State of New Jersey
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SEDIMENT TOXICITY TEST USING THE AMPHIPOD *Hyalella azteca*

Watershed Management Area 9 and 10 (South River and Millstone River)

New Jersey Department of Environmental Protection
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COMMISSIONER

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New Jersey Department of Environmental Protection
Division of Watershed Management
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WATER MONITORING MANAGEMENT
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April 1999

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Bureau of Freshwater and Biological Monitoring Biomonitoring Section

Assay Number(s): 99H002d, 99H002e, 99H002f

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EXECUTIVE SUMMARY

Toxicity tests using the amphipod *Hyalella azteca* were performed on sediments collected from three sites in the Raritan 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. One site tested is situated on the Millstone River within Watershed Management Area # 10, and two sites, one each on Manalapan Brook and McGellairds Brook, are within Watershed Management Area # 9, all within the Raritan AWater Region@. Suspected toxicity at two of the sites (Manalapan Brook and McGellairds Brook)was based on their Aseverely impaired@ biological assessments (i.e. degraded quality of benthic macroinvertebrate communities) found in previous survey(s) of New Jersey=s statewide Ambient Biomonitoring Network (AMNET). A reference site was selected at Millstone River because of its "non-impaired" AMNET assessment. The sediment toxicity tests were conducted to provide further data, which could be related to the previous assessments. Tests were conducted in accordance with the Bureau=s 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 sites did not exhibit acute toxicity, as measured by survival, or chronic toxicity as measured by growth, 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 Abenchmark@ species, which can be cultured in the laboratory with relative ease.

In December of 1998, the Bureau of Freshwater and Biological Monitoring conducted sediment toxicity tests on three stream sites, within an area of central New Jersey, which had exhibited varying degrees of impairment in previous AMNET sampling. The new initiative was designed to support management efforts in Watershed Management Areas (WMA) # 9 and # 10. Administratively, these include the Millstone and South river drainages, respectively, in the Raritan AWater 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 STATION#	BIOLOGICAL ASSESSMENT	LOCATION(see map)
AN0379	non-impaired	Millstone River @ Rt. 33 Bergan Mills
AN0439	severely impaired	Manalapan Bk. @ Federal Rd., Monroe Twp.
AN0447	severely impaired	McGellairds Bk. @ Rt. 527, Englishtown

Sediment samples were collected from all sites on December 4, 1998. 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:

98H006d = AN0379(reference, nonimpaired site)
98H006e = AN0439
98H006f = AN0447

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 overlying water 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 December 8, 1998 at 10:00 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 X 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 (December 18, 1998). 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 (98H006d), and the control. All test samples were 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 results for tests 98H006e and 98H006f both showed no significant difference in mortality from that of the reference sample. Dry weights of both test samples were then compared to that of the reference (see Table 3). The dry weight data was distributed normally as analyzed by the Shapiro-Wilks test, and therefore, an F-Test and T-Test were used when comparing test dry weight results. In this comparison the test samples showed no significant difference from the reference (see appendix B for statistical printout).

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 chambers 98H006d and 98H006e contained several chironomid, or midge, larvae.

DISCUSSION

The test sites (one each on Manalapan Brook and McGellairds Brook) in WMA 9 were 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 Millstone River in WMA 10, was chosen because of its prior Anonimpaired@ bioassessment in the AMNET survey(5), and because it is within the same Water Region (Raritan 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 AN0439 (Manalapan Brook), and site AN0447 (McGellairds Brook), were chosen for testing based on Aseverely impaired@ bioassessment results, upstream presence of NJPDES facilities (on McGellairds Brook), and the high agricultural land use in the area.

Survival and dry weight results showed no significant differences between the reference and the test site treatments. Since the test site results did not indicate acute toxicity, the severe impairment levels previously found may have been due to other factors including habitat degradation and/or various physiochemical parameters (e.g. temperature, turbidity, low dissolved oxygen, pH, etc...). 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.

REFERENCES

1. Plafkin, J.L., M.T. Barbour, K.D. Porter, S.K. Gross and R.M. Hughes. 1989. Rapid bioassessment protocols for use in streams and rivers - benthic macroinvertebrates and fish. EPA/440/4-89/001.United State Environmental Protection Agency. Washington, D.C. 143pp. and appendices.

