Sediment Toxicity Test Results

Alma Train Derailment 11/07/2015
Sediment Study

Tested December 2015 – January 2016

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Laboratory Workorder Number: 232374
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Reviewed Date: February 10, 2016

Introduction

Project: Alma Train Derailment 11/07/2015 – Sediment Toxicity Testing
Samples Collected By: Cardno (Jody Kubitz)
Sample Location: Mississippi River near Alma, Wisconsin
Sample Collection Date: 12/10/2015
Date *Hyalella azteca* Test Initiated: 12/29/2015
Date *Chironomus dilutus* Test Initiated: 1/12/2016
Test Type: 10 Day Solid Phase Freshwater Sediment Toxicity Test
Test Species:
Hyalella azteca (amphipod)
Age: 13 days
Source: WSLH Culture
Chironomus dilutus (larval midge)
Age: Second to third instar larvae
Source: WSLH Culture
Test Conditions: See Table 2 (USEPA, 2000)

Table 1. Sample Site Descriptions

WSLH Lab Number	Site Name	Description
232374001	Lab Control (LC)	Synthetic sediment
232374002	WDNR 1	Side Channel Upstream Sample
232374003	WDNR 2	Side Channel Middle Sample
232374004	WDNR 3	Side Channel Downstream Sample
232374005	WDNR 4	Backwater Contiguous Sample
232374006	WDNR 5	Backwater Contiguous Reference Sample
232374007	WDNR 6	Side Channel Reference Sample

Test Methods

Sediment tests are conducted using two freshwater organisms, *Hyalella azteca* and *Chironomus dilutus* (formally *Chironomus tentans*). These two organisms, which burrow and come into direct contact with the sediments, are recommended for use in sediment toxicity testing by the USEPA (2000).

Chironomus dilutus

Chironomus dilutus were exposed to sediment samples for ten days with ten organisms per beaker and eight replicates per sediment site. Dissolved oxygen, pH, and temperature of the overlying water were recorded daily. Hardness, alkalinity, ammonia, and conductivity of overlying water were measured at the beginning and at the end of the test (day 0 and day 10, respectively). On day 10, the organisms were recovered from the sediment to determine the number of survivors. Surviving organisms were subsequently dried overnight at 100°C and weighed to determine dry weight. The organisms were then ashed at 550°C for a minimum of 2 hours and weighed to determine ash-free dry weight (USEPA, 2000). There were no noted deviations from established test protocols.

Hyalella azteca

Hyalella azteca were exposed to sediment samples for ten days with ten organisms per beaker and eight replicates per sediment site. Dissolved oxygen, pH, and temperature of the overlying water were recorded daily. Hardness, alkalinity, ammonia, and conductivity of overlying water were measured at the beginning and at the end of the test (day 0 and day 10, respectively). On day 10, the organisms were recovered from the sediment to determine the number of survivors in each replicate. Survivors were subsequently dried overnight at 100°C and weighed to determine dry weight (USEPA, 2000). There were no noted deviations from established test protocols.

Statistical analyses

Statistical analyses were conducted using a PC-version of SAS® (SAS Institute, Cary, NC). One-way analysis of variance (ANOVA) followed by a multiple comparison test (Student-Newman-Keuls) was used to identify differences among treatments in survival and weight of survivors of *Chironomus dilutus* and *Hyalella azteca.* Results with $p < 0.05$ were considered significant.

Summary of Results

Overlying Water Chemical Parameters

Dissolved Oxygen and Temperature

Dissolved oxygen (DO) and temperature values in overlying water were within acceptable limits for most of the tests according to USEPA (2000) criteria, see Table 2. Initial DO and the minimum and maximum DO measured during the tests are presented in Figures 3 and 7. At the WDNR1 site, DO dropped below 2.5 mg/L in the *C. dilutus* test on days 1, 2, and 4 with a DO range of 0.83 - 1.71 mg/L; and on days 1 – 4 in the *H. azteca* test with a DO range of 0.62 - 2.02 mg/L. According to the USEPA (2000), periodic depressions of DO below 2.5 mg/L that are still above 1.5 mg/L are not likely to adversely affect test results. Temperatures in the *C. dilutus* overlying water were out of range on day 0 by a maximum of 1.6°C & on day 9 by a maximum of 0.6°C. However temperatures in the environmental chamber where the test was being conducted were within acceptable range for the entire ten day testing period.

pH

There are no required pH criteria but the pH of overlying waters were all in the neutral range with pH ranging from 7.90 to 8.71 s.u.

