# TOXICITY ASSESSMENT OF WABAMUN LAKE SEDIMENTS


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# Toxicity Assessment of Wabamun Lake Sediments

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# EXECUTIVE SUMMARY

In 2002 Alberta Environment (AENV) initiated several studies on Wabamun Lake in response to public concerns about murky water near the TransAlta Utilities Corporation ash lagoon outfall, and to follow-up on incidences of fish mortality. Although elevated metal levels were found in the water and sediments near the ash lagoon outfall, the cause of fish mortality was unrelated. However, contaminant levels in sediments remained a potential issue for aquatic ecosystem health.

HydroQual Laboratories Ltd. (HydroQual) was retained to start investigating the possibility that sediments in the affected areas contained substances harmful to species representative of the major trophic levels in aquatic systems (microbes, plants, invertebrates and fish).

AENV collected sediment samples from the following six sites: the ash lagoon (ALI), west basin of Wabamun Lake (WS1), near the ash lagoon outfall (WS2), east of the ash lagoon outfall (WS3), at the mouth of the inlet canal to the Wabamun Power Plant (WS4), and near the Wabamun Lake Water Treatment Plant outfall (WS5). Four additional samples were collected from Pigeon Lake (02SWE02287), Wizard Lake (02SWE02288), Isle Lake (02SWE02289) and Gull Lake (02SWE02290), as reference locations. Samples were code-labeled and their origin (i.e., exact sampling location or the fact that some samples originated from other lakes) was provided only after all testing was completed.

The toxicity investigation was carried out in sequential phases, starting with an initial screening of sediments that subsequently led to more intensive testing of positive acute or chronic results and a toxicity identification evaluation that was carried out on the most toxic sample.

Screening tests using luminescent bacteria, duckweed, *Lumbriculus*, *Daphnia*, and larval fathead minnows as test organisms were designed to determine the presence or absence of toxic substances. Definitive tests followed standard bioassay methods with luminescent bacteria, *Raphidocelis* (green alga), *Ceriodaphnia*, and larval fathead minnows and provided information on sample potency (acute and chronic endpoints). The tests were carried out on pore or interstitial water, and aqueous and methanol extracts of dried sediment.

The pore waters were not acutely toxic to plants and invertebrates. Acute effects were observed with aqueous and methanol extracts and the greatest effects were observed for the methanol extracts on luminescent bacteria and larval fathead minnow survival. The most consistently toxic sample was ALI. The least toxic sample was WS1. The order of decreasing overall toxicity amongst all extract samples was ALI>WS5>WS2>WS3=WS4>WS1.

Definitive tests were conducted on aqueous extracts with larval fathead minnow (WS2, WS5 and ALI) and *Ceriodaphnia* (WS1, WS2 and WS3). All extracts were lethal to both species and chronic endpoints could not be determined. Drying may have affected the nature and availability of sediment constituents, and for this reason, additional testing was done on the pore water.

The pore waters of samples WS1, WS2, and WS4 had moderate effects on growth of larval fathead minnows (IC25, or concentration which induces a 25% reduction in growth, occurred at concentrations ranging from 22 to 43% of the undiluted pore water), but pore water samples from WS3, WS5 and ALI had no effect at the concentrations tested. The pore waters from all samples also reduced reproduction in *Ceriodaphnia* (IC25 ranged from 8% for sample ALI to 42% for WS4).

Aqueous extracts of sediments from the other four lakes were tested with luminescent bacteria; pore water was tested with *Ceriodaphnia*, and larval fathead minnows. The aqueous extracts had moderate effects on light production by luminescent bacteria (relative to controls, full strength solutions reduced light emission to a range of 45 to 82%). These results were similar to those observed for the samples from Wabamun Lake. Pore water from Wizard Lake sediment was lethal to larval fathead minnow (LC25, or concentration needed to kill 25% of test animals was 39% of the undiluted pore water) and inhibited fish growth (IC25 of 12%). Pore water from Gull Lake sediments reduced young production in *Ceriodaphnia* (IC50 of 29% and IC25 of 8%). These effects were within the range of effects observed for the pore waters from the Wabamun Lake samples.

The ash lagoon sediment pore water and water and methanol sediment extracts were consistently more toxic than the lake sediment samples. For that reason a toxicity identification evaluation(TIE), was conducted on the ash lagoon sediment in an attempt to identify the compound(s) responsible for observed effects. Although the toxic constituents were not identified, the results indicated that they were likely organic compounds.

The results from this investigation suggest that sediments from the ash lagoon (ALI) may contain substances (organic in nature) that could adversely affect microbes and sensitive species of invertebrates and fish. The origin and identity of these substances could not be determined. It was also not possible to determine if these substances were the same as those responsible for the effects observed in some of the lake sediment samples.

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# 1.0 INTRODUCTION

In 2002 Alberta Environment (AENV) initiated several studies on Wabamun Lake in response to public concerns about murky water near the TransAlta Utilities Corporation ash lagoon outfall and to follow-up on incidences of fish mortality. Elevated metal levels were found in the water and sediments near the ash lagoon outfall, but the cause of fish mortality was unrelated to these metal levels (AENV 2002). However, contaminant levels in sediments remained a potential issue for aquatic ecosystem health.

HydroQual Laboratories Ltd. (HydroQual) was commissioned by AENV to conduct an initial toxicity assessment on sediments from Wabamun Lake. The objective was to determine if the sediments contained substances toxic to aquatic life forms (effects-based investigation).

A phased approach was taken in this study, whereby the objectives of each subsequent phase were determined by the findings of the previous phase (Figure 1). AENV was informed about each phase's results and consulted about the next phase's objectives. The phases included:

I. Trophic Level Screening Toxicity Assessment
II. Confirmation and Potency Testing (Modified Tests)
III. Formal Test Protocols (Acute and Chronic) on Sediment Extracts
IV. Formal Test Protocols (Acute and Chronic) on Porewater
V. Formal Testing on Sediments from Other Lakes
VI. Toxicity Identification Evaluation (TIE) on Toxic Samples
VII. Additional Investigative Testing

This report documents methods and presents results of each phase sequentially.

# 2.0 METHODS

Sample handling procedures and test methods are documented in this section.

## 2.1 Sample Collection

Surficial (top 5 cm) Wabamun Lake sediment samples were collected by AENV with an Ekman grab and placed in 20 L pails lined with plastic bags. Bags were closed with minimal headspace and the pails sealed with lids for shipment. A total of four sets of samples were collected for this investigation (three sets from the initial six sites and one set from other lakes). AENV provided coded labels for each sample and did not disclose details about sample locations (Table 1).

The first set of samples for Phases I and II was assigned sample numbers 20020659 through 20020663 and 20020673. Additional samples were collected from these sites for Phases III, IV, and VI. Four samples were collected from other lakes for testing in Phase V.

Individual samples are referred to in the body of this report by the last three characters in AENV's sample code. Hence, NESBL020618WS1 is referred to as WS1 (Table 1).

## 2.2 Sample Characterization and Preparation

Colour, odour, redox potential, moisture content and total organic matter were recorded for each sediment sample, and conductance and pH were measured on pore water and aqueous extracts. These measurements were used to determine if the samples were within the physiological tolerance range of the test organisms; they were also used to aid in the interpretation of results.

Aqueous and methanol extracts were prepared with sediment dried for 24 hours at 105°C (USEPA, 1989). The extracts consisted of four parts solvent to one part dried sediment by weight (Burton, 1992, Environment Canada, 1994). Aqueous extracts removed water-soluble materials from the sediments. Methanol extracts removed the more hydrophobic materials such as organics and non-polar metal complexes. Methanol is less toxic than other organic solvents and safer to use in the workplace.

Pore water was removed from the solids with low speed centrifugation of the sediment samples.

## 2.3 Test Methods

Reporting of results. Test results are reported as "percent of controls" or "point estimates" depending on whether the test was a screening test or a definitive test. Screening assays involved testing on single treatments or concentrations, and results are reported as a percent of the control response. Definitive tests involve multiple dilutions of the sample, and linear interpolation from the dose and response relationship are used to define the point estimates, or test concentration giving a defined change in the measured response (e.g., 25 or 50%). In this report, point estimates were defined for lethal (LC), inhibitory (IC), or effective (EC) concentration giving a 25 or 50% change in the response after a given exposure. For example, "LC50 @ 96 h" is the test concentration lethal to 50% of the test population at 96 h. In this

report, point estimates are expressed as the percentage volume of test solution needed to elicit the effect period.

