Geochemical and Toxicological Investigation of the Hogarth and Caland Pit Lakes, Former Steep Rock Iron Mine Site

by

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Abstract

The closure of the Steep Rock Iron Mines near Atikokan, Ontario, Canada in the late 1970s left behind an area of severe environmental concern. The subsequent flooding of the pit mines resulted in the formation of two adjacent pit lakes, Hogarth and Caland. As water levels in the two pit lakes continue to rise, the water columns are exposed to differing proportions of various wall rocks. The geochemical influence is likely the major control on the unique and dynamic water chemistries of the two pit lakes.

Although both pit lakes have near-neutral pH, Hogarth has greater concentrations of dissolved ions including Ca^{2+}, Mg^{2+}, and SO_4^{2-} than Caland. While dissolved ions increase in depth in both pit lakes, Caland is a meromictic lake with an anoxic sulfate-saline monimolimnion that is separated from the oxygenated freshwater mixolimnion by the chemocline. Both lakes have low concentrations of dissolved metals. Hogarth water produced chronic toxic effects in *Daphnia* sp. (Goold, 2008) while the freshwater lens of Caland was host to the Snow Lake Fish Farm which produced rainbow trout (*Oncorhynchus mykiss*) from the early 1980s until about 2008.

To investigate this geochemical influence of the wall rocks on pit lake water chemistry, a column experiment was conducted to empirically predict the influence of the varying types and proportions of wall rocks on the water chemistry of the two pit lakes as they fill and eventually merge. A mixing series of water from the two pit lakes was exposed to the relative types and proportions of rock lining the pits to mimic the filling and eventual joining of the two pit lakes. Although most water quality parameters varied little under the geological influence, the column experiment predicted that three main reactions will influence future pit lake water quality: (1) pyrite oxidation, (2) weathering of potassium feldspar, and (3) carbonate dissolution and precipitation. In the columns these reactions caused changes in pH indicative of buffering processes and increases in potassium, barium, and strontium concentrations.

To predict future impacts that pit lake water under geochemical influence would have on aquatic fauna, toxicity tests with the resultant column water were conducted using the duckweed, *Lemna minor.* After total frond counts were found to give a conservative estimate of toxic response, several alternative endpoint analyses were used: dry weights, chlorophyll a content, and total frond surface area. The dry weight measurements were determined to be inconclusive due to large variation among similar treatments. After controlling for the influence of dry weight using ANCOVA, chlorophyll a content was found to be significantly different from the controls in most treatments indicating that water from Hogarth impacts the growth rate of *Lemna minor.* The total frond surface area measurements confirmed that water from Hogarth significantly reduced the growth of the aquatic macrophye with water from a depth of 2 m (mixed with 30 m water from Caland) causing a greater reduction in overall frond surface area than water from 30 m (mixed with 2 m water from Caland).

These experiments show that if the water quality of the two pit lakes is allowed to progress naturally under the geological influence of the various rock types lining the pit lake walls, chronic toxic effects are very likely to be seen in aquatic flora and fauna that may become established in the pit lakes. If the combined pit overtops into the Seine River system along the historical flow path of the former Steep Rock Lake, the water quality of the combined pit lake will likely have a negative impact on downstream aquatic communities.

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i. General Introduction

i.i Characteristics of Pit Lakes

Once open pit mining operations cease, the resultant pits fill with water from groundwater inflow, surface run-off, and direct precipitation. Often the waters that fill the pits have low pH (2-4) and high sulphate and dissolved metal concentrations (Koschorreck et al., 2007). As the pits fill, weathering of the rock walls and other exposed surfaces releases solutes into the water column (Castro, 2000). In addition, pit lakes differ from natural lakes in having much greater depths relative to their surface area. Consequently, many pit lakes will stratify. Thermal and chemical stratification causes the chemical characteristics of the lake water to vary greatly with depth. Total dissolved solids and conductivity values tend to increase with depth in pit lakes (Castro 2000). If enough organic matter is present, the lower level of the stratified lake, the hypolimnion, will lose dissolved oxygen, often becoming completely anoxic. This will influence biological and chemical characteristics of the lake.

A pit lake's water quality parameters depend on the alkalinity of the infiltrating groundwater, the rock wall composition, and the amount and quality of surface run-off (Castro, 2000). Many iron ore deposits are located in host rocks containing accessory sulfide minerals which can be sources of acid sulfide water. Pyrite is a common sulfide mineral that produces acid in pit lakes as a result of its oxidation. Other sulfide minerals may be major sources of dissolved metals under acidic conditions. Other sources of dissolved metals are minerals that will react in low pH conditions including carbonates and silicates (Castro, 2000).

Not all pit lakes are acidic; they may be neutral or alkaline depending upon whether a source of acid-neutralizing carbonate is present in the wall rock (Davis, 2003; Banks, 1997). If the carbonate minerals are present in limited quantities, it is possible that an alkaline or neutral pit lake could become acidic once the carbonate source is depleted and further pyrite oxidation continues. In pit lakes with pH above 6, the pH buffering system is mainly controlled by the bicarbonate ion. Another contributor may be the hydrolysis of transition metals including iron (II), copper and zinc (Castro, 2000).

In addition to acidic conditions, pit lakes may have elevated levels of sulfate and dissolved metals and metalloids. The Guidelines for Canadian Drinking Water Quality (Health Canada, 2008) allows a maximum of 500 mg/L of sulfate in drinking water sources. Sulfate levels may be 4 or 5 times this concentration in pit lakes. Other elements such as certain essential nutrient metals including iron, copper, manganese, zinc, and cobalt and nonessential elements including lead, cadmium, arsenic, and mercury can exceed levels that are safe for aquatic life, or for agricultural, industrial, or domestic uses (Castro, 2000).

Favorable water quality is possible to achieve in pit lakes but depends on the following conditions: high carbonate availability, low pyrite and other metal sulphide availability, abundant inputs of organic matter and inorganic nutrients (Castro 2000).

Because mature pit lakes are found in a wide-range of environments which experience a variety of chemical conditions their individual water qualities will be ultimately dependent on their site-specific geologic, hydrologic, and geochemical variables (Davis, 2003) with other variables such as weather and climate change playing lesser roles.

There exist several remediation strategies that have improved the quality of acidic pit lakes including limestone drains, reactive barriers, ex-situ bioreactors, and constructed wetlands (Koschorreck et al., 2007). Another approach explored by Koschorreck et al. (2007) focused on the stimulation of naturally occurring microbial processes which would potentially generate in-lake alkalinity. However, pit lakes are often low in organic carbon which would limit these processes. By adding organic substrates the authors found that the pH of the sediment increased while there was not a lot of change in the water column. In a similar study Frömmichen et al. (2004) observed that after the addition of organic matter to mesocosms containing acidic mine water, the pH rose from 2.6 to around 6.5, and they detected alkalinity generation and bacterial growth.

i.ii Regional Geology

The Steep Rock Lake iron area, located in the Rainy River District, covers approximately 108 square kilometers (Shklanka, 1972). In 1964 and 1965, geological mapping of the area was completed. As well, air photographs were taken for use in topographic control. The maximum elevation is 472 m above sea level. The area is rugged and has many elongated ridges with steep, abrupt edges alternating with low-lying wet, swampy valleys. The minimum natural water level is found in the Atikokan River in the southwest and reaches 382 m above sea level. Detailed descriptions of the geology of the area can be found in the Ontario Department of Mines and Northern Affairs Geological Report 93 (Shklanka, 1972) and in a report by Kusky et al. (1999).

The Steep Rock area is located within the Superior (geological) Province. It covers an east-west belt of metasediments to the south and metavolcanics with a few metasediments to the north. The southern metasediments were invaded by granitic rocks in the south and southeastern portions of the area (Shklanka, 1972). The metavolcanic succession is bounded by granitic batholiths in the northeastern area.

The rocks in the area are structurally repeatedly folded and faulted. Three major metamorphic events resulted in three major periods of deformation. These coincide with the major orogenic events known as Kenorian, Hudsonian, and Grenville (Shklanka, 1972).

Lithology

There are five formations that make up the Archaean Steep Rock Group. These are the Wagita Formation (clastics), Mosher Carbonate, Joliffe Ore Zone, Dismal Ashrock, and Witch Bay Formation (metavolcanics) (Wilks et al., 1988).

Conglomerates of the Wagita Formation have clasts composed of quartz, mafic tonalite, tonalite, and fine-grained, dark grey mafic material (Wilks et al., 1988). There are metasandstones in this formation that are composed of quartz in a matrix of muscovite, chlorite, sericite, and calcite. There are minor amounts of recrystallized quartz and feldspar grains.

The Mosher Carbonate Formation consists of laminated carbonate composed of varying proportions of calcite, ankerite, and dolomite, as well as smaller proportions of quartz, pyrite, and kerogen. Stromatolites occur throughout the unit in both small-scale forms (laminae ranging from 0.5 mm to 4 cm) and large-scale forms ranging from domed structures with diameters of 3 m to tubular bodies up to 5 m long. The laminations are composed mainly of dolomite and calcite with some chert, kerogen, and minor detritus (Wilks et al., 1985).

The Jollife Ore Zone itself is made up of three members: the Magniferous Paint Rock Member, the Geothite Member, and the Pyritic Member. The Geothite Member contains pockets and lenses of brecciated and unbrecciated ore. The brecciated ore consists predominantly of goethite and hematite, and with a lesser amount of quartz and kaolinite (Wilks et al., 1988). The unbrecciated ore is composed of alternate layers of goethite and chert (MacIntosh, 1972). The Magniferous Paint Rock Member is an unconsolidated, earthy layer with fragments of goethite, hematite, chert, and quartz in a groundmass of the same minerals with kaolinite, illite, calcite, gibbsite, and pyrolusite. There are also blocks of carbonate up to 3 m across found throughout this formation. The Pyritic Member is comprised mainly of quartz, pyrite, with minor amounts of goethite (Cockerton, 2007).

The Dismal Ashrock Formation is comprised mainly of a mafic to ultramafic pyroclastic rock in which volcanic blocks occur and range in size from 4 cm to 7 m. The Dismal Ashrock Formation has high concentrations of MgO, Cr, and Ni. It is also unusually enriched in Fe, Ti, Zr, Mn, P, Ba, Nb, Rb, and Sr compared to other similar ultramafic volcanic rock units (Schaeffer et al. 1991). Within the Dismal Ashrock and the Geothite member there are bodies of massive, bedded pyrite which are closely associated with cherty carbonaceous-rich material.

The Witch Bay Formations consists of three lithologies. First, the mafic metavolcanics contain actinolite, epidote, chlorite, albite, calcite, and quartz. Secondly, the intermediate metavolcanics consist of tuffs and lavas. The tuffs contain muscovite, quartz, calcite, clays, and kerogen. The last lithology is the metasediments which are composed of carbon-rich phyllites, metasandstones, and metaconglomerates (Wilks et al., 1985).

i.iii History of the Steep Rock Mine Site

In 1938 the richest hematite deposit in North America to date was discovered below Steep Rock Lake. During the Second World War when the iron supply from South America was declining, the government allowed access and mining of the Steep Rock iron deposit under Canada's War Measures Act (Regional Engineering Services, 1986). An enormous water diversion project was undertaken in which the Seine River, which once flowed through Steep Rock Lake, was diverted from Marmion Lake through Raft and Finlayson Lakes into the West Arm of Steep Rock Lake. The water level in both Raft Lake and Finlayson Lake was lowered by 11 m in order to accomplish the diversion. At least 40 dams and other similar engineering structures were built to allow the Middle, East, and Southeast arms of Steep Rock Lake to be drained. These structures are now in need of repair, replacement or decommissioning (Sowa et al., 2001). The middle and eastern arms of the original lake, which now form the Hogarth and Caland pit lakes respectively, once had an overall surface area of 1300 ha and had a combined water volume of 450,000 Mm^3. Once the lake was drained, 210 Mm^3 of very soft lake bottom sediment was dredged in order to mine the ore bodies (Sowa et al., 2001). Dams built in the area between the Middle and West Arm of Steep Rock Lake contain much of the dredged material to such extent that the separated waters in the West Arm of Steep Rock Lake are filled with suspended sediments dredged from the drained portion of the lake.

The remainder of the dredged lake sediments was deposited in Marmion Lake (Northgate Minerals Corporation, 2004).

Pit mining of the ore continued until 1979 by which time 78 million tonnes of iron ore with an average grade of 63% metallic iron had been extracted (Sowa, 2003). Also at this time the pumps that had been keeping the pits dewatered were shut down, thus allowing the pits to begin filling with groundwater, surface runoff, and precipitation. Following closure of the mines in 1980, the site was returned to the Ministry of Natural Resources in 1985 (Regional Engineering Services, 1986). After a complete assessment of the condition of the dams, weirs, and tunnel systems, and a study of the dredging and overburden disposal it was determined that the Seine River could not be returned to its original course since it would then be flowing over the translocated overburden causing severe downstream disturbance through increased sediment loadings (Regional Engineering Services, 1986).

There were originally four pit lakes that formed on the site: Roberts, Errington, Caland and Hogarth. In the last few years Roberts Pit has joined with Hogarth pit forming one large pit lake that is approximately 3 kilometres long. The two pit lakes under study, referred to as Hogarth pit lake and Caland pit lake, are filling at such a rate that they are projected to join and continue filling until they ultimately begin spilling waters downstream into the Seine River between 2070 and 2100 (Jackson, 2007). The Seine River system flows into the International Boundary Waters of Rainy Lake and Lake of the Woods. Another foreseen hazard is that of extensive flooding which will affect the area surrounding the pits. Flooding will likely swamp Crown, municipal and private lands and roads, a section of Provincial Secondary Highway 622 and portions of the Ontario Power Generation (OPG) Atikokan Generating Station (AGS) site. Consequently, Steep Rock is currently one of the top three hazardous environmental sites in Ontario (Laderoute, pers. comm.).

In 2008, development of the Steep Rock Rehabilitation Project began. This project will serve to develop, over a period of three years, a long-term management plan for the rehabilitation and use of the Steep Rock site, and for the protection of source and downstream waters and public safety. This long-term management plan will ensure the environmental hazards and site management are properly addressed by about 2032 (Laderoute, pers. comm.).

i.iv Steep Rock Research at Lakehead University

Lakehead University undergraduate and graduate students have been studying the two pit lakes in cooperation with the Ministry of Natural Resources since 2000. Seasonal sampling of the water columns in both lakes shows that the Hogarth pit lake and the Caland pit lake, herein referred to as Hogarth and Caland respectively, differ in the concentration and distribution within the water column of various organic and chemical parameters. McNaughton (2001) carried out a complete limnological study of the two pit lakes to determine the potential of developing an aquaculture facility on Hogarth similar to the one on Caland. Vancook (2005) used GIS mapping to predict the path of overflow of Caland into Hogarth and subsequent outflow into the Seine River system. He also developed an approximate timeline for such events. Goold (2008) determined that the toxicity of Hogarth is due in large part to the elevated levels of dissolved ions in the water column and less so to heavy metals which are present in very low concentrations in both lakes. Cockerton (2007) explored the influence of water-rock interactions in the two pits and found that the main cause of the difference in water chemistry between the two pits is the proposed higher pyrite content of the Jolliffe ore zone below Hogarth. Perusse (2009) conducted a groundwater survey of the area of concern. Current research continues with a complete hydrodynamic model being developed by Larissa Stevens and a bioremediation investigation into the use of permeable reactive barriers to promote sulfate reduction being done by Simon Shankie, both of the Geology Department at Lakehead University. Ongoing monitoring of water quality in the two pit lakes also continues as detailed in the next section.

i.v Water Quality Monitoring

During the spring, summer, fall, and winter of 2006-2009, water samples (0.5 L) were collected from both Hogarth and Caland at two sampling locations, A and B, on each lake (Fig. i.i). The coordinates at each of the sampling locations are found in Table i.i. A Kemmerer bottle on a calibrated rope was used to take water samples at both stations A and B on each lake at depths of 2 m, 18 m, 40 m, and 1 m off the bottom ((x-1) m). To ensure quality control, a 1 L sample was taken at Caland 2 m to act as a lab duplicate. Also, a repeat sample at a randomly-chosen depth was taken from each lake to act as a field duplicate. The samples were collected in polyethylene bottles (HDPE #2) and were either kept cool or were kept from freezing depending upon the sampling season. These depths coincide with those sampled in previous studies (McNaughton (2001), Vancook (2005) and Goold (2008)). They also provide a sample from the mixolimnion, chemocline, and monimolimnion of Caland. All seeps flowing into the two lakes were also sampled seasonally when possible, though they often freeze during the winter months. The locations of these inflows are shown in Figure i.i.

For a separate study of stable isotopes in the two pit lakes, samples were also collected at depths of 2 m, 10 m, 18 m, 30 m, 40 m, 60 m, 80 m, 100 m, 125 m, and off bottom (x-1)m . See Conly et al. (2008).

In the fall of 2007 a Honda WX10 water pump was used to collect 25 L of unfiltered lake water from both Hogarth Pit Lake and Caland Pit Lake at depths of 2 m and 30 m. This water was stored in sealed dark blue polyethylene (HDPE #2) jugs in the walk-in cooler at Lakehead University where the temperature is kept near 4°C until it was to be used. A sample from each depth was analyzed for the chemical parameters listed in Table i.ii. This water was used in the column experiments which explored the geological influence on the water quality in the two pit lakes discussed in chapter 1.

Secchi Depths

Secchi depth measurements were made on the two pit lakes during the fall of 2006 under overcast skies with calm winds. The secchi depth at site A on Caland was 2.3 m while the secchi depth at site A on Hogarth was 4.05 m.

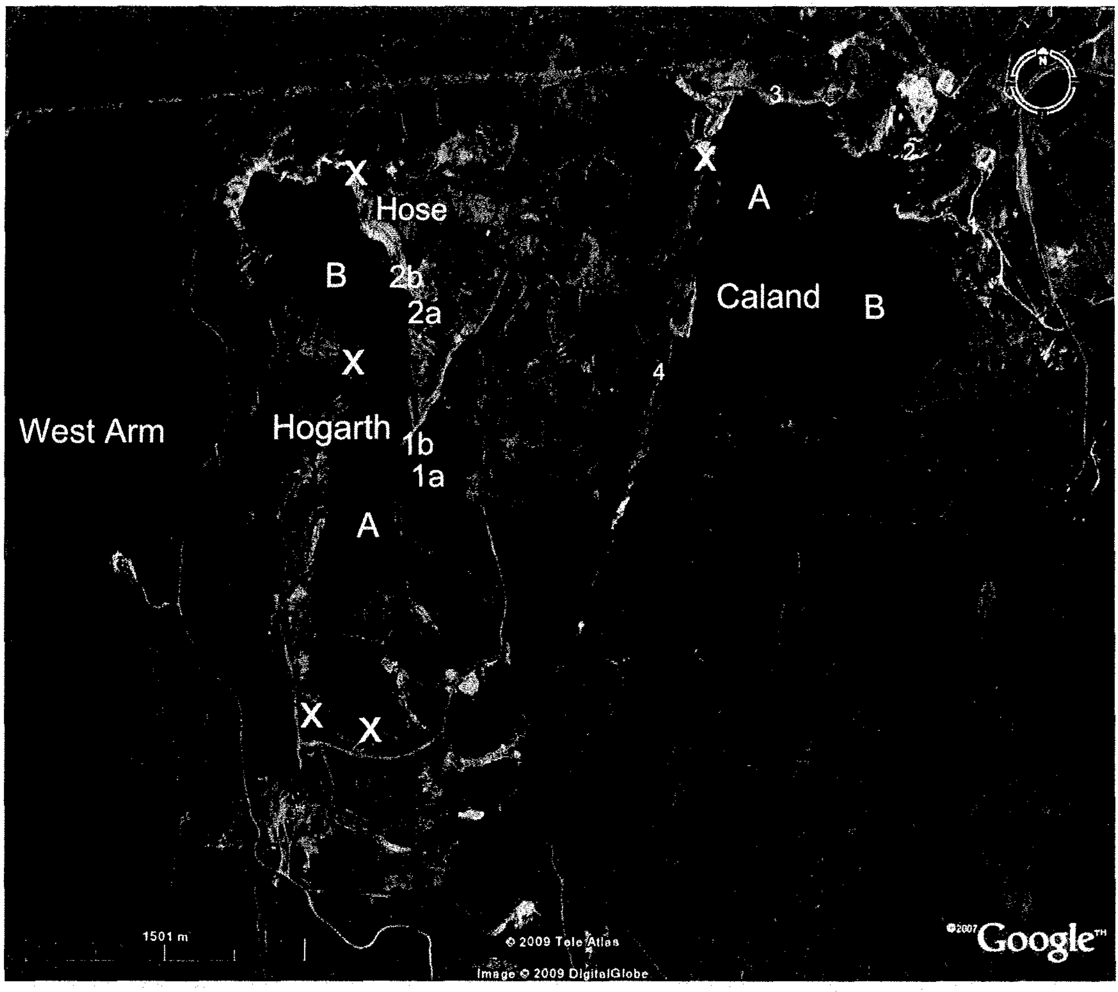

Figure i.i: Water sampling locations on each of the pit lakes (Google Earth, 2009). X denotes seeps that are present but are not regularly sampled.

Table i.i: Sampling Locations Coordinates on Hogarth Pit Lake and Caland Pit Lake

Hogarth A:	15U 0599515 5406841
Hogarth B:	15U 0599383 5408071
Caland A:	15U 0601696 5408724
Caland B:	15U 0601998 5408272

i.vi Laboratory Chemical Analysis

Field Samples

Water samples from all of the experiments conducted (field water samples, water mixing series samples, and column experiment samples discussed in chapter 1) were analyzed at the Lakehead University Environmental Lab (LUEL) on the Lakehead University Campus in Thunder Bay, Ontario, Canada adhering to strict Quality Assurance/ Quality Control (QA/QC) guidelines. For all test methods that were performed a blank sample of distilled deionized water (DDW) was analyzed followed by a standardized QC sample specific to the method. One water sample was analyzed twice as a laboratory repeat. The field duplicate sample was analyzed as a field repeat. LUEL standard operating procedures (SOPs) were followed for each test that was carried out (LUEL, 2003). These SOPs were adapted from Standard Methods for the Examination of Water and Wastewater 18th ed. (Greenberg, 1992).

Table i.ii: Chemical and physical features measured at each sampling station in both Hogarth and Caland Pit Lakes.

Physical Parameters:	depth (m)		secchi depth (m)	water temperature (°C)	
	total dissolved solids		total suspended soilds		
Chemical Parameters:	alkalinity	pH	conductivity		
Organics:	dissolved organic carbon				
	total nitrogen				
	ammonia				
	nitrate				
	total phosphorus				
Cations:	aluminum	arsenic	barium	beryllium	boron
	cadmium	calcium	cobalt	chromium	copper
	iron	lead	magnesium	manganese	
	molybdenum	nickel	potassium	sodium	
	strontium	sulfur	vanadium	zinc	
Anions:	chlorine	sulfate			

pH/ Alkalinity/ Conductivity

Within 24 hours of sampling, the pH, alkalinity, and conductivity are measured on each of the unfiltered water samples while they are at room temperature. Alkalinity and pH are determined simultaneously using a DL53 Mettler titrator, and a DL20 Autosampler while running LabX Lite Software version 1.1 to automate the analysis. Conductivity is measured using an Accumet XL60 multi meter with an Accumet conductivity probe. A temperature compensation probe is used to accurately determine the conductivity of the samples.

Total Suspended Solids/ Total Dissolved Solids

To determine the total suspended solids (TSS) a known volume of sample is passed through a preweighed 0.45μm glass fiber filter which is then dried to constant weight at 103-105°C. The proportion of the total solids retained by the filter is determined from the difference between the post weight and the pre weight of the filter determined using an analytical balance.

Total dissolved solids (TDS) are determined simultaneously with TSS. Once the sample is passed through a 0.45μm glass fiber filter, a known volume of the filtered sample is transferred to a preweighed evaporation beaker and then evaporated to dryness and constant weight at 180°C. The proportion of the total solids present in the sample after being filtered is determined from the difference between the post weight and the pre weight of the evaporation beaker using an analytical balance.

