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**Great  
Lakes  
Environmental  
Center**

Applied  
Environmental  
Sciences  
www.glec.com

Tony Coryell, Project Manager  
AECOM  
800 Lasalle Avenue, Suite 500  
Minneapolis, MN 55402  
Phone: 612.376.2000

**Traverse City  
Operations**

739 Hastings St.  
Traverse City  
MI 49686

231 941-2230  
231 941-2240 fax

**Columbus  
Operations**

1295 King Ave.  
Columbus  
OH 43212

614 487-1040  
614 487-1920 fax

**SUBJECT:** Whole Sediment Toxicity Testing Report  
10-day Toxicity Tests using *Hyalella azteca* and *Chironomus dilutus*.  
Pigs Eye Lake, St. Paul, Minnesota  
GLEC Project Number: 2391

Dear Mr. Coryell:

Great Lakes Environmental Center, Inc. (GLEC) has completed our analysis of the *Chironomus dilutus* (*C. dilutus*) and *Hyalella azteca* (*H. azteca*) 10-day survival and growth whole sediment toxicity tests. These tests were performed with six sediment samples that were collected by AECOM personnel on November 7, 2016 from Pigs Eye Lake located near St. Paul, Minnesota.

The sample identification numbers, survival, and growth test results for the six investigative sediment samples and a laboratory control are summarized and provided in the following tables:

- Table 1: 10-Day *Chironomus dilutus* (*C. dilutus*) Average Percent Survival
- Table 2: 10-Day *C. dilutus* Average Growth and Biomass Estimates (expressed as average ash-free-dry-weight (AFDW))
- Table 3: 10-Day *Hyalella azteca* (*H. azteca*) Average Percent Survival
- Table 4: 10-Day *H. azteca* Average Growth and Biomass Estimates

Water quality data for the overlying water for each sediment sample tested are summarized in Table 5 for the *C. dilutus* tests and Table 6 for the *H. azteca* tests. Summaries of the statistically significant differences ( $p=0.05$ ) between the laboratory control and all six investigative sediment samples collected from Pigs Eye Lake are provided in Table 7 for the *C. dilutus* tests and in Table 8 for the *H. azteca* tests.

Detailed summaries of the overlying water quality measurements are provided in Appendices B1 (*C. dilutus*) and B2 (*H. azteca*). The survival, growth, and statistical data sheets and summaries for the *C. dilutus* and *H. azteca* tests are shown in Appendices C1 through C2 and D1 through D2, respectively. The daily laboratory bench data sheets are kept on file at GLEC and are also provided on the enclosed compact diskette. Chain of Custody forms and reference toxicant data are provided in Appendices A and E, respectively.

## METHODS

The whole sediment toxicity tests were conducted at our Traverse City, Michigan laboratory following GLEC's written Standard Operating Procedures (SOPs) which are based on the procedures outlined in U.S. EPA Method, EPA/600/R-99/064 *Methods for Measuring the Toxicity and Bioaccumulation of Sediment-Associated Contaminants with Freshwater Invertebrates*, Second Edition and American Society for Testing and Materials (ASTM) 1706-05, *Standard Test Methods for Measuring the Toxicity of Sediment Associated Contaminants with Freshwater Invertebrates* (ASTM 2010).

Six sediment samples were collected and shipped to GLEC by AECOM personnel. Once received at GLEC, the sediment samples were assigned a unique GLEC laboratory identification number and stored between 0 and  $\leq 6^{\circ}\text{C}$  (but not frozen) until test initiation (see table below).

Sample I.D.	Investigative Sample Description	GLEC Lab. ID Number	Date Sampled	Date Received	Temperature Upon Receipt ( $^{\circ}\text{C}$ )
SE-04	Site Sample	11,135	November 07, 2016	November 08, 2016	4.9
SE-02	Site Sample	11,136	November 07, 2016	November 08, 2016	5.6
PEC-006	Site Sample	11,137	November 07, 2016	November 08, 2016	5.9
PEC-001	Site Sample	11,138	November 07, 2016	November 08, 2016	3.9
SE-03	Site Sample	11,139	November 07, 2016	November 08, 2016	5.7
PEC-21	Site Sample	11,140	November 07, 2016	November 08, 2016	5.7

The 10-day *C. dilutus* and *H. azteca* toxicity tests were initiated on December 02, 2016 for each of the six investigative sediment samples, one GLEC laboratory control sediment and one water only control, per test organism.