Qualification of results. Results are discussed in relative terms compared to control responses and with expert knowledge about the test organisms. The terminology that is applied to the specific group of samples that was tested in this study may not be applicable to other groups of samples. The detection limit for most biological effects is a change of 20 to 30% in the measured response relative to controls. This range reflects differences in test design (e.g., type of test, replicates, test duration) and organisms (e.g., sensitivity). A 'moderate effect' in the context of this investigation was considered a response significantly different from controls (detection limit) to a change of 70 to 80% of controls. Any change greater than 70 to 80% of controls was considered 'toxic'.

Testing media. In this initial assessment of lake sediment toxicity, tests were conducted on aqueous and methanol sediment extracts, and on pore water. For liquid test media a range of test organisms with relatively well-documented tolerance ranges is readily available, and testing protocols are well defined and straightforward. Actual sediment (liquid and solid media combined) was not tested, mainly because the variety of test organisms was a limiting factor at this stage.

### *2.3.1 Microbial Tests*

Microbes are an integral component of sediment systems. They play vital roles in the degradation of organic mater, the cycling nutrients and metals, and represent an important food source for many invertebrates. The microbial tests included in this study are the bacterial luminescence, and the enumeration of total aerobic and anaerobic heterotrophic bacteria and sulfur reducing bacteria.

The bacterial luminescence test is based on light output by the marine bacterium *Vibrio fischerii* (Environment Canada, 1992d). Substances that are toxic or stressful will reduce light output. Hence, light output is related to sample strength or toxicity. The samples were tested at 91% full strength. The slight dilution resulted from the addition of a salt solution to osmotically adjust the sample. The samples were incubated at 15$^{o}$C and light levels read at 5 and 15 minutes with a Microtox Model 500 Unit. The results were expressed as a percentage of the controls.

Sediment bacterial populations were enumerated with a most probable number (MPN) method (Carter, 1993). A one hundred-fold dilution of the porewater was prepared with growth media (trypticase soy broth). This working solution was dispensed into five wells (20 µL volumes) of a 96 well microplate containing 180 µL of media. These solutions were then serially diluted (factor of ten) an additional seven times (20 µL into 180 µL media).

The plates were incubated at room temperature for up to two weeks. Growth was scored by the presence of turbidity in the wells. The final cell densities were obtained from a table of most probable numbers with five replicates (Carter, 1993).

The plates for the enumeration of anaerobic bacteria were prepared in an identical fashion, but they were incubated in five percent carbon dioxide (no oxygen).

The sulfate reducing bacteria were enumerated in the same fashion, but with a medium obtained from Droycon Bioconcepts Inc. (SRB, sulfate reducing bacteria growth medium). Growth (activity) was assessed by the presence of a black colour in the wells.

### *2.3.2 Plant Tests*

The plant growth tests were performed on a floating macrophyte (*Lemna minor*, commonly known as duckweed) and a unicellular green alga (*Raphidocelis capricornutum*). The macrophyte and alga are both common aquatic plants found in most freshwater systems.

Duckweed is a fast growing plant found in ponds worldwide. The test was done with 30 mL volumes in 50 mL clear plastic containers. One three-frond plant was placed into each test vessel and growth (number of fronds) scored after a seven day incubation under continuous light at 4000 ±400 lux at 25 ±2$^{o}$C (Environment Canada, 1999). Growth was expressed as a percent of controls.

The algal growth inhibition test was carried out with the unicellular green alga *Raphidocelis capricornutum* (formerly *Selenastrum;* Environment Canada, 1992a). This species is common to many freshwater lakes and ponds throughout North America and Europe. The tests were done in 96 well microplates. The samples were spiked with nutrients and then inoculated with the alga. There were six replicates for each sample and controls. The plates were incubated under continuous light (4000 ±400 lux) at 25 ±2$^{o}$C. Growth was assessed by an increase in cell numbers (particle counts or turbidity).

Any substance or condition that is stressful will inhibit or retard growth, resulting in a lower final cell density. Increases in final cell densities over the controls may result from the presence of nutrients or other essential trace substances in the samples. The results were expressed as a percent of controls.

### *2.3.3 Invertebrate Tests*

The invertebrate test species included the freshwater microcrustaceans *Daphnia magna* and *Ceriodaphnia dubia*, and the sediment dwelling oligochaete *Lumbriculus* sp. These organisms feed on detritus and algae and are preyed upon by other invertebrates and fish. They are found throughout the world in freshwater systems.

The tests with *Daphnia* and *Lumbriculus* were done in 50 mL clear plastic containers containing 25 mL volumes of test solution. There were five organisms in each container with three replicates of each sample and the controls. The containers were incubated for four days at 25$^{o}$C with an 8 h dark and 16 h light photoperiod (intensity at the water surface of 800 lux). Survival was scored daily and the results expressed as a percent of controls.

The test with *Ceriodaphnia dubia* measures effects on survival and reproduction over a 7-d exposure period (Environment Canada, 1992c). The tests were done in 30 mL plastic containers containing 15 mL of sample. At start of test, test organisms must all be less than 24 h old and must have been released over an 8 h period. One animal was placed into each of ten test containers per treatment level. The organisms were fed a defined amount of algae and a fermented mixture of yeast, alfalfa powder, and trout chow. Test solutions were renewed daily to expose the organisms to fresh sample throughout the 7-d period. Survival was scored daily along with the number of live young released. *Ceriodaphnia* will typically produce 3 broods (20 to 40 neonates in total) over a 6 to 8 day period. The endpoints included lethality and reproduction (young produced per adult).

### *2.3.4 Fish Tests*

Fish are an integral part of aquatic ecosystems. They feed on plants and invertebrates and are preyed upon by other fish, birds and mammals.

Two types of tests were done with larval fathead minnows; short-term acute, and 7-d survival and growth. The short-term acute tests were done in 50 mL plastic containers containing 30 mL of solution (5 minnows per container and one replicate per treatment). The test containers were incubated for four days under ambient conditions of light and temperature. Survival and any signs of unusual behaviour were recorded daily.

The survival and growth tests were done in 400 mL plastic containers holding 250 mL of test solution (Environment Canada, 1992b). The test was started with organisms 24 h post-hatch (10 fish per test vessel). The sample was serially diluted to obtain five treatment levels (6.25, 12.5, 25, 50, and 100%). There were four replicates for each treatment. The fish were fed a defined ration of 24 h old *Artemia* daily (1,200 to 1,500 brine shrimp per day). The test solutions were replenished daily. Survival and any signs of unusual behaviour were recorded daily. The fish were dried and weighed at test termination to assess growth effects (ability to turn food into biomass).

# 3.0 RESULTS AND DISCUSSION

## 3.1 Phase I - Trophic Level Screening Toxicity Assessment

Phase I consisted of a series of screening tests on a range of different test species. Its intent was to obtain an initial indication of the level of toxicity in the sediment samples.

The screening tests were developed by HydroQual to provide preliminary toxicity data that could then serve as a guide for further testing. The tests were not intended as a substitute for standard test methods.

The pore water and aqueous extracts were tested at full strength or with minor dilution required by the test methods. The methanol extracts were tested at the no observed effect level (NOEC) for methanol for each species (NOEC; 5% for luminescent bacteria, 1% for *Lemna, Daphnia, Lumbriculus*, and larval fathead minnows, 0.1% for algae).

The following tests were conducted on the six sediment samples:

- microbes -
  - bacterial luminescence,
  - bacterial enumerations (aqueous samples only);
- plants -
  - algal growth inhibition,
  - *Lemna* growth inhibition;
- invertebrates -
  - *Lumbriculus* survival,
  - *Daphnia* survival;
- vertebrates -
  - larval fathead minnow survival.

### *3.1.1 Results*

Results of phase I are summarized in Tables 2 to 4.

All six samples submitted for testing in Phase I were dark green or grey and had odours of decaying organic matter. Pore water pH and conductance ranged from 7.3 to 7.7 and from 460 to 560 µS/cm, respectively. These values were well within the physiological tolerances of the test organisms. Two samples had distinctive sulfur odours (WS2 and ALI, Table 2); these two samples also had negative redox values. Moisture content of samples WS1 and WS5 was 30 and 49% by weight, respectively. The remaining samples had moisture contents ranging from 71 to 82%. These high moisture contents made it difficult to obtain enough dry weight for testing; when re-hydrated, little free water was available for the liquid extracts.

The total organic matter contents ranged from 3 to 26% by weight of the dried material (Table 2). Higher organic matter contents were measured in the samples with the higher moisture contents.