Total Phosphorus/ Total Nitrogen/ Nitrate and Nitrite/ Ammonia

Total phosphorus and total nitrogen are both determined using a Skalar autoanalyzer, which performs online all of the necessary reactions. To determine total phosphorus, the samples undergo acid hydrolysis by the addition of sulfuric acid to the sample stream and heating at 97°C. This converts polyphosphate and organophosphorus compounds to orthophosphate. The sample then undergoes further digestion under uv radiation through the addition of peroxodisulfate. Colourimetry determination is then used to determine the total phosphorus in the sample.

To measure total nitrogen, the sample is mixed with a potassium peroxodisulfate/ sodium hydroxide solution and heated to 90°C. It is then mixed with a borax buffer followed by the conversion of all nitrogen species to nitrate by uv radiation. Colourimetry determination is then used to determine the total nitrogen in the sample.

In a commercially packed cadmium column treated with copper sulfate as part of the scalar autoanalyzer, all nitrate is converted to nitrite. The solution is then diazotized with sulfanilamide and coupled with N-(1-phthyl)-ethylenediamine dihydrochloride which produces a highly coloured azo dye. The absorption of this solution is measured at 540nm to determine the nitrite in the sample (Joncas pers. comm., 2009).

The skalar autoanalyzer is used to determine the ammonia in the sample by colourimetry following the modified Berthelot reaction. The ammonia is chlorinated online forming monochloramine which is reacted with salicylate to form 5-aminosalycylate. A green coloured complex is formed after online oxidation and oxidative coupling of the sample. The absorption is measured at 660nm to determine the ammonia in the sample.

Dissolved Organic Carbon

The sample is filtered through a 0.45µm filter. A known volume of the filtered sample is then injected into a heated reaction vessel in which the water is vapourized and the organic carbon is oxidized to CO_2 and H_2O. The CO_2 from the organic carbon is transported in carrier gas streams and is measured by a nondispersive infrared analyzer.

Chemical Elements: Cation, Anions, and Metals

Ions and metals were determined at Lakehead University's Instrumentation Laboratory (LUIL) using ion chromatography (IC) and inductively coupled atomic emission spectrometry (ICP-AES). For IC analysis a Dionex Dx-120 was used. It is a dual channel IC which is used to measure both anions and cations including sulfate, chloride, nitrate, sodium, potassium, magnesium, and calcium. Samples with high known values of sulfate (samples from Hogarth and certain seeps) were diluted using distilled deionized water to lower concentrations to within IC detection limits (Table i.iii). ICP analysis was done using an ICP-AES Varian Vista Pro Radial spectrometer which measures element-specific characteristic emission spectra produced by a radio-frequency inductively coupled plasma by optical spectrometry. Samples were first digested with nitric acid and then concentrated five times using microwave pre-concentration. A list of these and all other physical measurements taken is found in Table i.ii.

Table i.iii: The detection limits of the measured water quality parameters.

Parameter	Al	As	B	Ba	Be	Ca	Cd	Cr	Cu
Detection Limit (ppm)	0.02	0.05	0.05	0.009	0.006	0.02	0.004	0.005	0.005
Parameter	Fe	K	Mg	Mn	Mo	Na	Ni	P	Pb
Detection Limit (ppm)	0.01	0.1	0.005	0.001	0.025	0.02	0.01	0.1	0.025
Parameter	S	Se	Si	Sr	Ti	V	Zn	Cl	SO4
Detection Limit (ppm)	0.1	0.05	0.1	0.01	0.01	0.03	0.05	0.05	0.05

i.vii Caland Pit Lake

Caland pit lake is a meromictic lake with a well-defined mixolimnion, chemocline and monimolimnion. Freshwater inflow has created a thinning, 20-metre-deep freshwater lens on a sulphate-saline, anoxic bottom layer. Carbonate wall rock including a large island of carbonate in the northern part of the lake, buffers the water's pH. From the early 1980s until 2007 the upper freshwater lens of Caland had been host to the Snow Lake Fish Farm which produced rainbow trout (*Onchorhyncus mykiss*) in a system of net pens. This practice added large amounts of organic matter to the water column every year.

There are numerous seeps flowing into Caland, four of which are sampled regularly (Fig. i.i). Seep 1 is the freshwater supply for the occupants of the fish farm. A holding area has been constructed which allows the seep to collect in a small, shallow pond like area. Seeps 2 and 3 flow down rock faces at the north and north-east ends of the lake, respectively. Seep 3 is rarely accessible and seldom sampled since it flows down a steep rock face. Seep 4 was discovered and first sampled during the summer of 2006. It flows down a steep bank at the southeast side of the pit. It likely drains from a tailing pile as it is orange in colour and has very low pH values.

i.viii Hogarth Pit Lake

The entire Hogarth water column is sulfate-saline and aerobic. However, it appears that it is beginning to stratify. In a past study (McNaughton, 2001) water from Hogarth was discovered to be acutely toxic and there were no apparent signs of aquatic life in the lake. A more recent study (Goold, 2008) found that it now appears to be chronically toxic. Snails and minnows were observed in shallow littoral zones of the lake during the summer of 2006. In 2004, Hogarth merged with Roberts' pit at the south end which appears to have a very similar chemical profile to Hogarth.

Like Caland, there are numerous inflows into Hogarth. Of these, five seeps are sampled regularly (Fig. i.i) which all enter from the north east banks of the lake. Seeps 1a and 2a are highly acidic, and appear orange in colour. There is a thick, orange precipitate lining each of these seeps. Conversely, seeps 1b and 2b are basic and have a white precipitate lining them. Seeps 1a and 2b often dry up during the summer months. Seeps 1b and 2a have always been flowing during the ice-free sampling periods. Seep 1a joins seep 1b before they enter the pit lake together. The hose seep originates far up the banks of Hogarth. A rubber hose has been inserted into the ground and water readily flows out of the hose. This water flows over a fair distance of ground before it reaches the lake. The seep is sampled directly from the hose and not any closer to the lake.

i.ix Comparison of the Hogarth and Caland Pit Lakes

The concentration of dissolved ions in Caland is lower than that of Hogarth especially in regards to major cations (Ca^{2+}, Mg^{2+}, Na^{2+}, K^{+}) and dissolved sulfate. All of these parameters increase with depth in each lake (Fig. i.ii). Goold (2008) found that chronic toxicological affects produced by water from the Hogarth pit were likely caused by the elevated levels of dissolved ions especially sulfate (SO_4^{2-}).

As a consequence of Hogarth having greater concentrations of dissolved ions than Caland, Hogarth also has greater conductivity, hardness, and TDS (total dissolved solids) levels than Caland. Likewise, these parameters all increase with depth in both pit lakes.

The pH of the two lakes is nearly always near-neutral to slightly basic, not typical of pit lakes which usually have acidic pH sometimes as low as 2-3 due to acid mine drainage (Gammons, 2006a and 2006b; Castro, 2000; Banks, 1997). The pH in Caland is on average 8.05 at 2 m and decreases with depth to an average of 7.50 near bottom. Hogarth has an average pH of 7.80 at 2 m and decreases with depth to 7.10 near bottom. The alkalinity is greater is Caland (130-173 mg/L $CaCO_3$) than in Hogarth (94-126 mg/L $CaCO_3$) and values increase with depth in both lakes. The higher alkalinity of Caland likely reflects the greater proportion of carbonate rock deposits lining the pit. Carbonate deposits are the major source of the buffering action seen in the two pits (Conly, 2007). Due to the interaction with carbonate wall rock the pH is maintained near neutral in the two pit lakes. For more historical data see Goold (2008), Vancook (2005), and McNaughton (2001).

The densities of the layers in each lake vary little (Table i.iv) and as such the two lakes will likely mix easily upon the joining of the two pit lakes.

Table i.iv: The density of each layer used in the modeling experiments.

Lake and depth	Density (g/cm^3)
Hogarth 2m	1.020
Caland 2m	1.016
Caland 30m	1.015

Because of the influence of the Snow Lake Fish Farm on Caland until recently, the levels of certain organic constituents are greater throughout the water column in Caland compared to Hogarth. Caland has greater dissolved organic carbon (DOC) concentrations than Hogarth (Caland: 2.9-3.2 mg/L; Hogarth: 1.0-1.3mg/L) with a maximum value in Caland at 18 m and a minimum value in Hogarth seen at the same depth. However, the average total nitrogen (Caland: 0.42-0.50 mg/L; Hogarth 0.40-0.75 mg/L) and total phosphorus (Caland: 0.032-0.037 mg/L; Hogarth: 0.029-0.037 mg/L) values from 2005-2008 do not differ greatly between the two pits (Fig. i.ii).

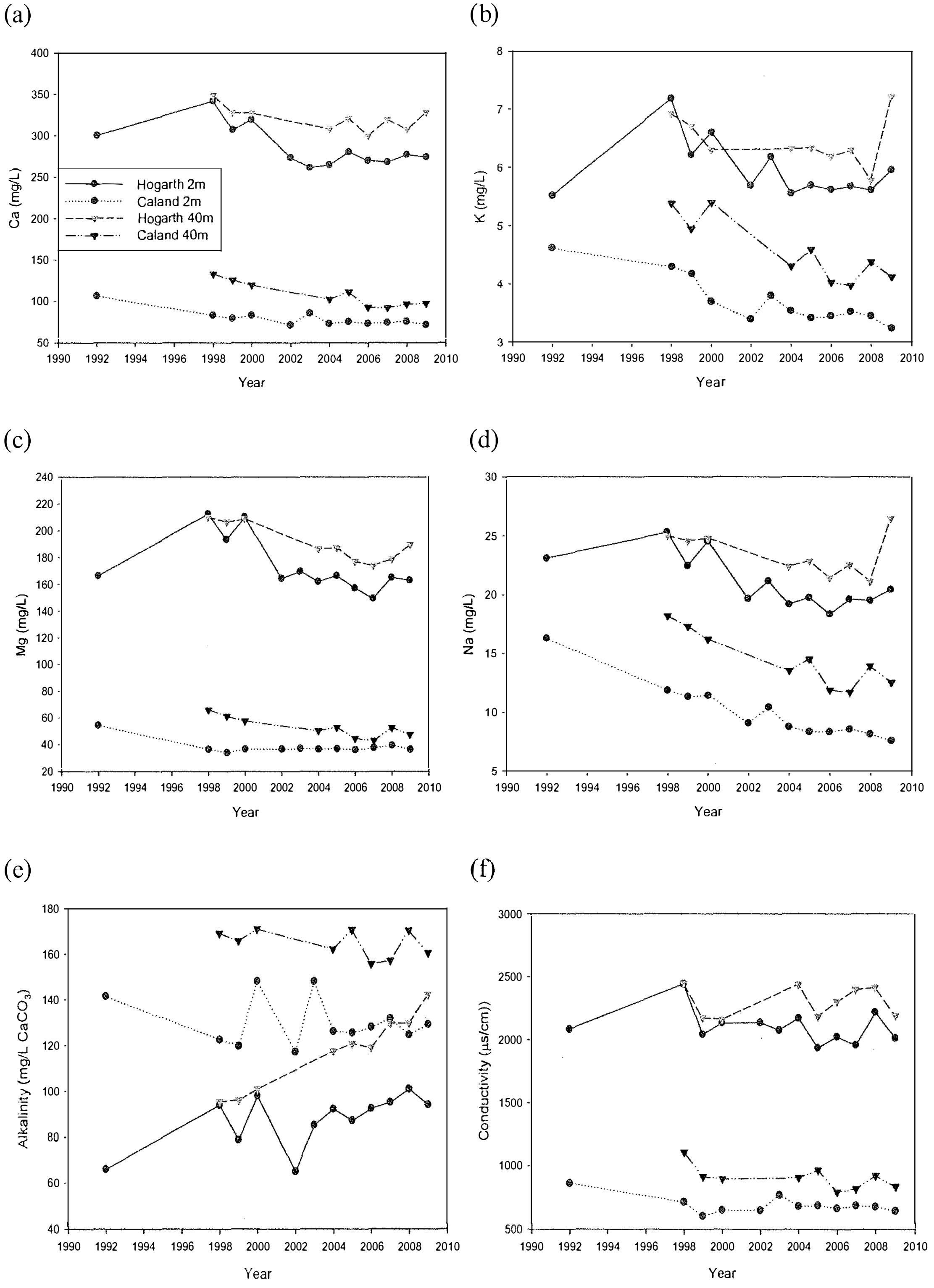

Figure i.ii: Annual averages of the chemical parameters measured in each pit lake at 2m and 40m depths. (continued on next page)

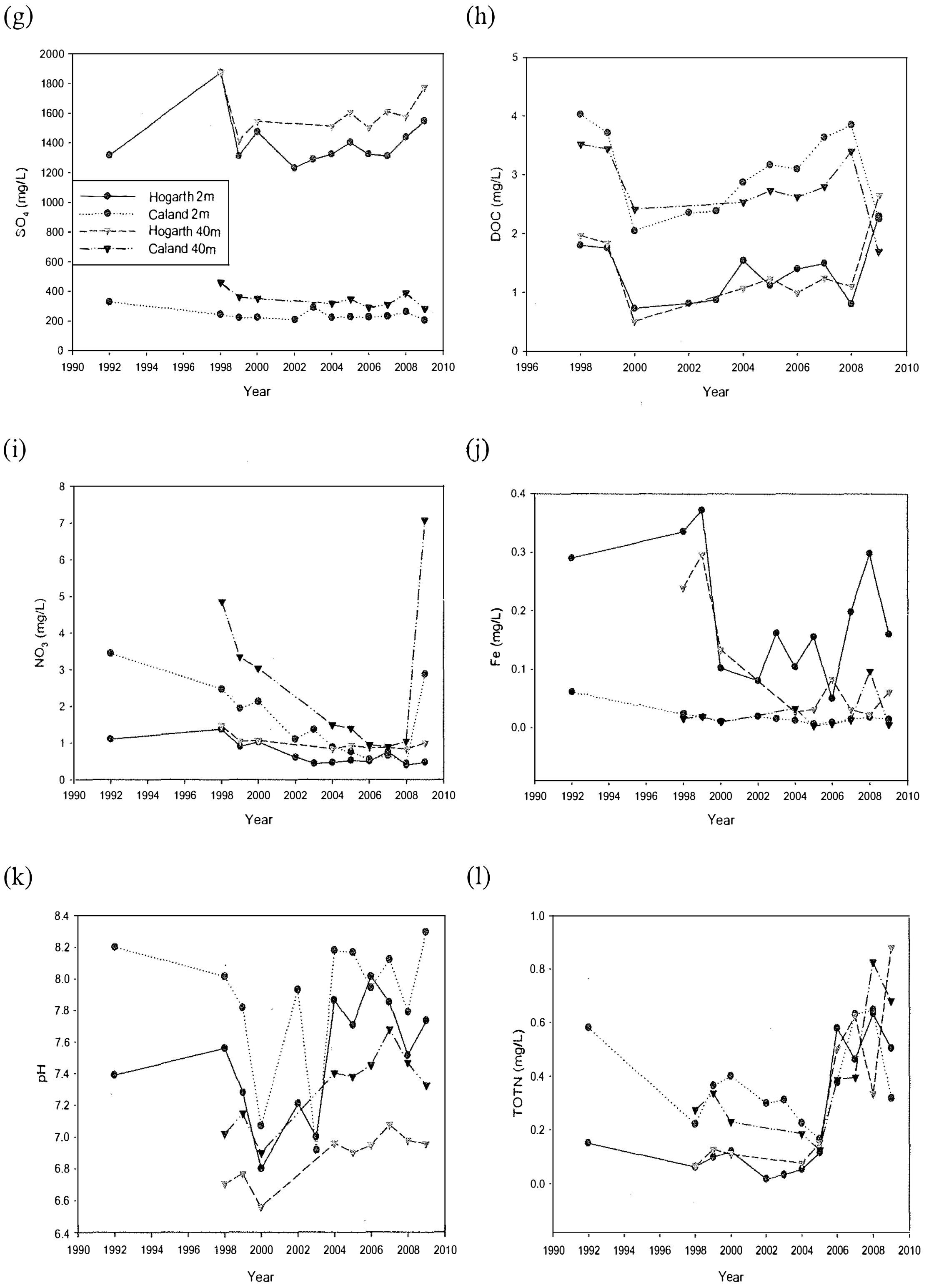

Figure i.ii continued: Annual averages of the chemical parameters measured in each pit lake at 2m and 40m depths

Chapter 1: Geochemical Effects on the Water Quality of the Steep Rock Pit Lakes

1.1 Introduction

1.1.1 Current Conditions at the Former Steep Rock Iron Mine Site

Both the Caland and Hogarth pit lakes on the former Steep Rock mine site are slowly filling with precipitation, run-off from tailing piles (seeps), and groundwater inflow. Vancook (2005) used GIS models to predict that the two lakes would mix by 2030 with subsequent outflow following shortly after. Work done by Jackson (2007) revealed this estimate to be optimistic in filling rates and predicted mixing and outflow would not occur until 2070 or later. Both of these studies predict that as the two pit lakes fill, Caland will flood the southwestern portion of the Caland pit and will slowly begin flowing into the Hogarth pit (Fig. 1.1). If these predictions are accurate, then in the initial stages of pit lake mixing there will be a small volume of water from the Caland pit flowing into the Hogarth pit. Another concern is that as the Caland pit continues filling, the upper freshwater lens will eventually fill an area that has a greater volume as well as a greater surface area. This will result in thinning of the freshwater lens. At present the upper lens is approximately 25 m deep but is anticipated to be much thinner by the time the two pit lakes join.

The water quality of the Caland upper freshwater lens varies greatly from that of its anoxic monimolimnion making it necessary to model the effects of mixing of each layer from Caland with water from the Hogarth pit lake. Also, since Hogarth appears to be beginning to stratify, water from both its epilimnion and hypolimnion was used in the modeling experiments. As such, the water used in the experiments was collected from depths of 2 m and 30 m from each lake, providing a sample from both the freshwater lens and the anoxic monimolimnion of Caland as well as the epilimnion and hypolimnion of Hogarth.

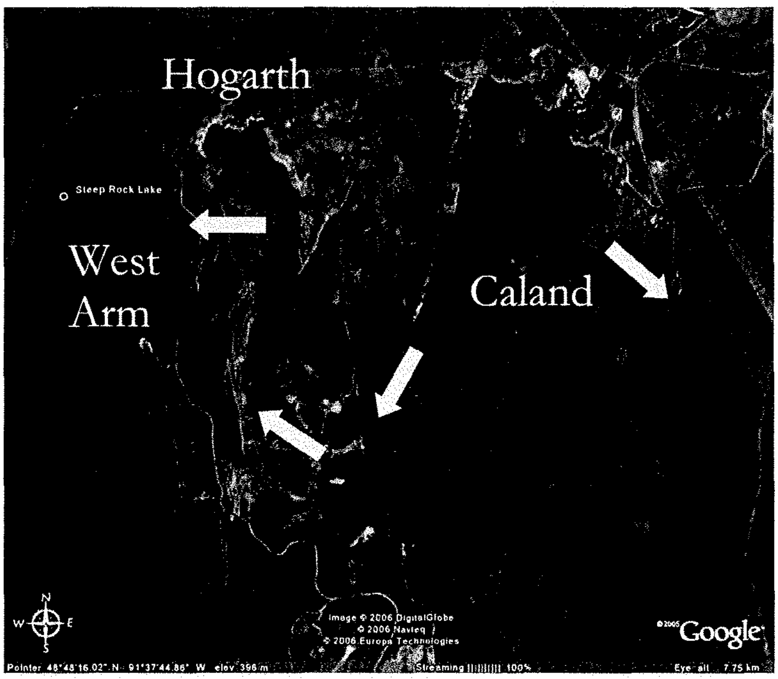

Figure 1.1: The original flow path of the former Steep Rock Lake. This also represents the direction of future filling and outflow into the West Arm.

The purpose of the following experiment was to empirically predict the future water quality of the Hogarth and Caland pit lakes as they fill, merge and reach the former Steep Rock Lake level before out flowing into the Seine River system. It was also to determine whether the toxic nature of the water in the Hogarth pit lake will change over time under the onsite geological influences, either ameliorating or worsening. The hypothesis was that the water quality in the two pit lakes will continue to change over time and that this change is caused by the geological influence of the various rock types lining the pit lake walls in both pits.

Chemical weathering is a major process influencing the global hydrogeochemical cycle of elements in which water acts as a reactant and as a transporting agent of both dissolved and particulate elements. The oxidants and carbon dioxide which are required in weathering reactions are readily available in the atmospheric reservoir. Through chemical weathering reactions, rocks and primary minerals are transformed into solutes and soils and then to sediments and sedimentary rocks (Stumm et al., 1996). Mining conditions result in the exposure of large quantities of reduced minerals to surface conditions (Banks, 1997). Unlike most chemical weathering processes that consume protons, pyrite oxidation produces protons which results in acid generation (Banks, 1997). If a pit lake is in contact with a carbonate source, chemical weathering of the carbonate can neutralize acid produced by pyrite oxidation (Castro, 2000; Appelo, 1998). Sulfide minerals other than pyrite may also be major sources of dissolved metals in acidic pit lakes (Castro, 2000). In pit lakes with rising water levels, the dissolution of soluble salts on weathered mine walls can contribute to poor pit lake water quality (Gammons, 2006a).

In order to understand the scenario of simple water mixing between the two lakes without any influence from geological sources, a water-mixing batch reaction experiment was conducted. This also established initial background concentrations for the mixing series that were used in the subsequent column experiments. The column experiments explored the geological influence on pit lake water quality. By comparing the results of the batch mixing experiment and the column experiment, it was possible to predict whether any future changes in water quality are likely to occur in the pit lakes due to the geological influence of the varying rock types lining the pit walls.

1.2 Methods

1.2.1 Density Differences versus Mixing

In order to better understand the dynamics of the mixing event, the densities of the each layer from each pit were determined during the winter of 2007. Since Hogarth was experiencing winter stratification at this time, one sample from 2 m was used for such measurements. Using a 1.00 ml pipette, samples from Hogarth, Caland 2 m, and Caland 30 m all at 3°C were weighed using an analytical balance to determine the density of each layer (Table i.iii). Although the density of Caland 2 m is slightly greater than that of Caland 30 m, the stratification of the lake is maintained due to the chemical gradient.

1.2.2 Geological Field Sampling Procedures

Using map 2217 Steep Rock Lake Area, Rainy River District published by the Ontario Department of Mines and Northern Development as part of Geological Report 93 (Shklanka, 1972) the relative rock amounts of each rock type that will be in contact with the water in each of the pits as they fill was determined; the original lake outline of the former Steep Rock Lake prior to mining developments was transcribed onto map 2217. This corresponds to an elevation of 390 m (Jackson, 2007). Once the two pit lakes join and reach this level they will achieve their maximum fill volume. This future predicted volume is used in the calculation of rock and water amounts used in the column experiment presented in section 1.2.4. From herein, this will be referred to as the maximum fill volume (MFV) of the combined pit lake. The area of each rock unit was measured using a Tamaya Planix 7 digital planimeter. The relative rock amounts in contact with the pit lake water in each pit prior to mixing was determined as well as the relative rock amounts in contact once mixing had occurred. These amounts are presented in Table 1.1.

Rock from each required rock type on map 2217 was collected from around the two pit lakes. These rocks were crushed and sorted by particle size. Rock of grain-size 0.5-2 mm was collected and used in column experiments that served to predict the geochemical effects on future pit lake water chemistry. The actual amount of each rock type used in the column experiments was determined from the mass ratio proportions in Table 1.1. These amounts are provided in Table 1.2.

Table 1.1: The amount of each rock type (m^2) lining each pit once they reach their maximum fill volumes (MFV) and the amount lining the combined pit.

