Sediment Toxicity Test Results

Manitowoc River Sediment Study

Tested: July – August 2014

University of Wisconsin - Madison
Wisconsin State Laboratory of Hygiene
Environmental Toxicology Department
Madison, Wisconsin

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Written By: Camille Turcotte (Danielson)
Reviewed by: Mallory Ballard
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Introduction

Project: Manitowoc River Sediment Study
Samples Collected By: Xiaochun Zhang (WDNR), Cheryl Bougie (WDNR), Tom Simmons (WDNR), Mallory Ballard (WSLH) and Camille Turcotte (WSLH)
Sample Location: Manitowoc River, Manitowoc, Wisconsin
Sample Collection Date: 07/21/2014
Date Test Initiated: Phase1 Acute *Daphnia magna* Trial 1, Trial 2, Trial 3 - 07/24/14
Phase1 Acute *Pimephales promelas* Trial 1, Trial 2 - 07/24/14
Phase 1 28-Day *Hyalella azteca* - 07/29/14
Phase 1 10-Day *Chironomus dilutus* - 08/01/14
Phase 2 Chronic *Pimephales promelas* - 07/31/14
Phase 2 Chronic *Daphnia magna* - 07/31/14
Phase 3 Chronic *Pimephales promelas* - 08/28/14

Test Species:

Daphnia magna
Age: < 24 hours for acute and chronic
Source: WSLH Culture

Pimephales promelas
Age: 13 days for acute; <24 hours for chronic
Source: WSLH Culture

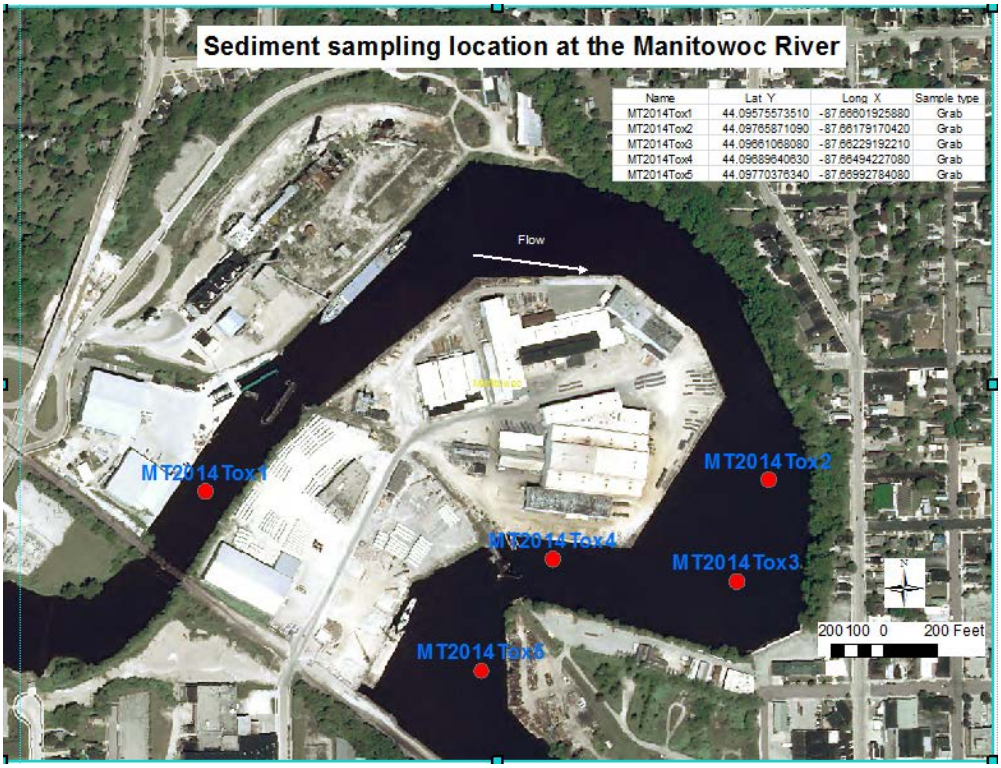
Hyalella azteca (amphipod)
Age: 7-8 days
Source: WSLH Culture

Chironomus dilutus (larval midge)
Age: 11-12 days ~2nd instar
Source: Aquatic Biosystems

Table 1. Sample Site Descriptions

WSLH Lab Number	**Site Name**	**Description**	**Elutriate Batches**
LC	Lab control	Synthetic sediment	Batch 1 made with Hard Water
FZ000017	Tox 1	Sediment upstream of MGP Site	Batch 1 made with Manitowoc River (MR) water
FZ000018	Tox 2	Sediment upstream from MGP Site	Batch 1 made with MR water
FZ000019	Tox 3	Sediment closest to MGP Site	Batch 1, 2, 3 and 4 all made with MR water
FZ000020	Tox 4	Sediment downstream from MGP site	Batch 1 made with MR water
FZ000021	Tox 5	Sediment downstream from MGP site	Batch 1 made with MR water
FZ000022	Tox 3 Water	Manitowoc River (MR) surface water collected at Tox 3 site	Used to make elutriate batches and for overlying water in *D. magna* and *P. promelas* tests

Figure 1. Map of Manitowoc River approximate sediment sampling locations (map created by Xiaochun Zhang prior to sampling). Actual collection sites differed slightly due to sampling logistics.



