

F-test and T-Test

BLANK Var	AN0709 Var	F-Value	Critical-F (Two-Tailed 0.05)	Variances	T-value	Deg. of Freedom	Critical-T (One-Tailed 0.05)	Result
0.0011	0.0015	1.3636	9.1172	Equal	-1.798	7	1.8946	No Significant Difference

Average Dry Weight per Replicate (in mg)

AN0709	AN0711
0.146	0.092
0.125	0.135
0.206	0.072
0.123	0.091
	0.106

Shapiro-Wilks Test for Normality

AN0709	AN0711	Pooled	Mean	Centered	Ordered	D-value	W-value	Critical-W (0.05)	Result
0.146	0.092	0.146		0.0242	-0.0498				
0.125	0.135	0.125		0.0032	-0.0308				
0.206	0.072	0.206	0.1218	0.0842	-0.0298	0.0124	0.924	0.829	Normal
0.123	0.091	0.123		0.0012	-0.0158				
	0.106	0.092		-0.0298	0.0012				
		0.135		0.0132	0.0032				
Mean	Mean	0.072		-0.0498	0.0132				
0.15	0.0975	0.091		-0.0308	0.0242				
		0.106		-0.0158	0.0842				

F-test and T-Test

AN0709 Var	AN0711 Var	F-Value	Critical-F (Two-Tailed 0.05)	Variances	T-value	Deg. of Freedom	Critical-T (One-Tailed 0.05)	Result
0.0011	0.0007	1.5714	6.5914	Equal	2.767	7	1.8946	Significantly Different