Bacterial luminescence tests were conducted on the aqueous and methanol extracts. The aqueous extracts had some moderate effects on light output (48 to 74% of controls). The methanol extracts were more toxic. The most toxic extract was sample ALI. A 0.6% dilution of the methanol extract reduced light output by 50% ($EC50_{@15min}$; this is a 0.03% volume/volume solution of the original methanol extract). The $EC50_{@15min}$ for sample WS5 was 2%. Both these results were considered highly toxic.

The bacterial luminescence results on the 5% methanol extracts for samples WS1 to WS4 had $EC50_{@15min}$ that ranged from 12 to 58%, respectively (Table 2). These values were also considered indicative of a moderate to toxic effect, but less so than for sample ALI.

The pore water was not toxic to luminescent bacteria (Table 3). There was some slight stimulation of light output observed for all six samples. The aqueous extracts had some moderate effects on light output. The results ranged from 55 to 69% of controls for samples WS1 to WS5. The aqueous extract of sample ALI was 22% of controls; this was considered borderline toxic.

The numbers of total heterotrophic aerobic bacteria were variable ranging from 100 to 2,000,000 per g (Table 3). The lowest levels were found in samples WS1, WS4, and ALI (ranged from 100 to 1,000 per g). The numbers of total heterotrophic anaerobic bacteria were low in all samples (< 25 per g, Table 3). This could be due to exposure to oxygen during sample handling. Oxygen will kill many obligate anaerobes. However, the levels of sulfate reducing bacteria (based on activity) were relatively high in all samples, except ALI (100,000 CFU/mL compared to <100 CFU/mL in ALI).

The aqueous extracts of samples WS1, WS3, WS4, and WS5 stimulated growth of the green alga, *Raphidocelis* (Table 3). The aqueous extracts of sediment samples WS2 and ALI had a moderately inhibitory effect on growth at the highest treatment level.

The aqueous extracts had no adverse effect on duckweed growth. In fact, the extracts stimulated growth and final frond numbers were almost double the controls.

The aqueous extracts were not toxic to invertebrates (*Daphnia* and *Lumbriculus*, Table 3). *Lumbriculus* survival was low in sample WS2 (40% of controls). The reason for this was unknown and was not correlated with the other test findings.

The aqueous extracts of WS1 and WS2 were moderately toxic to larval fathead minnows. The extract for WS3 was borderline highly toxic, whereas the extracts of WS4, WS5, and ALI were lethal to larval fathead minnows (Table 3).

The methanol extracts of the dried sediment were not toxic to plants and invertebrates. However, the extract from sample WS5 was moderately toxic to larval fathead minnows (47% of controls). The methanol extract of sample ALI was toxic to larval fathead minnows.

The methanol extracts of WS5 and ALI were highly toxic to luminescent bacteria followed by WS2 ($EC50_{@15min}$ was 0.2 and 0.9% respectively, Table 4). Samples WS3 and WS4 were also toxic ($EC50_{@15min}$ was 3.3 and 2.19%, respectively). The $EC50_{@15min}$ of the methanol extract of sample WS1 was 12%.

### *3.1.2 Discussion*

Screening tests were conducted on sediment pore water, and aqueous and methanol sediment extracts with species representative of major trophic levels in aquatic systems. The pore waters and aqueous extracts were not toxic to plants and invertebrates. The greatest effects were observed for the methanol extracts on luminescent bacteria and for the aqueous extracts on larval fathead minnow survival. The most consistently toxic sample was ALI. The least toxic sample was WS1. The order of decreasing overall toxicity for samples was ALI > WS3 > WS2 > WS3 = WS4 > WS1.

Luminescent bacteria and larval fathead minnows were considered the most sensitive test species based on Phase I results. These species were carried forward to Phase II.

A notable finding of Phase I was the low numbers of aerobic and anaerobic bacteria, but the relatively high numbers of sulphate reducing bacteria in the sediments. This requires further investigation.

## 3.2 Phase II – Confirmation and Potency Testing (modified tests)

Results of phase II are summarized in Table 5.

The objective of Phase II was to quantify effects on luminescent bacteria and larval fathead minnows. Tests were carried out on the aqueous sediment extracts, because phase I determined that these were most toxic to fish. The tests with larval fathead minnows were conducted over a range of sample dilutions (5 treatment levels plus a control). The sample was serially diluted (30 mL volume) by a factor of 2 to give the following test concentrations 100, 50, 25, 12.5, and 6.25% volume/volume. The test was conducted with one replicate and five organisms for each treatment level. The solutions were not replenished over the 96 h test period (static test).

Although invertebrates tested in phase I were not sensitive to the aqueous sediment extracts, a decision was made to include the 7-d survival and reproduction test with *Ceriodaphnia dubia* to assess potential chronic effects on reproduction. This standard test method is widely applied through environmental monitoring programs and legislation. *Ceriodaphnia* is also very sensitive to a wide range of contaminants and conditions. This test was conducted on samples WS1, WS2, and WS3, which were least toxic in phase I.

*3.2.1 Results*

The samples and extracts were stored at 4°C in darkness until tested. The aqueous extracts were moderately toxic (WS3 at 61% of controls) or not toxic to luminescent bacteria after six weeks storage (>80% of controls; Table 5).

The aqueous extracts were lethal to larval fathead minnow with $LC50_{@\ 96\ h}$ ranging from 29% (WS2) to 82% (WS1). These results were less toxic, but consistent with the findings from phase I. However, a slight decrease was noted in the aqueous extract of ALI following storage (Table 5).

The aqueous extracts of samples WS1, WS2, and WS3 were lethal to *Ceriodaphnia dubia* at the lowest concentration tested. For this reason, no point estimates were derived for effects on survival and reproduction (Table 5).

*3.2.2 Discussion*

The results for luminescent bacteria and larval fathead minnows indicated somewhat lower toxicity than observed in phase I, suggesting that the aqueous extracts had lost some toxicity as a result of their five to six weeks storage. Phase I was indicative of significantly higher toxicity for four out of six samples.

Only one of the six extracts was significantly toxic to luminescent bacteria (sample WS3). The effects on larval fathead minnows clearly indicated that the aqueous sediment extracts contained substances acutely lethal to fish. The interesting finding was that effects were detected in extracts of all samples. Low dissolved oxygen levels (4 mg/L) in the highest treatments (100% or undiluted extract) could have contributed to higher mortality in the 4-d test (test solutions were not replenished daily).

*Ceriodaphnia dubia* was very sensitive to the aqueous extracts. The lowest treatments (6.25% by volume) were acutely lethal to *Ceriodaphnia*. This was not a result of low dissolved oxygen levels.

## 3.3 Phase III - Formal Test Protocols (Acute and Chronic) on Sediment Extracts

Results of phase III are summarized in Table 6.

The objective was to quantify the toxicity measured in the aqueous samples with standard Environment Canada methods for both larval fathead minnows and *Ceriodaphnia*. The intent was to obtain acute and chronic endpoints for both species on the aqueous extracts.

The larval fathead minnow survival and growth tests were performed on aqueous sediment extracts of samples WS2, WS5, and ALI. The 7-d survival and reproduction test with *Ceriodaphnia* was repeated on aqueous sediment extracts of samples WS1, WS2, and WS3.

AENV collected fresh sediment samples for these tests because large sample volumes were required. The sediment was dried and then extracted as described in Section 2.2. However, the amount of free water present in the extracts was insufficient to conduct the full larval fathead minnow test following the Environment Canada method. The solutions were replenished daily throughout the seven day test. However, due to the insufficient volume, the test solutions were not replenished with fresh solutions the last two and three days of the tests for samples ALI and WS5, respectively. Instead, the solutions from the test vessels were removed, pooled, and then dispensed back to the containers.

### *3.3.1 Results*

Results of phase III are presented in Table 6.

Four of the aqueous sediment extracts were moderately toxic to luminescent bacteria (39 to 67% of controls for samples WS1 to WS4), but the extracts for samples WS5 and ALI were not toxic. The absence of a toxic response in ALI was unexpected as this was the most toxic sample in previous testing. The results for WS2, WS3, and WS5 were consistent with the findings of phase II.

The aqueous extracts of samples WS2, WS5, and ALI were lethal to larval fathead minnows. The $LC50_{@\,7\,d}$ on the fresh samples were consistent with the $LC50_{@\,4\,d}$ results obtained in phase II.

The results for *Ceriodaphnia* on samples WS1, WS2, and WS3 were consistent with the findings from phase II: the aqueous extracts were lethal at the lowest concentration tested (6.25% volume/volume).