### Summary of Test Procedures: 10-Day *Chironomus dilutus* and *Hyalella azteca* Whole Sediment Toxicity Tests

Second to third instar *C. dilutus* (approximately 10-11 days old at test initiation, provided by an outside supplier: Aquatic Bio Systems) and *H. azteca* (10-11 days old at test initiation, in-house culture) were used to initiate the 10-day whole sediment toxicity tests. *C. dilutus* and *H. azteca* were continuously exposed for 10 days to each of the

sediment samples, one laboratory control sediment and one water only control. In the water only controls, test organisms were exposed to the overlying water with no sediment but clean substrates: silica sand or nylon screen.

There were eight replicate test chambers for each sediment sample, water only control, and the laboratory control sediment. Each replicate contained 10 test organisms. The laboratory control sediment is a reference sediment that is collected from the Boardman River, a local river that has a primarily forested watershed in the Pere Marquette State Forest.

The *C. dilutus* and *H. azteca* were exposed in 470 mL glass test chambers, each containing 100 mL of whole sediment and 175 mL of overlying water.

Prior to adding the whole sediment to each test chamber, the control as well as each investigative sediment sample were thoroughly homogenized using a pre-cleaned stainless steel mixer or spoon until a uniform color and texture was achieved.

The homogenized sediment was then added to each test chamber using a pre-cleaned stainless steel spoon. After the addition of the sediment to the test chambers, overlying water was immediately added; this was considered test day -1, the test day prior to day 0 (December 01, 2016 for both the *C. dilutus* and *H. azteca* tests). Test organisms were randomly added to each replicate test chamber the following day; test day 0.

Overlying water was intermittently supplied to each test chamber at least twice daily (once every 12-hours) via a static-renewal water delivery system. The overlying water for each investigative sediment, the laboratory control sediment and the water only control consisted of de-chlorinated municipal (Traverse City, Michigan; Lake Michigan sourced) water, with an average hardness of 139 mg/L and an average alkalinity of 110 mg/L. Temperature, dissolved oxygen (DO), pH, and specific conductance of the overlying water was measured daily prior to use.

The *C. dilutus* test chambers were fed 1.5 mL of Tetrafin® goldfish food slurry (4 mg/mL dry solids) once daily. The *H. azteca* test chambers were fed a 1.0 mL mixture of yeast, trout food, and wheatgrass (YTC; ~1800 (1700-1900 +/- 5%) mg/L solids) once daily.

The test chambers were placed in a temperature controlled water bath under the specified conditions of  $23 \pm 1^\circ\text{C}$ ; photoperiod 16 hours light: eight hours dark; and light intensity of 100-1000 lux.

Temperature ( $23 \pm 1^\circ\text{C}$ ) and the DO ( $\geq 2.5$  mg/L) concentrations of the overlying water in the test chambers were measured daily in two alternating replicates for each test sediment, and the results were recorded on the laboratory bench data sheets. There were

no instances of decreased DO or temperature exceedances in either the *C. dilutus* or the *H. azteca* whole sediment toxicity tests.

Alkalinity, hardness, pH, conductance, and total ammonia (as N) were measured in the overlying water on test days 0 and 10 for both the *C. dilutus* (Table 5 and Appendix B1) and *H. azteca* (Table 6 and Appendix B2) tests. The results of these analyses were also recorded on the laboratory bench data sheets.

Observations of organism behavior and anomalies within the sediment were made daily for each test chamber and recorded on the laboratory bench data sheets.

The number of *C. dilutus* surviving in each replicate test chamber was recorded at test termination (10 days), and a summary of the percent survival at test termination is provided in Tables 1, 2, and 7. The average ash free dry weight [AFDW in milligrams (mg)] of the surviving organisms for each *C. dilutus* replicate, and the biomass [AFDW (mg) of the surviving organisms divided by the initial number of organisms] was also determined at test termination, and the results are summarized in Table 2.

The number of surviving *H. azteca* in each replicate chamber was recorded at test termination (10 days) and the survival data are summarized in Tables 3, 4, and 8. The average dry weight [in milligrams (mg)] of the surviving organisms for each *H. azteca* replicate, and the biomass [dry weight (mg) of the surviving organisms divided by the initial number of organisms] was also determined at test termination, and the data are summarized in Table 4.

A statistical procedure, using the program TOXCALC (version 5.0.32) and following statistical guidelines provided in U.S. EPA Method 600/R-99/064 and ASTM Method 1706-05 (2010), was used to compare the 10-day survival and growth data from the investigative sediment samples to survival and growth data from the laboratory control sediment. Prior to analysis, all percent survival data were transformed using an arc sine-square root transformation

All transformed data were then tested for normality and homogeneity of variances. Next, an analysis of variance (ANOVA) was conducted using the most appropriate parametric (e.g., Dunnett's or Bartlett's t-tests) or nonparametric (e.g., Steel's Many-One Rank or Wilcoxon with Bonferroni's) t-test. If the data failed to meet the assumptions of normality or homogeneity, the nonparametric tests were used to analyze the data. Additional statistical analysis would be conducted using homoscedastic or heteroscedastic t-tests, when an investigative sediment sample was significantly different from the laboratory control. The homoscedastic or heteroscedastic t-tests, are used for comparing a single treatment to a single control.