Summary of Project

Sediment and water column samples were collected from the Manitowoc River in Manitowoc, Wisconsin on July 21, 2014 to conduct toxicity tests and chemical analyses of contaminants of concern. The main contaminants suspected are polycyclic aromatic hydrocarbons (PAHs) and heavy metals that are potentially associated with former manufactured gas plant (MGP) plant operations in the area.

Four investigative sediment samples, one reference sediment sample and one surface water sample were collected. Samples were collected from a WDNR boat using a ponar grab sampler and placed into new aluminum pans and then scooped into new, clean 5 gallon HDPE buckets with stainless steel spoons. Water samples were grab samples collected from the boat using a plastic bucket, funnel and 20 L cubitainers. Sediment samples were mixed thoroughly and put into chemistry sample bottles in the field at the boat landing. All samples were put on ice and driven back to the Wisconsin State Laboratory of Hygiene on the same day as collection.

Sediment, surface water and elutriate samples were submitted for analyses of inorganic and organic contaminants. These included: PAHs, TOC, HEM, particle size, cyanide, and ammonia. The metals included: As, Cd, Cr, Cu, Pb, Hg, Ni, and Zn. Toxicity tests conducted included traditional sediment tests with the amphipod *Hyalella azteca* and chironomid *Chironomus dilutus* (formerly known as *Chironomus tentans*) as well as tests using the cladoceran *Daphnia magna* and the fathead minnow *Pimephales promelas*. *D. magna* and *P. promelas* were tested to see if the sediment samples would have effects on organisms that live above the sediments. Scouring and mixing occurs often in riverine systems and could allow for exposures in the waters above the sediments near the MGP site.

During collection, sheen was visible on the surface of the water at both Tox Site 2 and Tox Site 3. Tox Site 3 sediment samples and elutriate smelled like petroleum and elutriate samples had film/sheen on top

of the water. Sheen was also seen in Site 3 samples when ending Hyalella and Chironomid sediment tests. Small pieces of tar were also seen in sediment samples from Tox Site 3.

Photos showing test procedures and set ups are presented at the end of the report.

Test Methods

Surface Water and Elutriate Tests

Several different trial acute test protocols were designed in order to determine the best way to test sediment toxicity using species that are normally used for water column testing. Several of the trials used elutriate prepared from the sediment samples. Elutriate was used in an attempt to simulate conditions in the water column over the sediments due to bottom disturbances induced by wind and wave actions. Sediment disturbances can be responsible for release of dissolved and particulate-absorbed contaminants into the water column.

Elutriate was prepared following procedures adapted from Appendix G "Biological Effects Testing Procedures" from the Great Lakes Dredge Material Testing Manual. The manual defines elutriate as: "a suspension prepared by mixing specific volumes of sediment and water, used for chemical analysis and toxicity testing". Elutriate was made by mixing one part sediment to four parts water. Half gallon glass jars were filled with sediment and water, sample was shaken by hand for 1 minute and then on a shaker table at 150 rpm for 60 minutes. Samples were then settled overnight. Due to insufficient settling after 24 hours (see photos at end of report) it was decided that samples had to be filtered. This is because it is very difficult to set or renew tests in samples that are opaque from suspended particles, as you cannot see the test organisms. Therefore, the water overlying the settled sediment in the mixture was carefully poured off and then filtered using vacuum and GF/A 90mm glass fiber filters that had been pretreated in a muffle furnace. Centrifuging samples was not viable due to the large volumes of sample needed in order to conduct tests with both fish and daphnia and extra volumes needed to renew test solutions.

The elutriate samples prepared were used in toxicity testing by exposing aquatic test organisms to 100% elutriate or to elutriate overlying sediment treatments. A control elutriate was prepared with 1 part synthetic lab sediment and 4 parts synthetic hard water.

Elutriate Batch 1 consisted of elutriate prepared for each of the five sediment sites for use in Phase 1 of the testing. Sediment samples from each site were mixed with Manitowoc River surface water collected at Tox Site 3. Elutriate Batches 2-4 were prepared for only Tox Site 3 due to results from Phase 1 testing indicating primary toxicity was at Site 3. Batches of elutriate were used for 7 days. Therefore, Batch 2 was used for the entire Phase 2 *Pimephales promelas* chronic test (7 days), while Batches 2-4 were used to set and renew the Phase 2 *Daphnia magna* (21 day) chronic test. Chemistry samples (inorganics and organics) were taken for each of the batches as sample volumes allowed. Batch 2 chemistry samples were taken from aliquots of the elutriate that was poured off before filtering and also samples of elutriate after filtering. This was done in an attempt to assess how much of the possible toxicant the filtering might be removing. Those chemistry results were reported to the DNR separately from this report. If chemistry results differ greatly between before and after filtering, then tests with elutriate in the future should attempt to be set without filtering. An alternative to filtering might be settling elutriate jars for 48 hours. However, dissolved oxygen in the elutriate might be too low for organisms to survive. Prior to this project a preliminary elutriate preparation test was attempted with sediment from a different project. That elutriate was prepared in a similar manner but was settled for about 48 hours and resulted in dissolved oxygen below 5 mg/L.