### *3.3.2 Discussion*

For the most part, the results from Phase III confirmed the findings from Phase II. The lack of toxicity to luminescent bacteria in ALI was unexpected as this sample had been the most toxic in previous testing. This may be indicative of local variability in the distribution of toxic substances in sediments. However, similarl to previous results ALI was less toxic to fathead minnows than WS2 and WS5.

Although drying of sediments is a standard practice, it may physically and chemically alter the sediments in unpredictable ways. For this reason, it was decided, in consultation with AENV, to carry out further testing on pore water.

## 3.4 Phase IV - Formal Test Protocols (Acute and Chronic) on Pore Water

Unlike previous phases that focused on toxicity of sediment extracts, the objective of this phase was to determine the toxicity of pore water. Pore water is expected to give a measure of toxicity that is closer to what may be experienced in natural environments than extracts of heat-dried sediments.

The full Environment Canada test methods were followed and included 7-d survival and reproduction in *Ceriodaphnia*, 7-d survival and growth in larval fathead minnows, and growth inhibition in the green alga, *Raphidocelis capricornutum*. This species was included in Phase I, but the tests were only done on a single concentration (100%). Consequently, it was not possible to derive point estimates for assessing sample potency.

AENV collected fresh sediment samples for Phase IV testing. It was possible to isolate about 10L of pore water from each sample; this did not meet the 20L requirement to complete the testing for this phase. For this reason, the pore water was diluted to 50% with deionized water to generate enough volume for testing. A 50% dilution was the highest treatment tested for each sample.

### *3.4.1 Results*

Results of phase IV are presented in Table 7.

Bacterial luminescence tests were done on aqueous extracts of aliquots of dried sediments for comparison to results obtained in previous phases. The results were consistent with Phase I, but they were indicative of a higher level of toxicity than observed in Phase II. These results suggest that aging of the extracts over time reduced the amounts and or availability of the toxic constituents. The most toxic samples were WS5 and ALI with 24 and 25% of controls. WS3 and WS4 had a similar level of toxicity (48 and 53% of controls). The extracts from WS1 and WS2 were only moderately toxic to luminescent bacteria (83 and 72% of controls).

The pore waters from the six sediments did not inhibit growth of the green alga, *Raphidocelis*.

The pore waters from the six sediments were not lethal to larval fathead minnows after a 7-d exposure at the highest concentration tested (50% full strength). Some moderate effects on growth (weight gain) were detected in fish exposed to pore water from WS1, WS2, and WS4.

Unlike the aqueous extracts that were tested previously, the pore waters were not acutely lethal to *Ceriodaphnia* at the highest concentration tested (50% full strength). However, the pore waters reduced reproduction in *Ceriodaphnia* in most samples. The pore water from samples WS1, WS2, WS3, and WS5 reduced young production by 25% at concentrations ranging from 25 to 29% (volume/volume). The pore water from sample WS4 was less toxic (a 42% solution reduced young production by 25%). However, sample ALI was most toxic (an 8% solution reduced young production by 25% and an 18% solution by 50%).

### *3.4.2 Discussion*

The pore water was less toxic than the aqueous sediment extracts tested in phases II and III. The reason for this remains unclear, but it is suspected that drying increases the availability of the toxic constituents.

The pore waters from the six samples were not toxic to the unicellular green alga. This species and *Ceriodaphnia* are equally sensitive to metals, but not to organics. The absence of

phytotoxicity and the observed effects on young production would suggest that the toxic constituents were likely not metals but organic substances.

Although the pore waters were not acutely lethal to larval fathead minnows, some effects on growth were noted in three of the samples.

## 3.5 Phase V – Formal Testing on Samples from Other Lakes

Results of phase V are summarized in Table 8.

The objective of this phase was to obtain sediment toxicity data from four other lakes so that results from Wabamun Lake could be compared with those from other lakes.

Tests included bacterial luminescence on aqueous sediment extracts, and algal growth inhibition, 7-d larval fathead minnow survival and growth, and 7-d *Ceriodaphnia* survival and reproduction on pore water.

### *3.5.1 Results*

The aqueous sediment extracts were moderately toxic to luminescent bacteria (results ranged from 45 to 82% of controls; Table 8). These values were within the range obtained for aqueous extracts of dried sediments from Wabamun Lake.

The pore waters did not inhibit algal growth and they were not acutely lethal to larval fathead minnows (Table 8). However, pore water from sample 02SWE02288 had a moderate effect on larval fathead minnow growth.

Pore waters from samples 02SWE02287 and 02SWE02289 had no effect on survival and reproduction in *Ceriodaphnia* (Table 8), but pore water from sample 02SWE02290 had a significant effect on reproduction. A 29% solution reduced the number of young produced by 50%. Pore water from sample 02SWE02288 had a moderate effect on reproduction (a 30% solution reduced young production by 25%; Table 8).

### *3.5.2 Discussion*

Comparable responses were observed with samples tested in this phase compared to previous phases.

## 3.6 Phase VI – Toxicity Identification Evaluation (TIE) on Toxic Sediments

The objective of this phase was to separate and identify the water soluble toxic constituents present in the sediments.

A TIE is a systematic fractionation of a sample to characterize, isolate, and ultimately identify and confirm the nature of the toxic constituents. The treatments are based on conventional water

treatment practices. A unique aspect of the process is that the fractionation and analytical procedures are largely determined by how the effects are partitioned with each treatment.

The TIE fractionation was performed on the aqueous extract and pore water from ALI because sediments from that site yielded consistently more toxic responses than those from other sites.

### *3.6.1 Results*

Samples of the pore water and aqueous extract of dried sediment ALI were submitted for a chemical characterization and fractionation. The analyses included a metals scan and major parameters (major ions and nutrients). The results did not reveal any obvious parameters that could be contributing to the observed toxicity.

Fractionation procedures required each sample to be divided into three aliquots. One aliquot was adjusted to pH 3, the second to pH 10, while the third was not adjusted. Each aliquot was then passed through a C18 column (solid phase extraction). Aeration and filtration treatments, which are normally part of the TIE procedures, were not done because of sample volume limitations. The column effluent was then adjusted back to the original pH for testing. The columns were then eluted with 4 mL volumes of increasing concentrations of methanol in water (25, 50 and 75%) and finally with two 4 mL volumes of 100% methanol. This resulted in a total of 24 fractions for testing, eight at each of three pH values (aerated, filtered, column effluent, 25, 50, and 75% methanol in water, and two 100% methanol washes).

Laboratory dilution water was treated in the same fashion for quality assurance purposes. This was to verify that the manipulations did not impart toxicity to the sample.

All 18 fractions were generated for the pore water. However, only 17 fractions were received for the laboratory dilution water (the second 100% methanol at pH 11 elutriate was not done). For the aqueous sediment extract, the second 100% methanol elutriates at pH 3 and ambient pH were also not generated, and the pH 3 column effluent was not pH adjusted back to ambient pH for testing.

The fractions were tested with luminescent bacteria and larval fathead minnows (screening tests). The laboratory dilution water fractions were not lethal to larval fathead minnows and had no adverse effect on luminescent bacteria (Table 9a).

The fractions derived from the pore water and the aqueous sediment extracts had no adverse effect on luminescent bacteria and larval fathead minnows. These results were surprising because of the consistent and reproducible toxic effects measured in extracts of dried sediment (Table 9b and 9c).

*3.6.2 Discussion*

TIE fractionation and testing of pore water and aqueous sediment extracts from sample ALI yielded inconclusive results as none of the fractions was toxic to luminescent bacteria or fathead minnows. The results suggested that the toxic constituents were likely physically excluded from the column. However, this can not be confirmed because the aeration and filtration treatments in the standard TIE fractionation procedure were not done due to sample volume limitations.

## 3.7 Phase VII – Additional Investigative Testing

The objective of Phase VII was to investigate differences in toxicity observed between the pore water and the aqueous sediment extracts from ALI in an attempt to narrow down the nature of the toxic substances involved (organic versus inorganic).

*3.7.1 Results*

Bacterial luminescence was the only test included in Phase VII because it was rapid, required small volumes, and had been shown to be sensitive in previous testing.

Archived sample material and fresh extracts of wet and dried sediment were retested to confirm sample potency (Table 10). The ALI sediment was again confirmed as the most toxic and subjected to additional treatments including high speed centrifugation, ashing (550 $^{o}$C), and filtration (0.22 μm membrane filter). The filtration and high speed centrifugation treatments should have removed any large molecular weight substances and suspended solids. The ashing was done to remove organics

Aqueous extracts of the wet and oven dried sediment from ALI were moderately toxic to luminescent bacteria (36 and 53% of controls for the dried and wet sediments, respectively). The extract of the dried sediment was more toxic than the wet sediment.