The homoscedastic t-test assumes the data are normally distributed (Shapiro-Wilk Test or Kolmogorov D Test) and the variances are equal (F-test). If the variances are not

equal, the data are analyzed using the heteroscedastic t-test. If the data are not normally distributed, then the data are analyzed using a nonparametric t-test (e.g., Steel's Many-One Rank Test or Wilcoxon Rank Sum Test with Bonferroni's Adjustment).

Growth data were initially evaluated for normal distribution and homogeneity of variances. In those cases where the data were not normally distributed or homogenous, the data were analyzed using either the nonparametric test or the heteroscedastic t-test. In addition to growth being evaluated as average dry weight of the surviving organisms, growth was also analyzed as biomass (average dry weight of surviving organisms divided by the number of initial organisms).

The survival and growth data for *C. dilutus* and *H. azteca* for each Pigs Eye Lake investigative sample were compared statistically ( $p= 0.05$ ) to the laboratory control sediment sample.

Organisms exposed to the laboratory control sediment and the water only control achieved acceptable survival and growth, as specified in the U.S. EPA manual EPA/600/R-99/064. In this instance, the laboratory control sediment and water only control results confirmed test acceptability and the health of the test organisms.

## **RESULTS**

### **10-Day *Chironomus dilutus***

The organisms exposed to the laboratory control sediment and to the water only control exceeded the minimum survival (70 percent) and growth (0.48 mg AFDW at test termination) criteria for acceptable controls for the *C. dilutus* tests (Tables 1 and 2). The acceptability requirements for survival and growth for the *C. dilutus* test can be found in U.S. EPA manual EPA/600/R-99/064, Test Method 100.2, Table 12.1. There was 97.5 percent survival in both the GLEC laboratory control sediment and in the water only control.

The overlying water quality measurements (Table 5) were also within the acceptable limits following the U.S. EPA testing protocol. Daily mean temperatures were  $23^{\circ}\text{C} \pm 1^{\circ}\text{C}$ , dissolved oxygen (DO) was maintained above 2.5 mg/L in the overlying water; and there were no variations greater than 50% in overlying water hardness or alkalinity measurements within each test type. Total ammonia over ten days varied between 0.05 mg/L and 5.68 mg/L in the overlying water among all sediment types. Consequently, the *C. dilutus* whole sediment toxicity tests were conducted following the standard protocols and are valid assessments of sediment toxicity.

All test chambers were observed daily to assess organism behavior and no unusual observations were noted with the test organisms in these sediment samples.

### **Statistical Analysis for 10-Day *Chironomus dilutus* Tests**

#### **GLEC Laboratory Control Sediment Sample Compared to Pigs Eye Lake Investigative Sediment Samples**

*C. dilutus* survival and growth results from the GLEC laboratory control sediment sample were compared statistically to the six Pigs Eye Lake sediment samples (previously listed).

*C. dilutus* survival was not significantly reduced ( $p \geq 0.05$ ) in any of the six sediment samples when compared to the GLEC laboratory control sediment (see Tables 1, 2, and 7 and Appendix C2).

There was a significant difference ( $p < 0.05$ ) in *C. dilutus* growth (AFDW) between the laboratory control sediment and the sediment sample: SE-04 (GLC Number: 11,135) (see Tables 2 and 7 and Appendix C2).

Additionally, *C. dilutus* biomass (AFDW) was significantly reduced ( $p < 0.05$ ) in three sediment samples (listed below), when compared to the laboratory control sediment (see Tables 2 and 7 and Appendix C2).

- SE-04; (GLC Number: 11,135);
- PEC-006; (GLC Number: 11,137); and
- PEC-21; (GLC Number: 11,140).

Outputs for the survival and growth statistical analyses for the *C. dilutus* whole sediment toxicity tests are provided in Appendix C2.

#### **10-Day *Hyaella azteca***

The *H. azteca* test organisms exposed to the laboratory control sediment and to the overlying water exceeded the minimum survival criteria (80%), and displayed acceptable measurable growth (Tables 3 and 4). The requirements for acceptable survival and growth for the *H. azteca* can be found in U.S. EPA manual EPA/600/R-99/064, Test Method 100.1, Table 11.2. There was 100 percent survival in both the laboratory control sediment and in the water only control.