Standard acute and chronic test methods for *P. promelas* and *D. magna* were adapted to accommodate elutriate and sediment testing. All *P. promelas* and *D. magna* tests were conducted at 25°C (standard chronic test temperature). In samples with sediment on the bottom of the beakers it was sometimes difficult to find organisms (especially dead organisms) due to the sediment. At renewal and shut down the samples were poured out into watch glasses and searched for organisms but if they could not be found they were presumed dead. Table 2 summarizes the different phases of testing. Overall testing results are provided below in the narrative for each phase of the testing. Raw data, quality control information and statistical results are provided in two separate attached reports. One report covers the data for the *P. promelas* and *D. magna* tests while the second report covers *H. azteca and* C. *dilutus* results.

Table 2. Summary of Testing

Phase	Trial	Species	Test Type	Test Site
1	1	*D.magna*	Acute 48-hour test with elutriate overlying site sediment	Tox Sites 1-5 Elutriate and sediment
1	2	*D.magna*	Acute 48 hour test with Manitowoc River water overlying site sediment	Tox Sites 1-5 sediment
		P.promelas	Acute 96 hour test with Manitowoc River water overlying site sediment	Tox Sites 1-5 sediment
1	3	*D.magna*	Acute 48 hour test with elutriate only	Tox Sites 1-5 elutriate
		P.promelas	Acute 96 hour test with elutriate only	Tox Sites 1-5 elutriate
1		*H.azteca*	28 day sediment test	Tox Sites 1-5 sediment
1		*C.dilutus*	10 day sediment test	Tox Sites 1-5 sediment
2		*D.magna*	21 day chronic test with elutriate, elutriate/sediment and MR/sediment treatments	Tox Site 1 (reference) sediment and Tox Site 3 sediment and elutriate
		P.promelas	7 day chronic test with elutriate, elutriate/sediment and MR/sediment treatments	
3		*P.promelas*	7 day chronic test with MR overlying sediment with multiple dilutions of sediment	Tox Site 1 (reference) sediment and Tox Site 3 sediment

Phase 1 Acute Toxicity Testing Protocols and Summary of Results

Protocols for *Daphnia magna* Trial 1

- Sample treatment: 120 mL of sample elutriate (made by mixing Manitowoc River Tox Site 3 surface water and site sediment from each of the five sites) overlying 30 mL of site specific sediment in each beaker
- Control treatment: lab elutriate (prepared from hard water and synthetic lab sediment) overlying synthetic lab sediment
- Test chamber: 250 mL plastic beaker
- Number of organisms: 10 per beaker – 40 total per treatment
- Replicates: 4
- Renewal: Nonrenewal
- Duration: 48 hours
- Feeding: Fed 1mL YFC/1 mL *S. capricornutum* on day 0
- Endpoint: mortality

Overall Results of *Daphnia magna* Trial 1 (detailed results for all tests are provided in attached reports)

- Tox 3 elutriate overlying Tox 3 sediment resulted in 27.5% survival
- All other elutriate + sediment treatments of Tox 1, Tox 2, Tox 4, and Tox 5 had over 92% survival
- Control (synthetic hard water elutriate over synthetic sediment) had 100% survival

Protocols for *Pimephales promelas* Trial 1

- Elutriate plus sediment trial was not conducted for *Pimephales promelas* due to limited volume of elutriate

Protocols for *Daphnia magna* Trial 2

- Sample treatment: 120 mL of Manitowoc River Tox 3 surface water overlying 30 mL of sediment from each site
- Control treatment: Manitowoc river water overlying synthetic lab sediment
- Test chamber: 250 mL plastic beaker
- Number of organisms: 10 per beaker – 40 total per treatment
- Replicates: 4
- Renewal: Nonrenewal
- Duration: 48 hours
- Feeding: Fed 1mL YFC/1 mL *S. capricornutum* on day 0
- Endpoint: mortality

Overall Results of *Daphnia magna* Trial 2

- All treatments of Manitowoc River water overlying site sediments had 92% or greater survival
- Control of Manitowoc River water over synthetic lab sediment had 97.5% survival

Protocols for *Pimephales promelas* Trial 2

- Sample treatment: 160 mL of Manitowoc River Tox Site 3 surface water overlying 40 mL of sediment for each of the five sites
- Control treatments:
    - Manitowoc River water overlying synthetic lab sediment
    - Dechlorinated (DC) tap water control
- Test chamber: 250 mL plastic beaker

- Number of organisms: 10 per beaker – 40 total per treatment
- Replicates: 4
- Renewal: renewed with ~100mL of Manitowoc River water daily
- Duration: 96 hours
- Feeding: 0.1mL brine shrimp on Day 2
- Endpoint: mortality

Overall Results of *Pimephales promelas* Trial 2

- All site treatments of Manitowoc River water over site sediments resulted in 95% or greater survival
- Lab water control had 92.5% survival and the Manitowoc River water overlying lab sediment control had 97.5% survival

Protocols for *Daphnia magna* Trial 3

- Sample treatment: 120 mL of elutriate with no sediment in beaker (elutriate was made from Manitowoc River Tox Site 3 surface water and site sediment from each of the five sites)
- Control treatments:
    - Lab elutriate (made from hard water and lab control sediment)
    - Hard water control
- Test chamber: 250 mL plastic beaker
- Number of organisms: 10 per beaker – 40 total per treatment
- Replicates: 4
- Renewal: Nonrenewal
- Duration: 48 hours
- Feeding: Fed 1 mL YFC/1 mL *S. capricornutum* on day 0
- Endpoint: mortality