Centrifugation, filtration, and combustion at high temperature rendered the extract of the wet sediment non-toxic (Table 11). The toxicity of the extract of the dried sediment was not reduced following filtration or centrifugation. However, combustion at high temperature removed the toxicity.

*3.7.2 Discussion*

The results suggest that the toxic constituents are bound to larger particles suspended in the porewater under natural conditions. The particles are large enough to be removed with a 0.22 μm filter and centrifugation. Further, the sediment was rendered non-toxic following drying then combustion at high temperature (550 $^{o}$C). This treatment removes organic matter.

The results for the extract of the dry sediment suggested that the sediments contain a bioavailable, water soluble organic compound bound to the solid phase. It was also interesting to note that the extract of the dried sediment had a yellow color and the wet sediment extract was very pale yellow/gray in color. The colour was likely due to tannic acids in the sediment from

the decomposition of plant matter. Although present, the tannic acids had no effect on the optical properties of the test solution (colour correction was not required in the bacterial luminescence test).

# 4.0 SUMMARY

The objective was to determine if sediments from Wabamun Lake contained substances potentially harmful to species representative of major trophic levels in aquatic systems. The underlying premise was that effects on the more sensitive species in one or more trophic levels could impair normal food chain structure and function.

The study was conducted in seven phases on three separate sets of samples collected at different times (two sets from the same sites within Wabamun Lake and one set from other lakes in the area). Two samples from Wabamun Lake were anoxic when received (WS2 and ALI). The findings from each phase are summarized below.

Phase I involved a biological characterization of Wabamun Lake sediments. The sediments had a high organic matter and water contents. The pore water was not toxic to luminescent bacteria. Population densities of aerobic and anaerobic heterotrophic bacteria were lower than expected for organic sediments. However, levels of sulfur bacteria were relatively high as expected based on observations of sediment colour (black) and odour (rotten egg) at the time of sample receipt.

Aqueous extracts of the sediments were not toxic to plants and invertebrates. However, the extracts were lethal to larval fathead minnows and luminescent bacteria. Methanol extracts were not toxic to plants and invertebrates, but they were highly toxic to luminescent bacteria. The methanol extract of sample ALI was lethal to larval fathead minnows and the extract of sample WS5 caused some partial mortality. The extracts from the other sediments were not toxic to larval fathead minnows. In summary:

- Populations of heterotrophic bacteria were low in the pore water.
- The sediments contained non-polar organic substances lethal to bacteria.
- The water and methanol extractable substances were not lethal to plants and invertebrates.
- Water and methanol extractable substances present in the sediments were toxic to varying degrees to microbes and fish. Hence, the toxicity is due to polar and non-polar substances.
- It was not possible to determine if the observed effects were due to:
    - Inorganic or organic substances, or
    - One or more classes of compounds or physical conditions.

Phase II testing was done on aqueous extracts and included bacterial luminescence, acute lethality with larval fathead minnows, and survival and reproduction with the microcrustacean *Ceriodaphnia* (chronic endpoint). The extracts were not toxic to luminescent bacteria and only moderately toxic to fish. The results were consistent with Phase I. However, the extract from samples WS1, WS2, and WS3 (the other samples were not tested) were lethal to *Ceriodaphnia* at the lowest concentration tested (6.25% of a four to one aqueous extract). In summary:

- The decrease in toxicity to luminescent bacteria was unexpected. The compounds toxic to this species could be labile and different from those affecting fish and *Ceriodaphnia*.
- The results for the fish were consistent with Phase I.
- It was not possible to determine if the observed effects on the fish and *Ceriodaphnia* were due to:
  - Inorganic or organic substances, or
  - One or more classes of compounds or physical conditions (e.g., suspended matter that affects fish at the level of the gill or impedes feeding and normal development in *Ceriodaphnia*).
- The observed toxicity was likely due to polar substances (water soluble).

The tests in Phase II were repeated in Phase III with two differences. First, the larval fathead minnow test duration was extended from four to seven days. Second, larval fathead minnow tests were only done on samples WS2, WS5, and ALI. In summary:

- The bacteria luminescence results were different from those obtained in Phase II and Phase I. The compounds toxic to this species could be more labile and variable than those affecting fish and *Ceriodaphnia*.
- The effects on fish and *Ceriodaphnia* were consistent with Phase II (effects were reproducible).

Phase IV included the tests conducted in Phase III plus growth inhibition in the green alga, *Raphidocelis*. However, the tests with *Raphidocelis*, larval fathead minnows, and *Ceriodaphnia* were conducted on pore water as opposed to an aqueous extract. These results could not be compared to previous phases. However, the test with luminescent bacteria was done on an extract for comparison to previous results. Key findings included:

- The bacteria luminescence results were different from those obtained in previous phases (I to III). The compounds toxic to this species could be more labile and variable than those affecting algae, fish and *Ceriodaphnia*.
- The pore water was not toxic to algae or larval fathead minnows.
- Some moderate effects on reproduction in *Ceriodaphnia* were noted. It was not possible to determine if these effects were due to the same water soluble substances removed with the aqueous extract.

Phase V testing included the same species but was done on sediment from lakes in the region of Wabamun Lake. In summary:

- The aqueous sediment extracts were moderately toxic to luminescent bacteria. These effects were similar to those observed with Wabamun Lake sediment extracts.
- The pore water was not toxic to algae.
- Some moderate effects were observed on reproduction in *Ceriodaphnia*.

Phase VI involved a fractionation of the pore water and an aqueous extract of sediment from ALI in an attempt to separate out the toxic constituents. Key findings included:

- The toxicity was not recovered in the fractions off the C18 column (solid phase extraction). It was not possible to determine if the solubilities of the toxic substances were pH dependent.
- The toxic substances may have been physically excluded by the column.

Phase VII involved physical treatments of ALI sediment extracted wet (as received) and after oven drying. The treatments included centrifugation, filtration, and combustion (ashing). In summary:

- The toxicity was increased with drying and subsequent extraction in water or methanol.
- The toxic substances released after drying were not removed with centrifugation and filtration.
- The toxic substances in the pore water were removed with centrifugation and filtration (consistent with TIE fractionation results).
- Ashing removed all toxicity. This finding suggests that the toxic substances were likely organic in nature.

# 5.0 CONCLUSIONS AND RECOMMENDATIONS

The following conclusions were based on the findings from the seven phases of this study. Responses ranged from 'strong' in ash lagoon sediment samples to 'moderate' in sediment samples from Wabamun (ALI > WS5 > WS2 > WS3 = WS4 > WS1) and other lakes. The responses obtained for the four lake samples were considered moderate and similar to those obtained for samples from Wabamun Lake.

- The microbial populations in the sediments were lower than expected. This could indicate some level of trophic impairment. Microbes are vital for the decomposition of organic matter and the recycling of nutrients (convert chemical energy into biomass). They also serve as a food source for invertebrates.
- The sediments did not contain substances toxic to aquatic plants (growth does not appear to be impaired in Wabamun Lake).
- The sediments contained substances toxic to larval fish. These materials were present in the pore water and aqueous extracts. The results suggest that these substances were organic in nature. However, it was unclear whether or not the observed effects were chemical or physical (mode of action).
- The sediments contained non-polar compounds highly toxic to bacteria. This may be limiting the bacterial populations *in situ*.
- The sediments contained water-soluble substances that were toxic to *Ceriodaphnia*. Effects were detected at very low dilutions, which would suggest that the mode of action is due to chemical as opposed to physical interactions. At high dilutions, the test solution is primarily laboratory dilution water. This would tend to rule out potential physical effects from the test sample.
- The effects measured at multiple trophic levels suggest a potential for impairment of normal food chain structure and function in Lake Wabamun. Field data are required to confirm this supposition.
- Sediments from other lakes yielded responses similar to those observed in Wabamun Lake samples suggesting that substances toxic to microbes, fish, and invertebrates either occur naturally in lake sediments or are from man-made sources.
- Drying the sediment affects the availability of the water-soluble substances toxic to microbes, invertebrates and fish. It was not clear whether or not drying affected the same types of toxic substances present in the pore water. The results from Phase VII suggest different classes of substances (i.e., the toxicity in pore water, but not the aqueous extract, was physically removed with centrifugation and filtration).
- The sediments contained both water soluble (polar) and non-polar toxic substances. The observed effects are likely attributable to organic substances as opposed to metals.
- The sediment from the ash lagoon was consistently more toxic than the lake sediments. However, it was not possible to determine if the nature and cause of the toxicity in the ash lagoon sediment was the same or different from that present in the lake sediments.