The overlying water quality measurements (Table 6) were also within the acceptable limits following the U.S. EPA testing protocol. Daily mean temperatures were  $23 \pm 1$  °C, DO was maintained above 2.5 mg/L in the overlying water; and there were no variations greater than 50% in overlying water hardness or alkalinity measurements within each test type. Total ammonia over a ten day period varied between 0.03 mg/L and 5.68 mg/L in the overlying water among all sediment types.

All test chambers were checked daily to assess organism behavior and no unusual observations were noted with the test organisms in these sediment samples.

### **Statistical Analysis for 10-Day *Hyalella azteca* Tests**

#### **GLEC Laboratory Control Sediment Sample Compared to Pigs Eye Lake Investigative Sediment Samples**

*H. azteca* 10-day survival and growth results from the GLEC laboratory control sediment sample were compared statistically to the six Pigs Eye Lake sediment samples (previously listed).

*H. azteca* survival was not significantly reduced ( $p \geq 0.05$ ) in any of the six sediment samples, when compared to the laboratory control sediment (see Tables 3, 4, and 8 and Appendix D2).

*H. azteca* growth (mg) and biomass (mg) were both significantly reduced ( $p < 0.05$ ) in four sediment samples (listed below), when compared to laboratory control sediment (Tables 4 and 8 and Appendix D2).

- SE-04; (GLC Number: 11,135);
- PEC-006; (GLC Number: 11,137);
- PEC-001; (GLC Number: 11,138); and
- PEC-21; (GLC Number: 11,140).

Outputs for the survival and growth statistical analyses for the *H. azteca* whole sediment toxicity tests are provided in Appendix D2.

### **Summary**

In summary, GLEC completed whole sediment toxicity testing and analysis of six sediment samples. Each whole sediment toxicity test was performed following acceptable methods, without exception, and is accurate and complete. These whole sediment toxicity test results are in compliance with the requirements of the National Environmental Laboratory Accreditation Conference (NELAC).

Statistical analyses were completed for the whole sediment toxicity tests with *C. dilutus* and *H. azteca*. All data are summarized in the following tables and raw data are reported in the appendices to this report.

*C. dilutus* survival was not significantly reduced in any of investigative sediment samples when compared to the laboratory control sediment sample (Table 7). *C. dilutus*

**Tony Coryell, AECOM**  
**Pigs Eye Lake**  
**St. Paul, Minnesota**

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**January 24, 2017**

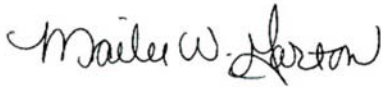
average growth was significantly reduced in one investigative sediment sample, while *C. dilutus* biomass was significantly reduced in three investigative sediment samples (Table 7).

*H. azteca* survival was not significantly reduced in any of the six investigative sediment samples when compared to the laboratory control sediment sample (Table 8).

*H. azteca* growth (expressed either as average dry weight or biomass) was significantly reduced in four sediment samples when compared to the laboratory control sediment sample (Table 8).

If you have any questions, or if you would like additional information, please contact either myself or Dennis McCauley at (231) 941-2230, or John Barkach at (248) 538-0900. Thank you for the opportunity to provide this service to AECOM. We look forward to continue providing environmental services to you in the future.

Sincerely,



Mailee W. Garton  
Laboratory Coordinator



John H. Barkach  
Senior Program Manager

MWG:mg







**TABLE 1. Comparison of Number of Surviving *Chironomus dilutus* per Replicate<sup>r</sup> and Percent Survival Between; Laboratory Control Sediment and the Investigative Sediment Samples; *Chironomus dilutus* 10-Day Whole Sediment Toxicity Tests Conducted December 02 - December 12, 2016; AECOM Pigs Eye Lake; St. Paul, Minnesota**

Replicate Number	Laboratory Control	SE-04	SE-02	PEC-006	PEC-001	SE-03	PEC-21	Water Only
<b>GLC Number</b>	<b>CS# 134</b>	<b>11,135</b>	<b>11,136</b>	<b>11,137</b>	<b>11,138</b>	<b>11,139</b>	<b>11,140</b>	<b>Secondary Control</b>
1	10	10	10	10	10	10	10	10
2	10	10	10	8	10	10	10	10
3	10	9	10	10	9	10	10	9
4	10	10	10	8	8	10	9	9
5	9	10	10	10	10	9	10	10
6	9	8	9	10	10	9	10	10
7	10	9	10	10	9	9	10	10
8	10	9	10	10	10	9	10	10
<b>10-Day Whole Sediment Toxicity Test Percent Survival<sup>r</sup></b>	<b>97.5</b>	<b>93.8</b>	<b>98.8</b>	<b>95.0</b>	<b>95.0</b>	<b>95.0</b>	<b>98.8</b>	<b>97.5</b>

<sup>r</sup>Replicates initiated with 10 organisms each replicate.