2. Hargrave, B.T. 1970. The utilization of benthic microflora by *Hyalella azteca*. J. Animal Ecology. 39:427-437.

3. de March, B.G.E. 1981. *Hyalella azteca* (Saussure). In: S.G. Lawrence (ed), Manual for the culture of selected freshwater invertebrates. Can. Spec. Pub. fish. Aquatic. Sc. No. 54, Department of Fisheries and Oceans.

4. Pennak, Robert William. 1978. Fresh-water invertebrates of the United States.John Wiley and Sons, Inc. New York, New York. 813pp.

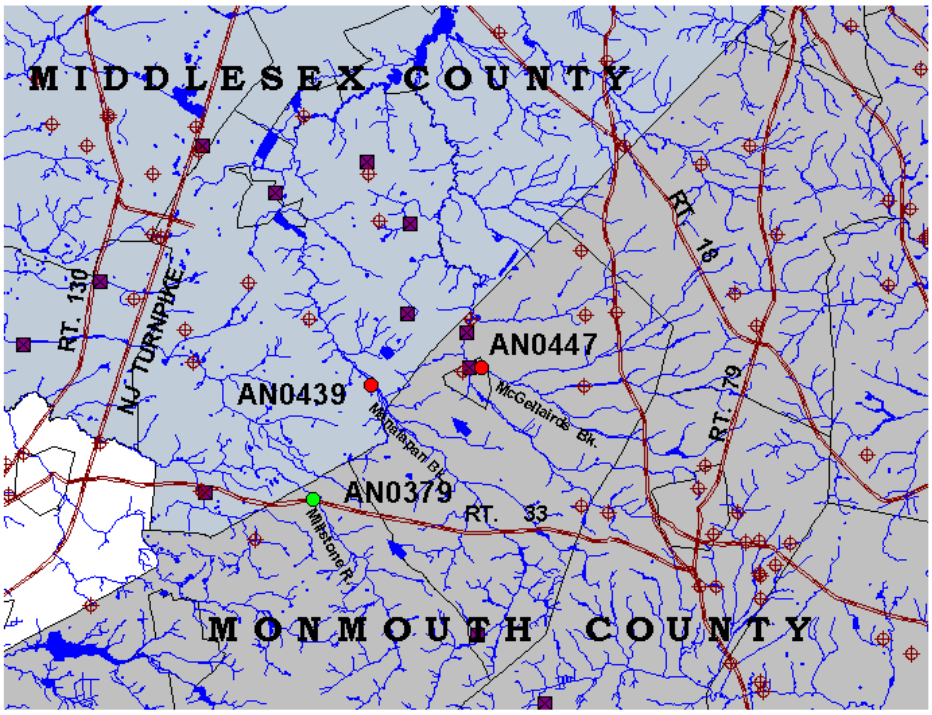
5. New Jersey Department of Environmental Protection. Data report. Ambient biomonitoring network, Raritan drainage basin, benthic macroinvertebrate data, 1993-94. 8pp. + maps and appendices.

6. New Jersey Department of Environmental Protection. 1992 Field sampling procedures manual, NJDEP, Trenton. 360pp..

7. New Jersey Department of Environmental Protection. Laboratory report. Standard operating procedures, culturing and sediment toxicity testing with *Hyalella azteca*. SM001.0795. 1995. Bureau of Freshwater & Biological Monitoring, Trenton. 7pp and appendices.

8. United State Environmental Protection Agency. 1991. Methods for measuring the acute toxicity of effluents and receiving waters to freshwater and marine organisms. EPA/600/4-90/027. Washington, D.C. 293pp.

9. New Jersey Department of Environmental Protection. 1996. New Jersey ECOMAP. State Forestry Services, Trenton.



SEDIMENT TOXICITY TEST 98H006d, 98H006e, 98H006f Millstone and South Rivers

3 0 3 6 Miles

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

NJDEP
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Sediment Toxicity Tests
Watershed Management Area # 9 and 10
Millstone and South Rivers

Table 1
Test Chamber Chemical/Physical Parameters

Control	HIGH	LOW	AVG.	STD. DEV.	% CV
pH	7.7	7.2	7.5	0.13	1.79
cond. Fmhos	154	136	146	6.05	4.14
D.O. mg/L	9.1	7.1	8.4	0.58	6.97

98H006d	HIGH	LOW	AVG.	STD. DEV.	% CV
pH	7.5	6.8	7.2	0.26	3.60
cond. Fmhos	156	135	150	6.43	4.30
D.O. mg/L	8.7	5.9	7.3	1.01	13.96

