Predicting toxicity of future combined pit lakes at the former Steep Rock Iron Mine near Atikokan, Ontario

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Abstract Caland and Hogarth pit lakes began filling with both groundwater and surface water after the closure of the former Steep Rock Iron Mines in 1979. Initial investigations in 1999 suggested that Hogarth contained little aquatic life and standard LC_{50} toxicity tests showed that Hogarth was acutely toxic to *Daphnia magna*. By 2004, Hogarth no longer exhibited acute toxicity, but using *Ceriodaphnia dubia* as an indicator species was shown to have chronic toxicity during winter months. Toxicity indicator evaluations (TIE) revealed that the most likely cause was elevated SO_4^{2-}, Ca^{2+}, and Mg^{2+}. In 2006, *Lemna minor* demonstrated a toxic response to Hogarth water and this organism was used to predict the toxic effect once the two pits join. Water from the hypolimnion and epilimnion of Hogarth was serially diluted with water from the hypolimnion and epilimnion of Caland to mimic the actual predicted mixing of the two pit lakes. A similar dilution series using water extracted from columns containing rock mixtures at amounts found in the two pit lakes was also tested for toxicity. The standard IC_{25} procedure for *Lemna* sp. of frond counts did not work although visually the fronds in treatments containing more than 50% of Hogarth water appeared smaller and had chlorotic areas. Using dry weight as a covariate, an ANCOVA of chlorophyll content showed a significant reduction in concentration with added Hogarth or Caland hypolimnion water. The best indicator of toxicity was surface area of the fronds which demonstrated conclusively the adverse effects of both added Hogarth water from any depth and Caland hypolimnion water. Given the depth regimes in the pit lakes, toxicity was predicted to continue after the pits joined.

Key Words aquatic toxicity, pit lakes, sulfate, *Ceriodaphnia dubia*, *Lemna minor*, geochemical modeling

Introduction

Caland and Hogarth pit lakes have been filling since the cessation of mining and dewatering efforts in 1979 of the former Steep Rock Iron Mines in northwestern Ontario. Groundwater and surface water inflows have contributed to the increasing depths (≈ 200 m in 2010) in both pits. The geochemical influence of the pit wall rocks has contributed to the distinct differences in the water quality in each of the pits. Hogarth has elevated levels of dissolved sulfate (1200—2000 mg/L) throughout the water column and shows little chemical stratification. Caland shows moderate levels of sulfate (200—500 mg/L) and is chemically stratified with an oxygenated fresh water mixolimnion, a distinct chemocline, and a sulfate-saline anoxic monimolimnion. From the early 1980s until 2007 the upper freshwater lens of Caland had been host to the Snow Lake Fish Farm, which contributed large amounts of organic matter to the water column and resulted in the anoxic nature of the monimolimnion. There are several acidic inflows into the two pits that originate from the numerous waste rock piles adjacent to the pits. However, pH remains circum-neutral in the two pits (Caland = 7.85; Hogarth = 7.50) due to buffering by carbonate wall rock. Other chemical differences include: i) Ca^{2+} and Mg^{2+} concentrations have remained constant, with high concentrations in Hogarth (287 mg/L; 175 mg/L) than in Caland (80 mg/L; 39 mg/L); ii) conductivity values in Hogarth (2107µS/cm) are higher than in Caland (695µS/cm) with few changes seen over the years; and, iii) alkalinity in both lakes has been increasing, with higher levels seen in Caland (131 mg/L$CaCO_3$) than Hogarth (89 mg/L$CaCO_3$). With continued filling, the two pits will merge and discharge into the adjacent Seine river system sometime between 2030 and 2070 (Jackson, pers comm., 2007). A major issue at the site is the toxicity of the Hogarth pit lake and its influence on the future merged pit lake waters. Toxicity experiments have been conducted over a ten year period and were designed to do the following: 1) explore the nature of toxicity in Hogarth using a *Daphnia magna* LC_{50} test; 2) determine the changing nature and source of toxicity in Hogarth using both a *Ceriodaphnia dubia* LC_{50} test and a Toxicity Indicator Evaluation (TIE); and 3) predict the future nature of toxicity of the merged pit lake in light of interaction with wall rocks using a *Lemna minor* IC_{25} test.