**TABLE 2.** Comparison of Average<sup>1</sup> Dry Weight (mg), Biomass<sup>2</sup> (mg) and Percent Survival Between; Laboratory Control Sediment and the Investigative Sediment Samples; *Chironomus dilutus* 10-Day Whole Sediment Toxicity Tests Conducted December 02 - December 12, 2016; AECOM Pigs Eye Lake; St. Paul, Minnesota.

GLC Number	Laboratory Control		SE-04		SE-02		PEC-006		PEC-001		SE-03		PEC-21		Water Only	
	CS#134		11,135		11,136		11,137		11,138		11,139		11,140		Secondary Control	
Replicate Number	Average <sup>1</sup> Weight (mg)	Biomass <sup>2</sup> Weight (mg)	Average <sup>1</sup> Weight (mg)	Biomass <sup>2</sup> Weight (mg)	Average <sup>1</sup> Weight (mg)	Biomass <sup>2</sup> Weight (mg)	Average <sup>1</sup> Weight (mg)	Biomass <sup>2</sup> Weight (mg)	Average <sup>1</sup> Weight (mg)	Biomass <sup>2</sup> Weight (mg)	Average <sup>1</sup> Weight (mg)	Biomass <sup>2</sup> Weight (mg)	Average <sup>1</sup> Weight (mg)	Biomass <sup>2</sup> Weight (mg)	Average <sup>1</sup> Weight (mg)	Biomass <sup>2</sup> Weight (mg)
1	1.11200	1.11200	1.04300	1.04300	1.33100	1.33100	1.22400	1.22400	1.06500	1.06500	1.39100	1.39100	1.08500	1.08500	0.85200	0.85200
2	1.18300	1.18300	0.90700	0.90700	1.24500	1.24500	1.25250	1.00200	1.24300	1.24300	0.86400	0.86400	1.08200	1.08200	0.97600	0.97600
3	0.99600	0.99600	1.02444	0.92200	1.31200	1.31200	1.01500	1.01500	1.33000	1.19700	1.09200	1.09200	0.93500	0.93500	1.18778	1.06900
4	1.26000	1.26000	0.89000	0.89000	1.20600	1.20600	1.13125	0.90500	1.21125	0.96900	1.13800	1.13800	1.17000	1.05300	0.99444	0.89500
5	1.13222	1.01900	1.12600	1.12600	1.16500	1.16500	1.03900	1.03900	1.17900	1.17900	1.28000	1.15200	1.13200	1.13200	1.05700	1.05700
6	1.41778	1.27600	1.32125	1.05700	1.38222	1.24400	0.94100	0.94100	1.01700	1.01700	1.30778	1.17700	0.98200	0.98200	0.88700	0.88700
7	1.23900	1.23900	1.04222	0.93800	1.18300	1.18300	1.00600	1.00600	1.28778	1.15900	1.29556	1.16600	1.01100	1.01100	0.91500	0.91500
8	1.16000	1.16000	0.92333	0.83100	1.36500	1.36500	0.90200	0.90200	1.16600	1.16600	1.15111	1.03600	0.98000	0.98000	0.87300	0.87300
Average <sup>1</sup> Ash-Free-Dry Weight (AFDW) (mg)	1.18750		1.03466 <sup>a</sup>		1.27365		1.06384		1.18738		1.18993		1.04713		0.96778	
Biomass <sup>2</sup> Weight (AFDW) (mg)			0.96425 <sup>a</sup>		1.25638		1.00425 <sup>a</sup>		1.12438		1.12700		1.03250 <sup>a</sup>		0.94050	
10-Day Percent Survival	97.5		93.8		98.8		95.0		95.0		95.0		98.8		97.5	

Note: Average Ash-Free-Dry Weight (AFDW) of *Chironomus dilutus* at test initiation = 0.30813 mg

<sup>1</sup> Average Ash-Free-Dry-Weight (AFDW) is the total ash-free-dry weight of surviving organisms

<sup>2</sup> Biomass weight is the total Ash-Free-Dry-Weight of surviving organisms divided by the initial number of organisms.