Overall Results of *Daphnia magna* Trial 3

- All site treatments of elutriate had 97% or greater survival
- The hard water control had 100% survival and lab elutriate control had 97.5% survival

Protocols for *Pimephales promelas* Trial 3

- Sample treatment: 140 mL of elutriate with no sediment in beaker (elutriate made from Manitowoc River Tox Site 3 surface water and site sediment from each of the five sites)
- Control treatments:
    - Lab elutriate (made from hard water and lab control sediment)
    - Dechlorinated (DC) tap water control
- Test chamber: 250 mL plastic beaker
- Number of organisms: 10 per beaker – 40 total per treatment
- Replicates: 4
- Renewal: Renewed with ~80 mL of elutriate daily
- Duration: 96 hours
- Feeding: 0.1 mL brine shrimp on Day 2
- Endpoint: mortality

Overall Results of *Pimephales promelas* Trial 3

- All site treatments of elutriate had 95% or greater survival
- The DC water control had 100% survival and lab elutriate control had 90% survival

After reviewing the results from Phase 1 testing and discussing them with Xiaochun Zhang at the DNR, we designed and began Phase 2 testing with *P. promelas* and *D. magna*. This phase involved chronic tests using Tox Site 3 since it was the only site that demonstrated toxicity in Phase 1 and was closest to the MGP contamination site. Treatments from the reference site (Tox Site 1) were also included in Phase 2.

Phase 2 Chronic Toxicity Testing Protocols and Summary of Results

Protocols for *Daphnia magna* Chronic Toxicity Testing Phase 2

- Treatments:
  - LC - Control 1 – hard water
  - 1 - Control 2 – hard water + lab sediment
  - 2 - Reference site – hard water + Tox Site 1 sediment
  - 3 - Tox Site 3 Manitowoc River water + sediment
  - 4 - Tox Site 3 elutriate
  - 5 - Tox Site 3 elutriate + Tox Site 3 sediment
- Test chamber: 30 mL plastic beaker - 20mL water only or 20 mL water over 5 mL sediment
- Number of organisms: 1 per beaker – 10 total per treatment
- Replicates: 10
- Renewal: Renewed three times per week by preparing fresh cups with sediment/water and transferring organisms
- Duration: 21 days
- Feeding: 0.15 mL YFC and 0.15 mL *S. capricornutum* daily
- Endpoint: mortality and reproduction

Overall Results of *Daphnia magna* Chronic Phase 2

- The treatment consisting of Tox Site 3 elutriate (no sediment) resulted in mean survival of 50% which was significantly different from the lab controls and reference site.
- The treatment of Tox Site 3 elutriate over Tox Site 3 sediment resulted in mean survival of 80% but was not statistically different from control or reference treatments.
- Reproduction was not statistically different among treatments or when compared to the lab water control.
- The synthetic sediment control had significantly reduced reproduction compared to the hard water control and the other treatments.
- Control and reference site survival were all over 90%.

Protocols for *Pimephales promelas* Testing Phase 2

- Treatments:
  - LC - Control 1 - dechlorinated tap water
  - 1 - Control 2 – dechlorinated tap water + synthetic lab sediment
  - 2 - Reference site – dechlorinated tap water + Tox Site 1 sediment
  - 3 - Tox Site 3 Manitowoc river water + sediment
  - 4 - Tox Site 3 elutriate
  - 5 - Tox Site 3 elutriate + Tox Site 3 sediment
- Test chamber: 30 mL plastic beaker – 20 mL water over 5 mL sediment or only 20 mL water
- Number of organisms: 2 per beaker – 20 total per treatment
- Replicates: 10

- Renewal: Renewed daily by preparing fresh cups with sediment/water and transferring the fathead minnows to the new cups
- Duration: 7 days
- Feeding: 0.1 mL brine shrimp 3x per day
- Endpoint: mortality and growth

Overall Results of *Pimephales promelas* Chronic Phase 2

- Chronic toxicity was observed in the treatment consisting of Manitowoc River water over Tox Site 3 sediment (treatment 3) which resulted in a mean survival of 20%.
- Chronic toxicity was also observed in the treatment consisting of Tox Site 3 elutriate over Tox Site 3 sediment (treatment 5) which resulted in mean survival of 5%.
- Control and reference site survival were all over 90%.
- There was a significant impact on *P. promelas* growth in treatments 3 through 5 which all had Tox Site 3 sediment or Tox Site 3 elutriate as part of the treatment. Growth of *P. promelas* was significantly reduced compared to both the control and the reference site in all treatments involving samples from Tox Site 3.
- Treatment 3 (Manitowoc River water overlying Tox Site 3 sediment) and treatment 5 (Tox Site 3 elutriate overlying Tox Site 3 sediment) were statistically the same as each other and both had much lower growth than the control and reference sites.
- Treatment 4 (Tox Site 3 elutriate only with no sediment) was also statistically lower than control and reference sites but not as low as the treatments with water (either Manitowoc River or elutriate) overlying the sediment.