Hardness, Alkalinity, and Ammonia

Results of hardness, alkalinity, and ammonia analyses from samples are summarized in Figures 4 and 8. According to USEPA (2000), values for hardness, alkalinity, and ammonia should not vary by more than 50% during the test. Values for hardness and alkalinity in both species varied less than 50%.

Ammonia values varied by greater than 50% in many of the sites for both species. However, the overall levels of ammonia in overlying water were low (≤ 4.00 mg/L) and not at levels that have been associated with toxicity in past sediment tests (20 - 310 mg/L, USEPA, 2000).

Conductivity

Conductivity ranged from 701 to 829 µS/cm. There are no established criteria for acceptability of conductivity results.

Overall Survival and Growth

Survival results are depicted in Figures 1 and 5. Growth results, as weights, are depicted in Figures 2 and 6. Statistical analyses indicated significant ($p < 0.05$) differences among sites in the growth of *C. dilutus* and of *H. azteca*. There was also a significant difference in survival of *C. dilutus* at one site.

Chironomus dilutus Survival and Growth

C. dilutus Survival

Survival data for *C. dilutus* is presented in Figure 1. Survival of *C. dilutus* was significantly lower in site WDNR1 than for the lab control. Survival at all other sites was not significantly different from the lab control.

C. dilutus Growth

Growth data for *C. dilutus*, as ash free dry weight (AFDW), is given in Figure 2. Larval weights ranged from 0.45 mg to 2.59 mg per surviving larval *C. dilutus* for all sediment sites. The lab control sediment averaged 1.69 mg per surviving larvae.

C. dilutus AFDW was significantly higher by 21.4% at site WDNR2 compared to the lab control.

C. dilutus AFDW was not significantly different between sites WDNR4, WDNR5, and the lab control.

The AFDW was not significantly different between sites WDNR3 and WDNR6, however both sites were significantly lower when compared to the lab control, WDNR2, WDNR4, and WDNR5.

The AFDW at site WDNR1 was significantly different from both the lab control and from all other sites with the exception of site WDNR6.

Hyalella azteca Survival and Growth

H. azteca Survival

Survival data for *H. azteca* is presented in Figure 5. Survival of *H. azteca* was not significantly different among any of the sites and the lab control.

H. azteca Growth

Growth data, as dry weight, for *H. azteca* is given in Figure 6. The weights ranged from 0.09 mg to 0.37 mg per surviving *H. azteca* for all of the sediment sites. The control sediment averaged 0.17 mg per surviving individual.

H. azteca dry weight was significantly higher at sites WDNR1, WDNR3, and WDNR4 compared to the lab control.

Dry weight of *H. azteca* was not significantly different between sites WDNR2, WDNR6, and the lab control.

The dry weight at site WDNR5 was significantly different from both the lab control and from all other sites.

Quality Control

Tests for both species met the minimum requirements for test acceptability at test termination (see Table 2). *C. dilutus* survival was 70% in the control sediment and the average weight per surviving individual was greater than 0.48 mg. *H. azteca* survival in the control sediment was 88.8% and the average weight increased over the length of the test.

Reference toxicity tests performed with both species resulted in a toxic response (LC_{50}) within control limits established during previous reference toxicant testing.

Conclusions

Some of the sediment collected in the Mississippi River near Alma, WI appears to have had an impact on the freshwater sediment test organisms, *Chironomus dilutus* and *Hyalella azteca*.