Methods

Daphnia magna acute toxicity tests: In May 1999 the Aquatic Toxicity Research Center (ATRC) at Lakehead University conducted a LC50 toxicity test on *D. magna*. A sample of Hogarth pit lake water was used for this test following Environment Canada (1996) test method.

Rainbow trout and *Daphnia magna* acute lethality tests: In June and July 2004, rainbow trout (*Onchorhyncus mykiss*) and *D. magna* acute lethality tests were conducted by the ATRC using water taken from 2 m in Hogarth according to Environment Canada (2000) and Environment Canada (1996), respectively.

Chronic toxicity tests using *Ceriodaphnia dubia* and *Lemna minor*: Chronic toxicity investigations based on reproduction and survival were conducted using neonate *C. dubia* according to the Environment Canada (1992) biological test method. Chronic Toxicity Identification Evaluation (TIE) Phase I tests and mock effluent chronic toxicity tests were conducted using water collected from 2 m in Hogarth during November 2004 with *C. dubia* as indicator species. Growth inhibition tests on L. minor using water from 2 m in Hogarth were carried out according to Environment Canada (1999) using modified APHA growth medium for the controls and the dilutions.

Predictive modeling of future merged pit lake toxicity using *Lemna minor*: *L. minor* was used to empirically predict the toxicity of the future merged pit lake under the geological influence of the pit lake wall rock through a series of static column experiments. Varying ratios of water from Hogarth and Caland at 2 m and 30 m depths were reacted with relative ratios of the various rock types found lining the two pits in order to simulate the future conditions of the pits as they fill and merge. The resultant water, which represented the future merged pit lake water quality, was analyzed using the 7-day growth inhibition toxicity test according to the Environment Canada Biological Test Method (1999). In this case water from Caland pit lake was used for the controls and the dilutions and *L.minor* served as the indicator species. The IC_{25} values for the tests were determined using ToxCalc v5.0. In addition, dry weights, chlorophyll a concentration, and frond surface area were used to analyze the results more thoroughly. One-way ANOVA tests were conducted using SPSS v. 17.0 to determine if there were any significant differences in the endpoints among any of the column water replicates and the controls (p=0.01). Post hoc Dunnett's tests were used to determine which column water treatments had significantly different means (p=0.05, 2-sided) compared to the controls for each of the endpoints showing significant differences after the ANOVA. An analysis of covariance (ANCOVA) was carried out to assess whether water from Hogarth caused a significant change in chlorophyll a content after controlling for differences in frond number and dry weight.

Results and Discussion

Daphnia magna acute toxicity tests: During 1999 there were no apparent signs of aquatic life in Hogarth. The LC_{50}, conducted on *D. magna* using full strength concentration, resulted in 100% mortality within a 48 hr period. The source of toxicity was likely due to fluctuations in SO_4^{2-}, Ni^{2+}, Mg^{2+}, and iron floc levels. No mortality was observed in either rainbow trout or D. magna acute lethality tests conducted in June and July 2004.

Chronic toxicity tests using *Ceriodaphnia dubia* and *Lemna minor*: The November 2004 7-day chronic test with *C. dubia* yielded an IC_{25} of 75% and an IC_{50} >100% Hogarth 2 m water. Subsequent toxicity tests throughout the following two years were intermittent. These tests were performed using water collected from Hogarth during January, May, June, and November 2005 and also during January, June, and July 2006. Every subsequent test except those conducted during the month of January in both years resulted in an IC_{25} of >100% suggesting that Hogarth water toxicity is pronounced during the winter months under ice cover. The January 2005 IC_{25} was 23.1% and the IC_{50} was 62.0%. The January 2006 IC_{25} was 32.1% and the IC_{50} was 66.9% Hogarth water. During these months it was noted that the conductivity, Ca^{2+}, Mg^{2+}, SO_4^{2-}, and total dissolved solids (TDS) were slightly higher than for the other months. The January 2005 TIE test baseline (full dilution series of Hogarth pit lake water without manipulations) had an IC_{25} of 23% and an IC_{50} of 62%. The results of all of the subsequent manipulation tests are reported in Table 1. Only the EDTA 8mg/L addition test resulted in a reduction in toxicity (from an IC_{50} of 62% to 100%). Reductions in toxicity due to EDTA addition are indicative of toxicity by cationic metals (e.g., Al^{3+}, Cd^{2+}, Cu^{2+}, Fe^{3+}, Pb^{2+}, Mn^{2+}, Ni^{2+}, and Zn^{2+}) and certain non-metal ions (Ca^{2+} and Mg^{2+}; Stumm and Morgan 1981; Sovari and Sillanpaa 1996). In January 2005 there was an isolated occurrence of elevated Pb