Phase 2 testing with *P. Promelas* resulted in toxicity in several Tox Site 3 treatments. Therefore, the DNR requested that we do another chronic test with diluted sediment from Tox Site 3 to see if there was a dose response. Phase 3 testing was conducted by diluting Tox Site 3 sediment with Tox Site 1 reference sediment on a wet weight basis. The lab control sediment was less preferable than the reference sediment for diluting because it was much finer and sandier than the Tox Site 3 sediment. It should be noted that the sediment from Tox Site 1 was different in particle size and composition (sandier) from the site sediment so it is difficult to have an extremely accurate dilution series.

Phase 3 Chronic *Pimephales promelas* Testing Protocols and Summary of Results

- Treatments:
  - LC - Control - dechlorinated tap water
  - 1 - Reference site – 100% Tox Site 1 sediment + Manitowoc River water
  - 2 - 25% Tox Site 3 sediment + 75% Tox Site 1 sediment + Manitowoc river water
  - 3 - 50% Tox Site 3 sediment + 50% Tox Site 1 sediment + Manitowoc river water
  - 4 - 75% Tox Site 3 sediment + 25% Tox Site 1 sediment + Manitowoc river water
  - 5 - 100% Tox Site 3 sediment + Manitowoc river water
- Test chamber: 30 mL plastic beaker – 20 mL water only or 20 mL water over 5 mL sediment
- Number of organisms: 2 per beaker – 20 total per treatment
- Replicates: 10
- Renewal: Renewed daily by preparing fresh cups with sediment/water and transferring organisms
- Duration: 7 days
- Feeding: 0.1 mL brine shrimp 3x per day
- Endpoint: mortality and growth

Overall Results of *Pimephales promelas* Chronic Phase 3

- Overall, the mean growth decreased as the concentration of Tox Site 3 sediment increased in the chronic 7 day test with *Pimephales promelas*. The exception to that was the 50% treatment which had higher mean biomass than the 25% concentration. It is possible this variation was due to sediment particles on the fathead minnows. The nature of this testing (having sediment in cups) made it difficult to ensure that the organisms were free of debris before drying to measure weight. After euthanizing, fish were dipped in Type 1 water but it was difficult to remove all particles.
- The reference site (Site 1) and lab water control had higher mean biomass than all the treatments.
- Mean survival of *P. promelas* ranged from 95% in the lab control to 50% survival in the 50% Tox Site 3 treatment. Treatments of 75 and 100% Tox Site 3 sediment resulted in 65% survival. We calculated a theoretical IC_{25} which is the "concentration" of Tox Site 3 sediment that theoretically would inhibit growth by 25%. The resulting IC_{25} was 16.5% Tox Site 3 sediment based on the mean biomass of the fathead minnows. The IC_{25} is normally used to calculate the amount of a water sample that is toxic. In this case, we did make dilutions of the sediment but it is difficult to know how accurate the sediment dilutions were. It is difficult to extrapolate sediment dilutions to an IC_{25} with great confidence.

Summary of *Daphnia magna* and *Pimephales promelas* Chemistry Results

Routine chemistry parameters of pH, DO, conductivity, ammonia, alkalinity and hardness are all presented in the reports. Due to the fact that elutriate samples were very limited in volume some of the analyses (such as alkalinity) could not be done on all the samples. The inorganic and organic chemistry samples being analyzed by other Departments of the Wisconsin State Laboratory of Hygiene will be reported to the DNR separately. Total suspended solids were measured in the Toxicology lab on the 2nd and 3rd batch of elutriates when it was being filtered for toxicity testing. It was not possible to filter more than 300 mL of samples through a single filter for TSS testing. For each batch of pre-filtered elutriate tested, two samples of 200- 300 mL each were filtered. The DNR had intended for a sample of post-filtered elutriate to be tested for TSS but this was not understood by the lab staff and therefore only pre-filter elutriate was tested for TSS. The TSS results are presented below in Table 2. Initial and final chemistries (DO, pH and conductivity) were also measured on treatments whenever feasible.

Table 3. Total suspended solids (TSS) in pre-filtration elutriate samples.

Elutriate Sample Treatment	Volume filtered (mL)	TSS (mg/L)
Tox Site 3 Pre-Filter Elutriate Batch 2	250	552
Tox Site 3 Pre-Filter Elutriate Batch 2 Duplicate	250	600
Average Tox Site 3 Batch 2		576
Tox Site 3 Pre-Filter Elutriate Batch 3	200	1,682
Tox Site 3 Pre-Filter Elutriate Batch 3 Duplicate	300	1,900
Average Tox Site 3 Batch 3		1,791

Phase 1 Chemistries

D. magna Trial 1
Initial chemistry samples taken from elutriate samples, final chemistry samples were taken by decanting off the top of each treatment at the end of the test.

- Initial DO ranged from 5.4 to 8.0
- Final DO ranged from 4.6 to 7.3
- Initial pH ranged from 7.8 to 8.3
- Final pH ranged from 7.8 to 8.0
- Initial Conductivity ranged from 542-717 uS

D. magna Trial 2
Initial chemistry samples were taken from an extra chemistry cup that was prepared for each treatment that had Manitowoc River water over sediment and was decanted off and measured. Final chemistry samples were taken by decanting off the top volume of water from beakers in each treatment at the end of test.

- Initial DO ranged from 6.1 to 9.3
- Final DO ranged from 6.4 to 7.8
- Initial pH ranged from 8.1 to 8.7
- Final pH ranged from 8.0 to 8.3
- Initial Conductivity ranged from 531 - 604 uS

D. magna Trial 3
Initial chemistry samples were taken from prepared elutriate, final chemistry samples were taken by decanting off the top volume of water from beakers in each treatment at the end of the test.