The results of this study provide initial information on the occurrence of toxicity in sediments from Wabamun Lake and other lakes. While responses indicative of toxicity have been recorded in some test species, the substance(s) that elicit the responses still need to be determined for Wabamun and other lakes. There is also a need to determine if benthic invertebrate species that live in or near the lake sediments and, in some cases ingest sediment, are affected. Further toxicity identification evaluations and benthic invertebrate community assessments would be valuable tools in dealing with these outstanding questions.

# 6.0 GENERAL REFERENCES

Alberta Environment, 2002. Wabamun Lake water quality and sediment survey. Preliminary report. ISBN No 0-778597-0 (On-line Edition) Pub. No 1/919.

Burton, G.A. Sediment Toxicity Assessment, 1992. Lewis Publishers, Boca Raton, FL.

Carter, M.R. (ed), 1993. Soil Sampling and Methods of Analysis. Lewis Publishers, Boca Raton.

Environment Canada, 1992a. Biological Test Method: Growth Inhibition Test Using the Freshwater Alga *Selenastrum capricornutum*, EPS 1/RM/25.

Environment Canada, 1992b. Biological Test Method: Test of Larval Growth and Survival Using Fathead Minnows, EPS 1/RM/22.

Environment Canada, 1992c. Biological Test Method: Test of Reproduction and Survival Using the Cladoceran *Ceriodaphnia dubia*, EPS 1/RM/21.

Environment Canada, 1992d. Biological Test Method: Toxicity Test using Luminescent Bacteria (*Vibrio fischeri*), EPS 1/RM/24.

Environment Canada, 1999. Biological Test Method: Test for Measuring the Inhibition of Growth Using the Freshwater Macrophyte*, Lemna minor*. EPS 1/RM/37.

Guidance Document on Application and Interpretation of Single-Species Tests in Environmental Toxicology, 1999. Environment Canada, EPS 1/RM/34.

Guidance Document on Collection and Preparation of Sediments for Physiochemcial Characterization and Biological Testing, 1994. Environment Canada, EPS 1/RM/29.

Hoke, R.A., et al. Toxicity of Sediments from Western Lake Erie and Maumee River at Toledo, Ohio, 1987: Implications for Current Dredged Material Disposal Practices, 1990. J. Great Lake Res., 16(3):457-470.

Protocols for Short Term Toxicity Screening of Hazardous Waste Sites, 1989. United States Environmental Protection Agency, USEPA 600/3-88-029.

## Table 1 Sample Identification

| Sample # | Common Reference | Client ID Batch 1 | Client ID Batch 2 | Client ID Batch 3 |
|---|---|---|---|---|
| 20020659 | WS1 | NESBL020618WS1 | 02SWE05004 | 02SWE6003 |
| 20020660 | WS2 | NESBL020618WS2 | 02SWE05001 | 02SWE6001 |
| 20020661 | WS3 | NESBL020618WS3 | 02SWE05003 | 02SWE6002 |
| 20020662 | WS4 | NESBL020618WS4 | 02SWE05002 | 02SWE6004 |
| 20020663 | WS5 | NESBL020618WS5 | 02SWE05000 | 02SWE6000 |
| 20020673 | ALI | NESBL020619ALI | 02SWE05005 | 02SWE6005 |
| 20021165-1 | n/a | 02SWE02287 | - | - |
| 20021165-2 | n/a | 02SWE02288 | - | - |
| 20021165-3 | n/a | 02SWE02289 | - | - |
| 20021165-4 | n/a | 02SWE02290 | - | - |

## Table 2 Phase I Initial Characterization of Sediments

| Sample # | Client ID | Color | Odour | pH (units) | Conductance (uS/cm) | ReDox (mV) | Moisture Content (%) | Total Organic Matter (%) | Bacterial Luminescence Aqueous Extract Screen (% ctls) | Bacterial Luminescence Methanol Extract | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | EC50 (%) | EC20(%) |
| 20020659 | NESBL020618WS1 | dark grey | organic (fishy) | 7.5 | 500 | 160 | 30 | 3 | 74 | 58 | 31 |
| 20020660 | NESBL020618WS2 | dark grey green | organic/sulfur | 7.4 | 560 | -5 | 82 | 22 | 57 | 12 | 5 |
| 20020661 | NESBL020618WS3 | dark grey green | strong organic | 7.3 | 550 | 55 | 79 | 16 | 53 | 19 | 10 |
| 20020662 | NESBL020618WS4 | dark green brown | none | 7.3 | 510 | 80 | 82 | 26 | 74 | 26 | 13 |
| 20020663 | NESBL020618WS5 | dark grey black | mild organic | 7.3 | 460 | 105 | 49 | 12 | 75 | 1.9 | 0.75 |
| 20020673 | NESBL020619ALI | very dark grey | mild sulfur | 7.7 | 510 | >-80 | 71 | 17 | 48 | 0.63 | 0.21 |

Notes:
Bacterial luminescence aqueous extract screens run at 91% full strength, bacterial luminescence methanol EC50 run with full dilutions at 5% strength of methanol extract
EC - effect concentration; % ctls - percent of control response

## Table 3 Phase I - Sediment Health Results Summary - Aqueous Extract

| Sample # | Client ID | Bacterial Luminescence PW (% ctls) | Bacterial Luminescence AQ-XT (% ctls) | THB Aerobic (MPN/g) | THB Anaerobic (MPN/g) | SRB CFU/ml | Algal Growth Inhibition (% ctls) | Lemna Growth Inhibition (% ctls) | Daphnia Survival (% ctls) | Lumbriculus Survival (% ctls) | Fathead Survival (% ctls) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 20020659 | NESBL020618WS1 | 145 | 69 | $7.8x10^{1}$ | $1.3x10^{1}$ | $1x10^{5}$ | 172 | 203 | 100 | 100 | 70 |
| 20020660 | NESBL020618WS2 | 134 | 61 | $1.8x10^{6}$ | $2.3x10^{1}$ | $1x10^{5}$ | 56 | 173 | 100 | 40 | 47 |
| 20020661 | NESBL020618WS3 | 120 | 62 | $1.8x10^{5}$ | $7.8x10^{0}$ | $5x10^{4}$ | -155 | 186 | 100 | 100 | 23 |
| 20020662 | NESBL020618WS4 | 127 | 62 | $3.3x10^{2}$ | $2.3x10^{1}$ | $1x10^{5}$ | 111 | 198 | 100 | 100 | 0 |
| 20020663 | NESBL020618WS5 | 118 | 55 | $2.3x10^{4}$ | $1.3x10^{1}$ | $1x10^{5}$ | 166 | 120 | 100 | 100 | 0 |
| 20020673 | NESBL020619ALI | 116 | 22 | $1.3x10^{3}$ | $2.0x10^{0}$ | $<1x10^{2}$ | 23 | 186 | 100 | 100 | 0 |

Notes:

Bacterial luminescence aqueous extract and porewater screens run at 91% full strength; PW - porewater; AQ-XT - aqueous extract; THB - total heterotrphic bacteria;

MPN/g - most probable number per gram; SRB - sulfur reducing bacteria; CFU - colony forming units; % ctls - percent of controls.

**Table 4 Phase I - Sediment Health Results Summary - Methanol Extract**

| Sample # | Client ID | Bacterial Luminescence (EC50%) | Algal Growth Inhibition (% ctls) | Lemna Growth Inhibition (% ctls) | Daphnia Survival (% ctls) | Lumbriculus Survival (% ctls) | Fathead Survival (% ctls) |
|---|---|---|---|---|---|---|---|
| 20020659 | NESBL020618WS1 | 12 | 93 | 80 | 100 | 100 | 116 |
| 20020660 | NESBL020618WS2 | 0.9 | 85 | 97 | 100 | 100 | 93 |
| 20020661 | NESBL020618WS3 | 3.3 | 85 | 88 | 100 | 100 | 93 |
| 20020662 | NESBL020618WS4 | 2.1 | 97 | 150 | 100 | 100 | 93 |
| 20020663 | NESBL020618WS5 | 0.18 | 91 | 142 | 100 | 100 | 47 |
| 20020673 | NESBL020619ALI | 0.17 | 95 | 150 | 100 | 100 | 0 |

Notes:
Bacterial luminescence run at 5% of methanol extract; EC - effect concentration; %ctls - percent of control response.