98H006e	HIGH	LOW	AVG.	STD. DEV.	% CV
pH	7.3	6.8	7.1	0.23	3.29
cond. Fmhos	155	115	141	10.85	7.71
D.O. mg/L	8.7	5.8	7.5	1.03	13.66

98H006f	HIGH	LOW	AVG.	STD. DEV.	% CV
pH	7.4	6.8	7.3	0.19	2.61
cond. Fmhos	157	120	147	11.98	8.12
D.O. mg/L	8.7	6.8	8.0	0.63	7.84

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Sediment Toxicity Tests
Watershed Management Area # 9 and 10
Millstone and South Rivers

TABLE 2

MORTALITY DATA
(number surviving)

ASSAY #	REP. A	REP. B	REP. C	REP. D	REP. E	%survival
Control	9	10	10	10	10	98
98H006d	10	10	9	9	9	94
98H006e	9	10	10	10	10	98
98H006f	9	10	10	9	10	96

Statistical Analysis

Test Endpoint: Survival
Test Used: Wilcoxan Rank Sum Test
Results: 98H006d: no significant difference from control
98H006e: no significant difference from reference station
98H006f: no significant difference from reference station

*see appendix B for statistical printout

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Sediment Toxicity Tests
Watershed Management Area # 9 and 10
Millstone and South Rivers

TABLE 3
WEIGHT DETERMINATION

Drying Oven Temperature: 105EC Duration: 2 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 A	25.01	25.25	0.24	9	0.027	
B	20.72	21.27	0.55	10	0.055	
C	23.34	23.94	0.60	10	0.060	0.047
D	21.53	21.98	0.45	10	0.045	
E	26.55	27.03	0.48	10	0.048	
99H002d A	20.65	21.97	1.32	10	0.132	
B	13.10	14.17	1.07	10	0.107	
C	18.14	19.73	1.59	9	0.177	0.146
D	17.14	18.48	1.34	9	0.149	
E	17.63	19.12	1.49	9	0.166	
99H002e A	22.24	23.74	1.50	9	0.167	
B	24.37	26.53	2.16	10	0.216	
C	20.23	22.05	1.82	10	0.182	0.194
D	20.15	22.19	2.04	10	0.204	
E	16.57	18.60	2.03	10	0.203	
99H002f A	20.63	22.12	1.49	9	0.166	
B	23.16	24.87	1.71	10	0.171	
C	14.56	16.54	1.98	10	0.198	0.181
D	17.34	19.16	1.82	9	0.202	
E	18.62	20.31	1.69	10	0.169	

Statistical Analysis
Test Endpoint: Growth
Results:

F-test and T-test	98H006d:	no significant difference from control
F-test and T-test	98H006e:	no significant difference from reference station
F-test and T-test	98H006f:	no significant difference from reference station

*see appendix B for statistical printout

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APPENDIX A

AMNET DATA(5)

Sediment Toxicity Tests
Watershed Management Area # 9 and 10
Millstone and South Rivers

Appendix A

Raritan Basin - Jamesburg USGS Quadrangle
Station AN0379
Millstone River, Route 33, Bergen Mills
July 25, 1990

Family	Number of Individuals	Family Tolerance Value (FTV)
Hydropsychidae	25	4
Chironomidae	6	6
Heptageniidae	15	4
Tubificidae	7	10
Dytiscidae	4	4
Bryozoa	18	7
Cordulegastridae	1	3
Gastropoda	3	7
Nemertea	5	5
Ephemerellidae	1	1
Corydalidae	2	0
Tipulidae	4	3
BloodRedChironomidae	3	8
Sphaeriidae	2	8
Gomphidae	1	1
Aeshnidae	2	3
Elmidae	1	4

Statistical Analysis

Number of Taxa: 17
Total Number of Individuals: 100
% Contribution of Dominant Family: 25.00
Family Biotic Index: 5.21
Scraper/Filterer Collector Ratio: 0.02
Shredder/Total Ratio: 0.22
E+P+T*: 3 *(Ephemeroptera, Plecoptera and Trichoptera)
%EPT: 41.00
EPT/C*: 4.56 *(Chironomidae)
NJIS Score: 24
Biological Condition: non-impaired
Deficiency(s) noted: none

Observations

Streamwater: slightly turbid...Flow: moderate...Width/Depth(ft): 15/<1...Substrate: gravel...Streambank Vegetation/Stability: good/good...Canopy: open...Other: brush-lined/commercial