- Initial DO ranged from 5.4 to 8.0
- Final DO ranged from 7.4 to 9.7
- Initial pH ranged from 7.8 to 8.3
- Final pH ranged from 8.0 to 8.6
- Initial Conductivity ranged from 542-717 uS

P. promelas Trial 2 and 3
Initial chemistry samples were taken from elutriate only samples on each preparation day and also by measuring Manitowoc River water and lab control water before renewal. Final chemistry samples were taken by decanting off the top volume of water from beakers in each treatment at renewal and the end of the test.

- Initial DOs ranged from 5.4 to 10.4
- Final DO ranged from 4.9 to 7.6
- Initial pH ranged from 7.8 to 8.9
- Final pH ranged from 7.7 to 8.4
- Initial Conductivity ranged from 489-802 uS

Hyalella azteca and *Chironomus dilutus* Sediment Tests

In addition to the water (elutriate or Manitowoc River water/sediment) tests conducted with *D. magna* and *P. promelas,* traditional sediment tests were also conducted. The sediment tests were conducted using two 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 28 days with ten organisms per beaker and eight replicates per sediment site. One replicate from each site was ended at Day 10 which is the normal timeframe for Hyalella tests at our lab. By ending one replicate we were able to decide if we should continue the test, or if there was significant toxicity we could choose to end the entire test at day 10. Due to a lack of mortality the test was continued the entire 28 days for the remaining 7 replicates at each 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 28, respectively). Conductivity was also recorded on days 8, 16, and 22. On day 28, 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 other noted deviations from established test protocols aside from the extended duration of the test.

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.* SAS was also used to assess the significant differences in treatments of the *Daphnia magna* and *Pimephales promelas* acute and chronic tests. Results with $p < 0.05$ were considered significant.

Summary of *Hyalella azteca* and *Chironomus dilutus* Test Results

Overlying Water Chemical Parameters in Sediment Tests

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. A few temperatures were slightly outside of the ranges but not enough to significantly affect the test. Temperatures ranged from 21.6°C to 24.2°C as presented in Figures 5 and 11. Initial DO and the minimum and maximum DO measured during the tests are presented in Figures 3 and 9.

pH

There are no required pH criteria but the pH of overlying waters were all in the neutral range with pH ranging from 8.0 to 8.8, as shown in Figures 4 and 10.

Conductivity, Hardness, Alkalinity, and Ammonia

Results of conductivity, hardness, alkalinity, and ammonia analyses from samples are summarized in Figures 6 and 12. 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 for 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 (≤ 1.58 mg/L) and not at levels that have been associated with toxicity in sediment tests in the past (20 - 310 mg/L, USEPA, 2000).

Conductivity

Conductivity ranged from 697 to 905 µS/cm. There are no established criteria for acceptability of conductivity results.

Overall Survival and Growth

Survival results are depicted in Figures 1 and 7. Growth results are depicted in Figures 2 and 8. Survival of *Chironomus dilutus* and *Hyalella azteca* was not significantly negatively affected by any of the treatments compared to the lab control ($p < 0.05$). Statistical analyses indicated a significant decrease in growth of *C. dilutus* at Tox Site 2 compared to the lab control. Growth of *Hyalella azteca* was not significantly lower than the lab control in any of the treatments. The reference site (Site 1) had significantly higher growth for both *Hyalella azteca* and *Chironomus dilutus*. The reference sediment was sandier and coarser in particle size than the control and all the treatments.

***Chironomus dilutus* Survival and Growth**

C. dilutus Survival

Survival data for *C. dilutus* is presented in Figure 1. The survival of the control was significantly lower than the sediment sites but still met the required criteria with 80% survival. Survival at all the treatment sites was 88% or greater and there was no significant difference between the sites.

C. dilutus Growth

Growth data for *C. dilutus* is given in Figure 2. The mean larval weights (ash-free dry weight) ranged from 1.47 mg to 1.98 mg per larval *C. dilutus* for all of the sediment sites. The control sediment averaged 1.84 mg per larvae. Tox Site 1 (the reference site) had significantly higher growth than all the other sites but was not statistically different from the control. Tox Site 2 showed a significantly lower larval weight when compared to both the control and the reference site (Tox Site 1). Tox Sites 3-5 all had significantly lower growth when compared to the reference site but not significantly lower than the lab control.

***Hyalella azteca* Survival and Growth**

The Hyalella test was continued for 28 days after ending one replicate at Day 10. Weights and data from the replicate at each site that was ended on Day 10 are not included. At Day 10 all treatments had 90% or greater survival.

H. azteca Survival

Survival data for *H. azteca* is presented in Figure 7. *H. azteca* showed no significant difference in survival between the laboratory control and the sediment sites. All treatments had 90% or greater survival.

H. azteca Growth

Growth data for *H. azteca* is given in Figure 8. For the 28 day test the mean larval weights ranged from 0.22 mg to 0.35 mg per larval *C. dilutus* for all of the sediment sites. The control sediment averaged 0.22

mg per larvae. The control had the lowest mean dry weight while the reference site had the highest weight. Dry weight of *H. azteca* was not significantly different between sites 2, 3, 4 and 5 but they were all significantly lower than the reference site. Site 3 was statistically different than the control due to higher mean dry weight. Sites 2, 4 and 5 were not statistically different from the lab control.