Rock Type	Caland at MFV	Hogarth at MFV	Combined at MFV
Marmion Complex	2705760	669600	3350880
Mosher Carbonate (Dolomite)	1363700	794880	2158580
Magniferous paint rock		not sampled	
Geothite (Hematite)	144090	214560	372330
Pyritic iron formation	36000	53280	90720
Dismal Ashrock Formation	705600	604800	1310400
Witch Bay Formation	2554560	1118880	3673440
Mafic intrusive	158400	4320	174240
Late mafic intrusive	970560	115200	1032480

Table 1.2: The weight of each rock type representing the relative amount of rock in contact with the pit lake water upon reaching maximum fill volume (MFV) and mixing.

Rock Type	Hogarth at MFV	Caland at MFV	Combined
Marmion Complex	75g	125g	200g
Mosher Carbonate (Dolomite)	89g	63g	152g
Geothite (Hematite)	24g	7g	31g
Pyritic iron formation	6g	2g	8g
Dismal Ashrock Formation	68g	33g	101g
Witch Bay Formation	126g	118g	244g
Mafic intrusive	0.5g	7g	7.5g
Late mafic intrusive	13g	45g	58g

1.2.3 Batch Mixing Series – No Geological Influence

Water used in the initial water mixing series was collected from sampling stations Hogarth B and Caland A (see Table i.i for coordinates) at depths of 2 m and 30 m during the summer of 2007 using a Kemmerer bottle on a calibrated rope. The samples were collected in polyethylene bottles (HDPE #2). The samples were kept cool at all times. The unfiltered samples were mixed in the proportions shown in Table 1.4 in polyethylene bottles before analysis.

During October 2007 water from these initial mixing series was also placed in brown polyethylene 0.5 L Nalgene bottles. These sample bottles were tightly sealed and stored at ambient room temperature for 18 months to determine if there were any changes in water chemistry over an extended period of time. Unfiltered water samples from both the initial mixing series in October 2007, and from the final mixes left in brown nalgene bottles for 18 months until April 2009 were thoroughly mixed before they were analyzed for metals and ions using IC and ICP at Lakehead University's Instrumentation Laboratory (LUIL). Alkalinity, pH, and conductivity were also measured at LUEL. These results are presented in Table 1.4.

1.2.4 Column Experiments: Predicting Pit Lake Water Chemistry at Maximum Fill Volume

Columns were built using 10.2 cm inner diameter transparent PVC piping. Black end caps were attached to one end using Sluyter PVC/ABS Transition Solvent Cement. On the other end, a black schedule 80 female adaptor with a male end were glued to allow the column to be sealed or opened with ease (Cockerton, 2007). A small hole was drilled in the bottom end cap and a syringe lure and valve were inserted to allow the liquid to be sampled from the columns at their lowest point. These columns were mounted onto a stand to maintain a vertical orientation. For more details on construction of the columns see Cockerton (2007).

Sleeves to hold the small rock particles were sewn using Nitex bolting cloth with an aperture of 100 μm. These were suspended to submerse all rock within the water in each column by attaching them to the column lids. Using the rock amounts predicted to be in contact with the pit lake water once maximum fill volume is reached, the representative amount of each rock type by weight was determined. This was based on the ratio of water to rock at the point of maximum fill volume, which is estimated to be a very approximate ratio of 10:1. This ratio was based on the volume of each pit and the surface area of the pit walls. There are many factors that would need to be taken into account to determine a more precise water to rock ratio. As filling continues, there will initially be water infiltration into the rock, but as the water table rises this infiltration will slow. Also, groundwater flow into the lake should decrease as the recharge area shrinks (Fralick, pers. comm.). However, a 10:1 water to rock ratio satisfies the purposes of the column experiment.

Each column used in the experiment held a volume of 4 L of water weighing 4 kg. Using this weight and the 10:1 water to rock ratio the weight of each rock type required was determined (Table 1.2). For this experiment, the relative rock amounts from the Hogarth pit at its maximum fill volume (MFV) were used to determine the amount of each sorted rock type to be placed in each sleeve in each column. The Hogarth MFV rock amounts were weighed out, mixed thoroughly and placed in the Nitex sleeves.

The water added to the columns consisted of a varying ratio of water from each of the pit lakes at either 2 m or 30 m depth. This allowed a rough modeling of the overflow of Caland into Hogarth, which was predicted to occur based on the flooding model by Jackson (2007). Overflow of Caland into Hogarth also follows the original flow path of the Seine River through the former Steep Rock Lake (Fig. 1.1). To model this, an increasing ratio of Caland to Hogarth water was used in the individual columns. The predicted rock amounts in contact with the Hogarth pit lake at the point when the lake reaches the former lake level, referred to herein as the maximum fill volume (MFV), were used to determine the amount and types of rock added to columns 3 - 8 and 10 to observe whether there would be a shift in water chemistry parameters towards those of present Hogarth conditions. The predicted MFV rock amounts in the Caland pit were used to determine the amount and types of rock added to column 9, containing only water from Caland 30 m. This would show the influence of the rock types lining the Caland pit as the pit lake fills. The water ratios, depths, and rock amounts in each column are presented in Table 1.3.

Table 1.3: The volume and depth of water from each lake used in each column and the columns to which a sleeve of rock was added. Rock added to the columns corresponds to the amount and types of rock lining the Hogarth pit at MFV as determined in Table 1.2 except in column 9*.

Column	Caland	Hogarth	Caland : Hogarth	Rock added
1	2m – 4L	N/A	1:0	none
2	N/A	2m – 4L	0:1	none
3	2m – 0.4L	30m – 3.6L	1:9	yes
4	2m – 1L	30m – 3L	1:3	yes
5	2m – 2L	30ml – 2L	1:1	yes
6	30m – 0.4L	2m – 3.6L	1:9	yes
7	30m – 1L	2m – 3L	1:3	yes
8	30m – 2L	2m – 2L	1:1	yes
9	30m – 4L	N/A	1:0	yes*
10	N/A	30m – 4L	0:1	yes

*Rock added corresponds to the types and proportions lining the Caland pit.

1.2.4a Column Experiment Samples

The water in the columns was sampled at one hour, one day, three days, seven days, and then each week for the next 20 weeks. Approximately 50 ml of water was extracted at each sampling using a 10 ml syringe which locked onto the column valve apparatus. The sample was filtered using 0.45 μm PTFE filter membranes. The pH of each sample was measured and recorded. Approximately 0.005 ml of trace metal grade nitric acid was added to a 15 ml portion of each sample in a sealed centrifuge tube. These samples were analyzed for metals and cations (Al, As, B, Ba, Be, Ca, Cd, Cr, Cu, Fe, K, Mg, Mn, Mo, Na, Ni, P, Pb, S, Sb, Se, Si, Sr, Ti, V, Zn) by ICP-AES at LUIL. A filtered, non-acidified 10 ml aliquot of each sample was used for the determination of chloride and sulfate concentrations using IC analysis. Due to the elevated concentration of sulfate in the samples, it was necessary to dilute each sample by ten times with distilled, deionized water to achieve concentrations within the detection limits of the ICP-AES. The remaining portion of the sample was used to measure the pH of the column. Approximately 50 ml of a stock solution of each column water mix was returned to each column after sampling to maintain the water volume in the columns to ensure the water to rock ratio remained constant throughout the experiment.

On day 139 when the experiment was complete, the columns were all drained and the water was collected for use in other experiments. Column 8 was refilled with water from the original stock solutions (50% Hogarth 2 m water; 50% Caland 30 m) and the rock sleeve was then returned to the column. The objective was to see if the addition of a new batch of water to the previously reacted rock would result in the same reactions even though equilibrium was reached in the first part of the experiment for most parameters.

Column 8 appeared to give the best estimate of the water ratios in the future joined pit lake since it contained a 1:1 ratio of water from Caland 30 m and water from Hogarth 2 m with Hogarth MFV rock amounts. As filling continues and Caland's freshwater lens thins, most of the volume of water from Caland that will flow into Hogarth when the pit lakes merge will have water quality similar to that of the hypolimnion, represented in the column experiment by Caland 30 m water.

1.2.5 Data Analysis

In order to identify the parameters which had similar patterns in concentration fluctuations during the column experiment, hierarchical cluster analysis was carried out using SPSS Statistics v. 17.0. Many authors have successfully used this multivariate analysis to examine the spatio-temporal variations in water quality at various study sites (Qadir et al., 2008; Singh et al., 2008; Zhou et al., 2007; Kazi et al., 2009). The final water quality values from the column experiment were analyzed using Pearson Correlation values as the distance measure for between groups linkage analysis. The analysis excluded those parameters that were below detection limits for at least 90 % of the measurements. Chlorine and sulfate values were not included because of the large variation in their measurements, which could skew the results. The parameters included in the analysis are pH, barium, calcium, magnesium, potassium, sodium, strontium, and sulfur. To determine the degree of dissimilarity to use as a means of determining the number of clusters, a scatter plot was created of dissimilarity distance versus the number of clusters. The point at which the slope of the graph showed little change determined the number of clusters of parameters present.

1.3 Results

1.3.1 Batch Mixing Series – No Geological Influence

The results of the initial water mixes are presented in Table 1.4 as 'Initial'. For comparison, this table also contains the water quality values of the water mixes left in brown Nalgene bottles at ambient temperature for 18 months. These are the 'Final' values. When the initial mixes are compared to those that were stored in brown Nalgene bottles for 18 months (Table 1.4), there do not appear to be any major changes in any of the parameters over time. In most cases the linear relationship between concentration and proportion of Hogarth water was maintained (Fig. 1.9).

Table 1.4: Initial and final water mixing results for batch mixing experiments.

	Caland 2 m		Caland 30 m		Hogarth 2 m	Hogarth 30 m		H30 10% C30 90%	
	Initial	Final	Initial	Final	Initial	Initial	Final	Initial	Final
B	<DL	0.03	0.05	0.05	<DL	0.05	0.05	0.05	0.04
Ba	<DL	0.022	<DL	0.022	0.008	<DL	<DL	<DL	0.02
Ca	71.48	72.71	88.18	89.22	256.2	306.89	303.99	109.29	111.19
K	3.5	3.4	4.2	4	5.3	6.6	6.4	4.4	4.1
Mg	34.34	36.174	40.54	43.994	109.5	148.09	169.064	51.83	58.564
Mn	<DL	0.002	<DL	<DL	0.147	<DL	<DL	<DL	<DL
Na	7.28	7.9	10.7	10.81	18.44	22.05	22.76	11.27	11.7
Ni	<DL	<DL	<DL	<DL	0.02	0.02	0.03	<DL	<DL
S	74.9	64.8	91.2	81	438.4	503.2	456.2	131.1	118.8
Si	<DL	0.7	<DL	2.4	<DL	<DL	4	<DL	2.5
Sr	<DL	0.47	<DL	0.66	<DL	<DL	1.33	<DL	0.71
Conductivity	728.6	642.3	728.2	781.2	208.6	2338	2293		984.6
pH	8.03	8.09	8.04	8.15	8.05	7.21	7.89		8.13
Alkalinity	134.2	123.16	134.6	149.12	91.4	129.5	105.62		147.93
SO4	236.6	221.95	237.85	272.96	1292	1637.69	1566.31		387.53
Cl	4.54	3.71	4.54	6.47	10.9	13.64	7.89		6.9

*H2 and H30 refer to water from Hogarth at a depth of 2 m and 30 m, respectively. C2 and C30 refer to water from Caland at a depth of 2 m and 30 m,
*The percentages of H2, H30, C2, and C30 refer to the relative proportion of each water used in the batch reaction in each treatment.
*Al, As, Be, Cd, Co, Cr, Cu, Mo, Fe, P, Pb, Sb, Se, Ti, V, Zn were <DL for at least 90% of measurements.
*Initially, Hogarth 2 m had Al=0.061mg/L, Fe=0.087mg/L, all other samples were <DL for Al and Fe.
*There is no Hogarth 2 m final sample.
*Values appearing blank were not measured at the time.

1.3.2 Column Experiment

The results of the column experiment are presented in Appendices A and B. In terms of absolute increases in concentration, potassium and sulfate showed the greatest increases during the column experiment. In terms of the percent increase compared to starting values, barium, boron, potassium, strontium, and sulfate showed the greatest increases.

The water quality parameter concentrations from the 1 hour sampling and the final sampling on day 139 of the column experiment are presented in Table 1.5.

Table 1.5: 1 hour and final column experiment water quality parameter values. All concentrations are

Column	1 - 1hour	1 - Final	2 - 1 hour	2 - Final	3 - 1 hour	3 - Final	4 - 1 hour	4 - Final	5 - 1 hour
pH	8.21	8.24	8.10	8.15	8.10	8.17	8.07	8.38	8.17
Al	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL
B	0.04	0.04	0.04	0.04	0.07	0.12	0.09	0.11	0.08
Ba	0.021	0.022	0.009	0.009	0.012	0.023	0.016	0.022	0.016
Be	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL
Ca	68.89	69.10	252.00	252.33	280.60	267.73	255.90	233.43	190.30
Cd	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL
Cr	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL
Cu	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL
Fe	0.01	<DL	0.01	<DL	0.01	<DL	0.02	<DL	0.01
K	3.2	3.3	5.4	5.5	6.7	10.3	6.8	9.7	5.7
Mg	34.34	35.16	142.57	146.29	153.67	154.09	139.27	137.09	105.37
Mn	<DL	<DL	<DL	<DL	0.015	<DL	0.018	<DL	0.013
Na	7.33	6.87	17.90	17.64	20.80	20.18	19.00	18.03	14.90
Ni	<DL	<DL	<DL	<DL	0.03	<DL	0.02	<DL	<DL
S	66.7	68.9	401.3	406.6	435.7	450.6	387.3	393.0	274.2
Si	0.6	0.4	2.5	1.8	3.2	1.5	2.9	0.1	2.1
Sr	0.46	0.46	1.08	1.05	1.29	1.34	1.22	1.20	0.95
Cl^-	4.38	3.89	10.15	9.64	12.69	13.36	12.13	11.67	9.60
SO_4^{2-}	198.75	218.33	1315.95	1293.80	1411.94	1418.60	1234.65	1223.80	869.94

1.3.2a Effect of Rock on Parameter Concentrations

Over the course of the 139 day column experiment, the water quality parameters in the two columns containing only water and under no influence from water-rock interactions (Column 1 containing Caland 2 m water; Column 2 containing Hogarth 2 m water) showed very little change. The variance for all measured parameters was less than 5% with the following exceptions: sodium decreased 6.3% in column 1; chloride decreased 11.1% in column 1; and sulfate increased 9.9% in column 1. The other columns also showed little variation in many of the measured parameters.

pH

The pH values showed less than a 5% change from 1 hour values to the final values taken on day 139 in all ten columns. When the pH values from one hour are compared to the final values taken on day 139 the overall change is very small, with the greatest change seen in column 5 (50% Hogarth 30 m water + 50% Caland 2 m water + Hogarth MFV rock) in which the final pH was 0.4 units greater than the starting values. However, over the course of the 139 days, the pH values in the columns containing rock (3-10) did change in such a way that they lend insight into the geochemical reactions that occurred. In all of the columns under geological influence, there was an initial drop in pH that is not seen in those containing only water (1 and 2). Then the pH appears to equilibrate until there is a slight increase near the end of the experiment (Fig 1.2).

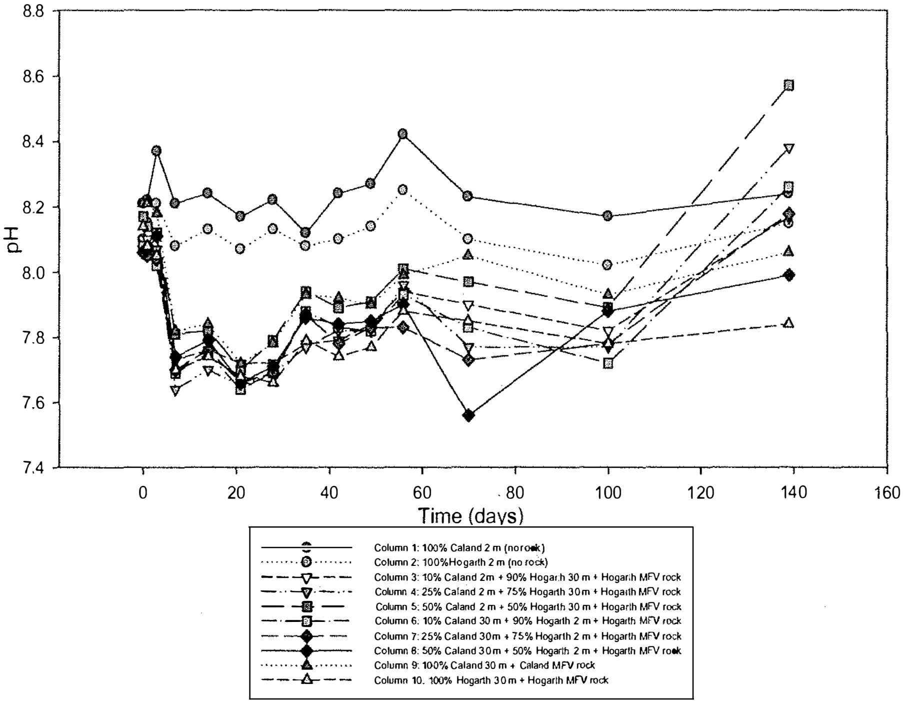

Figure 1.2: The pH levels in each column over time. The pH of the columns containing rock all showed an initial decrease while those with only water (columns 1 and 2) showed little change with time. Note the initial drop is not more than 0.5 pH units.

Major Cations

Final calcium concentrations changed less than 5% from initial values in all columns except for an 8.8% decrease in column 4 (75% Hogarth 30 m water + 25% Caland 2 m water + Hogarth MFV rock) and a 11.4% decrease in column 5 (50% Hogarth 30 m water + 50% Caland 2 m water + Hogarth MFV rock).

Magnesium showed less than a 5% change from 1 hour values to the final values taken on day 139 in all ten columns (Fig 1.3). Sodium showed less than a 10% change (Fig 1.4).

Potassium concentrations showed the greatest change over the 139 day experiment. The values in columns 1 and 2 containing only water changed less than 3% over the course of the experiment. The potassium concentrations increased in all of the other columns (3-10) which all contained rock. The average increase in potassium was 3.3 mg/L while the greatest increase of 3.9 mg/L occurred in column 10 (Hogarth 30 m water + Hogarth MFV rock). These increases are shown in Figure 1.5.

Sulfate and Total Sulfur

Sulfate concentrations increased in all columns containing rock except column 4 which showed a decrease of 0.9% representing a drop in concentration of 10.85 mg/L. The greatest increases in sulfate concentration were seen in columns 8, 9, and 10. Column 8 (50% Caland 30 m + 50% Hogarth 2 m + Hogarth MFV rock) increased by 79.96 mg/L (+11.1%), column 9 (Caland 30 m + Caland MFV rock) increased by 26.33 mg/L (+10.0%), and column 10 (Hogarth 30 m water + Hogarth MFV rock) had the greatest total increase of 98.67 mg/L representing a relative increase of 6.8%. However, sulfur changed less than 5% in all columns other than column 9 which increased by 5.8% (Fig 1.6).

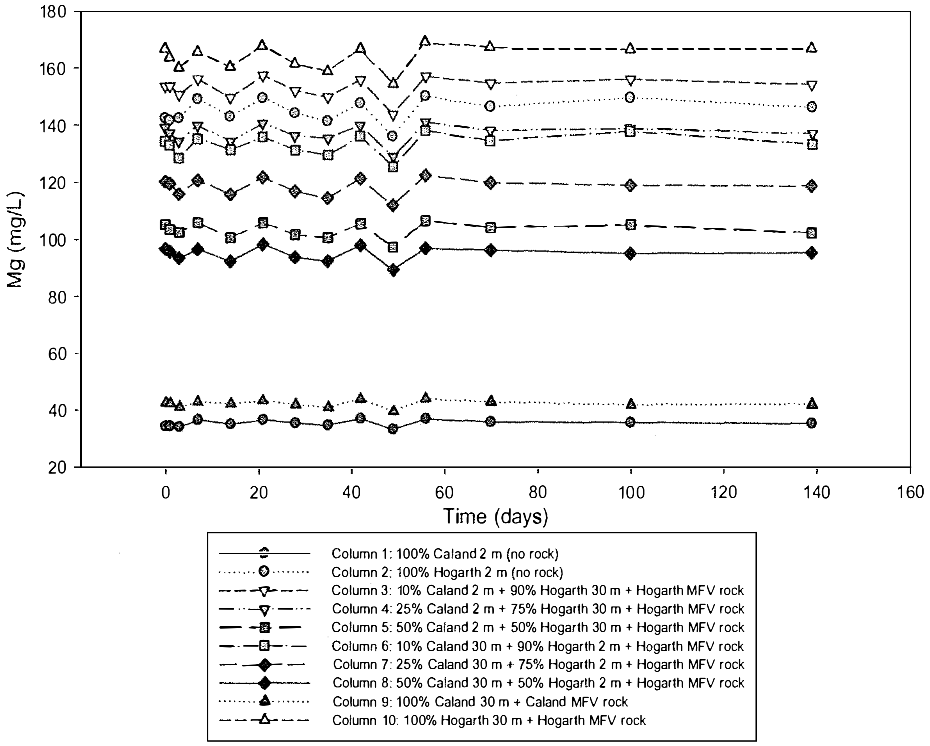

Figure 1.3: Magnesium concentrations in each column over time.

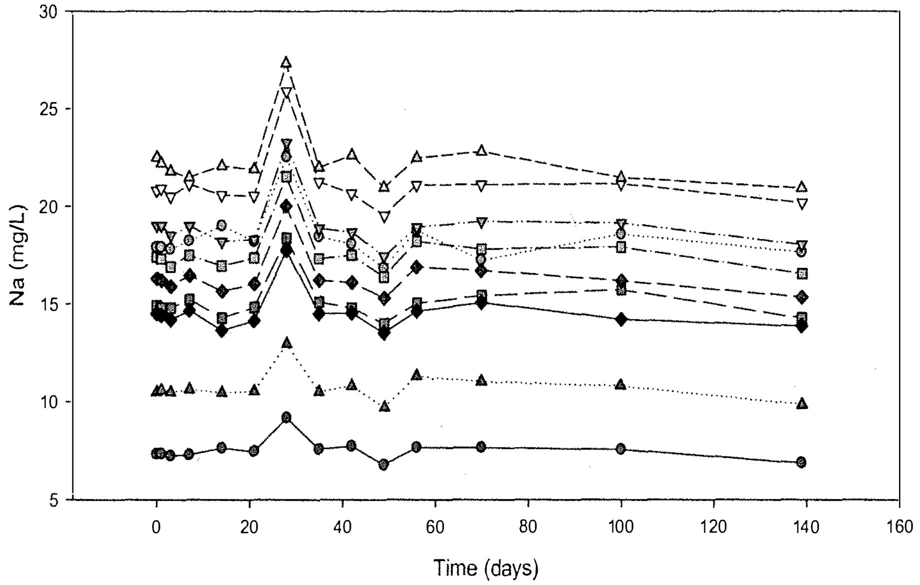

Figure 1.4: Sodium concentrations in each column over time.