<sup>a</sup> Significantly different (p < 0.05) from laboratory control sediment (CS# 134)



**TABLE 3. Comparison of Number of Surviving *Hyalella azteca* per Replicate<sup>r</sup> and Percent Survival Between; Laboratory Control Sediment and the Investigative Sediment Samples; *Hyalella azteca* 10-Day Whole Sediment Toxicity Tests Conducted December 02 - December 12, 2016; AECOM Pigs Eye Lake; St. Paul, Minnesota.**

Replicate Number	Laboratory Control	SE-04	SE-02	PEC-006	PEC-001	SE-03	PEC-21	Water Only
GLC Number	CS# 134	11,135	11,136	11,137	11,138	11,139	11,140	Secondary Control
1	10	8	10	10	10	10	10	10
2	10	10	9	10	10	10	9	10
3	10	10	10	6	10	9	10	10
4	10	9	8	9	10	10	10	10
5	10	10	10	10	10	10	9	10
6	10	10	10	9	10	10	6	10
7	10	10	10	9	10	10	10	10
8	10	10	9	9	10	10	8	10
<b>10-Day Whole Sediment Toxicity Test Percent Survival<sup>r</sup></b>	<b>100.0</b>	<b>96.3</b>	<b>95.0</b>	<b>90.0</b>	<b>100.0</b>	<b>98.8</b>	<b>90.0</b>	<b>100.0</b>

<sup>r</sup> Replicates initiated with 10 organisms each replicate.



**TABLE 4** Comparison of Average<sup>1</sup> Dry Weight (mg), Biomass<sup>2</sup> (mg) and Percent Survival Between; Laboratory Control Sediment and the Investigative Sediment Samples; *Hyalella azteca* 10-Day Whole Sediment Toxicity Tests Conducted December 02 - December 12, 2016; AECOM Pigs Eye Lake; St. Paul, Minnesota.

GLC Number	Laboratory Control		SE-04		SE-02		PEC-006		PEC-001		SE-03		PEC-21		Water Only	
	CS#134		11,135		11,136		11,137		11,138		11,139		11,140		Secondary Control	
Replicate Number	Average <sup>1</sup> Weight (mg)	Biomass <sup>2</sup> Weight (mg)	Average <sup>1</sup> Weight (mg)	Biomass <sup>2</sup> Weight (mg)	Average <sup>1</sup> Weight (mg)	Biomass <sup>2</sup> Weight (mg)	Average <sup>1</sup> Weight (mg)	Biomass <sup>2</sup> Weight (mg)	Average <sup>1</sup> Weight (mg)	Biomass <sup>2</sup> Weight (mg)	Average <sup>1</sup> Weight (mg)	Biomass <sup>2</sup> Weight (mg)	Average <sup>1</sup> Weight (mg)	Biomass <sup>2</sup> Weight (mg)	Average <sup>1</sup> Weight (mg)	Biomass <sup>2</sup> Weight (mg)
1	0.11500	0.11500	0.06250	0.05000	0.07400	0.07400	0.08000	0.08000	0.06200	0.06200	0.10600	0.10600	0.06900	0.06900	0.09300	0.09300
2	0.08800	0.08800	0.06600	0.06600	0.10444	0.09400	0.05700	0.05700	0.07100	0.07100	0.08000	0.08000	0.06444	0.05800	0.10000	0.10000
3	0.09700	0.09700	0.05900	0.05900	0.09200	0.09200	0.08500	0.05100	0.06300	0.06300	0.11667	0.10500	0.07200	0.07200	0.09800	0.09800
4	0.08100	0.08100	0.07889	0.07100	0.08875	0.07100	0.08111	0.07300	0.07700	0.07700	0.08700	0.08700	0.06600	0.06600	0.09100	0.09100
5	0.08800	0.08800	0.06500	0.06500	0.08000	0.08000	0.06600	0.06600	0.07100	0.07100	0.09800	0.09800	0.04889	0.04400	0.09300	0.09300
6	0.08700	0.08700	0.05900	0.05900	0.08400	0.08400	0.07444	0.06700	0.05000	0.05000	0.06600	0.06600	0.07833	0.04700	0.09300	0.09300
7	0.09900	0.09900	0.07300	0.07300	0.08500	0.08500	0.07889	0.07100	0.06400	0.06400	0.07200	0.07200	0.07700	0.07700	0.09100	0.09100
8	0.09400	0.09400	0.04700	0.04700	0.08556	0.07700	0.06667	0.06000	0.07800	0.07800	0.09900	0.09900	0.06125	0.04900	0.09200	0.09200
Average <sup>1</sup> Dry Weight (mg)	0.09363		a	0.06380	0.08672		a	0.07364	0.06700		0.09058		a	0.06711	0.09388	
Average Biomass <sup>2</sup> Weight (mg)	0.09363		a	0.06125	0.08213		a	0.06563	0.06700		0.08913		a	0.06025		0.09388
10-Day Percent Survival	100.0		96.3		95.0		90.0		100.0		98.8		90.0		100.0	

Note: Average Dry Weight of *Hyalella azteca* at test initiation = 0.01675 mg

<sup>1</sup> Average Dry Weight is the total dry weight of surviving organisms

<sup>2</sup> Biomass weight is the total dry weight of surviving organisms divided by the initial number of organisms.