Chironomus dilutus survival was not significantly different at five of the six sites tested when compared to the lab control. The one site where significantly lower survival was observed was at WDNR1. In addition, the growth of *C. dilutus* larvae appears to have been significantly reduced at this same site (WDNR1) as well as at sites WDNR3 and WDNR6.

Hyalella azteca survival was not impacted at any of the test sites when compared to the lab control. There were, however, some significant differences in growth among the test sites. Specifically, growth at site WDNR5 was significantly lower when compared to both the lab control and all other sites tested.

The differences in the impact on the two species may be a result of the fact that *C. dilutus* larvae burrow into the sediment and actually ingest the sediment to some degree while *H. azteca* reside in the pore water at the sediment-water interface and do not ingest the sediment.

It should be noted that at test set-up, the lab staff observed that some sediment collected at the sites contained indigenous organisms and staff made an effort to remove them prior to testing. Organisms included: mussels (WDNR1, WDNR2, WDNR3, WDNR6), native *Hyalella sp.* (WDNR1, WDNR2, WDRN6), dragon fly larvae (WDNR5), midges (WDNR3), and leeches (WDNR4).

References

Shobanov NA, II Kiknadze, MG Butler. Palearctic and Nearctic Chironomus (Camptochironomus) dilutus (Fabricius) are different species (Diptera: Chironomidae). 1999. Entomologica Scandinavica, 30, 3, 311-322.

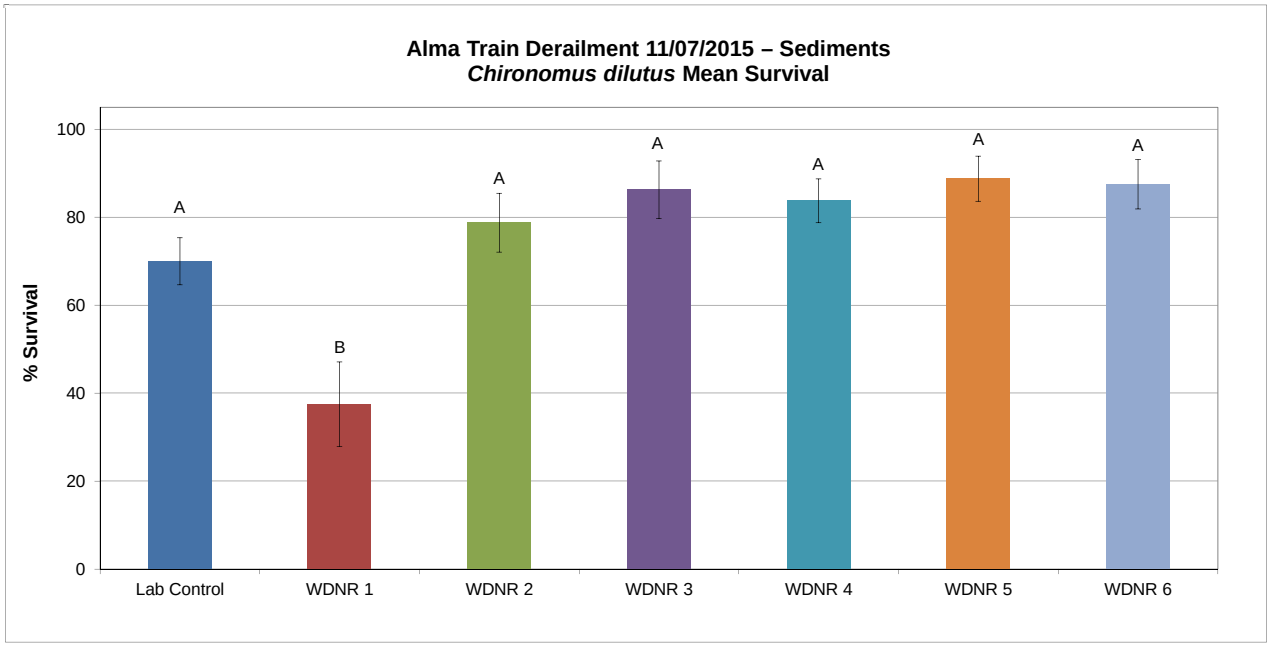
United States Environmental Protection Agency (USEPA). Methods for measuring the toxicity and bioaccumulation of sediment-associated contaminants with freshwater invertebrates. Second Edition. 2000. EPA/600/R-99/064. Office of Research and Development, U.S. Environmental Protection Agency, Washington, D.C.