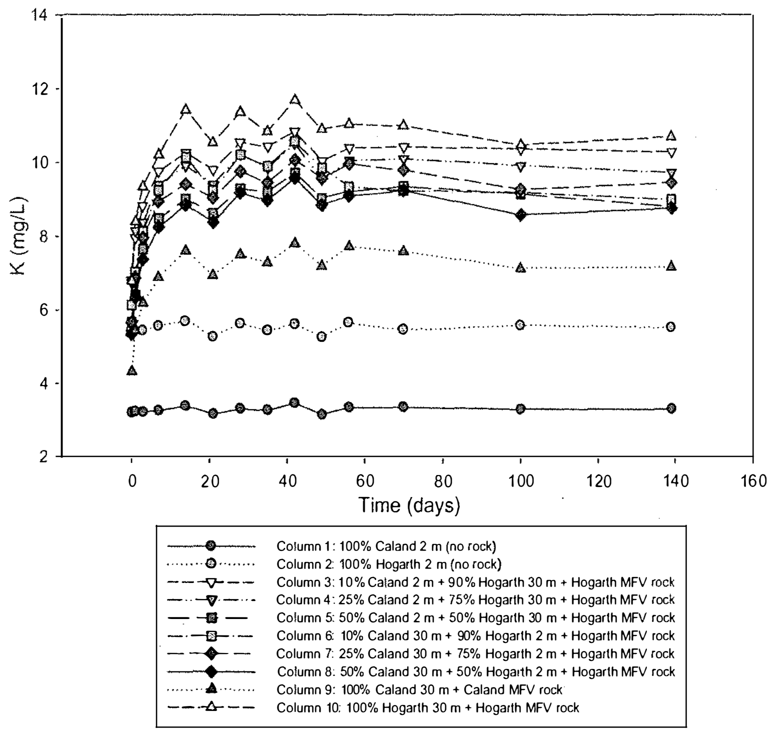

Figure 1.5: The potassium concentration in each column over time. Potassium levels in the columns containing rock all show an initial increase in potassium levels followed by a state of equilibrium.

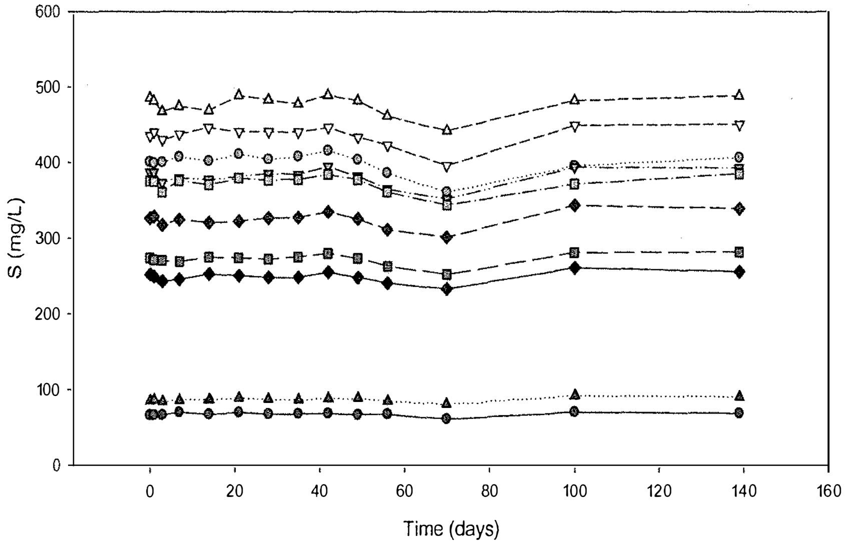

Figure 1.6: Sulfur concentration in each column over time.

Trace Metals

Similar to columns 1 and 2 containing only water, the columns containing rock showed little variation in trace metal concentrations. Most trace metals were below their respective detection limits for the analysis methods used throughout the experiment (Table i.iii).

Other Significant Changes

A notable change was seen in the concentrations of barium. Actual barium concentrations remained fairly low in all columns. However, they did show an average increase of 0.010 mg/L in all columns containing rock (3-10) with the greatest increase of 0.0161 (+203.8%) observed in column 10 (Hogarth 30 m water + Hogarth MFV rock). This is similar to the increase in potassium concentrations. These trends are presented in Figure 1.7. Barium concentrations show a decrease on day 70 from day 56 values in a similar magnitude in all ten columns. As such, this drop is likely due to an analytical error.

Boron concentrations remained low in all columns. They showed an average increase of 0.04 mg/L in columns 3-10 with a maximum increase in concentration of 0.07 mg/L seen in both column 9 (Caland 30 m water + Caland MFV rock) and column 10 (Hogarth 30 m water + Hogarth MFV rock). Although these concentrations are low, they represent a 103.3% and a 113.1% increase respectively.

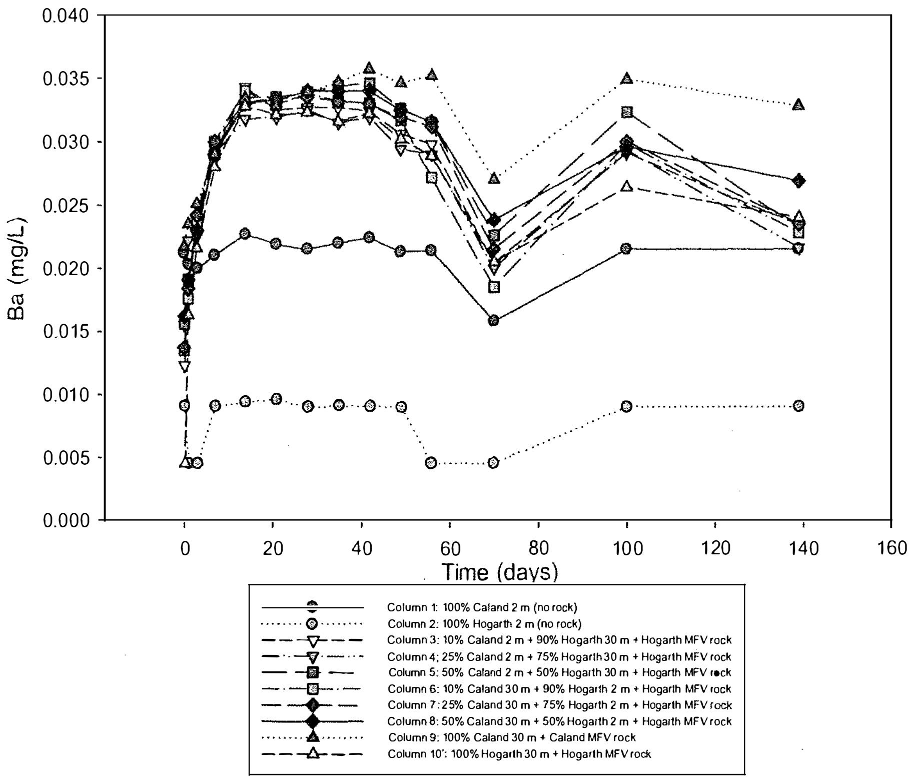

Figure 1.7: The barium concentration in each column over time. Barium levels in the columns containing rock appear slightly higher than those with only pit lake water. However, the magnitude of the increase is quite small.

1.3.2b Patterns of Parameter Fluctuations

Cluster analysis revealed that there were five main patterns of concentration fluctuations in the column parameters that could be divided into five clusters (Fig 1.8) The first cluster included the pH values from all ten columns. This cluster was subdivided into two smaller groups that distinguished the pH values from columns 1 and 2 from the other columns. Columns 1 and 2 contained only water and no rock and showed little change in pH during the experiment. The pH values in column 3-10 containing rock all showed an initial drop followed by a period where buffering occurred to stabilize the pH values (Fig 1.2). The same overall trend occurred in each column and was unique to the pH values compared to all other water quality parameters analyzed.

The next cluster grouped the sulfur values from all ten columns with the barium concentrations from columns 1 and 2 containing only water and no rock (Fig 1.6). Sulfur values in all ten columns changed very little over the course of the 139-day experiment, as did the barium concentrations in columns 1 and 2.

The third cluster included just the magnesium values from all ten columns (Fig 1.3). These values showed slight variations throughout the experiment but the overall change in concentration was less than 3% in all columns. Although the magnesium values changed very little overall like sulfur (columns 1-10) and barium (columns 1 and 2), there was more variation in magnesium concentration seen throughout the experiment, causing the magnesium values to be distinguished from these sulfur and barium values.

The fourth cluster included just the sodium values from all ten columns (Fig 1.4). Sodium values were the only concentrations to decrease in all ten columns during the experiment. The drop was not very large with the greatest decrease of 1.57 mg/L representing a 7% drop seen in column 10 (Hogarth 30 m water + Hogarth MFV rock). However, the decrease did occur consistently in all ten columns unlike other parameters such as calcium which decreased in some columns but increased in others.

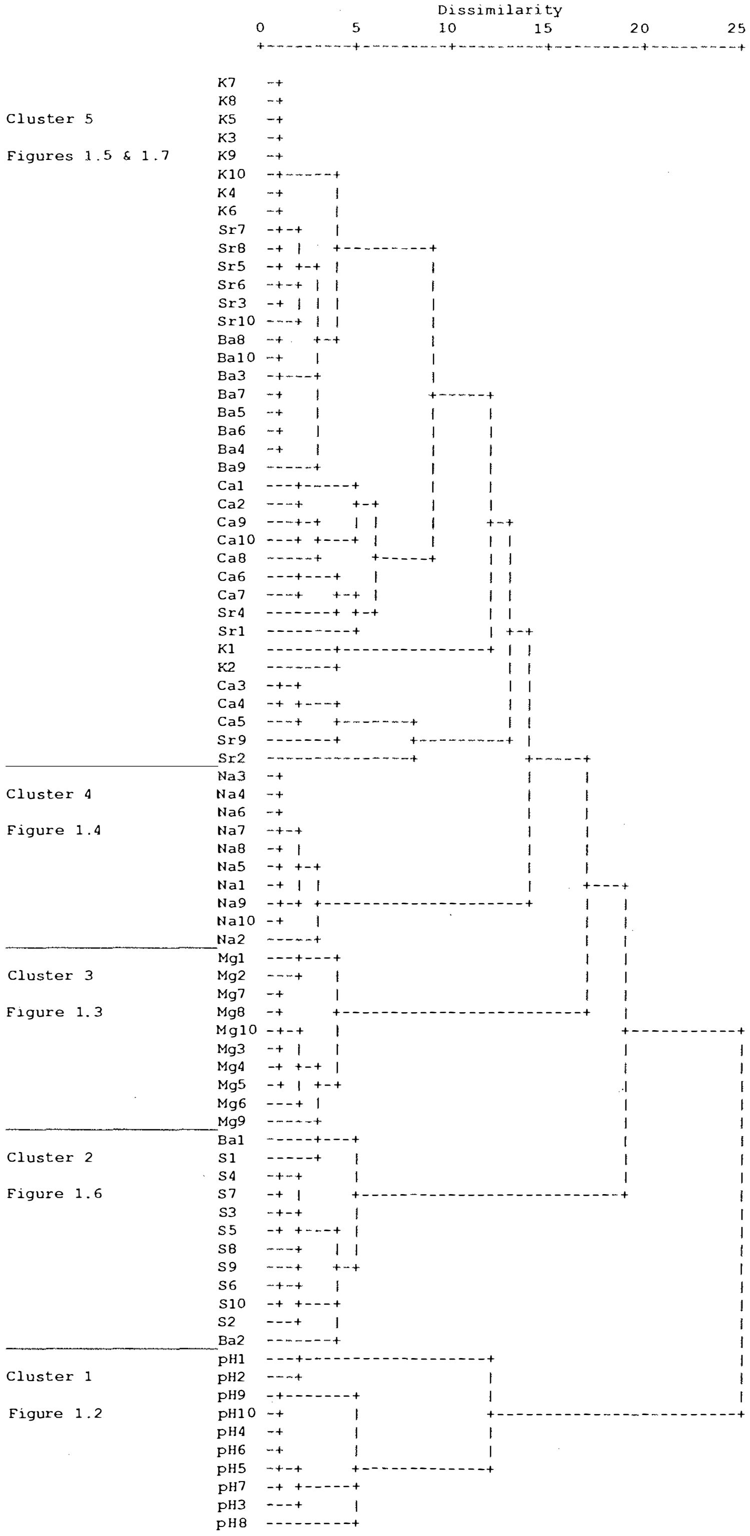

Figure 1.8: Dendrogram showing the results of hierarchical cluster analysis done on final water quality values from the column experiment. The parameters are labeled according to which column they correspond to (1-10).

The final cluster contains the most complex grouping of parameters. It includes the potassium (Fig.1.5), strontium, and calcium values from all ten columns as well as the barium values from columns 3-10. The potassium values from columns 1 and 2 were distinguished from the potassium values from the other columns (3-10). The potassium concentrations in these two columns which contained only water varied little throughout the experiment. The potassium concentrations showed an overall increase in the other columns (3-10) which all contained rock with a maximum increase of 3.8 mg/L (67%) in column 7 (75% Hogarth 2 m water + 25% Caland 30 m water + Hogarth MFV rock). During the course of the experiment, the greatest concentration increases were seen in potassium, barium, and strontium and so it is expected that they would be clustered together. Similarly, calcium showed an initial increase in most columns, but concentration slowly decreased during the experiment and by the 139 day sampling, calcium concentrations were lower than starting concentrations in several columns (3, 4, and 5). The other columns showed little overall change in calcium concentrations.

1.3.3 Column 8 Refill Results

Although the rock in the column had been previously reacted with water from the two pit lakes, with the addition of a new batch of water to the column, similar changes in the measured parameters were seen indicating that similar reactions must be occurring. These results are presented in Table 1.6 with the comparable data from the column experiment. The percent change in each parameter from one hour values to 100 day values for the column experiment data and to 90 days for the post refill data are also presented for comparison.

Table 1.6: The original (O) and post refill (R) values from column 8 at one hour and 100 days (original) and 90 days (post refill) for comparison. The original data is from the column experiment data set in Appendix A.

	O - 1 hour	R - 1 hour	O - 100 days	R - 90 days	O - % change	R - % change
pH	8.06	7.88*	7.88	7.83	-2.23	-0.63
Al	<DL	<DL	<DL	<DL	N/A	N/A
B	0.07	<DL	0.11	<DL	68.36	N/A
Ba	0.016	0.015	0.030	0.018	82.716	21.053
Ca	174.30	181.03	176.24	162.32	1.11	-10.34
Cu	<DL	<DL	<DL	0.005	N/A	100
Fe	0.01	<DL	<DL	0.40	-100	100
K	5.3	4.8	8.6	6.6	60.9	37.5
Mg	96.816	101.990	95.076	94.095	-1.797	-7.741
Mn	0.011	<DL	<DL	<DL	-100	N/A
Na	14.50	14.33	14.21	14.86	-2.01	3.70
Ni	<DL	<DL	<DL	0.02	N/A	100
S	252.4	242.3	260.8	272.2	3.3	12.3
Si	2.3	1.6	2.4	0.7	5.4	-56.3
Sr	0.89	0.88	0.99	0.82	10.92	-6.03
Cl	8.21	8.17	10.59	8.30	28.99	1.59
SO4	718.6	802.4	806.6	767.0	12.2	-4.4

*Value from 14 days post refill; no 1 hour measurement taken

1.3.4 Batch Mixes Compared to the Column Results

In order to compare the results of the column experiments to the initial and final batch water mixes, the results of each parameter measured for the Hogarth 30 m and Caland 2 m mixing series were plotted (Fig. 1.9). In increasing proportion of Hogarth 30 m water, this mixing series was represented by columns 1, 5, 4, 3, 10. The final values from these columns were, therefore, included in the figure to show changes that may have occurred in the columns due to the geological influence of the various rock types in the columns.

For many parameters (calcium, magnesium, sodium, sulfur, sulfate, and chlorine) the linear relationship between concentration and proportion of water from the Hogarth pit lake was maintained during the column experiment. This concentration fluctuation pattern is illustrated by the graph of the magnesium concentrations in Figure 1.9a. Column calcium concentrations were slightly lower than in the water mixes. As seen in Figure 1.9a the column magnesium concentrations were comparable to those in the batch water mixes. Column sodium concentrations were slightly lower than in the batch water mixes (Fig. 1.9c). Both sulfur and sulfate column concentrations were almost identical to the concentrations in the batch mixes. Column chlorine concentrations were slightly greater than in the water mixes beyond the 50% Hogarth water mix. With less than 50% Hogarth water, the concentrations are highly comparable. Potassium concentrations (Fig. 1.9b) were elevated in the columns compared to the batch water mixes. Column pH values (Fig. 1.9d) did not follow the linear trend shown by the water mixes.

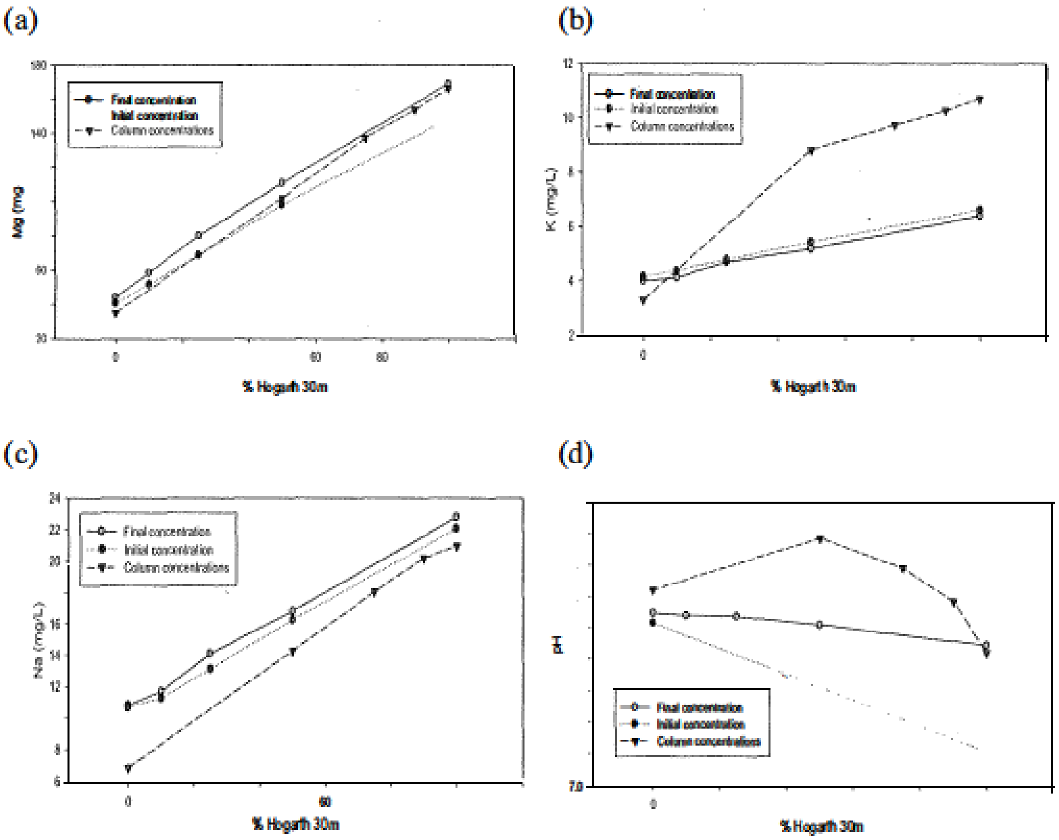

Figure 1.9: Water quality values of the initial and final water mixes and the final column data.

1.4 Discussion

1.4.1 Density and Mixing

The hydrodynamic behaviour of the two lakes at the time of joining is unknown. Based on the current water density measurements if mixing does occur in the future, the density stratification will not be an issue. Mixing experiments show that water from each layer of Caland readily mixes with water from each layer of Hogarth with slight mechanical mixing under laboratory conditions. There is very little resistance to mixing due to density differences and, therefore, fall and spring overturn of the joined pit lake will very likely occur. Thus, as Caland flows into Hogarth, their waters will likely mix and the resultant water chemistry will be a mixture of that of the two original pit lakes. This is shown by the water mixing series and the column experiment.

1.4.2 Batch Mixing Experiment

The initial mixes were carried out to evaluate any reactions that could occur due to simple mixing of the water from the two pits, Hogarth and Caland, under the present conditions. Given that many constituents are present in such high concentrations (SO_4^{2-}, Ca^{2+}, Mg^{2+}) there was the possibility of causing precipitation reactions that would remove certain elements from solution. However, the results of the simple mixing experiments show that for all parameters the relationship between the proportion of Hogarth water and the parameter concentration was linear (Fig. 1.9 & Table 1.4). When the initial mixes were compared to those that were stored in brown Nalgene bottles for 18 months, there do not appear to be any major changes in any of the parameters over time. In most cases the linear relationship between concentration and proportion of Hogarth water was preserved.

1.4.3 Column Experiment

When the initial and final mixes were compared there were no major apparent changes in the water quality over time (Fig. 1.9). As such, any deviation from this relationship in any of the columns could be attributed to the geological influences. Over the course of the column experiment very few changes in the measured parameters were observed. The influence of the rock in eight of the ten columns affected only a few parameters. When the final values are compared to the starting values in terms of absolute concentration, potassium, sulfur, and sulfate showed the greatest increases during the experiment. In terms of the percent increase (compared to starting values) barium, boron, potassium, strontium, and sulfate showed the greatest increases.

Although the overall change in pH from one hour until day 139 is very little in all columns, the pattern of pH change over the course of the experiment showed that considerable changes occurred. In the columns containing rock (3-10) there was an initial decrease in pH, which was not observed in columns 1 or 2 (water only). This initial decrease occurred over the same interval that the initial increase in potassium occurred. The pH values remained fairly constant until day 28 when the values began to increase to day 35 levels. pH values again remained relatively constant until a slight increase in values was seen from the values measured on day 100 to those measured on day 139.

By examining the parameters that changed during the experiment, the three main reactions likely taking place simultaneously in the columns are pyrite oxidation, weathering of potassium feldspar, and carbonate dissolution and precipitation.

Oxidation reactions of metal sulfide minerals such as pyrite are usually acid-generating processes. When pyrite is oxidized, sulfate is created in the process causing sulfate concentrations to rise. There was an increase in sulfate in all of the columns containing rock during the experiment except in column 4 which showed a decrease in sulfate of less than 1%. The oxidation of pyrite is summarized by the following reaction:

$$4FeS_{2\ \text{pyrite}} + 14H_2O + 15O_2 \rightarrow 4Fe(OH)_{3\ (aq)} + 8SO_4^{2-} + 16H^+$$

Most sulfide mineral oxidation reactions occur in the surface weathering environment since most groundwater supplies contain little oxygen, which is necessary to the process (Eby, 2004).

Although acid was generated through the oxidation of pyrite, the pH of the columns remained near-neutral. The initial drop in pH seen in columns 3-10 (Fig. 1.3) containing rock was likely caused by the acid generated through the oxidation of pyrite in the columns. However, the final pH values of the columns indicated that the acidic products of pyrite oxidation were neutralized. Neutralization of acid produced by pyrite oxidation can be accomplished through weathering of alkali feldspar and/or carbonate dissolution.

When alkali feldspar ($(K,Na)AlSi_3O_8$) undergoes weathering the following reaction occurs to produce the kaolinite (aluminum silicate hydroxide), silicon dioxide (which is dissolved reactive silica), and potassium ions:

$$2KAlSi_3O_8 + 2H^+ + H_2O \rightarrow Al_2Si_2O_5(OH)_4 + 4SiO_2 + 2K^+$$

This reaction could account for the increase in potassium concentration seen in all columns containing rock from the two pits since alkali feldspar is one of the minerals present in the Marmion complex as well as the Witch Bay formation (Conly, pers. comm., 2009). Barium and strontium make up minor components of alkali feldspars. Thus, when feldspar weathering occurs, it releases these elements causing simultaneous increases in potassium, barium, and strontium.

Carbonate minerals also act to buffer the addition of acid mine drainage according to the following reaction:

$$CaCO_{3(s)} + H^+ \rightarrow HCO_3^- + Ca^{2+}$$

The carbonate involved can be either calcite or dolomite or a combination of the two (Eby, 2004). As the reaction proceeds, calcium concentrations would increase in solution. However, in many columns the final calcium concentrations were actually lower than starting values. Some other process must have forced this reaction to proceed to the left causing carbonate precipitation to occur, which would lower calcium concentrations. Also, if calcium concentrations reached an equilibrium state in the column water, carbonate dissolution reactions would decline. If a source of bicarbonate was present in solution, this reaction could proceed to the left, causing carbonate to precipitate, thus lowering calcium and magnesium concentrations in solution.