Appendix A

Raritan Basin - Jamesburg USGS Quadrangle
Station AN0439
Manalapan Brook, Federal Road, Monroe Township
September 29, 1993

Family	Number of Individuals	Family Tolerance Value (FTV)
Hemiptera	45	8
Chironomidae	5	6
Aeshnidae	2	3
Simuliidae	1	6
Libellulidae	1	9
Sphaeriidae	1	8
Pyralidae	1	5
Nemertea	1	5
Gastropoda	1	7

Statistical Analysis

Number of Taxa: 9
Total Number of Individuals: 58
% Contribution of Dominant Family: 77.59
Family Biotic Index: 7.52
Scraper/Filterer Collector Ratio: 0.00
Shredder/Total Ratio: 0.00
E+P+T*: 0 *(Ephemeroptera, Plecoptera and Trichoptera)
%EPT: 0.00
EPT/C*: 0.00 *(Chironomidae)
NJIS Score: 3
Biological Condition: severely impaired
Deficiency(s) noted: Hemiptera overwhelmingly dominant, paucity of clean water organisms, significant organic pollution

Observations

Streamwater: turbid...Flow: moderate...Width/Depth(ft): 20/1...Substrate: sand/gravel/concrete stones...Streambank Vegetation/Stability: good/good...Canopy: open...Other: wooded/grass fields

Appendix A

Raritan Basin - Freehold USGS Quadrangle
Station AN0447
McGellairds Brook, Route 527, Englishtown
September 14, 1993

Family	Number of Individuals	Family Tolerance Value (FTV)
Tubificidae	80	10
Sphaeriidae	1	8
Chironomidae	8	6
Gastropoda	2	7
Tipulidae	2	3
Nemertea	4	5
Sialidae	1	4
Hydropsychidae	1	4
Coenagrionidae	3	9
BloodRedChironomidae	1	8
Anthomyiidae	1	6
Elmidae	1	4

Statistical Analysis

Number of Taxa: 12
Total Number of Individuals: 105
% Contribution of Dominant Family: 76.19
Family Biotic Index: 9.04
Scraper/Filterer Collector Ratio: 1.00
Shredder/Total Ratio: 0.07
E+P+T*: 1 *(Ephemeroptera, Plecoptera and Trichoptera)
%EPT: 0.95
EPT/C*: 0.11 *(Chironomidae)
NJIS Score: 6
Biological Condition: severely impaired
Deficiency(s) noted: paucity of clean water organisms, Tubificidae overwhelmingly dominant, significant organic pollution

Observations

Streamwater: turbid...Flow: slow...Width/Depth(ft): 30/1...Substrate: sand/mud...Streambank Vegetation/Stability: fair/fair...Canopy: open...Other: tree-lined/suburban/residential; fish

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APPENDIX B
STATISTICAL DATA

Sediment Toxicity Tests
Watershed Management Area # 9 and 10
Millstone and South Rivers

Appendix B

Sediment Toxicity Tests
Watershed Management Area # 9 and 10

SURVIVAL RESULTS

CONTROL VS. AN0379 (reference)

Survival Proportions with Arc Sine Square Root Transformation

Blank	AN0379	Blank Trans	AN0379 Trans
0.9	1.0	1.249	1.4127
1.0	1.0	1.4127	1.4127
1.0	0.9	1.4127	1.249
1.0	0.9	1.4127	1.249
1.0	0.9	1.4127	1.249

Shapiro-Wilks Test for Normality

Blank Trans	AN0379 Trans	Pooled	Mean	Centered	Ordered	D-value	W-value	Critical-W (0.05)	Result
1.249	1.4127	1.249		-0.0982	-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
1.4127	1.249	1.4127		0.0655	-0.0982				
1.4127	1.249	1.4127		0.0655	0.0655				
		1.4127		0.0655	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				
		1.249		-0.0982	0.0655				

Appendix B

Sediment Toxicity Tests
Watershed Management Area # 9 and 10

SURVIVAL RESULTS continued...

CONTROL VS. AN0379 (reference)
Wilcoxan Rank Sum Test

Pooled	Sorted	Point	Wilcoxan Rank	Blank	AN0379	Critical(from Table K=1)	Result
1.249	1.249	10	2.5	0	2.5	19	No Significant Difference
1.4127	1.249	9	2.5	0	2.5		
1.4127	1.249	8	2.5	0	2.5		
1.4127	1.249	1	2.5	2.5	0		
1.4127	1.4127	7	7.5	0	7.5		
1.4127	1.4127	6	7.5	0	7.5		
1.4127	1.4127	5	7.5	7.5	0		
1.249	1.4127	4	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		
				Sum	Sum		
				32.5	22.5		

Appendix B

Sediment Toxicity Tests
Watershed Management Area # 9 and 10

SURVIVAL RESULTS continued...