Table 2. Summary of Test Conditions for Conducting Sediment Toxicity Tests with *C. dilutus* and *H. azteca*

	Parameter	**Conditions**
1.	Test Type	Whole sediment toxicity test with renewal of overlying water
2.	Temperature	23 ± 1°C
3.	Light Quality	Wide-spectrum fluorescent lights
4.	Illuminance	100 to 1,000 lux
5.	Photoperiod	16hr Light: 8hr Dark
6.	Test Chamber	470 mL polypropylene Beaker
7.	Sediment Volume	100 mL
8.	Overlying Water Volume	175 mL
9.	Renewal of Overlying Water	2 volume additions/day
10.	Age of Organisms	Second to third instar larvae (*C. dilutus*) 7 to 14 day old, within a 1 to 2 day range (*H. azteca)*
11.	Number of organisms/chamber	10
12.	Number of replicates/treatment	8
13.	Feeding	1.0 mL YFC (1,800 mg/L stock) daily to each test chamber (*H. azteca*) 1.5 mL Tetramin flake fish food mixture (1.5 mL contains 6.0 mg of dry solids) to each test chamber (*C. dilutus*)
14.	Aeration	None, unless dissolved oxygen in overlying water drops below 2.5 mg/L
15.	Overlying water	Dechlorinated tap water
16.	Test chamber cleaning	If screens become clogged during a test, gently brush outside of screen
17.	Overlying water quality	Hardness, alkalinity, ammonia, DO, pH, and conductivity at the beginning and end of a test. Temperature, pH, and DO daily.
18.	Test duration	10 day
19.	Endpoints	Survival and growth (dry weight) (*H. azteca*) Survival and growth (ash free dry weight) (*C. dilutus*)
20.	Test acceptability	Minimum mean control survival of 70%, minimum mean weight per surviving control organism of 0.48 mg ash free dry weight (*C. dilutus*); Minimum mean control survival of 80% and measurable growth of test organisms in the control sediment (*H. azteca*)

Figure 1
Alma Train Derailment 11/07/2015 – Sediment Toxicity Testing
***Chironomus dilutus* Survival**
Sediment Collected: December 10, 2015
Test Date: January 12, 2016

			Percent Survival per Replicate									
Lab Number	**Site Name**	**Description**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**Mean**	**Std Dev**
232374001	Lab Control	Synthetic sediment	80	60	70	60	50	90	60	90	70.0	5.3
232374002	WDNR 1	WDNR 1	10	30	40	30	40	20	100	30	37.5	9.6
232374003	WDNR 2	WDNR 2	70	90	90	80	40	90	100	70	78.8	6.7
232374004	WDNR 3	WDNR 3	100	100	100	80	50	90	100	70	86.3	6.5
232374005	WDNR 4	WDNR 4	60	100	90	70	80	100	80	90	83.8	5.0
232374006	WDNR 5	WDNR 5	80	100	90	100	100	80	60	100	88.8	5.2
232374007	WDNR 6	WDNR 6	60	80	100	100	90	100	100	70	87.5	5.6