Quality Control

Tests for all species met the minimum requirements for test acceptability. Replicates and controls were run with all tests. All controls met the survival and weight requirements as applicable. Chemistry and environmental parameters were within the requirements throughout the tests. Reference toxicity tests conducted in July/August performed with all species resulted in a toxic response (IC_{25}/LC_{50}) within control limits established during previous reference toxicant testing.

Conclusions

The sediments collected in the Manitowoc River in Manitowoc, WI demonstrated toxicity to some of the organisms tested. The traditional sediment testing organisms, *Hyalella azteca* and *Chironomus dilutus*, did not show very significant effects. Overall growth of both species was significantly less than the reference site at Sites 2-5 but not significantly less than control sediment. Survival of *H. azteca* and *C. dilutus* was not significantly affected by any of the treatments.

Phase 1 acute tests with *Daphnia magna* and *Pimephales promelas* only demonstrated toxicity in the *D. magna* treatment that consisted of Tox Site 3 elutriate overlying sediment from Tox Site 3 which had 72.5% mortality.

In the next phase of testing, Phase 2, chronic tests with both *D. magna* and *P. promelas* were conducted.

Chronic toxicity to *P. promelas* was found in the treatments that consisted of Manitowoc River water over Tox Site 3 and the Tox Site 3 elutriate (no sediment) treatment which both resulted in significantly reduced survival compared to the control and reference site. Growth of *P. promelas* was significantly reduced in all the treatments involving Tox Site 3 when compared to the controls and the reference site.

Phase 2 *Daphnia magna* chronic tests using Tox Site 3 elutriate treatments resulted in significantly decreased survival only in the treatment consisting of Tox Site 3 elutriate with no sediment. *D. magna* reproduction was not statistically different among any of the treatments.

Phase 3 testing consisted of a chronic test done with *P. promelas* using a "serial dilution" of the Tox Site 3 sediment. Overall, the mean growth decreased as the concentration of Tox Site 3 sediment increased in the chronic 7 day test with *Pimephales promelas*. Survival also generally decreased as the concentration of Tox Site 3 sediment increased. A theoretical IC_{25} (based on mean biomass) was calculated to be 16.5% of Tox Site 3 sediment.

References

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United States Environmental Protection Agency, Washington, D.C. Great Lakes Dredge Material Testing and Evaluation Manual. September 30, 1998. U.S. Environmental Protection Agency Regions 2, 3, 5, and Great Lakes National Program Office and U.S. Army Corps of Engineers Great Lakes & Ohio River Division.

Table 4. 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	$23 \pm 1^{\circ}C$
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*)

Project Photos

Photo1. Sediment and water chemistry samples being taken to other Departments at WSLH for analyses.

Photo 2. Jars of elutriate Batch 1 for all sites and the synthetic lab sediment control (far right) on preparation day (prior to extended settling).

Photo 3. Example of elutriate sample after 24 hours of settling.

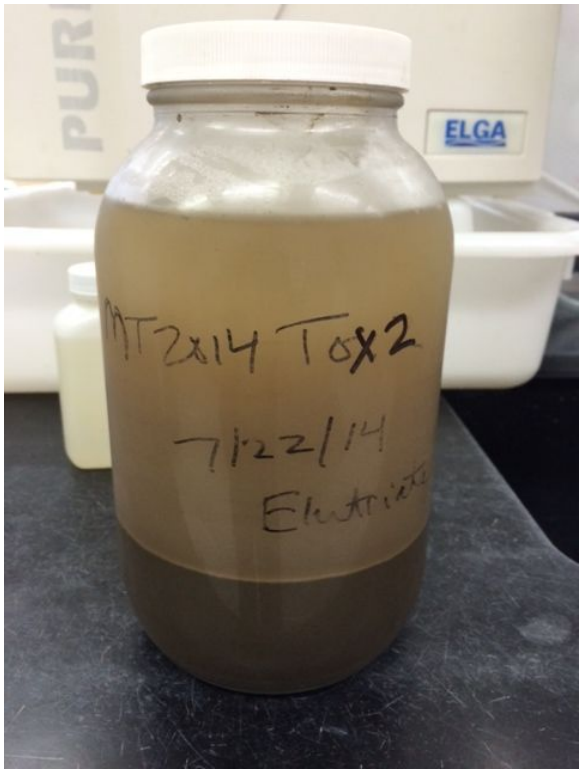

Photo 4. Filtering of elutriate from Tox Site 3.

Photo 5. Acute *Pimephales promelas* test beaker before renewal with fish visible.

Photo 6. Acute *P. promelas* tests randomly set up in testing chambers.