AN0379 (reference) VS. AN0439

Survival Proportions with Arc Sine Square Root Transformation

AN0379	AN0439	AN0379 Trans	AN0439 Trans
1.0	0.9	1.4127	1.249
1.0	1.0	1.4127	1.4127
0.9	1.0	1.249	1.4127
0.9	1.0	1.249	1.4127
0.9	1.0	1.249	1.4127

Shapiro-Wilks Test for Normality

AN0379 Trans	AN0439 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				
1.249	1.4127	1.249	1.3472	-0.0982	-0.0982	0.0643	0.6403	0.842	Not Normal
1.249	1.4127	1.249		-0.0982	-0.0982				
1.249	1.4127	1.249		-0.0982	0.0655				
		1.249		-0.0982	0.0655				
Mean	Mean	1.4127		0.0655	0.0655				
1.3145	1.38	1.4127		0.0655	0.0655				
		1.4127		0.0655	0.0655				
		1.4127		0.0655	0.0655				

Appendix B

Sediment Toxicity Tests
Watershed Management Area # 9 and 10

SURVIVAL RESULTS continued...

AN0379 (reference) VS. AN0439

Wilcoxan Rank Sum Test

Pooled	Sorted	Point	Wilcoxan Rank	AN0379	AN0439	Critical(from Table K=1)	Result
1.4127	1.249	6	2.5	0	2.5	19	No Significant Difference
1.4127	1.249	5	2.5	2.5	0		
1.249	1.249	4	2.5	2.5	0		
1.249	1.249	3	2.5	2.5	0		
1.249	1.4127	10	7.5	0	7.5		
1.249	1.4127	9	7.5	0	7.5		
1.4127	1.4127	8	7.5	0	7.5		
1.4127	1.4127	7	7.5	0	7.5		
1.4127	1.4127	2	7.5	7.5	0		
1.4127	1.4127	1	7.5	7.5	0		
				Sum	Sum		
				22.5	32.5		

Appendix B

Sediment Toxicity Tests
Watershed Management Area # 9 and 10

SURVIVAL RESULTS continued...

AN0379 (reference) VS. AN0447

Survival Proportions with Arc Sine Square Root Transformation

AN0379	AN0447	AN0379 Trans	AN0447 Trans
1.0	0.9	1.4127	1.249
1.0	1.0	1.4127	1.4127
0.9	1.0	1.249	1.4127
0.9	0.9	1.249	1.249
0.9	1.0	1.249	1.4127

Shapiro-Wilks Test for Normality

AN0379 Trans	AN0447 Trans	Pooled	Mean	Centered	Ordered	D-value	W-value	Critical-W (0.05)	Result
1.4127	1.249	1.4127		0.0818	-0.0819				
1.4127	1.4127	1.4127		0.0818	-0.0819				
1.249	1.4127	1.249	1.3309	-0.0819	-0.0819	0.067	0.6547	0.842	Not Normal
1.249	1.249	1.249		-0.0819	-0.0819				
1.249	1.4127	1.249		-0.0819	-0.0819				
		1.249		-0.0819	0.0818				
Mean	Mean	1.4127		0.0818	0.0818				
1.3145	1.3472	1.4127		0.0818	0.0818				
		1.249		-0.0819	0.0818				
		1.4127		0.0818	0.0818				

Appendix B

Sediment Toxicity Tests
Watershed Management Area # 9 and 10

SURVIVAL RESULTS continued...