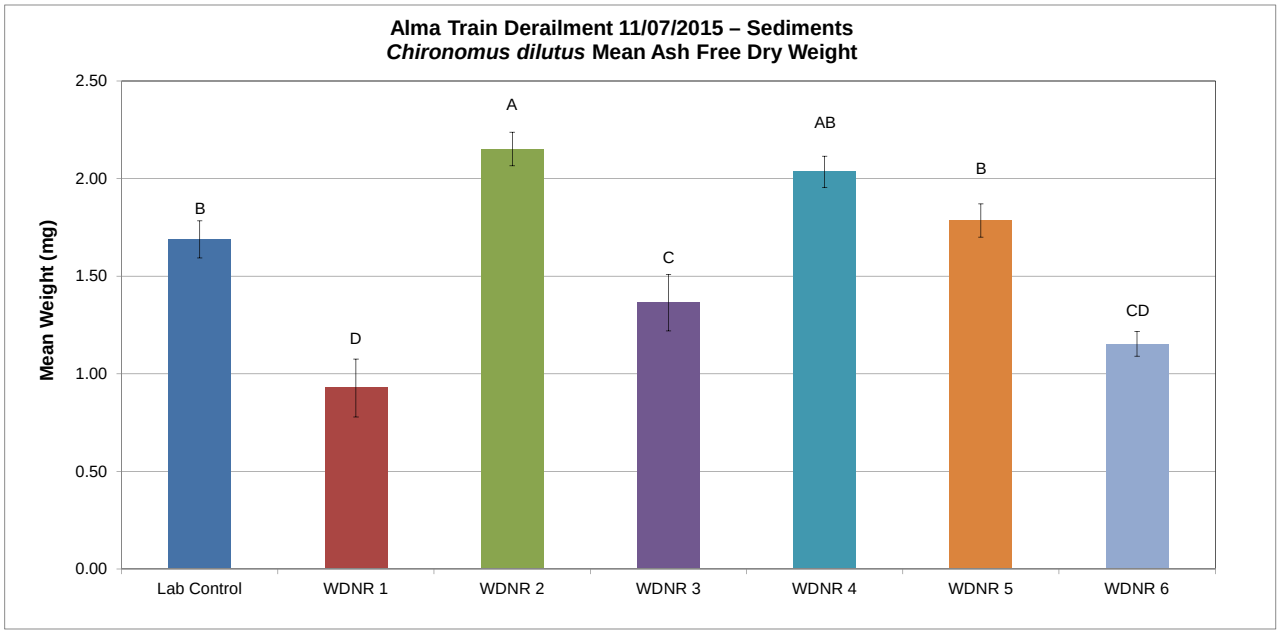


Results with $p < 0.05$ are considered significant.
Graph bars with the same letter are not statistically different from one another.

Figure 2
Alma Train Derailment 11/07/2015 – Sediment Toxicity Testing
***Chironomus dilutus* Ash Free Dry Weight (AFDW)**
Sediment Collected: December 10, 2015
Test Date: January 12, 2016

mg/Surviving Larva per Replicate

Lab Number	Site Name	Description	1	2	3	4	5	6	7	8	Mean	Std Dev
232374001	Lab Control	Synthetic sediment	1.80	1.86	1.76	1.14	1.66	1.49	2.03	1.77	1.69	0.10
232374002	WDNR 1	WDNR 1	0.92	0.45	0.51	0.95	0.72	1.33	0.82	1.72	0.93	0.15
232374003	WDNR 2	WDNR 2	2.59	1.82	2.19	2.11	2.07	2.14	1.92	2.38	2.15	0.09
232374004	WDNR 3	WDNR 3	1.47	1.35	0.74	1.41	2.06	1.34	0.90	1.64	1.36	0.14
232374005	WDNR 4	WDNR 4	2.29	1.80	1.80	2.20	2.03	1.73	2.22	2.21	2.03	0.08
232374006	WDNR 5	WDNR 5	1.97	1.69	2.24	1.80	1.78	1.50	1.81	1.51	1.79	0.09
232374007	WDNR 6	WDNR 6	1.18	1.48	0.95	1.10	1.25	1.01	1.27	0.98	1.15	0.06



Results with $p < 0.05$ are considered significant.
Graph bars with the same letter are not statistically different from one another.