<sup>a</sup> Significantly different (p < 0.05) from laboratory control sediment (CS# 134)



**TABLE 5. Summary of Mean Water Quality Parameters of Overlying Water Collected Prior to Renewal; *Chironomus dilutus* 10-Day Whole Sediment Toxicity Tests Conducted December 02 - December 12, 2016; AECOM Pigs Eye Lake; St. Paul, Minnesota.**

<b>Sample ID GLC No.</b>	<b>Temperature (°C) (range)  n=22</b>	<b>pH (s.u.) (range)  n=4</b>	<b>Dissolved Oxygen (mg/L) (range)  n=22</b>	<b>Specific Conductivity (µmhos/cm) (range)  n=4</b>	<b>Hardness (CaCO3 mg/L) (range) n=2, n=4 GLC No. 11,135</b>	<b>Alkalinity (CaCO3 mg/L) (range) n=2, n=4 GLC No. 11,135</b>	<b>Ammonia (mg/L as N) (range) n=2, n=4 GLC No. 11,135</b>
<b>Laboratory Control CS#134</b>	<b>22.6</b> (22.4-22.9)	<b>7.61</b> (7.54-7.74)	<b>5.0</b> (3.8-7.4)	<b>345</b> (341-355)	<b>152</b> (152-152)	<b>123</b> (120-126)	<b>0.88</b> (0.18-1.57)
<b>Water Only Control NA</b>	<b>22.7</b> (22.4-23.0)	<b>8.01</b> (7.63-8.34)	<b>6.0</b> (4.9-8.4)	<b>327</b> (312-342)	<b>146</b> (140-152)	<b>109</b> (102-116)	<b>0.08</b> (0.05-0.1)
<b>SE-04 11135</b>	<b>22.7</b> (22.4-22.8)	<b>7.63</b> (7.57-7.69)	<b>5.5</b> (4.4-7.8)	<b>460</b> (356-566)	<b>191</b> (156-228)	<b>154</b> (130-178)	<b>0.10</b> (0.07-0.12)
<b>SE-02 11136</b>	<b>22.6</b> (22.3-22.9)	<b>7.59</b> (7.46-7.73)	<b>4.5</b> (2.9-6.6)	<b>448</b> (353-569)	<b>204</b> (156-252)	<b>172</b> (132-212)	<b>0.26</b> (0.16-0.35)
<b>PEC-006 11137</b>	<b>22.6</b> (22.2-22.8)	<b>7.56</b> (7.43-7.68)	<b>4.7</b> (3.6-6.9)	<b>381</b> (351-412)	<b>142</b> (132-152)	<b>130</b> (126-134)	<b>2.95</b> (0.23-5.68)
<b>PEC-001 11138</b>	<b>22.6</b> (22.1-22.9)	<b>7.54</b> (7.44-7.62)	<b>4.6</b> (3.5-6.7)	<b>383</b> (350-416)	<b>144</b> (140-148)	<b>129</b> (126-132)	<b>2.61</b> (0.25-4.96)
<b>SE-03 11139</b>	<b>22.5</b> (22.0-22.8)	<b>7.62</b> (7.52-7.71)	<b>4.8</b> (3.4-6.5)	<b>463</b> (358-571)	<b>214</b> (164-264)	<b>174</b> (126-222)	<b>0.39</b> (0.14-0.63)
<b>PEC-21 11140</b>	<b>22.5</b> (22.0-22.7)	<b>7.55</b> (7.51-7.58)	<b>4.9</b> (3.8-7.0)	<b>447</b> (347-552)	<b>188</b> (144-232)	<b>166</b> (124-208)	<b>0.76</b> (0.11-1.41)

n= Number of measurements



**TABLE 6. Summary of Mean Water Quality Parameters of Overlying Water Collected Prior to Renewal; *Hyalella azteca* 10-Day Whole Sediment Toxicity Tests Conducted December 02 - December 12, 2016; AECOM Pigs Eye Lake; St. Paul, Minnesota.**