Dissolved carbon dioxide is usually in equilibrium with carbon dioxide in the atmosphere. When dissolved carbon dioxide reacts with water, bicarbonate and hydrogen ions are produced in the following acid-generating reaction:

$$H_2O + CO_{2\,(g)} \leftrightarrow H_2CO_3 \leftrightarrow HCO_3^- + H^+$$

In the column experiment, the bicarbonate that formed in this reaction allowed the carbonate precipitation reaction to proceed. The acid that was generated through carbonate precipitation and the reaction of carbon dioxide with water allowed the feldspar weathering reaction to proceed, which in turn increased potassium, barium, and strontium concentrations in solution.

Three acid-generating reactions were occurring simultaneously in the columns: reaction of carbon dioxide with water, pyrite oxidation, and carbonate mineral precipitation. The reaction of carbon dioxide with water allowed carbonate precipitation to proceed by producing bicarbonate ions. Although acid was generated through these three processes which caused an initial drop in pH in the columns containing rock, the additional acid was buffered and pH values increased as equilibrium was reached. The additional acid allowed alkali feldspar weathering reactions to proceed, which in turn buffered the pH in the columns. Potassium, barium, and strontium concentrations increased due to alkali feldspar weathering. The major source of pH buffering in the experiment was the uptake of hydrogen ions during alkali feldspar weathering.

The increase in pH from day 100 to day 139 did not coincide with any notable changes in any of the other parameters and thus the cause of this pH increase cannot be determined through the chemical data. Further study would be needed to determine the cause of the final pH increase seen in some columns.

When the results of the column 8 refill experiment were compared to the data from the original column experiment (Table 1.5), similar trends were observed in both data sets. For the duration of the experiment the pH drop was not as great (less than 1%) indicating that pyrite oxidation is occurring to a much lesser degree in the refill water.

The pyrite source may have been exhausted in the first half of the experiment since sulfate levels show less than 5% variance in the refill waters. This amount of variation in sulfate levels was seen in water samples left in storage over time and thus this change may not be due to any reaction. Similar to the first part of the experiment, calcium and magnesium concentrations decrease in the refill water indicating that they are likely precipitating out of solution as carbonate minerals. Potassium and barium concentrations increased slightly whereas strontium decreased slightly. The increases in potassium and barium suggest that feldspar weathering continued in the second half of the experiment and that the alkali feldspar source was not exhausted in the first half. Although potassium and barium concentrations reached equilibrium in the first part of the experiment, when a new batch of water was added, the new water was no longer in equilibrium with the mineral and so weathering of alkali feldspar could continue. The strontium concentrations decreased perhaps because the strontium sources within the feldspar rock were depleted during the first part of the experiment.

These reactions are the most likely ones occurring under the conditions of the column experiment based on the changes seen in the water quality parameters over the 139 days. To be more certain of the reactions involved, further research would be needed. These could include X-ray diffraction and SEM-EDS (Scanning Electron Microscopy-Energy Dispersive Spectroscopy) analysis of the various mineral types at the Steep rock site and a much longer mineral weathering experiment. Computer-based modeling programs such as AquaChem or phreeqC could be used to predict further changes based on the input of water quality data.

Some water quality parameters showed changes over the course of the column experiment which are not observed in the trends from seasonal sampling (Fig. i.ii). Seasonal trends and stable isotope analyses (Goold, 2008) indicate that to date the two primary reactions influencing water chemistry have been pyrite oxidation and carbonate dissolution (Conly, pers. comm.). However, exposure to potassium-bearing rocks will only increase as the pits continue to fill, providing the conditions necessary for alkali feldspar weathering reactions similar to what was observed in the column experiment. This shows the need for continued monitoring of the two pit lakes. The water quality of the two pit lakes is undergoing continuous changes that may not be revealed by a short-term geochemical modeling experiment. More prolonged experiments are likely to give more accurate insight into the future water quality of the two lakes as they fill and merge.

This experiment determined the geochemical effect of the wall rock of each pit and did not account for other major influences on water quality including but not limited to the influence of the various acidic and basic inflows to both pits; the effect of inundation of sulfide tailings and waste rock piles that would produce acid mine drainage; the addition of organic matter as the surrounding trees, grasses, and plants are flooded; as well as other unknown influences that may be present or develop over time as a result of climate change. Another consideration is that the small grain size of rock used in the column experiments may be an exaggeration of the surface area available for weathering reactions of the wall rocks. In fact, the small grain size, in particular fine dust produced during crushing and sieving, may explain the some of the rapid changes observed early in the column experiment. The permeability of the wall rock may be lower than what is represented by the grain size used in the experiment. Thus, chemical changes in the actual pit lakes would either be smaller or occur at a much slower rate than what occurred in the experiment.

Finally, the experiment conducted in this chapter showed that increases in certain chemical parameters will occur which may affect aquatic life in the pit lakes. The effects of these elevated parameters on the survival and growth of an aquatic macrophyte is further examined in Chapter 2.

Chapter 2: *Lemna minor* as a Toxicity Testing Tool of Future Pit Lake Water Quality

2.1 Introduction

Aquatic and terrestrial macrophytes are fundamental elements of all ecosystems. Most life forms depend heavily on the oxygen and organic substances produced by plants. Any change in plant function or habitat in an ecosystem will likely have a severe impact on the structure and function of the entire system. As such they are routinely used as a critical portion of many environmental monitoring and assessment programs (Wang, 1991). Macrophytes are becoming more vital in the testing of many substances believed to be harmful to either human health or the environment including herbicides, mining and mill effluents, and other dangerous chemicals. This includes both the use of plants in laboratory tests as well as their use as indicators during field monitoring programs. Phytotoxicity tests are in fact required by many regulatory agencies and industries in the United States, Canada, and international organizations (Wang, 1991).

Phytotoxicity testing is also a very useful practice in the environmental assessment of effluents from mining or mill waste. Many of the various components of these effluents found to be nontoxic to fish and invertebrates could harm or eradicate aquatic plants upon discharge into the environment. Given many plants' capacity for bioaccumulation of certain substances, components with non-lethal effects could ultimately adversely affect food production as well as impact organisms higher up the food chain (Wang, 1995).

Sensitivity varies widely between toxicants, among taxonomic groups and even within taxa (Wang, 1995). Fenske *et al.* (2006) compared the sensitivity to various heavy metals of seven different biological test methods using bacteria, human cells, protozoa, nematodes, aquatic plants (*Lemna minor*), and fishes and found the test with *L. minor* to be the most sensitive with copper producing the strongest effects. *L. minor* reacted to the lowest concentration of toxicants in the least amount of time.

The family *Lamnaceae* has about 40 species in four genera. Commonly referred to as duckweed, the many species are groups of widespread, fast growing, free floating aquatic angiosperms (Wang, 1991). Because of these growth characteristics, *Lemna* species are commonly used in various toxicity tests involving aquatic pollutants with *L. minor* and *L. gibba* being most frequently used. One such test is the test for measuring the inhibition of growth using the freshwater macrophyte, *Lemna minor* developed by Environment Canada (2007).

Toxicity experiments completed by Goold (2008) found that the water in the Hogarth pit lake caused toxic responses in *Ceriodaphnia dubia.* The high levels of dissolved ions released through weathering reactions involving the various rock units lining the pits was suggested as the most likely cause of such a response. As filling of the pit lakes continues, the water in the two pits will come into contact with different rock types in varying ratios, the result of which could be further mineral weathering which could cause a change in certain physical and chemical parameters in the water of each pit lake.

The purpose of this experiment was to assess whether the future predicted water quality of the two pit lakes that was determined from the column experiment described in the previous chapter would pose ecological threats to aquatic life. The test organism used was the freshwater macrophyte *Lemna minor.* The hypothesis was that the future water quality of the two pit lakes would continue to severely impact the growth of aquatic macrophytes due to the high concentrations of dissolved ions (Ca^{2+}, Mg^{2+}, and SO_4^{2-}) in the water column.

2.2 Methods

2.2.1 Toxicity Testing using *Lemna minor*

Studies by Vancook (2005) and Jackson (2007) predict that as the two pit lakes fill, Caland will flood the southwestern portion of the pit and will eventually begin flowing into the Hogarth pit. Thus, during the initial stages of pit joining there will be small volumes of Caland water flowing into the Hogarth pit. As well, as Caland fills, the greater surface area of the upper portion of the lake will reduce the freshwater lens to less than its present 25 m depth. The exact depth that the lens will reach at the mixing point is unknown; however, both the freshwater lens and the monimolimnion were included in the present experiments.

Because the water quality of the freshwater lens varies greatly from that of the anoxic monimolimnion, it was necessary to explore the effects of mixing of both layers from Caland with water from the Hogarth pit lake. Since the concentration of most water quality parameters increase with depth in both lakes, water from both the epilimnion and the hypolimnion of Hogarth were used in the modeling experiments. For consistency, water used in the experiments was collected from depths of 2 m and 30 m from each lake. This provided a sample from both the freshwater lens and the anoxic monimolimnion of Caland as well as the epilimnion and hypolimnion of Hogarth.

All of the toxicity tests that were conducted followed the procedure found in the L*emna minor* growth inhibition test developed by Environment Canada (2007) and the results were analyzed for IC25 using ToxCalc v5.0. To model the mixing of the two pits, a mixing series of water from each pit and from two depths (2m and 30m) in each was used in the toxicity tests. Normally, when following the method developed by Environment Canada (2007) a sample of an effluent or discharge water (the toxicant) is serially diluted using distilled deionized water (DDW) to determine an IC25 value from the toxicity test. An IC25 (inhibition concentration 25) is the concentration of the toxicant that causes a twenty five percent reduction in growth relative to that of the control treatment.

In this experiment, water from Hogarth (the toxicant) was serially diluted with water from Caland instead of DDW to mimic the actual predicted mixing of the two pit lakes. Although the method followed the standard toxicity test developed by Environment Canada (2007), the substitution of water from Caland instead of DDW resulted in a non-standard procedure being conducted. Because of this the results could not be analyzed properly with the standard analysis method of using ToxCalc software to determine the IC25 values. This is explained in section 2.4.1.

To remove any algae in the samples, the water was filtered using a glass fibre filter with pore size of ~ 1μm and a negative pressure pump system. A combination of three nutrient stock solutions was added to each sample according to Environment Canada (2007). In order to equilibrate the samples with the added nutrients and to stabilize the sample pH, oil-free compressed air was dispensed through each sample through disposable pipette tips on plastic tubing for thirty minutes. The pH values were then measured and were corrected using sodium hydroxide (NaOH) or hydrochloric acid (HCl) if the pH values did not fall in the range 6.5 to 9.5 (Env. Can., 2007). These filtered, nutrient-spiked, pH-corrected samples were then used in a series of toxicity tests using *Lemna minor* at the Lakehead University Toxicity Investigation and Evaluation (TIE) Laboratory.

New cultures of *Lemna minor* strain CPCC 490 were obtained from the Canadian Phycological Culturing Centre at the University of Waterloo. These plants were cultured according to the methods in Environment Canada (2007) for three weeks before a one week health test was performed to ensure the viability of the plants. The plants passed the health test with an average 10.1-fold increase in frond number over the 7 day period. A minimum 8-fold increase is required to pass the health test.

Two types of toxicity tests were conducted: a test with a simple water mixing series and a test using the resultant water from the column experiment described in detail in the previous chapter.

2.2.1a Toxicity Tests on Water Mixing Series

Tests were conducted using water sampled from the two lakes and mixed in varying proportions to create a dilution series of Hogarth water with water from Caland. Since toxicity was found to vary by season (Goold, 2008), this test was carried out using water sampled in winter 2007, summer 2007, and fall 2007. The winter and summer tests used the mixing ratios in Table 2.1. As well, with the summer 2007 tests, the following two additional mixing series were tested using the same ratios as in Table 2.1: Hogarth 2 m x Caland 30 m; Hogarth 30 m x Caland 2 m. When the toxicity tests using the fall 2007 water were conducted the mixing ratios were changed to coincide to those used in the column experiment from the previous chapter (Table 2.2). This would provide a baseline toxicity response to which the results of the column toxicity tests could be compared.

Table 2.1: The mixing ratios of water from Hogarth and Caland, at depths of 2 m and 30 m used in toxicity tests using *Lemna minor* in water sampled during winter 2007 and summer 2007. There were two additional mixing series tested with the summer 2007 samples with the same ratios: Hogarth 2 m x Caland 30 m; Hogarth 30 m x Caland 2 m.

Hogarth 2 m : Caland 2 m	Hogarth 30 m : Caland 30 m
1:0	1:0
3:1	3:1
1:1	1:1
1:3	1:3
0:1	0:1

Table 2.2: The mixing ratios of water from Hogarth and Caland, at depths of 2 m and 30 m used in toxicity tests using *Lemna minor* in water sampled during fall 2007. These coincide with the ratios used in the column experiment from chapter 1, so that the results of the fall 2007 toxicity tests could be directly compared to the results of the column water toxicity tests.

Hogarth 2 m : Caland 30 m	Hogarth 30 m : Caland 2 m
1:0	1:0
9:1	9:1
3:1	3:1
1:1	1:1
0:1	0:1

After one week of incubation, all fronds in each replication were counted regardless of colour or size to determine the total number of fronds. General qualitative observations about the overall appearance and growth of the fronds were recorded. For the tests done on the water sampled during fall 2007 the total number of fronds as well as the number of fronds experiencing chlorosis and necrosis were counted. Additionally, more detailed observations regarding the appearance of the fronds were made including relative frond sizes in each set of replicates, colony destruction, and abnormal growth patterns. For all of the tests IC25 values were determined based on total frond numbers using ToxCalc v5.0.

2.2.1b Toxicity Tests on Resultant Water from the Column Experiment

The column toxicity tests used the resultant water from the column experiment which represented a dilution series similar to those used in the water-only toxicity tests.

As described in the first chapter, a column experiment was conducted to empirically predict the future water quality of the Hogarth and Caland pit lakes as they fill, merge and reach the former Steep Rock lake level. They may outflow into the Seine river system if they overtop the pits. Columns were set up containing varying ratios of water from a depth of 2 m and 30 m from each of the two pit lakes. Rock was added to most of the columns in relative proportion to those types lining the Hogarth pit lake (Table 2.3) to determine the geological influence that contact with the pit walls will have on water quality as the lakes fill and merge. Rock in relative proportion to those lining the Caland pit were added to a column with Caland 30 m water to predict the future water quality of the Caland pit lake. The contents of each of the ten columns used in the experiment are presented in Table 2.4. The water in the columns was sampled at regular intervals over the course of the 139-day experiment.

Upon completion of the column experiment, the resultant water was used in the column water toxicity tests using the freshwater macrophyte *Lemna minor* that modeled the initial stages of mixing between the Hogarth and Caland pit lakes. The water from the column experiment was stored for several months before it was used in these toxicity tests. The water chemistry was analyzed just prior to the toxicity experiments. This water quality data representing the starting concentrations in the column water toxicity tests is presented in Table 2.5.

Table 2.3: The amount of each rock type (m^2) for each pit once they reach their maximum fill volumes (MFV) and the amount in the combined pit.

Rock Type	Caland at MFV	Hogarth at MFV	Combined at MFV
Marmion Complex	2705760	669600	3350880
Mosher Carbonate (Dolomite)	1363700	794880	2158580
Magniferous paint rock	not sampled - none present in pit walls		
Geothite (Hematite)	144090	214560	372330
Pyritic iron formation	36000	53280	90720
Dismal Ashrock Formation	705600	604800	1310400
Witch Bay Formation	2554560	1118880	3673440
Mafic intrusive	158400	4320	174240
Late mafic intrusive	970560	115200	1032480

Table 2.4: The volume and depth of water from each lake in each column and the columns to which a sleeve of rock was added. Rock added corresponds to the amount of rock lining the Hogarth pit at MFV as determined in Table 2.3 except in column 9*.

Column	Caland	Hogarth	Caland : Hogarth	Rock added
1	2m – 4L	N/A	1:0	none
2	N/A	2m – 4L	0:1	none
3	2m – 0.4L	30m – 3.6L	1:9	yes
4	2m – 1L	30m – 3L	1:3	yes
5	2m – 2L	30ml – 2L	1:1	yes
6	30m – 0.4L	2m – 3.6L	1:9	yes
7	30m – 1L	2m – 3L	1:3	yes
8	30m – 2L	2m – 2L	1:1	yes
9	30m – 4L	N/A	1:0	yes*
10	N/A	30m – 4L	0:1	yes

*Rock added corresponds to the types and proportions lining the Caland pit (Table 2.3).

Table 2.5: Starting water quality parameter values for the column water toxicity tests using *Lemna minor*. All concentrations are in mg/L except pH (pH units).

Column	1	2	3	4	5	6	7	8	9	10
pH	8.25	8.03	7.85	7.70	7.65	7.78	7.87	8.08	8.17	7.99
Al	<DL	<DL	<DL	<DL	<DL	<DL	0.03	<DL	<DL	<DL
B	0.04	0.04	0.12	0.11	0.11	0.10	0.10	0.11	0.14	0.13
Ba	0.022	0.009	0.025	0.023	0.026	0.024	0.039	0.034	0.039	0.028
Be	<DL	<DL	<DL	<DL	<DL	<DL	0.012	<DL	<DL	<DL
Ca	73.63	255.96	275.76	239.96	172.16	244.46	219.06	180.86	88.73	307.46
Cd	<DL	<DL	<DL	<DL	<DL	<DL	0.012	<DL	<DL	<DL
Cr	<DL	<DL	<DL	<DL	<DL	<DL	0.013	<DL	<DL	<DL
Cu	<DL	<DL	<DL	<DL	<DL	<DL	0.014	0.006	0.007	<DL
Fe	<DL	<DL	0.01	0.01	<DL	<DL	0.02	<DL	<DL	<DL
K	3.3	5.6	11.0	10.5	9.9	9.7	10.7	9.9	8.3	12.2
Mg	37.00	149.09	159.59	142.49	106.79	138.79	122.99	98.25	43.15	169.79
Mn	<DL	<DL	<DL	<DL	<DL	<DL	0.012	0.003	<DL	<DL
Na	7.92	19.16	22.62	20.38	16.76	18.70	18.53	16.40	12.27	25.59
Ni	<DL	0.02	0.02	0.02	<DL	0.02	0.04	0.02	<DL	0.04
S	68.4	393.2	442.9	387.6	280.0	379.5	338.0	252.9	89.0	486.5
Sb	0.006	0.011	0.007	0.01	0.007	0.021	0.011	0.005	<DL	0.011
Si	0.7	2.7	2.3	0.2	<DL	0.2	0.2	3.8	3.4	4.7
Sr	0.49	1.13	1.50	1.35	1.12	1.26	1.28	1.14	0.74	1.70
Cl^-	3.44	8.36	11.56	10.75	8.40	9.33	8.33	7.41	5.82	12.40
SO_4^{2-}	191.54	1217.90	1350.40	1184.03	863.80	1176.68	1025.67	784.05	267.30	1472.56

The water mixing series represented by the ten columns that were used in the second type of toxicity test are presented in Table 2.6.

Table 2.6: The water mixing series used in the column toxicity tests using *Lemna minor* to show the effects of the reacted column water on the growth of the macrophyte.

Column	Hogarth 2 m: Caland 30 m	% Hogarth 2 m
9	0:1	0%
8	1:1	50%
7	3:1	75%
6	9:1	90%
2	1:0	100%

Column	Hogarth 30 m: Caland 2 m	% Hogarth 30 m
1	0:1	0%
5	1:1	50%
4	3:1	75%
3	9:1	90%
10	1:0	100%

Since the water used in the column experiment was collected during fall 2007, the results of the toxicity test on this water could be directly compared to the water-only toxicity tests using just the water sampled in fall 2007. This would show whether the geological influence of the rock lining the pit walls on future water quality could change the toxicity of the pit lake water.

Again, after one week of incubation total frond number, fronds experiencing chlorosis and necrosis, and single fronds were counted. The total frond numbers were analyzed using ToxCalc v5.0. Because the procedure was modified by using a mixing series instead of a simple dilution series, the results were also analyzed using dry weights and chlorophyll a analysis as alternate endpoints. As well, the total frond surface area in each treatment was measured using a digital planimeter and colour photographs.

Dry weights

After counting, the colonies (including the roots) from each test vessel were dried in a drying oven. The plants were first rinsed with DDW, blotted dry and then transferred to oven- and dessicator-dried, pre-weighed numbered glass test tubes. These tubes containing the plants were placed in a drying oven for 24 hours at 55°C and then in a dessicator overnight (approx. 16 hours). The tubes containing the dried plants were then weighed on an analytical balance to ± 0.0001 mg adhering to strict quality control standards. Final weights were calculated and the average weights of the replicates were determined.

Chlorophyll a analysis

Following weighing, the dried samples were analyzed for chlorophyll a content. Acetone (90%) was added to the samples which were then mixed for one hour on a shaker table. The tubes were covered in aluminum foil and left in the dark at ambient temperature to digest for 24 hours. The samples were each diluted with 90% acetone to a total volume of 50 ml in order to keep the samples within the method detection limits. Using the Varian Cary 5E spectrophotometer in the Lakehead University Instrumentation Lab (LUIL), the wavelengths of each sample were measured at 630, 645, 663, and 750 nm. These were used to calculate chlorophyll a according to the equation in Appendix C.

Frond Surface Area Calculation

At the end of the 7 day toxicity test using the drained column water, the resulting fronds were photographed for digital image analysis. The plants in each replicate were poured with their sample water into a plastic petrie dish. This dish was placed on a grey card which was used in an attempt to calibrate the pigmentation level. A ruler was placed beside the petrie dish on the card for scale. Using a constant external light source, the plants were photographed using a Pentax K10D with a 55-200mm lens on a tripod at a fixed height. A remote was used to trigger the shutter to eliminate camera shake for clear photographs.

The software program Image Pro Plus was used in an attempt to analyze the digital photographs to measure frond surface area and to quantify overall pigmentation of each replication. An attempt was also made at doing the analysis using Adobe Photoshop. Unfortunately after many failed attempts, this digital analysis method was abandoned and replaced by manually measuring the surface area using a planimeter. The colour digital images were enlarged and printed. The fronds were then outlined and distinguished into four colour classes: dark green, light green, yellow (chlorosis), and white (necrosis). A Tamaya Planix 7 digital planimeter was used to measure the surface area covered by each colour class for each treatment.

2.2.2 Data Analysis

2.2.2a One-Way ANOVA

One-way ANOVA tests were conducted using SPSS v. 17.0 to determine whether there were any significant differences in the mean total frond numbers, the mean dry weights, the mean chlorophyll a concentrations, and the mean total surface areas 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.

2.2.2b ANCOVA

Using the results of the *Lemna minor* growth inhibition test, 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. ANCOVA has the same assumptions as ANOVA but with two other important considerations: (1) independence of the covariate and the treatment effect and (2) homogeneity of regression slopes. The first assumption can be tested using Levene's test of equality of variances while the second assumption is tested using a test of between-subjects effects using the combined effect of the covariate and the treatment effect. This analysis and the assumption tests were done using SPSS v. 17.0.

2.3 Results

2.3.1 ToxCalc Results of the Toxicity Tests

2.3.1a Water Mixes from Winter, Summer, and Fall 2007

The IC25 values for all tests were determined using ToxCalc v5.0 (Table 2.7). Because the procedure was modified to use a mixing series of the two lakes, the initial data analysis reported unexpected results such as an IC25 value of 0.0% Hogarth 30 m for the summer 2007 mix of Hogarth 30 m x Caland 30 m water. This value suggests that Caland 30 m water itself causes at least a 25% reduction in frond number compared to the B controls and that no Hogarth water (0.0%) is required to produce such a response.

Table 2.7: The results of the water-only toxicity tests using a water mixing series from winter, summer, and fall 2007 samples.

Lake and Depth	Season and Date	IC25 (%)
Hogarth 2 m x Caland 2 m	Winter 2007	57.8
Hogarth 30 m x Caland 30 m	Winter 2007	99.9
Hogarth 2 m x Caland 2 m	Summer 2007	84.6
Hogarth 30 m x Caland 30 m	Summer 2007	0.0
Hogarth 2 m x Caland 30 m	Summer 2007	17.7
Hogarth 30 m x Caland 2 m	Summer 2007	30.9
Hogarth 2 m x Caland 30 m	Fall 2007	40.1
Hogarth 30 m x Caland 2 m	Fall 2007	38.0

IC25 value: Inhibition concentration causing a 25% reduction in growth

It should be noted that for the winter and summer 2007 mixes the plants did not pass the minimum growth requirements for the test to be valid. The B controls in these mixing series did not produce the required number of fronds by the end of the test for the results to be valid. Only half of the B controls used in the fall 2007 mixes met the health criterion.