Figure 3
Alma Train Derailment 11/07/2015 – Sediment Toxicity Testing
***Chironomus dilutus* Dissolved Oxygen (mg/L)**
Sediment Collected: December 10, 2015
Test Date: January 12, 2016

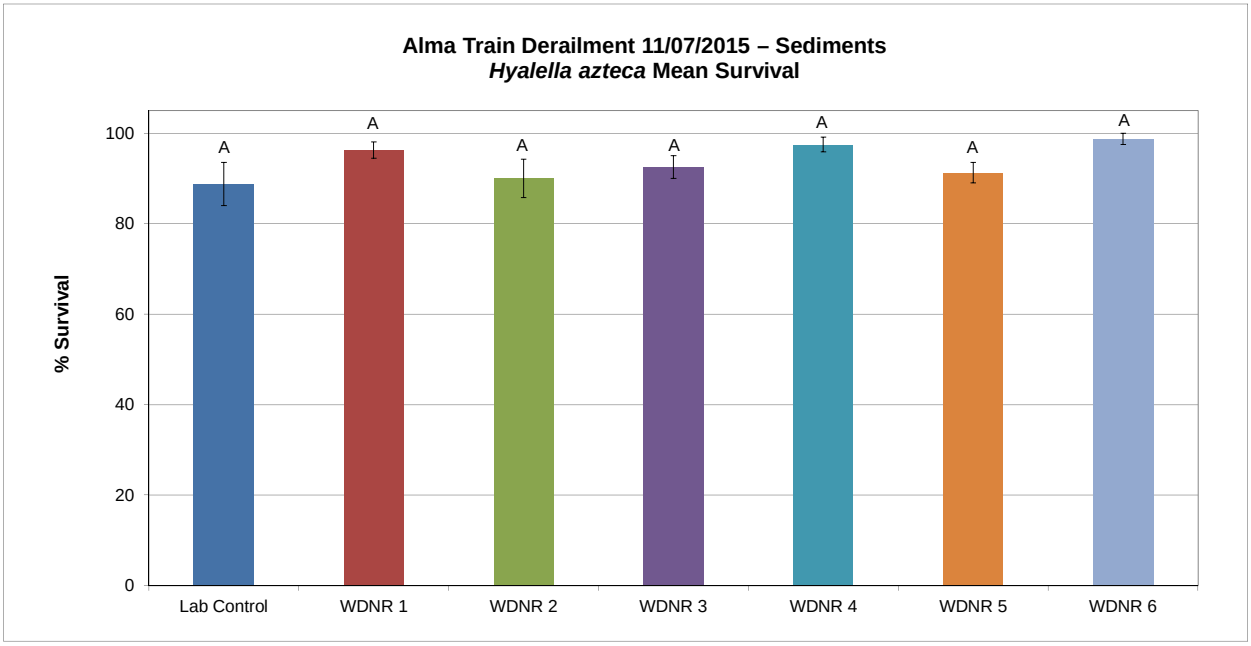
Lab Number	Site Name	Initial	Minimum	Maximum
232374001	Lab Control	8.44	3.68	8.44
232374002	WDNR 1	4.53	0.83	4.97
232374003	WDNR 2	8.13	3.42	8.13
232374004	WDNR 3	8.26	3.15	8.26
232374005	WDNR 4	7.97	3.04	7.97
232374006	WDNR 5	7.70	3.27	7.70
232374007	WDNR 6	7.73	4.04	7.73

Figure 4
Alma Train Derailment 11/07/2015 – Sediment Toxicity Testing
Chironomus dilutus
Hardness, Alkalinity, and Ammonia
Sediment Collected: December 10, 2015
Test Date: January 12, 2016