<b>Sample ID GLC No.</b>	<b>Temperature (°C) (range)  n=22</b>	<b>pH (s.u.) (range)  n=4</b>	<b>Dissolved Oxygen (mg/L) (range)  n=22</b>	<b>Specific Conductivity (µmhos/cm) (range)  n=4</b>	<b>Hardness (CaCO3 mg/L) (range) n=2, n=4 GLC No. 11,135</b>	<b>Alkalinity (CaCO3 mg/L) (range) n=2, n=4 GLC No. 11,135</b>	<b>Ammonia (mg/L as N) (range) n=2, n=4 GLC No. 11,135</b>
<b>Laboratory Control CS#134</b>	<b>23.1</b> (22.4-23.5)	<b>7.79</b> (7.68-7.92)	<b>6.8</b> (5.9-7.5)	<b>348</b> (342-353)	<b>156</b> (152-160)	<b>126</b> (126-126)	<b>0.81</b> (0.04-1.57)
<b>Water Only Control NA</b>	<b>23.2</b> (22.8-23.6)	<b>8.20</b> (8.15-8.24)	<b>7.7</b> (6.8-8.5)	<b>330</b> (314-346)	<b>142</b> (140-144)	<b>110</b> (102-118)	<b>0.07</b> (0.05-0.09)
<b>SE-04 11135</b>	<b>23.2</b> (22.9-23.6)	<b>7.80</b> (7.65-7.95)	<b>7.1</b> (4.8-8.2)	<b>468</b> (362-581)	<b>190</b> (152-228)	<b>154</b> (128-178)	<b>0.06</b> (0.04-0.07)
<b>SE-02 11136</b>	<b>23.2</b> (22.5-23.6)	<b>7.84</b> (7.69-7.97)	<b>6.9</b> (6-8.1)	<b>456</b> (370-544)	<b>208</b> (164-252)	<b>174</b> (136-212)	<b>0.19</b> (0.03-0.34)
<b>PEC-006 11137</b>	<b>23.1</b> (22.4-23.5)	<b>7.69</b> (7.68-7.71)	<b>6.4</b> (5.6-7.1)	<b>386</b> (354-422)	<b>142</b> (132-152)	<b>127</b> (120-134)	<b>2.86</b> (0.03-5.68)
<b>PEC-001 11138</b>	<b>23.1</b> (22.5-23.5)	<b>7.66</b> (7.52-7.81)	<b>6.4</b> (5.8-7.4)	<b>385</b> (353-423)	<b>146</b> (140-152)	<b>129</b> (126-132)	<b>2.50</b> (0.04-4.96)
<b>SE-03 11139</b>	<b>23.1</b> (22.4-23.5)	<b>7.90</b> (7.56-8.19)	<b>6.6</b> (5.6-7.5)	<b>475</b> (368-593)	<b>216</b> (168-264)	<b>179</b> (136-222)	<b>0.34</b> (0.04-0.63)
<b>PEC-21 11140</b>	<b>23.1</b> (22.3-23.5)	<b>7.73</b> (7.54-7.92)	<b>6.7</b> (5.4-7.6)	<b>462</b> (353-580)	<b>194</b> (156-232)	<b>169</b> (130-208)	<b>0.73</b> (0.04-1.41)

n= Number of measurements



**TABLE 7. Summary of Statistically Significant Differences (p< 0.05) Between the Laboratory Control Sediment and the Investigative Sediment Samples; *Chironomus dilutus* 10-Day Whole Sediment Toxicity Tests Conducted December 02 - December 12, 2016; AECOM Pigs Eye Lake; St. Paul, Minnesota.**

<b>Sample ID</b> GLC Number	<b>10-Day Survival</b>	<b>10-Day Growth<sup>1</sup> (AFDW)</b>	<b>10-Day Biomass<sup>2</sup> (AFDW)</b>
<b>SE-04</b> 11,135		<b>a</b>	<b>a</b>
<b>SE-02</b> 11,136			
<b>PEC-006</b> 11,137			<b>a</b>
<b>PEC-001</b> 11,138			
<b>SE-03</b> 11,139			
<b>PEC-21</b> 11,140			<b>a</b>

<sup>1</sup>Growth is average Ash-Free-Dry-Weight (AFDW) of surviving organisms

<sup>2</sup>Biomass weight is the total Ash-Free-Dry-Weight of surviving organisms divided by the initial number of organisms

<sup>a</sup> Significantly different (p< 0.05) from laboratory control sediment (CS# 134)



**TABLE 8. Summary of Statistically Significant Differences ( $p < 0.05$ ) Between the Laboratory Control Sediment and the Investigative Sediment Samples; *Hyalella azteca* 10-Day Whole Sediment Toxicity Tests Conducted December 02 - December 12, 2016; AECOM Pigs Eye Lake; St. Paul, Minnesota.**

Sample ID GLC Number	10-Day Survival	10-Day Growth <sup>1</sup>	10-Day Biomass <sup>2</sup>
SE-04 11,135		a	a
SE-02 11,136			
PEC-006 11,137		a	a
PEC-001 11,138		a	a
SE-03 11,139			
PEC-21 11,140		a	a

<sup>1</sup>Growth is the total dry weight of surviving organisms

<sup>2</sup>Biomass is the total dry weight of surviving organisms divided by the initial number of organisms.

<sup>a</sup> Significantly different ( $p < 0.05$ ) from laboratory control sediment (CS# 134)