2.3.1b Toxicity Tests using Resultant Column Water

The B control replicates had a final average frond number of 59 (9.8-fold increase). The Caland 2m 100% replicates had a final average frond number of 67 (11.2-fold increase) while the Caland 30m 100% replicates had a final average frond number of 50 (8.3-fold increase). The Caland 2m 100% replicates actually had a greater proportional increase in frond number than the B control replicates. Both the Caland 2m and the 30m 100% replicates met the health criteria to be considered controls in the toxicity test analysis with ToxCalc. These quantitative results were analyzed using ToxCalc v5.0. Analysis was done using both the total number of fronds and the number of live fronds only (Table 2.8). The dose response curves are presented in Figure 2.1.

Table 2.8: The results of the *Lemna minor* growth inhibition test using column water.

Mixing Series - Lake and Depth	Columns	IC25 value (%)	Greatest IC value calculated (%)
Hogarth 2m x Caland 30m (total # fronds)	9,8,7,6,2	>100	IC10 = 28.32
Hogarth 2m x Caland 30m (live only)	9,8,7,6,2	>100	IC20 = 41.13
Hogarth 30m x Caland 2m (total # fronds)	1,5,4,3,10	>100	IC20 = 97.30
Hogarth 30m x Caland 2m (live only)	1,5,4,3,10	93.10	N/A

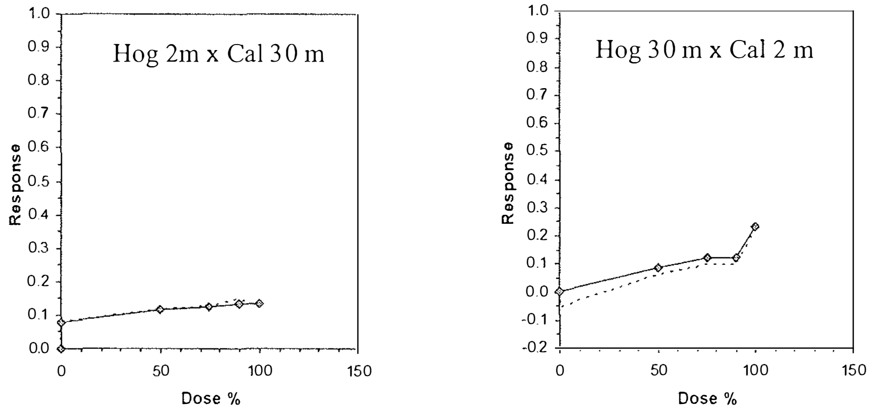

Figure 2.1: The dose response curves for the Hogarth 2 m x Caland 30 m mixing series (left) and the Hogarth 30 m x Caland 2 m mixing series (right). A dose response curve relates the magnitude of a stressor (concentration of Hogarth water) to the response of the organism under study (*Lemna minor*).

2.3.2 Alternate Endpoints

2.3.2a Dry Weights and Chlorophyll a Analysis

The final dry weights and chlorophyll a content of each treatment from the column toxicity tests are presented in Tables 2.9 and 2.10. The total frond number from each treatment is included for comparison. For each of the three quantitative parameters, the mean and standard deviation for each set of replicates were determined for the two mixing series represented by the column water ratios.

Table 2.9: Final frond number, dry weight and chlorophyll a data for *Lemna minor* toxicity tests using column water representing Hogarth **2 m** water.

% Hogarth 2 m	Total Frond Number	Dry weight (mg)	Chlorophyll a (μg/g)
0 (Column 9)	53	4.7	1383.76
0	51	4.4	1514.65
0	45	4.6	1058.62
Mean ± standard deviation	49.7 ± 4.2	4.6 ± 0.2	1319.01 ± 234.81
50 (Column 8)	42	5.2	720.11
50	49	4.8	1012.89
50	45	4.1	1078.76
Mean ± standard deviation	45.3 ± 3.5	4.7 ± 0.6	937.25 ± 190.91
75 (Column 7)	41	3.6	1636.77
75	48	5.9	947.67
75	45	3.8	1602.79
Mean ± standard deviation	44.7 ± 3.5	4.4 ± 1.3	1395.74 ± 388.42
90 (Column 6)	43	3.5	1579.59
90	43	4.3	1618.84
90	41	3.5	1450.73
Mean ± standard deviation	42.3 ± 1.2	3.8 ± 0.3	1549.72 ± 97.95
100 (Column 2)	45	2.3	1777.92
100	43	2.2	2468.92
100	45	4.6	1333.15
Mean ± standard deviation	44.3 ± 1.2	3.0 ± 1.4	1860.00 ± 572.32

Table 2.10: Final frond number, dry weight and chlorophyll a data for *Lemna minor* toxicity tests using column water representing Hogarth **30 m** water. The values of the controls are included for comparison.

% Hogarth 30 m	Total Frond Number	Dry weight (mg)	Chlorophyll a (μg/g)
0 (Column 1)	64	5.7	1868.76
0	75	4.5	2822.11
0	63	2.5	4750.50
Mean ± standard deviation	67.3 ± 6.7	4.3 ± 1.6	3147.12 ± 1468.10
50 (Column 5)	50	3.5	3111.26
50	53	4.0	2455.74
50	52	4.0	2691.50
Mean ± standard deviation	51.7 ± 1.5	3.8 ± 0.3	2752.83 ± 332.04
75 (Column 4)	51	4.5	2021.48
75	49	4.2	2045.19
75	43	2.2	3915.35
Mean ± standard deviation	47.7 ± 4.2	3.6 ± 1.3	2660.67 ± 1086.65
90 (Column 3)	52	2.0	4315.78
90	45	3.9	2065.38
90	46	3.6	1983.79
Mean ± standard deviation	47.7 ± 3.8	3.2 ± 1.0	2788.32 ± 1323.45
100 (Column 10)	42	3.9	1266.53
100	31	3.6	1558.10
100	38	2.9	1564.53
Mean ± standard deviation	37.0 ± 5.6	3.5 ± 0.5	1463.05 ± 170.23
Control	54	4.1	2339.71
Control	59	4.3	1692.41
Control	65	3.2	2741.73
Mean ± standard deviation	59.3 ± 5.5	3.9 ± 0.6	2257.95 ± 529.41

As the percentage of Hogarth increased, the dry weight decreased. However, the standard deviation values of most measurements were so large that none of the dry weight values were significantly different from the others (Tables 2.9 and 2.10; Fig 2.2).

The mean chlorophyll a values were lower than the controls in the Hogarth 30 m x Caland 2 m mixing series while they were greater than the controls in the Hogarth 2 m x Caland 30 m mixing series with a concentration of ≤75% Hogarth 2 m. Again, large standard deviations caused many of the differences in chlorophyll a content to not be significantly different from the control treatments (Tables 2.9 and 2.10; Fig 2.3).

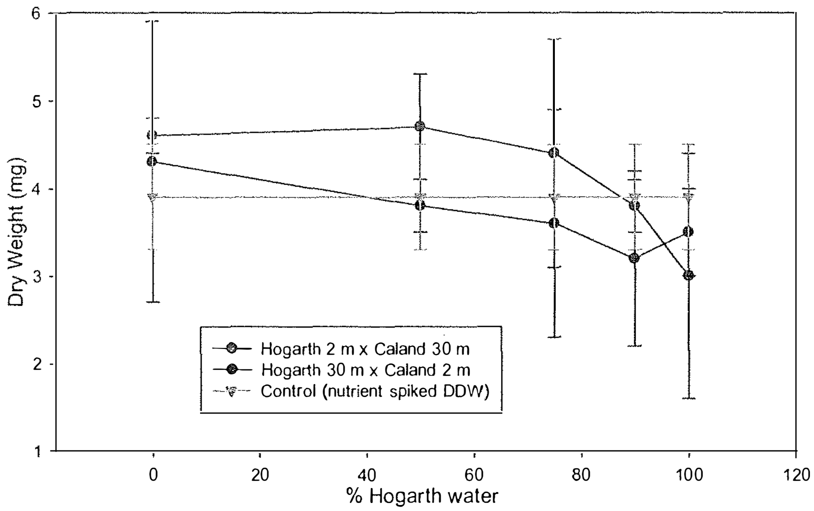

Figure 2.2: The results of the dry weight measurements of *Lemna minor* following the seven day growth inhibition test with column water.

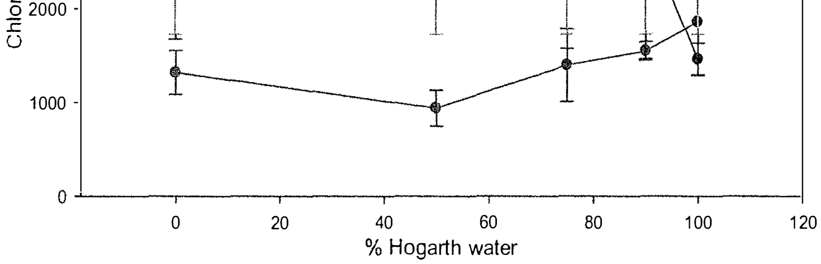

Figure 2.3: The results of the chlorophyll a analysis of *Lemna minor* following the seven day growth inhibition test with column water.

2.3.2b Frond Surface Area Calculation

The total surface area of all fronds in each replicate was measured. The surface area representing dark green, light green, yellow, and white were distinguished. These results are presented in Table 2.11 and Figure 2.4.

Table 2.11: The average *Lemna minor* frond surface area (cm^2) at the end of the seven day growth inhibition test including total surface area and that distinguished by colour.

Column	Dark Green	Light Green	Yellow	White	Total SA
1	0.1953123	0.5045568	1.0807279	0.0032552	1.7838521
2	0.2246092	0.3417964	0.4573560	0.1969396	1.2207012
3	0.2018227	0.1757810	0.4101555	0.2620437	1.0498030
4	0.2473956	0.3938798	0.6201163	0.3515618	1.6129534
5	0.3320309	0.6155669	0.9309883	0.0065104	1.8850965
6	0.1985675	0.3027340	0.2766922	0.2425124	1.0205060
7	0.2799477	0.2343947	0.3190099	0.2473953	1.0807475
8	0.1757811	0.3873694	0.2115881	0.3190097	1.0937483
9	0.1920571	0.5240878	0.3710933	0.2180984	1.3053366
10	0.1855467	0.2734371	0.1302080	0.2539056	0.8430974
Control	0.3938798	2.0996075	0.0651041	0.0097656	2.5683569

Column 1: 100% Caland 2 m water
Column 2: 100% Hogarth 2 m water
Column 3: 10% Caland 2 m water + 90% Hogarth 30 m water + Hogarth MFV rock
Column 4: 25% Caland 2 m water + 75% Hogarth 30 m water + Hogarth MFV rock
Column 5: 50% Caland 2 m water + 50% Hogarth 30 m water + Hogarth MFV rock
Column 6: 10% Caland 30 m water + 90% Hogarth 2 m water + Hogarth MFV rock
Column 7: 25% Caland 30 m water + 75% Hogarth 2 m water + Hogarth MFV rock
Column 8: 50% Caland 30 m water + 50% Hogarth 2 m water + Hogarth MFV rock
Column 9: 100% Caland 30 m water + Caland MFV rock
Column 10: 100% Hogarth 30 m water + Hogarth MFV rock

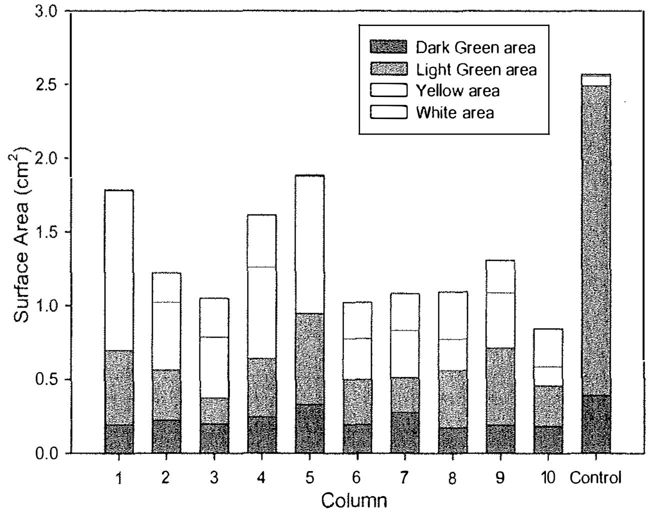

Figure 2.4: The average *Lemna minor* frond surface area (cm^2) at the end of the seven day growth inhibition test including total surface area and that distinguished by colour.

Figure 2.5 shows a comparison of one of the treatments from column 10 which had the lowest total surface area and one of the control treatments.

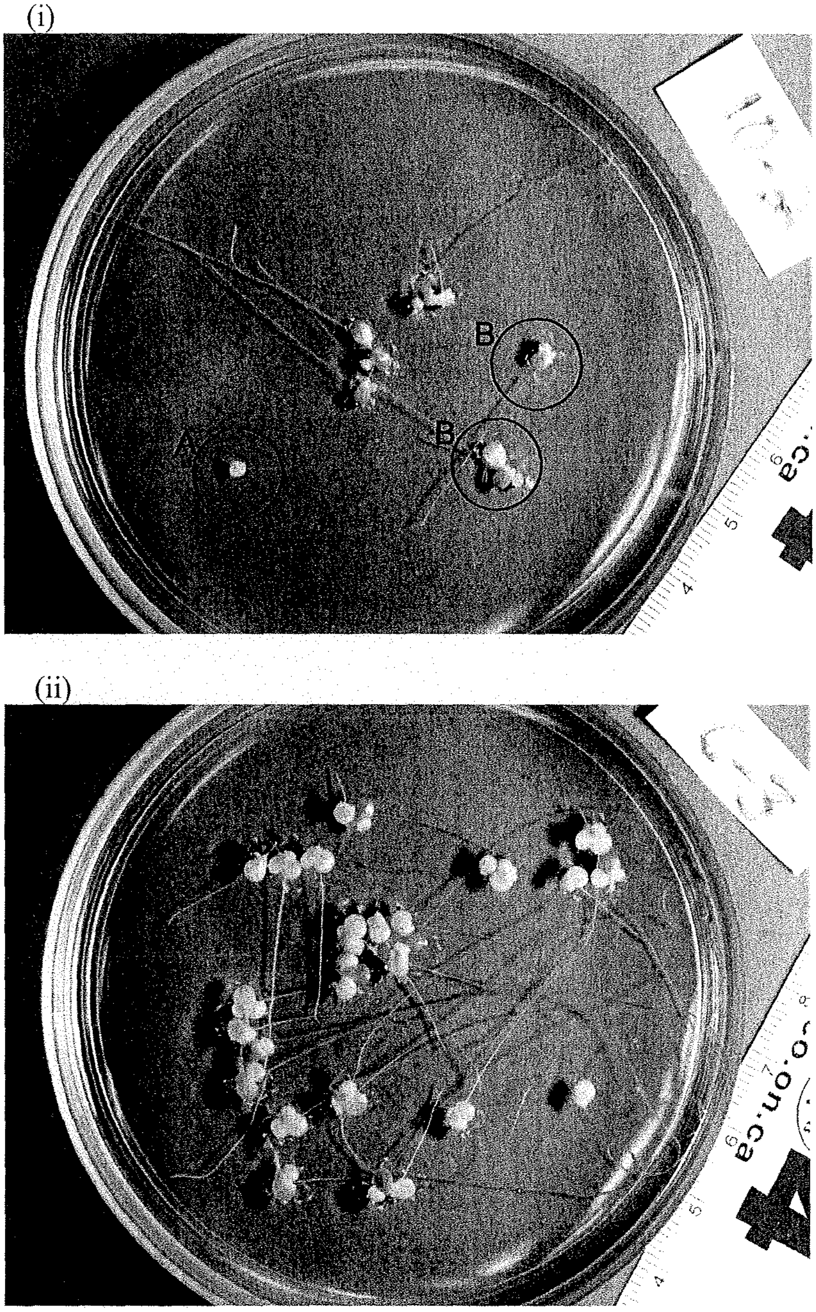

Figure 2.5: Photographs taken at the end of the seven day growth inhibition test. The plants in (i) were grown in 100% Hogarth 30 m water with Hogarth MFV rock, while the plants in (ii) were grown in the control water (DDW + nutrients).

2.3.3 One-Way ANOVA

A one-way ANOVA revealed that there were significant differences in total frond number between the treatments, F(10,22)=12.461, p<0.001. Dunnett's comparisons revealed that columns 2, 3, 4, 6, 7, 8, and 10 were all significantly different from the controls (p=0.05, 2-sided). Column 1 (100% Caland 30 m), column 5 (50% Hogarth 30 m water; 50% Caland 2 m water; Hogarth MFV rock), and column 9 (Caland 30 m water; Caland MFV rock) were not significantly different from the controls in regards to total frond number. All of the columns other than column 1, 5, and 9 produced significantly (p=0.05, 2-sided) fewer fronds than the control samples. Water from column 1 produced more fronds (67 versus 59) than the controls, however the 2-sided significance was p=0.154, making this result not significant according to Dunnett's test. Effect size measures were calculated (Appendix D) for the columns that showed a significantly lower number of total fronds. To understand the relative magnitude of the difference in total frond number, the *d* family effect size measures were determined (Table 2.12). The greatest effect was seen in column 6 (*d*=5.106, p=0.006).

Table 2.12: Effect size measures (*d*) calculated from the total frond numbers in the column water toxicity tests. *d* effect values show that the greatest difference in total frond number was seen in column 6.

Column	Column contents	*d* Effect Size	Sig. (2 tailed)
8	50% Hogarth 2 m, 50% Caland 30 m	3.1	0.021
7	75% Hogarth 2 m, 25% Caland 30 m	1.626	0.018
6	**90% Hogarth 2 m, 10% Caland 30 m**	**5.106**	**0.006**
2	100% Hogarth 2 m	4.503	0.01
4	75% Hogarth 30 m, 25% Caland 2 m	2.413	0.043
3	90% Hogarth 30 m, 10% Caland 2 m	2.511	0.039
10	100% Hogarth 30 m	4.033	0.008

A one-way ANOVA revealed that there were no significant differences in the dry weights of the *Lemna minor* plants in any of the treatments, F(10,22)=1.013, $p>0.01$. Likewise, a one-way ANOVA revealed that there were no significant differences in the chlorophyll a per unit mass measurements in any of the treatments, F(10,22)=2.969, $p>0.01$.

A one-way ANOVA established that there were significant differences in total frond surface area between the treatments, F(10,22)=13.037, $p<0.001$. Dunnett's comparisons revealed that the plants in all of the column water treatments had significantly different total frond surface areas compared to the controls ($p=0.05$, 2-sided). Furthermore, the total frond surface area was significantly lower than the controls in all column water treatments according to Dunnett's test.

A one-way ANOVA showed that there were no significant differences in the dark green surface areas in any of the treatments, F(10,22)=1.954, $p>0.01$. One-way ANOVA tests revealed that there were significant 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 significantly lower light green surface area measurements compared to the controls ($p=0.05$, 2-sided). Plants grown in water from columns 1, 2, 4, and 5 had significantly 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 (Caland 2 m water) and column 5 (50% Hogarth 30 m water + 50% Caland 2 m water + Hogarth MFV rock) had significantly greater white surface areas than the controls ($p=0.05$, 2-sided).

2.3.4 Analysis of Covariance (ANCOVA)

Both the assumption of independence of the covariate and the treatment effect and of homogeneity of regression slopes were not violated for this analysis. The ANCOVA found that frond number did not have a significant effect on chlorophyll a content for either of the two mixing series considered (Hogarth 2 m x Caland 30 m; Hogarth 30 m x Caland 2 m). However, there was a significant effect on chlorophyll a content due to the differences in dry weight between treatments in both mixing series. The differences in the dry weights of each treatment were controlled for by recalculating the chlorophyll a values as if the dry weight of every treatment was equal to one another. After controlling for the effect of dry weight it was found that water from Hogarth 2 m had a significant effect on the chlorophyll a content of the plants in the test, $F(5,11)=5.26$, $p<0.05$. As seen in Figure 2.6 the plants in all treatments with Hogarth 2 m water and Caland 30 m water produced significantly ($p=0.05$, 2-tailed) less chlorophyll a per unit mass than the controls.

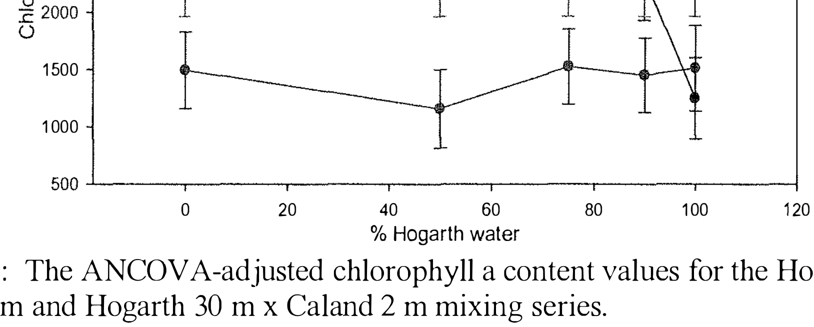

Figure 2.6: The ANCOVA-adjusted chlorophyll a content values for the Hogarth 2 m x Caland 30 m and Hogarth 30 m x Caland 2 m mixing series.

Similarly, after controlling for the effect of dry weight it was found that water from Hogarth 30 m had a significant effect on the chlorophyll a content of the plants in the test, *F*(5,11)=22.03, $p<0.05$, compared to the controls (Fig. 2.6). The plants grown in 0% Hogarth 30 m water in this series, which was actually 100% Caland 2 m water, produced significantly more chlorophyll a per unit mass than the controls while the plants in 100% Hogarth 30 m water produced significantly less chlorophyll a per unit mass than the controls. The plants growing between 50% and 75% Hogath 30 m water, inclusive, did not show a significant ($p=0.05$) difference in chlorophyll a per unit mass compared to the controls.

2.3.5 Qualitative Observations

Qualitative observations made regarding the overall growth and appearance of the duckweed at the end of the seven day toxicity test using column water indicated results that were contradictory to the calculated IC25 results. The plants in the test water from column 2 which contained 100% Hogarth 2 m water showed signs of reduced growth. They had very small side fronds (Fig. 2.5i – B) which showed signs of chlorosis and in most cases complete necrosis. As well, the plants showed signs of colony destruction; there were single fronds (Fig. 2.5i – A) with very small, white side fronds. The average number of fronds experiencing chlorosis was 22.6% while another 18.0% of the total number of fronds were dead. These values are both below the critical IC25 value of 25% reduction in growth, hence the IC25 value calculated by ToxCalc of >100% Hogarth 2 m. There was an average of 4 single fronds per test vessel.

The plants in the test water from column 10 which contained 100% Hogarth 30 m water had similar results (Fig.2.5i). The fronds were small with an overall reduced surface area. There were many white or pale green areas. Again, there were signs of colony destruction in the way of single fronds (Fig. 2.5i – A) with very small dead side fronds (Fig. 2.5i – B). The average number of fronds experiencing chlorosis was 13.5 % while another 29.7 % of the total number of fronds was dead. Because the number of dead fronds exceeds 25%, the IC25 value that was calculated by ToxCalc was 93.1% Hogarth 30 m when only the live fronds were considered (Table 2.8). There was an average of 2 single fronds per test vessel.