AN0379 (reference) VS. AN0447

Wilcoxan Rank Sum Test

Pooled	Sorted	Point	Wilcoxan Rank	AN0379	AN0447	Critical(from Table K=1)	Result
1.4127	1.249	9	3	0	3	19	No Significant Difference
1.4127	1.249	6	3	0	3		
1.249	1.249	5	3	3	0		
1.249	1.249	4	3	3	0		
1.249	1.249	3	3	3	0		
1.249	1.4127	10	8	0	8		
1.4127	1.4127	8	8	0	8		
1.4127	1.4127	7	8	0	8		
1.249	1.4127	2	8	8	0		
1.4127	1.4127	1	8	8	0		
				Sum	Sum		
				25	30		

Appendix B

Sediment Toxicity Tests
Watershed Management Area # 9 and 10

GROWTH

CONTROL VS. AN0379 (reference)

Average Dry Weight per Replicate (in mg)

BLANK	AN0379
0.027	0.132
0.055	0.107
0.060	0.177
0.045	0.149
0.048	0.166

Shapiro-Wilks Test for Normality

BLANK	AN0379	Pooled	Mean	Centered	Ordered	D-value	W-value	Critical-W (0.05)	Result
0.027	0.132	0.027		-0.0696	-0.0696				
0.055	0.107	0.055		-0.0416	-0.0516				
0.06	0.177	0.06	0.0966	-0.0366	-0.0486	0.0283	0.8914	0.842	Normal
0.045	0.149	0.045		-0.0516	-0.0416				
0.048	0.166	0.048		-0.0486	-0.0366				
		0.132		0.0354	0.0104				
Mean	Mean	0.107		0.0104	0.0354				
0.047	0.1462	0.177		0.0804	0.0524				
		0.149		0.0524	0.0694				
		0.166		0.0694	0.0804				

F-Test and T-Test

BLANK Var	AN0379 Var	F-Value	Critical-F (Two-Tailed 0.05)	Variances	T-value	Deg. of Freedom	Critical-T (One-Tailed 0.05)	Result
0.0002	0.0008	4	6.3882	Equal	-7.0145	5	2.015	No Significant Difference

Appendix B

Sediment Toxicity Tests
Watershed Management Area # 9 and 10

GROWTH continued...

AN0379 (reference) VS. AN0439

Average Dry Weight per Replicate (in mg)

AN0379	AN0439
0.132	0.167
0.107	0.216
0.177	0.182
0.149	0.204
0.166	0.203

Shapiro-Wilks Test for Normality

AN0379	AN0439	Pooled	Mean	Centered	Ordered	D-value	W-value	Critical-W (0.05)	Result
0.132	0.167	0.132		-0.0383	-0.0633				
0.107	0.216	0.107		-0.0633	-0.0383				
0.177	0.182	0.177	0.1703	0.0067	-0.0213	0.0104	0.9648	0.842	Normal
0.149	0.204	0.149		-0.0213	-0.0043				
0.166	0.203	0.166		-0.0043	-0.0033				
		0.167		-0.0033	0.0067				
Mean	Mean	0.216		0.0457	0.0117				
0.1462	0.1944	0.182		0.0117	0.0327				
		0.204		0.0337	0.0337				
		0.203		0.0327	0.0457				

F-Test and T-Test

AN0379 Var	AN0439 Var	F-Value	Critical-F (Two-Tailed 0.05)	Variances	T-value	Deg. of Freedom	Critical-T (One-Tailed 0.05)	Result
0.0008	0.0004	2	6.3882	Equal	-3.1113	7	1.8946	No Significant Difference

Appendix B

Sediment Toxicity Tests
Watershed Management Area # 9 and 10

GROWTH continued...

AN0379 (reference) VS. AN0447

Average Dry Weight per Replicate (in mg)

AN0379	AN0447
0.132	0.166
0.107	0.171
0.177	0.198
0.149	0.202
0.166	0.169

Shapiro-Wilks Test for Normality

AN0379	AN0447	Pooled	Mean	Centered	Ordered	D-value	W-value	Critical-W (0.05)	Result
0.132	0.166	0.132		-0.0317	-0.0567				
0.107	0.171	0.107		-0.0567	-0.0317				
0.177	0.198	0.177	0.1637	0.0133	-0.0147	0.0073	0.943	0.842	Normal
0.149	0.202	0.149		-0.0147	0.0023				
0.166	0.169	0.166		0.0023	0.0023				
		0.166		0.0023	0.0053				
Mean	Mean	0.171		0.0073	0.0073				
0.1462	0.1812	0.198		0.0343	0.0133				
		0.202		0.0383	0.0343				
		0.169		0.0053	0.0383				

F-Test and T-Test

AN0379 Var	AN0447 Var	F-Value	Critical-F (Two-Tailed 0.05)	Variances	T-value	Deg. of Freedom	Critical-T (One-Tailed 0.05)	Result
0.0008	0.0003	2.6667	6.3882	Equal	-2.3597	6	1.9432	No Significant Difference