***Table 1** Results from the January 2005 TIE Phase I characterization tests (in percent Hogarth water)*

Endpoint	Baseline	Filtration	Aeration	pH 6 Buffered	pH7 Buffered	SPE
IC_{25}	23.1	27	28.8	28.7	29.5	10.6
IC_{50}	62	52.1	59.2	61.3	34.2	49.2
7-day EC_{50}	61.6	35.3	61.6	77.1	77.1	53.6
Endpoint	$Na_2S_3O_3$ (10 mg/L)	$Na_2S_3O_3$ (25 mg/L)	EDTA (3.0 mg/L)	EDTA (8.0 mg/L)	Methanol Elute	
IC_{25}	9	42.5	10.3	84	<12.5*	
IC_{50}	60.6	64.1	58.8	>100	<12.5*	
7-day EC_{50}	70.2	46.6	89.1	>100	>100	

*Indicates IC estimate less than the lowest concentration
Note: SPE denotes solid-phase extraction

in the water (0.0885 mg/L). Such levels exceed the Canadian Water Quality Guideline of 0.007 mg/L (for hardness >180 $mgCaCO_3$/L) and suggest that cationic metal toxicity may contribute to winter toxicity. Bioaccumulation studies with *Eleocharis smallii* and *Pyganodon grandis* found that Ni and S are the only parameters that occurred at elevated levels. The January 2006 TIE test baseline had an IC_{25} of 32.1% and an IC_{50} of 66.9%, with none of the manipulation tests resulting in a decrease in toxicity.

Further investigations into TDS-related toxicity were conducted using mock effluents. Hogarth water data shows that 93% of the effluent TDS could be accounted for by the sum of Ca^{2+}, Mg^{2+}, and SO_4^{2-}. Therefore, the mock effluent was likely a good representative of TDS in Hogarth water (Table 2). The mock effluent was less toxic than Hogarth water producing IC_{25} values of 33.9% and 38.2% compared to 23.1% and 32.1% and IC_{50} values of 80.3% and 66.5% compared to 62.0% and 66.9%. The elevated TDS levels in Hogarth account for the majority of the toxicity. However, the small reduction in toxicity due to EDTA addition manipulation tests indicates that metals may be a minor toxicant.

L. minor toxicity tests indicated that both the test effluents (Hogarth pit lake water and mock effluent) were less toxic to this species than the others tested. Higher IC_{25} values, indicating lower toxicity levels, were observed: Mock test #1 had an $IC_{25}$of 90.74%; Mock test #2 had an IC_{25} of 88.7%; the August 2006 test had an IC_{25} of >100%; the September 2006 test had an IC_{25} of 93.4%; and the October 2006 test had an IC_{25} of 85.1%. Less than 50% reduction in frond number compared to the controls occurred in all cases so no IC_{50} values exist. The plants in the higher concentrations (50% and 100%) of both effluents showed signs of stress including smaller, unhealthy-looking fronds that were chlorotic and had shorter roots. The toxic effect on *L. minor* was determined to be due to elevated TDS levels (approximately 2000 mg/L), with SO_4^{2-} as the major contributing ion.