2.4 Discussion

2.4.1 Frond Number

A few concerns arise with the use of total frond number and IC25 calculations as an estimate of toxic effect. First, frond count is irrelevant to frond size or biomass. Under stress, small buds may protrude and be included in the final frond counts despite the fact that they may be less than 5% of the biomass of a healthy frond grown in one of the controls (Mohan et al., 1999). In total frond counts these two fronds are considered equal. Consequently, total frond counts and IC25 calculations greatly underestimate toxic responses. A second concern is that frond counts do not distinguish between plants that are alive or dead, or experiencing chlorosis (Mohan et al., 1999). These are important considerations which should be included when assessing plant health. As discussed previously, the unexpected IC25 values that resulted from the water-only toxicity tests were due to this conservative estimate of toxicity based on total frond number. Consequently, the ToxCalc calculations of IC25 values for both the water-only and the column toxicity tests were found to be inconclusive due to both poor plant health and the inclusion of dead fronds in the final calculations which grossly underestimates toxic effect (Mohan et al., 1999).

The one-way ANOVA uses a higher significance level (p=0.01) than the calculated IC25 values (p=0.25) and therefore the ANOVA found that the total frond numbers were significantly lower (p=0.05) than the controls in all column water treatments except columns 1, 5 and 9. Plants grown in Caland 2 m water (column 1) showed similar growth rates in regards to total frond number to the controls.

Unfortunately, total frond counts were the only toxicity endpoints analyzed for the water-only mixes. Since total frond counts were determined to be inconclusive because they underestimate toxic response, a direct comparison could not be made between the results of the fall 2007 water-only tests and the column water tests.

2.4.2 Dry Weights

The results of the dry weight measurements were also inconclusive. According to the one-way ANOVA with post hoc Dunnett's tests that were conducted, there were no significant differences (p=0.05, 2-tailed) in the dry weights of the *Lemna minor* plants in any of the columns compared to the dry weights of the control plants. Although it appeared that the plants followed a trend of decreasing dry weight with increasing Hogarth water (both 2 m and 30 m) concentration (Fig 2.2), the large standard deviations caused all of the differences to be considered non-significant. An interesting note: though not significant, the dry weights in the treatments containing 100% Caland water (both 2 m and 30 m) are greater than the dry weight of the controls. It appears that water from Caland at both depths stimulates growth in *Lemna minor* compared to the controls, with 30 m water providing the greatest opportunity for biomass production. Although the results were not significant, the overall trends in dry weight suggest that Hogarth 30 m water reduces growth in *Lemna minor* to a greater degree than Hogarth 2 m water.

2.4.3 Chlorophyll a

From the results of the ANCOVA-adjusted chlorophyll a analysis it appears that water from Hogarth does reduce the growth of *Lemna minor* in the toxicity tests in both mixing series examined; the Hogarth 2 m x Caland 30 m mixing series which best represents the future joined pit lake conditions appears to cause a greater reduction in chlorophyll a content than the Hogarth 30 m x Caland 2 m series (Fig.2.6). Despite the fact that the Hogarth 30 m x Caland 2 m mixing series reduced the biomass production of *Lemna minor* below that of the controls, this series had mean chlorophyll a values above those of the controls. One hypothesis is that the plants may have been investing more energy into biomass production rather than into chlorophyll a production. That is, if the rate of biomass production was greater in the Hogarth 2 m series than the Hogarth 30 m series, then if the rate of chlorophyll a production did not change, then the chlorophyll a concentrations per unit mass would be lower in the series which had higher biomass production. This is similar to studies of nutrient uptake in plants under stress. In general, plants under stress are still capable of accumulating the basic nutrients necessary for optimal biomass production if they are available in their environment. However, the rate of mobilization of these nutrient supplies is often insufficient to maintain normal biomass production rates and thus growth is reduced (Burns, 1992). These nutrients therefore concentrate in plant matter as biomass production is reduced. A similar phenomenon is likely happening with the chlorophyll a concentrations in the toxicity tests with *Lemna minor*.

2.4.4 Frond Surface Area

All of the columns produced plants with a significantly (p=0.05, 2-sided) lower total surface area than the controls. The Hogarth 30 m x Caland 2 m mixing series had greater total surface areas than the comparable mixes in the Hogarth 2 m x Caland 30 m mixing series. Although the results of the dry weights and the chlorophyll a analyses indicate that Hogarth 30 m water produced a greater reduction in growth in *Lemna minor* than Hogarth 2 m water, the results of the total frond surface area indicate the opposite. However, the most important conclusion is that both mixing series caused a significant reduction in surface area compared to the control treatments.

Many authors have used plant surface area and pigmentation as tools to assess a plant's growth rate (Wiesnerová, 2008; Cedergree et al., 2007; Jupsin et al., 2005). There is a small possibility of error in this simple method compared to the other methods previously examined such as photosynthetic pigment extraction with subsequent absorption measurements. Like the chlorophyll a analysis discussed previously, the analysis of the surface area measurements indicates that the future pit lake water quality will negatively impact the growth of aquatic macrophytes by significantly reducing the total frond surface area. This is concerning since a reduction in surface area will likely reduce photosynthesis given that leaf area represents the area available for light absorption for photosynthesis. There could also be an impact on photosynthesis in the remaining green leaf tissue which would reduce overall biomass production (Bastiaans, 1991).

2.5 Conclusions

These results indicate that there may be a chronic toxic effect from exposure to the water in the joined pit lake in aquatic macrophytes due to the high levels of sulfate. Given the importance of aquatic plants in all aquatic ecosystems, this could affect downstream aquatic communities with an overall decline in primary producers (Wang, 1991).

In future toxicity tests more sensitive and accurate test end points should be used to reduce the uncertainty and sometimes contradictory results determined through some of the end points examined here. These include measurements of viable biomass and physiological activity such as adenosine triphosphate content, fluorescence emission, ^{14}C uptake (Lockhart, 1983), enzyme activity, and oxygen production (Mohan et al., 1999)

General Conclusions

The column experiment revealed that few changes in water quality may occur as pit filling continues. The parameters that changed during the experiment, as well as their patterns of change over time revealed that three major reactions were likely occuring simultaneously during the experiment. The pattern of pH changes along with increases in potassium, barium, and strontium indicated that alkali feldspar weathering occurred. Initial decreases in pH along with increases in sulfate indicated that pyrite oxidation had occurred. Although most of the pH changes were buffered as a consequence of alkali feldspar weathering reactions, carbonate dissolution and precipitation reactions may have occurred to a lesser extent.

The column 8 refill experiment revealed that the sources of alkali feldspar and pyrite were not exhausted in the first part of the experiment even though equilibrium was reached for most parameters.

The toxicity tests revealed that water from Hogarth causes a significant reduction in growth in *Lemna minor*, particularly in terms of chlorophyll a content and total frond surface area. Continued monitoring and testing is needed to accurately predict the ecological effects of the future mixed pit lake waters. Although these tests show that there will not likely be severe acute effects in aquatic macrophytes, if the water quality is allowed to progress naturally under the geological influence of the various rock types lining the pit lake walls, chronic toxic effects are very likely to be seen in aquatic flora and fauna in the lakes and living downstream of the area of concern. Remediation strategies must be explored and developed to improve the water quality of the future combined pit lake with the purpose of mitigating future negative impacts on downstream aquatic communities.

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Appendix A

Complied Column Experiment Data

Table C1: Column experiment pH data.

	Column 1	Column 2	Column 3	Column 4	Column 5	Column 6	Column 7	Column 8	Column 9	Column 10
1 hour	8.21	8.10	8.10	8.07	8.17	8.08	8.06	8.06	8.21	8.14
24 hours	8.22	8.15	8.10	8.06	8.14	8.06	8.05	8.07	8.21	8.08
72 hours	8.37	8.21	8.07	8.07	8.12	8.02	8.04	8.11	8.18	8.05
7 days	8.21	8.08	7.73	7.64	7.81	7.69	7.70	7.74	7.82	7.70
14 days	8.24	8.13	7.76	7.70	7.82	7.75	7.76	7.79	7.84	7.74
21 days	8.17	8.07	7.72	7.65	7.70	7.64	7.66	7.66	7.72	7.68
28 days	8.22	8.13	7.72	7.69	7.79	7.69	7.69	7.71	7.78	7.66
35 days	8.12	8.08	7.78	7.77	7.94	7.88	7.87	7.86	7.93	7.79
42 days	8.24	8.10	7.79	7.82	7.89	7.83	7.78	7.84	7.92	7.74
49 days	8.27	8.14	7.84	7.82	7.91	7.82	7.83	7.85	7.90	7.77
56 days	8.42	8.25	7.94	7.96	8.01	7.93	7.83	7.90	7.99	7.88
70 days	8.23	8.10	7.90	7.77	7.97	7.83	7.73	7.56	8.05	7.85
100 days	8.17	8.02	7.82	7.77	7.89	7.72	7.78	7.88	7.93	7.78
139 days	8.24	8.15	8.17	8.38	8.57	8.26	8.18	7.99	8.06	7.84

Table C2: Column experiment barium data (mg/L).

	Column 1	Column 2	Column 3	Column 4	Column 5	Column 6	Column 7	Column 8	Column 9	Column 10
1 hour	0.021	0.009	0.012	0.016	0.016	0.014	0.014	0.016	0.022	0.008
24 hours	0.020	0.008	0.019	0.022	0.019	0.018	0.018	0.019	0.024	0.016
72 hours	0.020	0.008	0.023	0.023	0.024	0.024	0.024	0.023	0.025	0.022
7 days	0.021	0.009	0.029	0.029	0.029	0.030	0.030	0.029	0.029	0.028
14 days	0.023	0.009	0.034	0.032	0.034	0.034	0.033	0.033	0.033	0.033
21 days	0.022	0.010	0.033	0.032	0.034	0.033	0.033	0.033	0.033	0.032
28 days	0.022	0.009	0.033	0.033	0.034	0.034	0.034	0.034	0.034	0.032
35 days	0.022	0.009	0.033	0.032	0.034	0.033	0.033	0.034	0.035	0.032
42 days	0.022	0.009	0.032	0.032	0.035	0.033	0.033	0.034	0.036	0.032
49 days	0.021	0.009	0.031	0.029	0.033	0.032	0.032	0.033	0.035	0.030
56 days	0.021	0.003	0.030	0.029	0.032	0.027	0.031	0.032	0.035	0.029
70 days	0.016	0.003	0.020	0.020	0.023	0.019	0.022	0.024	0.027	0.021
100 days	0.022	0.009	0.029	0.029	0.032	0.030	0.030	0.030	0.035	0.026
139 days	0.022	0.009	0.023	0.022	0.023	0.023	0.024	0.027	0.033	0.024

Table C3: Column experiment calcium data (mg/L).

	Column 1	Column 2	Column 3	Column 4	Column 5	Column 6	Column 7	Column 8	Column 9	Column 10
1 hour	68.89	252.00	280.60	255.90	190.30	240.20	214.30	174.30	86.80	307.30
24 hours	68.78	249.60	285.10	256.80	189.00	240.80	217.90	175.60	88.17	307.70
72 hours	68.43	251.60	281.40	250.50	191.20	236.80	214.20	174.50	87.46	302.30
7 days	71.13	257.60	287.00	256.90	195.20	247.10	220.50	175.90	89.94	309.30
14 days	70.25	258.82	289.72	260.92	195.02	254.62	225.82	180.92	93.22	315.12
21 days	71.11	258.81	290.71	261.81	196.81	252.91	227.51	184.71	92.40	315.81
28 days	71.15	258.47	290.67	261.37	195.47	251.97	224.77	182.67	91.81	313.07
35 days	70.20	254.80	288.90	261.80	195.30	252.70	221.90	182.60	91.20	311.40
42 days	71.10	256.94	288.84	260.54	196.04	255.04	227.04	185.44	92.97	314.24
49 days	66.81	243.23	274.53	248.13	186.33	241.73	215.33	173.93	86.37	297.93
56 days	71.62	259.21	287.51	261.01	197.51	250.81	226.91	181.51	92.02	313.51
70 days	69.25	251.42	280.42	251.52	188.52	243.12	220.82	178.12	89.88	306.52
100 days	69.75	257.03	280.83	253.13	188.93	252.43	221.53	176.23	85.55	304.73
139 days	69.10	252.33	267.73	233.43	168.53	237.33	215.63	179.43	88.13	307.73

Table C4: Column experiment potassium data (mg/L).

	Column 1	Column 2	Column 3	Column 4	Column 5	Column 6	Column 7	Column 8	Column 9	Column 10
1 hour	3.2	5.4	6.7	6.8	5.7	6.1	5.7	5.3	4.3	6.8
24 hours	3.2	5.4	8.0	8.1	6.4	7.0	6.9	6.3	5.4	8.4
72 hours	3.2	5.4	8.8	8.4	7.7	8.1	8.0	7.4	6.2	9.3
7 days	3.3	5.6	9.8	9.4	8.5	9.2	8.9	8.2	6.9	10.2
14 days	3.4	5.7	10.3	9.9	9.0	10.2	9.4	8.9	7.6	11.4
21 days	3.2	5.3	9.8	9.4	8.6	9.2	9.0	8.4	6.9	10.5
28 days	3.3	5.6	10.6	10.2	9.3	10.2	9.8	9.2	7.5	11.4
35 days	3.3	5.4	10.4	9.9	9.2	9.9	9.5	9.0	7.3	10.8
42 days	3.5	5.6	10.8	10.5	9.7	10.6	10.1	9.6	7.8	11.7
49 days	3.1	5.3	10.0	9.5	9.0	9.8	9.6	8.8	7.2	10.9
56 days	3.3	5.6	10.4	10.1	9.2	9.3	10.0	9.1	7.7	11.0
70 days	3.3	5.4	10.4	10.1	9.3	9.2	9.8	9.2	7.6	11.0
100 days	3.3	5.6	10.4	9.9	9.1	9.2	9.3	8.6	7.1	10.5
139 days	3.3	5.5	10.3	9.7	8.8	9.0	9.5	8.8	7.2	10.7

Table C5: Column experiment magnesium data (mg/L).

	Column 1	Column 2	Column 3	Column 4	Column 5	Column 6	Column 7	Column 8	Column 9	Column 10
1 hour	34.34	142.57	153.67	139.27	105.37	134.47	120.37	96.82	42.71	167.67
24 hours	34.38	141.77	153.77	137.37	103.67	133.07	119.67	95.82	42.41	164.57
72 hours	34.19	142.57	150.87	134.47	102.67	128.57	116.07	93.61	41.27	161.07
7 days	36.54	149.30	156.10	140.10	106.10	135.40	120.90	96.60	42.87	166.30
14 days	35.12	143.10	149.70	134.40	100.80	131.50	115.90	92.42	42.31	161.20
21 days	36.65	149.68	157.38	140.78	105.98	135.88	121.78	98.40	43.31	168.48
28 days	35.46	144.30	152.10	136.40	101.90	131.40	117.00	93.83	42.01	162.20
35 days	34.77	141.59	149.99	135.59	100.89	129.79	114.49	92.54	41.10	159.39
42 days	37.02	147.68	155.88	139.98	105.78	136.28	121.38	98.03	43.96	167.18
49 days	33.23	136.18	143.88	128.88	97.50	125.38	111.98	89.45	39.73	154.58
56 days	36.91	150.18	157.08	141.08	106.78	138.18	122.48	96.93	44.07	169.48
70 days	35.80	146.58	154.68	138.28	104.18	134.68	119.88	96.25	43.00	167.48
100 days	35.55	149.69	155.99	138.99	105.19	137.99	118.99	95.08	41.78	166.39
139 days	35.16	146.29	154.09	137.09	102.29	133.39	118.69	95.20	42.08	166.19

Table C6: Column experiment sodium data (mg/L).

	Column 1	Column 2	Column 3	Column 4	Column 5	Column 6	Column 7	Column 8	Column 9	Column 10
1 hour	7.33	17.90	20.80	19.00	14.90	17.40	16.30	14.50	10.50	22.50
24 hours	7.34	17.90	20.90	19.00	14.80	17.30	16.20	14.40	10.60	22.20
72 hours	7.24	17.80	20.50	18.50	14.80	16.90	15.90	14.20	10.50	21.80
7 days	7.29	18.22	21.12	18.98	15.23	17.48	16.44	14.67	10.63	21.47
14 days	7.63	18.99	20.57	18.18	14.29	16.95	15.65	13.65	10.46	22.07
21 days	7.46	18.21	20.52	18.31	14.83	17.34	16.02	14.14	10.53	21.88
28 days	9.17	22.52	25.89	23.25	18.37	21.52	20.01	17.73	12.96	27.33
35 days	7.57	18.42	21.23	18.85	15.09	17.30	16.19	14.50	10.49	21.97
42 days	7.73	18.07	20.66	18.67	14.79	17.50	16.10	14.53	10.82	22.62
49 days	6.78	16.84	19.53	17.45	14.00	16.38	15.31	13.52	9.70	20.93
56 days	7.66	18.76	21.08	18.93	15.04	18.21	16.89	14.63	11.31	22.48
70 days	7.66	17.23	21.10	19.22	15.42	17.80	16.70	15.06	11.02	22.79
100 days	7.55	18.57	21.15	19.14	15.72	17.91	16.19	14.21	10.79	21.46
139 days	6.87	17.64	20.18	18.03	14.28	16.54	15.34	13.87	9.86	20.93

Table C7: Column experiment nickel data (mg/L).

	Column 1	Column 2	Column 3	Column 4	Column 5	Column 6	Column 7	Column 8	Column 9	Column 10
1 hour	<DL	<DL	0.03	0.02	<DL	<DL	<DL	<DL	<DL	0.03
24 hours	<DL	0.02	0.03	0.02	0.02	0.02	<DL	<DL	<DL	0.03
72 hours	<DL	<DL	0.03	0.03	<DL	<DL	<DL	<DL	<DL	0.03
7 days	<DL	0.02	0.02	0.02	0.01	0.01	0.01	<DL	<DL	0.02
14 days	<DL	<DL	0.03	0.02	<DL	0.02	<DL	<DL	<DL	0.03
21 days	<DL	<DL	0.03	0.03	0.02	0.02	<DL	<DL	<DL	0.04
28 days	<DL	<DL	0.03	0.03	<DL	0.02	<DL	<DL	<DL	0.03
35 days	<DL	0.02	0.02	0.02	0.02	0.02	0.02	0.02	<DL	0.03
42 days	<DL	0.02	0.03	0.03	0.02	0.02	0.02	0.02	<DL	0.03
49 days	<DL	<DL	0.03	0.02	0.02	<DL	<DL	<DL	<DL	0.03
56 days	<DL	<DL	0.03	0.03	<DL	0.02	<DL	<DL	<DL	0 03
70 days	<DL	<DL	0.02	0.02	0.02	0.02	0.02	<DL	<DL	0.03
100 days	<DL	<DL	0.03	0.02	0.02	0.03	0.03	<DL	<DL	0.03
139 days	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL

Table C8: Column experiment sulfur data (mg/L).

	Column 1	Column 2	Column 3	Column 4	Column 5	Column 6	Column 7	Column 8	Column 9	Column 10
1 hour	66.7	401.3	435.7	387.3	274.2	375.7	326.8	252.4	85.5	485.0
24 hours	66.8	399.5	440.8	387.3	271.7	375.4	329.0	249.6	87.1	481.1
72 hours	66.3	401.0	430.7	374.0	271.3	361.3	317.9	242.8	84.9	467.4
7 days	69.9	407.3	437.4	379.4	269.0	376.1	324.6	245.7	86.0	474.4
14 days	67.6	402.1	446.6	377.6	275.2	370.8	320.8	253.0	87.5	469.2
21 days	69.5	410.7	440.7	381.0	273.7	379.1	322.1	250.2	88.5	488.5
28 days	67.8	404.3	441.0	385.6	272.4	376.7	326.8	248.3	87.1	483.2
35 days	67.9	407.7	440.0	383.8	274.9	377.2	327.2	248.0	87.0	477.5
42 days	68.7	415.6	446.3	394.6	279.8	384.3	334.8	255.1	88.5	489.4
49 days	66.9	404.0	433.7	381.8	273.8	376.8	325.9	248.2	87.9	481.6
56 days	67.4	386.0	422.7	364.9	262.7	361.1	311.2	240.5	84.8	461.6
70 days	61.1	360.8	395.8	351.9	252.1	343.7	301.2	232.7	80.4	442.0
100 days	70.4	395.5	449.2	393.9	281.1	371.7	343.5	260.8	91.8	482.1
139 days	68.9	406.6	450.6	393.0	281.8	385.3	339.2	255.9	90.5	488.4

Table C9: Column experiment sulfate data (mg/L).

	Column 1	Column 2	Column 3	Column 4	Column 5	Column 6	Column 7	Column 8	Column 9	Column 10
1 hour	198.75	1315.95	1411.94	1234.65	869.94	1207.88	1044.06	718.64	263.27	1451.53
24 hours	196.74	1143.58	1319.48	1195.90	830.59	1151.69	1005.00	774.38	262.18	1380.78
72 hours	201.30	1268.89	1391.16	1207.07	844.77	1154.09	1015.38	763.66	263.82	1522.34
7 days	213.39	1175.34	1391.20	1175.21	830.67	1180.92	1025.50	773.67	270.43	1461.46
14 days	202.12	1247.33	1234.14	1171.80	715.18	1178.04	871.67	757.40	263.99	1052.71
21 days	210.96	987.20	1467.34	1115.32	845.28	1226.29	1078.21	753.91	278.98	1608.39
28 days	215.64	1315.61	1450.94	1267.69	914.30	1257.08	1077.59	819.42	276.24	1578.04
35 days	213.44	1335.18	1450.45	1452.80	871.51	1251.14	1067.06	792.34	279.92	1602.72
42 days	209.08	1286.33	1388.52	1213.97	860.26	1191.72	1019.95	786.51	279.03	1507.20
49 days	220.07	1286.83	1511.77	1236.10	869.45	1194.88	1030.17	794.82	258.76	1552.95
56 days	214.88	1284.86	1400.34	1229.96	871.58	1196.68	1072.00	795.69	280.88	1550.32
70 days	200.42	1231.72	1364.71	1161.68	872.96	1177.24	1033.06	803.89	281.40	1502.91
100 days	213.26	1024.81	1390.32	1232.42	890.81	1202.93	1042.49	806.61	277.97	1515.36
139 days	218.33	1293.80	1418.60	1223.80	874.60	1224.60	1048.60	798.60	289.60	1550.20

Table C10: Column experiment silicon data (mg/L). Blank values were not measured.

	Column 1	Column 2	Column 3	Column 4	Column 5	Column 6	Column 7	Column 8	Column 9	Column 10
1 hour	0.6	2.5	3.2	2.9	2.1	2.5	2.4	2.3	2·0	3.5
24 hours	0.6	2.5	3.3	3.1	2.2	2.6	2.6	2.4	2·2	3.6
72 hours	0.6	2.5	3.4	3.1	2.5	2.8	2.7	2.6	2.3	3.7
7 days										
14 days										
21 days										
28 days										
35 days	0.2	1.1	1.8	1.6	1.4	1.6	1.5	1.5	1.3	1.8
42 days	0.2	1.0	1.7	1.6	1.4	1.6	1.6	1.5	1.3	1.8
49 days	0.2	1.0	1.7	1.6	1.4	1.5	1.5	1.5	1.3	1.8
56 days	0.2	1.0	1.7	1.6	1.4	1.4	1.5	1.4	1.3	1.8
70 days	0.2	1.0	1.6	1.5	1.3	1.3	1.4	1.4	1.2	1.7
100 days	0.4	1.7	2.8	2.5	2.1	2.4	2.5	2.4	2.2	3.0
139 days	0.4	1.8	1.5	0.1	0.1	0.1	0.1	2.5	2.2	3.0

Table C11: Column experiment strontium data (mg/L).