**Table 5 Phase II Results - 4:1 Aqueous Extracts - 2002/07/29**

| Sample # | Client ID | Microtox Confirmation Screens (% ctls) | 4d Acute Fathead Minnow Survival | | 7d Ceriodaphnia Survival - LC50 (%) | 7d Ceriodaphnia Reproduction - IC25 (%) |
|---|---|---|---|---|---|---|
| | | | LC50 (%) | LC25 (%) | | |
| 20020659 | NESBL020618WS1 | 110 | 82 | 62 | <6.25 | <6.25 |
| 20020660 | NESBL020618WS2 | 82 | 29 | 20 | <6.25 | <6.25 |
| 20020661 | NESBL020618WS3 | 61 | 45 | 30 | <6.25 | <6.25 |
| 20020662 | NESBL020618WS4 | 115 | 32 | 26 | nd | nd |
| 20020663 | NESBL020618WS5 | 115 | 31 | 23 | nd | nd |
| 20020673 | NESBL020619ALI | 87 | 69 | 55 | nd | nd |

Notes:
Tests run on 4:1 aqueous extract; FM fathead minnow; LC - lethal concentration; IC - inhibitory concentration; % ctls - percent of controls;
Fathead minnow test run as modified test with replicates and dilutions
Low DO recorded in top concentrations for both FM and CD tests
nd = no test

**Table 6 Phase III Results - 4:1 Aqueous Extracts - 2002/09/06**

| Sample # | Client ID | Microtox Confirmation Screens (% ctls) | EC Fathead Minnow Survival LC50 (%) | EC Fathead Minnow Survival LC25 (%) | 7d Ceriodaphnia Survival - LC50 (%) | 7d Ceriodaphnia Reproduction - IC25 (%) |
|---|---|---|---|---|---|---|
| 20020659 | NESBL020618WS1 | 39 | nd | nd | <6.25 | <6.25 |
| 20020660 | NESBL020618WS2 | 58 | 19 | 15 | <6.25 | <6.25 |
| 20020661 | NESBL020618WS3 | 38 | nd | nd | <6.25 | <6.25 |
| 20020662 | NESBL020618WS4 | 67 | nd | nd | nd | nd |
| 20020663 | NESBL020618WS5 | 124 | 36 | 28 | nd | nd |
| 20020673 | NESBL020619ALI | 108 | 43 | 25 | nd | nd |

Notes:
Tests run on 4:1 aqueous extract; EC - Environment Canada; LC - lethal concentration; IC - inhibitory concentration
WS2 met EC test requirements
WS5 used the same solution for replenishing on days 4-7
ALI used the same solution for replenishing on days 5-7
Low DO recorded in top concentrations for both FM and CD tests
nd = no test

**Table 7 Phase IV Results - Porewater - 2002/10/10**

| Sample # | Client ID | Microtox Screens | 72 h Algal Growth | | 7 d Fathead Minnow | | | | 7d Ceriodaphnia | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | (% ctls) | IC50 (%) | IC25 (%) | LC50 (%) | LC25 (%) | IC50 (%) | IC25 (%) | LC50 (%) | LC25 (%) | IC50 (%) | IC25 (%) |
| 20020659 | NESBL020618WS1 | 83 | >50 | >50 | >50 | >50 | >50 | **43** | >50 | >50 | >50 | **29** |
| 20020660 | NESBL020618WS2 | 72 | >50 | >50 | >50 | >50 | >50 | **31** | >50 | >50 | >50 | **26** |
| 20020661 | NESBL020618WS3 | 48 | >50 | >50 | >50 | >50 | >50 | >50 | >50 | >50 | **45** | **25** |
| 20020662 | NESBL020618WS4 | 53 | >50 | **49** | >50 | >50 | >50 | **22** | >50 | >50 | >50 | **42** |
| 20020663 | NESBL020618WS5 | 25 | >50 | >50 | >50 | >50 | >50 | >50 | >50 | >50 | >50 | **27** |
| 20020673 | NESBL020619ALI | 24 | >50 | >50 | >50 | >50 | >50 | >50 | >50 | >50 | **18** | **8** |

Notes:
Microtox tests run on 4:1 aqueous extract; all other tests run on 50% porewater; LC - lethal concentration; IC - inhibitory concentration
% ctls - percent of controls

**Table 8 Phase V Results - Porewater - 2002/11/26**

| Sample # | Client ID | Microtox Screens | 72 h Algal Growth | | 7 d Fathead Minnow | | | | 7d Ceriodaphnia | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | (% ctls) | IC50 (%) | IC25 (%) | LC50 (%) | LC25 (%) | IC50 (%) | IC25 (%) | LC50 (%) | LC25 (%) | IC50 (%) | IC25 (%) |
| 20021165-1 | 02SWE02287 | 59 | >50 | >50 | >50 | >50 | >50 | >50 | >50 | >50 | >50 | >50 |
| 20021165-2 | 02SWE02288 | 45 | >50 | >50 | >50 | **39** | >50 | **12** | >50 | >50 | >50 | **30** |
| 20021165-3 | 02SWE02289 | 62 | >50 | >50 | >50 | >50 | >50 | >50 | >50 | >50 | >50 | >50 |
| 20021165-4 | 02SWE02290 | 82 | >50 | >50 | >50 | >50 | >50 | >50 | >50 | >50 | **29** | **8** |

Notes:
Microtox tests run on 4:1 aqueous extract; all other tests run on 50% porewater; LC - lethal concentration; IC - inhibitory concentration
% ctls - percent of controls

## Table 9a Toxicity Results in TIE Fractions for Laboratory Dilution Water

| Fraction | pH (units) | Conductivity (uS/cm) | Bacterial Luminescence (% ctls) | Fathead Minnow (48h) (% ctls) | Fathead Minnow (72h) (% ctls) |
|---|---|---|---|---|---|
| lab control | 8.3 | 312 | 93 | 100 | 100 |
| **pH3** | | | | | |
| 25% MeOH | 8.0 | 402 | nd | 106 | 106 |
| 50% MeOH | 8.1 | 386 | nd | 106 | 106 |
| 75% MeOH | 8.1 | 381 | nd | 106 | 106 |
| 100% MeOH | 8.1 | 385 | nd | 85 | 64 |
| 100% MeOH | 8.1 | 392 | nd | 85 | 85 |
| C18 effluent | 3.0 | 850 | nd | 106 | 106 |
| **ambient pH** | | | | | |
| 25% MeOH | 8.1 | 391 | 111 | 106 | 106 |
| 50% MeOH | 8.1 | 394 | 115 | 106 | 106 |
| 75% MeOH | 8.0 | 387 | 146 | 106 | 106 |
| 100% MeOH | 8.1 | 388 | 113 | 64 | 64 |
| 100% MeOH | 8.1 | 391 | 134 | 106 | 106 |
| C18 effluent | 8.3 | 398 | 81 | 106 | 106 |
| **pH11** | | | | | |
| 25% MeOH | 8.0 | 393 | nd | 106 | 64 |
| 50% MeOH | 8.1 | 396 | nd | 106 | 106 |
| 75% MeOH | 8.1 | 383 | nd | 106 | 106 |
| 100% MeOH | nd | nd | nd | nd | nd |
| 100% MeOH | 8.0 | 387 | nd | 106 | 106 |
| C18 effluent | 7.3 | 599 | nd | 85 | 64 |

Notes:
nd - not done; %ctls - percent of control response; MeOH - methanol.

## Table 9b Toxicity Results in TIE Fractions for ALI Porewater

| Fraction | pH (units) | Conductivity (uS/cm) | Bacterial Luminescence (% ctls) | Fathead Minnow (48h) (% ctls) | Fathead Minnow (72h) (% ctls) |
|---|---|---|---|---|---|
| lab control | 8.3 | 312 | 93 | 100 | 100 |
| **pH3** | | | | | |
| 25% MeOH | 8.0 | 393 | 120 | 100 | 125 |
| 50% MeOH | 7.9 | 392 | 96 | 100 | 125 |
| 75% MeOH | 8.0 | 393 | 133 | 100 | 125 |
| 100% MeOH | 8.1 | 388 | 124 | 100 | 75 |
| 100% MeOH | 8.0 | 381 | 148 | 100 | 100 |
| C18 effluent | 3.3 | 703 | 83 | 80 | 50 |
| **ambient pH** | | | | | |
| 25% MeOH | 8.0 | 404 | 142 | 100 | 125 |
| 50% MeOH | 8.1 | 388 | 138 | 100 | 75 |
| 75% MeOH | 8.1 | 381 | 124 | 100 | 100* |
| 100% MeOH | 8.1 | 388 | 108 | 100 | 125 |
| 100% MeOH | 8.1 | 382 | 139 | 100 | 125 |
| C18 effluent | 6.9 | 528 | 148 | 80 | 80 |
| **pH11** | | | | | |
| 25% MeOH | 8.1 | 400 | 130 | 80 | 100 |
| 50% MeOH | 8.0 | 388 | 127 | 100 | 100 |
| 75% MeOH | 8.1 | 383 | 129 | 100 | 100 |
| 100% MeOH | 8.0 | 375 | 121 | 80 | 75 |
| 100% MeOH | 8.1 | 384 | 143 | 100 | 100 |
| C18 effluent | 8.4 | 790 | 130 | 100 | 100 |

Notes:
* - repeated value; %ctls - percent of control response; MeOH - methanol.