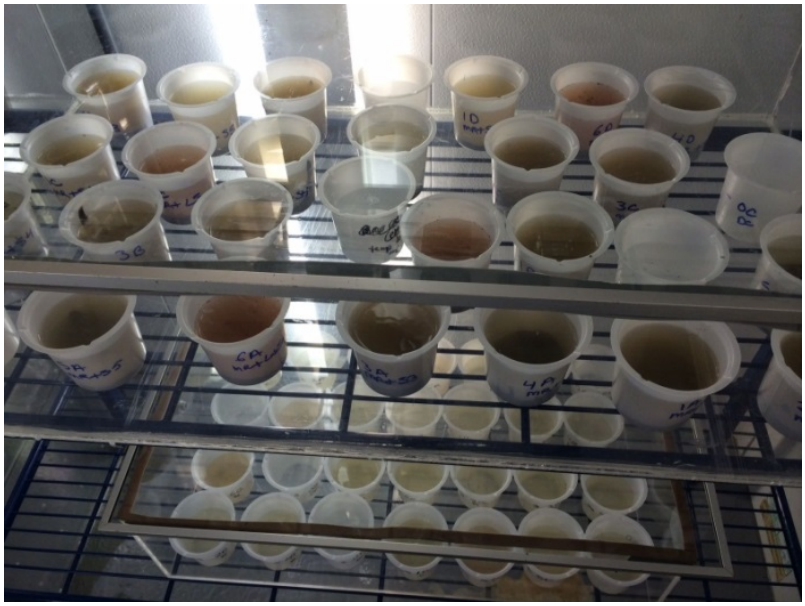

Photo 7. Acute *P. promelas* tests set up with organisms – orange colored sample on top left is synthetic sediment elutriate control, bottom right is lab culture water control. Note difficulty in seeing fish in some of the samples.

Photo 8. Chronic *Daphnia magna* and *Pimephales promelas* test set up.

Photo 9. Settled Tox Site 1 elutriate over sediment sample with film on top.

Photo 10. Tox Site 3 (top beaker) and Site 2 (bottom beaker) with sheen/film on top of sample made from Manitowoc River water over site sediment.

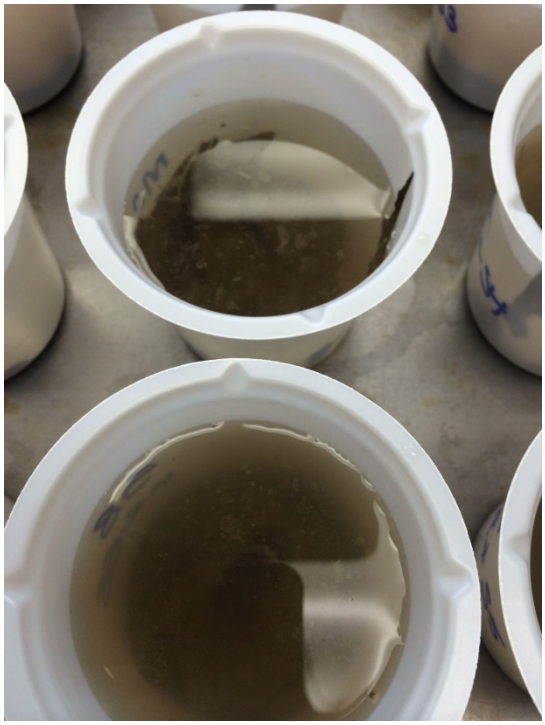

Photo 11. Phase 1, Trial 2 prepared beakers (prior to adding organisms and randomizing) for all Sites and controls. These are made up of Manitowoc River overlying sediment. Lab water control is at the top, then Sites 1-5 and then Treatment 6 (synthetic lab control) is at the bottom of the picture.

Photo 12. Sediment testing with *Hyalella azteca*.

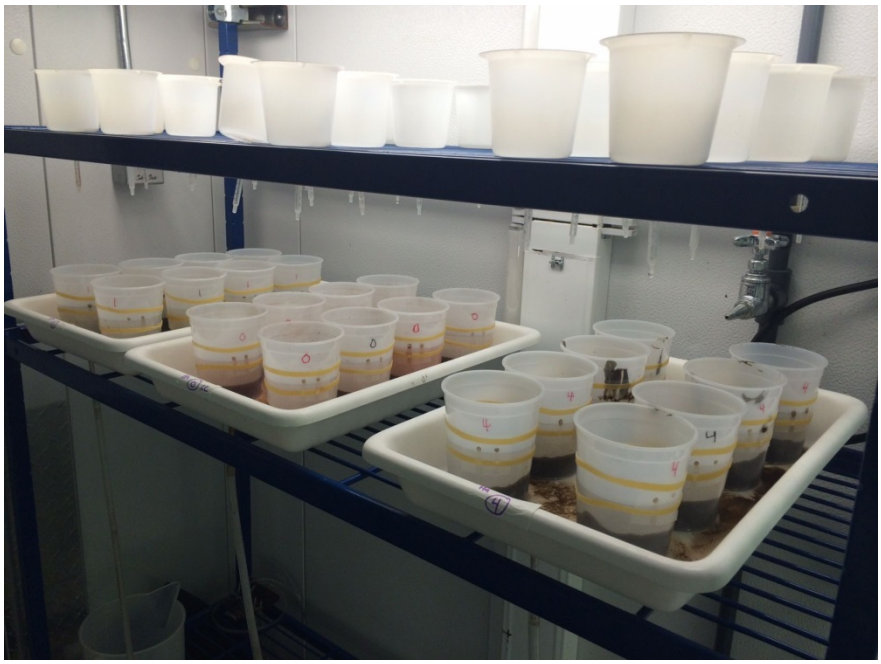

Photo 13. Sheen on sediment sieve observed during shutdown of 28 day *Hyalella* test at Tox Site 3.