			Hardness (mg/L)		Alkalinity (mg/L)		Ammonia (mg/L)	
Lab Number	**Site Name**	**Description**	Initial (day 0)	Final (day 10)	Initial (day 0)	Final (day 10)	Initial (day 0)	Final (day 10)
232374001	Lab Control	Synthetic sediment	204	184	323	318	0.14	1.52
232374002	WDNR 1	WDNR 1	220	164	367	311	2.99	4.00
232374003	WDNR 2	WDNR 2	164	160	288	291	0.64	1.40
232374004	WDNR 3	WDNR 3	192	196	lab error - no sample collected	286	1.21	0.20
232374005	WDNR 4	WDNR 4	188	148	298	282	1.16	1.20
232374006	WDNR 5	WDNR 5	212	212	311	278	1.79	1.43
232374007	WDNR 6	WDNR 6	188	156	309	276	1.18	0.36

Figure 5
Alma Train Derailment 11/07/2015 – Sediment Toxicity Testing
***Hyalella azteca* Survival**
Sediment Collected: December 10, 2015
Test Date: December 29, 2015

			Percent Survival per Replicate									
Lab Number	**Site Name**	**Description**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**Mean**	**Std Dev**
232374001	Lab Control	Synthetic sediment	70	70	100	100	100	90	100	80	88.8	4.8
232374002	WDNR 1	WDNR 1	100	100	90	90	100	100	100	90	96.3	1.8
232374003	WDNR 2	WDNR 2	90	80	100	100	100	100	70	80	90.0	4.2
232374004	WDNR 3	WDNR 3	90	90	80	90	100	100	90	100	92.5	2.5
232374005	WDNR 4	WDNR 4	90	100	100	100	100	100	90	100	97.5	1.6
232374006	WDNR 5	WDNR 5	90	90	90	90	100	90	80	100	91.3	2.3
232374007	WDNR 6	WDNR 6	90	100	100	100	100	100	100	100	98.8	1.3



Results with $p < 0.05$ are considered significant.
Graph bars with the same letter are not statistically different from one another.

Figure 6
Alma Train Derailment 11/07/2015 – Sediment Toxicity Testing
***Hyalella azteca* Dry Weight per Surviving Individual (mg)**
Sediment Collected: December 10, 2015
Test Date: December 29, 2015

mg/Surviving *H. azteca* per Replicate

Lab Number	Site Name	Description	1	2	3	4	5	6	7	8	Mean	Std Dev
232374001	Lab Control	Synthetic sediment	0.20	0.21	0.16	0.17	0.17	0.13	0.16	0.19	0.17	0.01
232374002	WDNR 1	WDNR 1	0.27	0.33	0.32	0.21	0.28	0.22	0.21	0.19	0.25	0.02
232374003	WDNR 2	WDNR 2	0.14	0.19	0.17	0.17	0.17	0.14	0.19	0.14	0.16	0.01
232374004	WDNR 3	WDNR 3	0.32	0.35	0.35	0.28	0.26	0.37	0.30	0.21	0.30	0.02
232374005	WDNR 4	WDNR 4	0.24	0.25	0.26	0.26	0.26	0.26	0.23	0.18	0.24	0.01
232374006	WDNR 5	WDNR 5	0.11	0.14	0.10	0.11	0.11	0.12	0.11	0.09	0.11	0.01
232374007	WDNR 6	WDNR 6	0.16	0.16	0.14	0.15	0.13	0.16	0.18	0.10	0.15	0.01

Results with $p < 0.05$ are considered significant.
Graph bars with the same letter are not statistically different from one another.

Figure 7
Alma Train Derailment 11/07/2015 – Sediment Toxicity Testing
***Hyalella azteca* Dissolved Oxygen (mg/L)**
Sediment Collected: December 10, 2015
Test Date: December 29, 2015

Lab Number	Site Name	Initial	Minimum	Maximum
232374001	Lab Control	8.24	6.05	8.24
232374002	WDNR 1	5.58	0.62	7.77
232374003	WDNR 2	8.40	6.09	8.40
232374004	WDNR 3	7.86	5.16	7.86
232374005	WDNR 4	7.93	5.66	7.93
232374006	WDNR 5	8.16	5.47	8.16
232374007	WDNR 6	8.10	4.70	8.10