Predictive modeling of future merged pit lake toxicity using *Lemna minor*: The initial data analysis of the baseline toxicity tests using mixed water from the two pit lakes reported unexpected results (i.e., IC_{25} value of 0.0% Hogarth 30 m). Suspect results were also seen with the column water toxicity tests with IC_{25} values of >100% in three of the four mixing series examined. Despite these results, the plants in these mixes showed definite signs of stress such as small fronds, chlorotic and necrotic tissue, and single fronds indicating colony destruction; however, toxicity was not detected with the standard Environment Canada protocols. A one-way ANOVA showed that there were no differences in the dry weights or the chlorophyll contents of the *L. minor* plants in any of the treatments, but there were differences in total frond surface area between the treatments [$F(10,22)=13.037$, $p<0.001$]. Dunnett's comparisons showed that the plants

***Table 2** Ionic composition of Hogarth water and mock effluent*

Source	Ca^{2+}	K^+	Mg^{2+}	Na^{2+}	Alk.*	Cl^-	NO_3^-	SO_4^{2-}	TDS
Mock Effluent	298	n/a	179	n/a	n/a	n/a	n/a	1443	1920
Hogarth - Nov. 2004	296	6	181	22	101	10	1	1436	2053
Annual Average 2005	280	6	166	20	87	11	0.52	1403	2068
Annual Average 2006	270	6	157	18	92	10	0.50	1321	1986

Values expressed as mg/L, except * from total alkalinity ($mgCaCO_3$/L) in all of the columns had lower total frond surface areas versus the controls ($p=0.05$, 2-sided; Fig. 1). A one-way ANOVA showed that there were no differences in the dark green surface areas in any of the treatments, but there were differences in the light green [$F(10,22)=27.528$, $p<0.001$], yellow [$F(10,22)=15.287$, $p<0.001$], and white [$F(10,22)=9.586$, $p<0.001$] surface areas between the treatments. Dunnett's test revealed that all of the column treatments had lower light green surface area measurements versus the controls ($p=0.05$, 2-sided). Plants grown in water from columns 1, 2, 4, and 5 had greater yellow surface area measurements than the controls according to Dunnett's test ($p=0.05$, 2-sided). Plants grown in water from all columns except column 1 (100% Caland 2 m water) and column 5 (50% Hogarth 30 m water + 50% Caland 2 m water + rock) had greater white surface areas than the controls ($p=0.05$, 2-sided; Fig. 1). An ANCOVA found that there was an effect on chlorophyll a content due to the differences in dry weight between treatments in both mixing series. After controlling for the effect of dry weight it was found that water from Hogarth 2 m had an effect on the chlorophyll a content of the plants in the test [$F(5,11)=5.26$, $p<0.05$ (Fig. 2)].

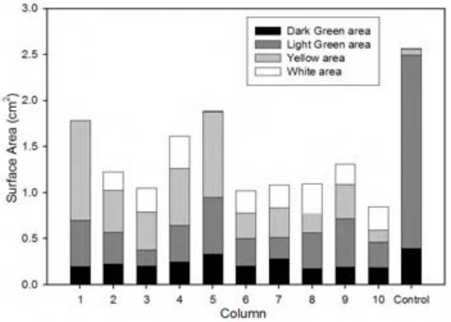

Figure 1 *Resultant frond surface areas by colour*

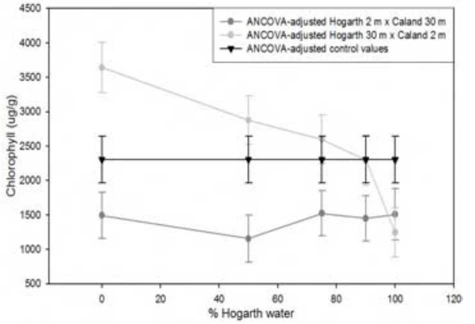

Figure 2 *ANCOVA-adjusted chlorophyll a values*

Conclusions

Frond counts and IC_{25} calculations were found to greatly underestimate toxicity because they include both small fronds and dead or chlorotic fronds in the analysis. Alternatively, chlorophyll a and surface area measurements better estimate the actual toxic response, and revealed that the future pit lake water quality will negatively impact the growth of aquatic macrophytes. The cause of toxicity was determined to be elevated Ca^{2+}, Mg^{2+}, and SO_4^{2-}. These tests show that there will not be acute effects. Changes in the nature of toxicity at the site indicate that if the water quality is allowed to progress naturally, chronic toxic effects will likely occur in aquatic flora and fauna in the lakes.

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