## Table 9c Toxicity Results in TIE Fractions for ALI 4:1 Extract on Dried Sediment

| Fraction | pH (units) | Conductivity (uS/cm) | Bacterial Luminescence (% ctls) | Fathead Minnow (48h) (% ctls) | Fathead Minnow (72h) (% ctls) |
|---|---|---|---|---|---|
| lab control | 8.3 | 312 | 93 | 100 | 100 |
| **pH3** | | | | | |
| 25% MeOH | 8.0 | 404 | 172 | 106 | 106 |
| 50% MeOH | 8.0 | 389 | 154 | 106 | 106 |
| 75% MeOH | 8.1 | 384 | 129 | 106 | 106 |
| 100% MeOH | nd | nd | nd | nd | nd |
| 100% MeOH | 8.1 | 386 | 114 | 85 | 85 |
| C18 effluent | 3.1 | 1033 | 118 | 106 | 85 |
| **ambient pH** | | | | | |
| 25% MeOH | 8.0 | 402 | 129 | 106 | 106 |
| 50% MeOH | 8.1 | 395 | 150 | 106 | 85 |
| 75% MeOH | 8.1 | 395 | 143 | 106 | 106 |
| 100% MeOH | nd | nd | nd | nd | nd |
| 100% MeOH | 8.0 | 388 | 114 | 106 | 106 |
| C18 effluent | 8.0 | 641 | 118 | 64 | 64 |
| **pH11** | | | | | |
| 25% MeOH | 8.0 | 405 | 144 | 106 | 106 |
| 50% MeOH | 8.1 | 397 | 147 | 106 | 106 |
| 75% MeOH | 8.1 | 397 | 147 | 106 | 85 |
| 100% MeOH | 8.1 | 398 | 125 | 106 | 106 |
| 100% MeOH | 8.1 | 388 | 152 | 85 | 85 |
| C18 effluent | 9.1 | 1071 | 98 | 64 | 64 |

Notes:
nd - not done; %ctls - percent of control response; MeOH - methanol.

## Table 10 Phase VII - Comparison of Bacterial Luminescence Screens Over Time

| Sample # | Client ID | Microtox Screens | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 (% ctls) | 2 (% ctls) | 3 (% ctls) | 4 (% ctls) | 5 (% ctls) | Porewater-1st batch (% ctls) | Porewater-3rd batch (% ctls) | fresh 4:1-1st batch (% ctls) | fresh 4:1-3rd batch (% ctls) |
| 20020659 | NESBL020618WS1 | 74 | 69 | 110 | 39 | 83 | 129 | 103 | 52 | 50 |
| 20020660 | NESBL020618WS2 | 57 | 61 | 85 | 58 | 72 | no sample | 132 | no sample | 53 |
| 20020661 | NESBL020618WS3 | 53 | 62 | 61 | 38 | 48 | no sample | 114 | no sample | 36 |
| 20020662 | NESBL020618WS4 | 74 | 62 | 115 | 67 | 53 | 133 | 129 | 63 | 43 |
| 20020663 | NESBL020618WS5 | 75 | 55 | 115 | 124 | 25 | 133 | 110 | 28 | 31 |
| 20020673 | NESBL020619ALI | 48 | 22 | 87 | 108 | 24 | 129 | 141 | 28 | 36 |

Notes:
Microtox tests run on 4:1 aqueous extract
% ctls - percent of controls

## Table 11 Summary of Wet and Dried ALI Extracts and Treatments

| Sample | Treatment | Color | Bacterial Luminescence (% ctls) |
|---|---|---|---|
| Wet Sediment | initial | very pale yellow/gray | 53 |
| | centrifugation | clear | 95 |
| | filtration | clear | 115 |
| | ashing | clear | 136 |
| Dried Sediment | initial | yellow | 36 |
| | centrifugation | clear yellow | 53 |
| | filtration | clear yellow | 43 |
| | ashing | clear | 136 |

Notes:
Microtox tests run on 4:1 aqueous extract
% ctls - percent of controls

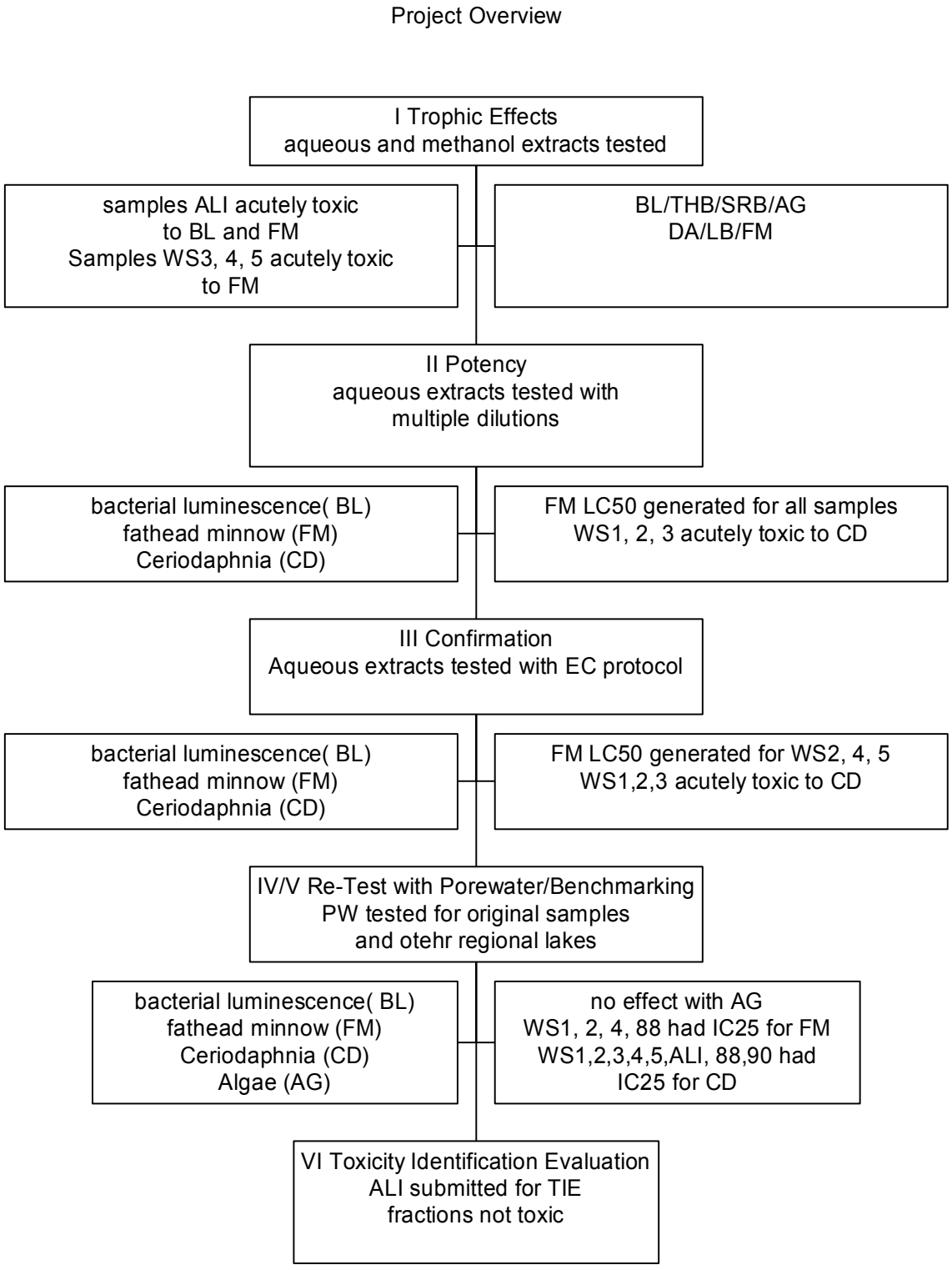


**Figure 1 Project Overview**