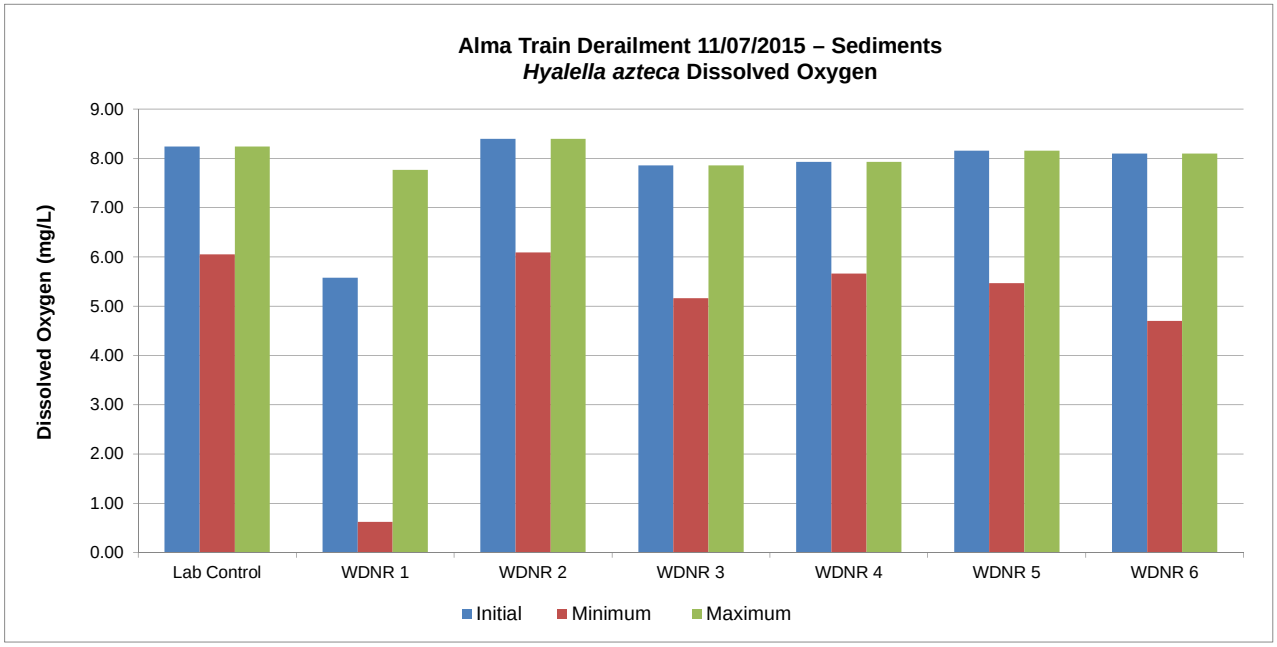


Figure 8
Alma Train Derailment 11/07/2015 – Sediment Toxicity Testing
Hyalella azteca
Hardness, Alkalinity, and Ammonia
Sediment Collected: December 10, 2015
Test Date: December 29, 2015

			Hardness (mg/L)		Alkalinity (mg/L)		Ammonia (mg/L)	
Lab Number	**Site Name**	**Description**	Initial (day 0)	Final (day 10)	Initial (day 0)	Final (day 10)	Initial (day 0)	Final (day 10)
232374001	Lab Control	Synthetic sediment	208	172	323	306	0.12	0.25
232374002	WDNR 1	WDNR 1	220	176	343	322	0.57	2.36
232374003	WDNR 2	WDNR 2	172	152	300	286	0.69	0.13
232374004	WDNR 3	WDNR 3	184	152	298	284	0.88	0.17
232374005	WDNR 4	WDNR 4	184	172	303	276	0.80	0.18
232374006	WDNR 5	WDNR 5	216	164	315	283	1.10	0.09
232374007	WDNR 6	WDNR 6	184	164	310	294	0.60	0.22