	Column 1	**Column 2**	**Column 3**	**Column 4**	**Column 5**	**Column 6**	**Column 7**	**Column 8**	**Column 9**	**Column 10**
1 hour	0.46	1.08	1.29	1.22	0.95	1.08	1.00	0.89	0.65	1.38
24 hours	0.46	1.08	1.36	1.29	0.96	1.11	1.04	0.92	0.66	1.46
72 hours	0.45	1.07	1.38	1.27	1.01	1.15	1.08	0.96	0.66	1.49
7 days	0.46	1.08	1.43	1.31	1.06	1.21	1.15	1.00	0.67	1.50
14 days	0.47	1.10	1.46	1.34	1.09	1.28	1.18	1.04	0.70	1.60
21 days	0.47	1.09	1.45	1.33	1.10	1.23	1.18	1.04	0.68	1.56
28 days	0.46	1.07	1.45	1.33	1.08	1.24	1.16	1.04	0.68	1.55
35 days	0.47	1.09	1.47	1.33	1.10	1.25	1.17	1.06	0.68	1.55
42 days	0.47	1.08	1.46	1.35	1.10	1.27	1.18	1.07	0.70	1.58
49 days	0.45	1.05	1.39	1.27	1.06	1.21	1.15	1.02	0.66	1.52
56 days	0.46	1.07	1.41	1.29	1.06	1.20	1.16	1.02	0.69	1.51
70 days	0.46	1.06	1.41	1.30	1.07	1.18	1.15	1.02	0.67	1.51
100 days	0.46	1.09	1.41	1.28	1.05	1.18	1.10	0.99	0.63	1.47
139 days	0.46	1.05	1.34	1.20	0.97	1.12	1.08	0.98	0.06	1.46

Table C12: Column experiment chloride data (mg/L).

	Column 1	**Column 2**	**Column 3**	**Column 4**	**Column 5**	**Column 6**	**Column 7**	**Column 8**	**Column 9**	**Column 10**
1 hour	4.38	10.15	12.69	12.13	9.60	10.39	9.59	8.21	6.91	13.00
24 hours	4.21	9.17	12.41	12.15	9.44	10.08	9.79	8.86	8.21	13.01
72 hours	4.26	10.02	13.11	12.05	9.61	10.12	9.66	9.28	7.01	13.88
7 days	4.77	10.26	13.77	12.47	9.99	10.58	10.13	9.17	7.28	13.85
14 days	3.99	9.43	11.82	11.33	8.16	9.78	8.40	8.39	6.64	10.47
21 days	3.93	8.34	13.01	11.09	9.24	10.10	9.65	8.43	6.88	13.84
28 days	4.18	11.43	15.66	14.12	10.85	12.27	11.79	8.02	6.73	16.88
35 days	4.42	12.44	15.47	16.05	9.59	12.23	11.65	8.75	6.84	16.68
42 days	4.05	9.54	12.96	11.56	9.18	9.67	9.35	8.46	6.59	13.71
49 days	4.17	9.95	14.60	12.18	9.55	10.06	9.70	8.72	6.82	14.55
56 days	4.01	9.64	13.05	11.74	9.25	9.71	9.48	8.51	6.63	14.13
70 days	3.67	9.10	12.62	11.02	8.89	9.45	9.08	10.21	8.69	15.43
100 days	3.68	7.59	15.12	13.84	10.07	10.19	9.24	10.59	6.86	15.85
139 days	3.89	9.64	13.36	11.67	9.67	9.88	9.80	8.44	6.61	14.17

Table C13: Column experiment copper data (mg/L).

	Column 1	Column 2	Column 3	Column 4	Column 5	Column 6	Column 7	Column 8	Column 9	Column 10
1 hour	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL
24 hours	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL
72 hours	0.005	<DL	<DL	<DL	<DL	<DL	<DL	<DL	0.005	<DL
7 days	<DL	<DL	<DL	<DL	<DL	<DL	<DL	0.005	0.007	<DL
14 days	<DL	<DL	0.005	0.006	0.005	0.006	<DL	0.006	0.007	<DL
21 days	<DL	<DL	<DL	0.006	<DL	<DL	<DL	<DL	0.007	0.007
28 days	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	0.007	0.006
35 days	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	0.006	<DL
42 days	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	0.006	<DL
49 days	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	0.007	<DL
56 days	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	0.007	<DL
70 days	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	0.007	<DL
100 days	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL
139 days	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	0.006	<DL

Table C14: Column experiment iron data (mg/L).

	Column 1	Column 2	Column 3	Column 4	Column 5	Column 6	Column 7	Column 8	Column 9	Column 10
1 hour	0.01	0.01	0.01	0.02	0.01	0.02	0.02	0.01	0.01	0.03
24 hours	<DL	0.02	0.01	<DL	0.02	0.02	<DL	0.02	0.02	<DL
72 hours	0.01	<DL	<DL	0.01	<DL	<DL	<DL	<DL	0.01	0.02
7 days	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL
14 days	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL
21 days	<DL	<DL	<DL	0.01	0.02	0.03	<DL	<DL	0.02	<DL
28 days	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL
35 days	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL
42 days	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL
49 days	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL
56 days	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL
70 days	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL
100 days	<DL	<DL	<DL	0.01	0.01	0.01	0.01	<DL	<DL	<DL
139 days	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL

Table C15: Column experiment manganese data (mg/L).

	Column 1	Column 2	Column 3	Column 4	Column 5	Column 6	Column 7	Column 8	Column 9	Column 10
1 hour	<DL	<DL	0.015	0.018	0.013	0.023	0.016	0.011	0.005	0.009
24 hours	<DL	<DL	0.023	0.024	0.013	0.030	0.021	0.013	0.008	0.018
72 hours	<DL	<DL	0.026	0.024	0.015	0.033	0.024	0.015	0.011	0.023
7 days	<DL	<DL	0.031	0.025	0.009	0.016	0.022	0.014	0.012	0.028
14 days	<DL	<DL	0.004	0.001	<DL	<DL	<DL	<DL	0.003	0.021
21 days	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL
28 days	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL
35 days	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL
42 days	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL
49 days	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL
56 days	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL
70 days	<DL	0.002	<DL	<DL	<DL	<DL	<DL	<DL	0.002	<DL
100 days	<DL	0.001	<DL	0.002	0.002	0.002	0.001	<DL	0.002	<DL
139 days	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL

Appendix B

Statistics from the results of the column experiment

pH statistics

	column 1	column 2	column 3	column 4	column 5	column 6	column 7	column 8	column 9	column 10
mean	8.237857	8.122143	7.888571	7.869286	7.980714	7.871429	7.854286	7.858571	7.967143	7.835714
median	8.225	8.115	7.83	7.795	7.925	7.83	7.805	7.855	7.93	7.785
std. dev	0.076979	0.058333	0.159656	0.211568	0.218754	0.17948	0.16327	0.161191	0.15755	0.152352
std.err.	0.020574	0.01559	0.04267	0.056544	0.058464	0.047968	0.043636	0.04308	0.042107	0.040718
95% conf.	0.044448	0.033681	0.092185	0.122158	0.126307	0.103631	0.094271	0.093071	0.090969	0.087967
99% conf.	0.061979	0.046966	0.128544	0.17034	0.176126	0.144505	0.131454	0.12978	0.126849	0.122663
size	14	14	14	14	14	14	14	14	14	14
total	115.33	113.71	110.44	110.17	111.73	110.2	109.96	110.02	111.54	109.7
min.	8.12	8.02	7.72	7.64	7.7	7.64	7.66	7.56	7.72	7.66
max.	8.42	8.25	8.17	8.38	8.57	8.26	8.18	8.11	8.21	8.14
min. pos.	8.12	8.02	7.72	7.64	7.7	7.64	7.66	7.56	7.72	7.66

Sulphate Statistics

	column 1	column 2	column 3	column 4	column 5	column 6	column 7	column 8	column 9	column 10
mean	209.17	1228.388	1399.351	1222.741	854.4214	1199.656	1030.767	781.3957	273.3193	1488.351
median	212.11	1276.875	1395.77	1218.885	869.695	1195.78	1037.775	789.425	277.105	1518.85
std. dev	7.768008	108.3273	66.94975	76.75115	45.94695	31.80524	51.48236	26.46777	9.399565	139.5757
std.err.	2.076088	28.95168	17.89307	20.51261	12.27984	8.500309	13.75924	7.07381	2.512139	37.30316
95% conf.	4.48521	62.54762	38.65646	44.31574	26.52954	18.36419	29.72566	15.28236	5.427263	80.5903
99% conf.	6.254278	87.21783	53.90344	61.79487	36.9934	25.60744	41.45014	21.31007	7.567898	112.3769
size	14	14	14	14	14	14	14	14	14	14
total	2928.38	17197.43	19590.91	17118.37	11961.9	16795.18	14430.74	10939.54	3826.47	20836.91
min.	196.74	987.2	1234.14	1115.32	715.18	1151.69	871.67	718.64	258.76	1052.71
max.	220.07	1335.18	1511.77	1452.8	914.3	1257.08	1078.21	819.42	289.6	1608.39
min. pos.	196.74	987.2	1234.14	1115.32	715.18	1151.69	871.67	718.64	258.76	1052.71

Sulphur Statistics

	column 1	column 2	column 3	column 4	column 5	column 6	column 7	column 8	column 9	column 10
mean	67.57427	400.1736	436.5164	381.1521	272.4093	372.5164	325.0736	248.8021	86.95427	476.5307
median	67.71	403.05	440.3408	382.7908	273.75	375.9	326.35	248.95	87.055	481.35
std. dev	2.240294	13.32624	13.91698	11.68254	7.646244	10.83406	10.75165	7.032761	2.736992	13.01644
std.err.	0.598744	3.561588	3.719469	3.12229	2.043545	2.895525	2.8735	1.879584	0.731492	3.47879
95% conf.	1.293535	7.694507	8.035594	6.745441	4.414904	6.255534	6.207952	4.060682	1.580326	7.515628
99% conf.	1.803735	10.7294	11.20502	9.405996	6.156242	8.722859	8.656509	5.662307	2.203642	10.47996
size	14	14	14	14	14	14	14	14	14	14
total	946.0398	5602.43	6111.23	5336.13	3813.73	5215.23	4551.03	3483.23	1217.36	6671.43
min.	61.11	360.8	395.8	351.9	252.1	343.7	301.2	232.7	80.38	442
max.	70.41	415.6	450.6482	394.6	281.8482	385.3482	343.5	260.8	91.84	489.4
min. pos.	61.11	360.8	395.8	351.9	252.1	343.7	301.2	232.7	80.38	442

Chloride Statistics

	column 1	column 2	column 3	column 4	column 5	column 6	column 7	column 8	column 9	column 10
mean	4.115	9.764286	13.54643	12.38571	9.506429	10.32214	9.807857	8.86	7.05	14.24643
median	4.11	9.64	13.08	12.09	9.57	10.09	9.655	8.615	6.85	14.005
std. dev	0.29516	1.182304	1.201618	1.389586	0.610946	0.86781	0.90655	0.739667	0.62778	1.641527
std.err.	0.078885	0.315984	0.321146	0.371382	0.163282	0.231932	0.242286	0.197684	0.167781	0.438716
95% conf.	0.170424	0.682656	0.693808	0.80234	0.352757	0.50107	0.523438	0.42708	0.362477	0.947809
99% conf.	0.237643	0.951911	0.967462	1.118801	0.491893	0.698703	0.729893	0.59553	0.505446	1.321647
size	14	14	14	14	14	14	14	14	14	14
total	57.61	136.7	189.65	173.4	133.09	144.51	137.31	124.04	98.7	199.45
min.	3.67	7.59	11.82	11.02	8.16	9.45	8.4	8.02	6.59	10.47
max.	4.77	12.44	15.66	16.05	10.85	12.27	11.79	10.59	8.69	16.88
min. pos.	3.67	7.59	11.82	11.02	8.16	9.45	8.4	8.02	6.59	10.47

Potassium Statistics

	column 1	column 2	column 3	column 4	column 5	column 6	column 7	column 8	column 9	column 10
mean	3.273164	5.495521	9.759093	9.42645	8.542379	9.083021	8.942736	8.337379	6.907521	10.33059
median	3.271	5.4723	10.2758	9.7873	9.022	9.239	9.4395	8.8038	7.1793	10.7608
std. dev	0.087145	0.133583	1.158506	1.004479	1.179329	1.238641	1.270905	1.203091	0.982282	1.334089
std.err.	0.02329	0.035701	0.309624	0.268458	0.315189	0.331041	0.339664	0.32154	0.262526	0.35655
95% conf.	0.050317	0.07713	0.668916	0.579981	0.680939	0.715185	0.733815	0.694659	0.567165	0.770296
99% conf.	0.070163	0.107552	0.932752	0.808739	0.949517	0.997271	1.023248	0.968648	0.790868	1.074119
size	14	14	14	14	14	14	14	14	14	14
total	45.8243	76.9373	136.6273	131.9703	119.5933	127.1623	125.1983	116.7233	96.7053	144.6283
min.	3.144	5.261	6.72	6.8	5.66	6.12	5.66	5.33	4.31	6.78
max.	3.454	5.724	10.84	10.52	9.721	10.57	10.07	9.579	7.802	11.69
min. pos.	3.144	5.261	6.72	6.8	5.66	6.12	5.66	5.33	4.31	6.78

Barium Statistics

	column 1	column 2	column 3	column 4	column 5	column 6	column 7	column 8	column 9	column 10
mean	0.021036	0.008014	0.027214	0.027029	0.028529	0.027257	0.0279	0.028571	0.031021	0.026057
median	0.02145	0.00885	0.02945	0.02915	0.0319	0.0299	0.0306	0.0306	0.0331	0.02845
std. dev	0.001669	0.002359	0.006558	0.005489	0.006405	0.006876	0.006471	0.00592	0.004802	0.00733
std.err.	0.000446	0.00063	0.001753	0.001467	0.001712	0.001838	0.001729	0.001582	0.001283	0.001959
95% conf.	0.000964	0.001362	0.003786	0.003169	0.003698	0.00397	0.003736	0.003418	0.002773	0.004232
99% conf.	0.001344	0.001899	0.00528	0.004419	0.005157	0.005536	0.00521	0.004766	0.003866	0.005901
size	14	14	14	14	14	14	14	14	14	14
total	0.2945	0.1122	0.381	0.3784	0.3994	0.3816	0.3906	0.4	0.4343	0.3648
min.	0.0158	0.0025	0.0123	0.0155	0.0156	0.0135	0.0137	0.0162	0.0217	0.0079
max.	0.0227	0.0096	0.0343	0.0325	0.0346	0.034	0.0335	0.034	0.0357	0.0328
min. pos.	0.0158	0.0025	0.0123	0.0155	0.0156	0.0135	0.0137	0.0162	0.0217	0.0079

Silicon Statistics

	column 1	column 2	column 3	column 4	column 5	column 6	column 7	column 8	column 9	column 10
mean	0.38372	1.60998	2.2638	1.95624	1.5882	1.76901	1.7865	1.9373	1.7532	2.5769
median	0.3353	1.4025	1.7425	1.6475	1.4425	1.556	1.547	1.8935	1.6885	2.402
std. dev	0.159182	0.671549	0.80445	0.920115	0.683693	0.799882	0.782425	0.533804	0.477647	0.861676
std.err.	0.050338	0.212362	0.254389	0.290966	0.216203	0.252945	0.247425	0.168804	0.151045	0.272486
95% conf.	0.113874	0.480408	0.575482	0.658225	0.489095	0.572214	0.559726	0.381869	0.341696	0.616419
99% conf.	0.163604	0.690206	0.826799	0.945678	0.702687	0.822104	0.804163	0.548634	0.490917	0.885615
size	10	10	10	10	10	10	10	10	10	10
total	3.8372	16.0998	22.638	19.5624	15.882	17.6901	17.865	19.373	17.532	25.769
min.	0.2282	0.9608	1.517	0.1134	0.05	0.1151	0.142	1.366	1.237	1.676
max.	0.5907	2.492	3.42	3.063	2.462	2.771	2.736	2.581	2.339	3.684
min. pos.	0.2282	0.9608	1.517	0.1134	0.05	0.1151	0.142	1.366	1.237	1.676

Strontium Statistics

	column 1	column 2	column 3	column 4	column 5	column 6	column 7	column 8	column 9	column 10
mean	0.462264	1.075214	1.406857	1.293429	1.046143	1.193143	1.126286	1.003114	0.626459	1.509643
median	0.46035	1.077	1.408	1.2925	1.057	1.2035	1.146	1.0185	0.66645	1.5105
std. dev	0.007264	0.015438	0.050948	0.044776	0.052637	0.060449	0.054735	0.052375	0.163337	0.058986
std.err.	0.001941	0.004126	0.013616	0.011967	0.014068	0.016156	0.014629	0.013998	0.043654	0.015765
95% conf.	0.004194	0.008914	0.029417	0.025853	0.030393	0.034903	0.031604	0.030241	0.09431	0.034058
99% conf.	0.005849	0.01243	0.04102	0.036051	0.04238	0.04867	0.044069	0.042168	0.131508	0.047491
size	14	14	14	14	14	14	14	14	14	14
total	6.4717	15.053	19.696	18.108	14.646	16.704	15.768	14.0436	8.77042	21.135
min.	0.4513	1.047	1.294	1.197	0.9473	1.08	1.003	0.8901	0.06282	1.379
max.	0.4745	1.103	1.47	1.349	1.103	1.28	1.178	1.068	0.7023	1.603
min. pos.	0.4513	1.047	1.294	1.197	0.9473	1.08	1.003	0.8901	0.06282	1.379

Sodium Statistics

	column 1	column 2	column 3	column 4	column 5	column 6	column 7	column 8	column 9	column 10
mean	7.519264	18.36255	21.08809	18.96524	15.11166	17.60952	16.37452	14.54381	10.72616	22.31666
median	7.5062	18.13815	20.85	18.89005	14.865	17.37	16.19055	14.45	10.565	22.0221
std. dev	0.555164	1.326404	1.456381	1.329809	1.042336	1.231076	1.13845	1.006122	0.76124	1.555567
std.err.	0.148374	0.354496	0.389234	0.355406	0.278576	0.329019	0.304264	0.268897	0.20345	0.415743
95% conf.	0.320549	0.765859	0.840907	0.767825	0.60184	0.710817	0.657336	0.58093	0.439536	0.898177
99% conf.	0.44698	1.067932	1.17258	1.070673	0.839219	0.99118	0.916604	0.810061	0.6129	1.252438
size	14	14	14	14	14	14	14	14	14	14
total	105.2697	257.0757	295.2333	265.5133	211.5633	246.5333	229.2433	203.6133	150.1663	312.4333
min.	6.777	16.843	19.533	17.453	14.003	16.383	15.313	13.523	9.696	20.9298
max.	9.1716	22.5206	25.8906	23.2506	18.3706	21.5206	20.0106	17.7306	12.9606	27.3306
min. pos.	6.777	16.843	19.533	17.453	14.003	16.383	15.313	13.523	9.696	20.9298

Calcium Statistics

	column 1	column 2	column 3	column 4	column 5	column 6	column 7	column 8	column 9	column 10
mean	69.82581	254.418	283.8537	255.268	191.0108	246.968	221.0108	178.9894	89.70727	309.0465
median	69.97495	255.8687	286.0477	256.8477	193.1093	248.9561	221.1777	178.7763	89.9102	308.5161
std. dev	1.364629	4.618421	6.72267	7.795519	7.418636	6.703621	4.919434	4.004831	2.620203	5.2053
std.err.	0.364712	1.234325	1.796709	2.08344	1.982714	1.791618	1.314774	1.070336	0.700279	1.391175
95% conf.	0.78793	2.666653	3.881636	4.501094	4.283484	3.870638	2.840457	2.31237	1.512893	3.005515
99% conf.	1.098708	3.718442	5.412642	6.276428	5.972987	5.397305	3.960798	3.224421	2.109612	4.190958
size	14	14	14	14	14	14	14	14	14	14
total	977.5613	3561.851	3973.951	3573.751	2674.151	3457.551	3094.151	2505.851	1255.902	4326.651
min.	66.8056	243.2256	267.7321	233.4321	168.5321	236.7953	214.1953	173.9256	85.5454	297.9256
max.	71.6221	259.2121	290.713	261.813	197.5121	255.0423	227.513	185.4423	93.2233	315.813
min. pos.	66.8056	243.2256	267.7321	233.4321	168.5321	236.7953	214.1953	173.9256	85.5454	297.9256

Magnesium Statistics

	column 1	column 2	column 3	column 4	column 5	column 6	column 7	column 8	column 9	column 10
mean	35.36521	145.1052	153.2266	137.3338	103.5066	133.2838	118.5409	95.0695	42.32879	164.4409
median	35.31005	145.2951	153.9278	137.8244	103.9244	133.9278	119.3257	95.5078	42.35775	166.2451
std. dev	1.138244	4.078262	3.673957	3.283909	2.638552	3.651701	3.053272	2.471044	1.157507	4.195627
std.err.	0.304209	1.089961	0.981906	0.877662	0.705183	0.975958	0.816021	0.660414	0.309357	1.121328
95% conf.	0.657217	2.354769	2.121325	1.896113	1.523487	2.108474	1.762944	1.426768	0.668339	2.422534
99% conf.	0.916438	3.283543	2.958023	2.643983	2.124385	2.940104	2.45829	1.989518	0.931947	3.378037
size	14	14	14	14	14	14	14	14	14	14
total	495.113	2031.473	2145.173	1922.673	1449.093	1865.973	1659.573	1330.973	592.603	2302.173
min.	33.233	136.183	143.883	128.883	97.503	125.383	111.983	89.453	39.733	154.583
max.	37.0231	150.1786	157.3844	141.0786	106.7786	138.1786	122.4786	98.4044	44.0686	169.4786
min. pos.	33.233	136.183	143.883	128.883	97.503	125.383	111.983	89.453	39.733	154.583

Hardness Statistics

	column 1	column 2	column 3	column 4	column 5	column 6	column 7	column 8	column 9	column 10
mean	322.4646	1242.982	1350.496	1212.558	910.4398	1174.871	1048.313	845.0875	401.272	1460.368
median	322.6838	1244.16	1352.448	1219.025	911.23	1176.998	1050.254	843.6364	401.9765	1463.19
std. dev	7.941969	26.88417	25.60332	27.07163	24.27973	26.5236	20.8874	16.60554	10.10407	26.27618
std.err.	2.12258	7.185097	6.842775	7.235197	6.489031	7.08873	5.582392	4.438017	2.700427	7.022603
95% conf.	4.585654	15.52279	14.78323	15.63103	14.019	15.3146	12.06028	9.587956	5.834043	15.17173
99% conf.	6.39434	21.64533	20.61407	21.79626	19.54841	21.35502	16.81713	13.36966	8.135122	21.15581
size	14	14	14	14	14	14	14	14	14	14
total	4514.504	17401.75	18906.94	16975.81	12746.16	16448.2	14676.39	11831.23	5617.808	20445.15
min.	305.9934	1177.669	1288.072	1157.013	849.2156	1128.693	1006.653	808.9214	382.0567	1391.314
max.	333.4136	1276.201	1385.036	1243.352	940.3765	1208.214	1079.54	873.612	416.2693	1494.198
min. pos.	305.9934	1177.669	1288.072	1157.013	849.2156	1128.693	1006.653	808.9214	382.0567	1391.314

Appendix C

Chlorophyll a Calculations

In terms of ug CH_a/g dry weight.

Each absorbance value must be corrected for turbidity using the following turbidity correction:

$A_{corr} = A_n - A_{750}$

The corrected values are then used in the following equation to calculate chlorophyll a concentration.

Chlorophyll a = $[11.64\ A_{663}(corr) - 2.16\ A_{645}(corr) + 0.1\ A_{630}(corr)] \times X/Y*1/L$

Where:

X = extract volume in ml (total diluted volume was 50ml)
Y = quartz cell length in centimetres (cell length used was 1cm)
L = original sample volume in litres (instead used dry weight of *Lemna*, = g)

Since in this case Y=1, and the dry weight of *Lemna* (DW) is used instead of using the original sample volume, the equation can be rewritten as:

Chlorophyll a = $[11.64\ A_{663}(corr) - 2.16\ A_{645}(corr) + 0.1\ A_{630}(corr)] \times X/DW$

Appendix D

Calculating the *d* family of effect size measures

Adapted from Leech et al. (2008)

When comparing two groups, the effect size (*d*) is calculated by subtracting the mean of the second group from the mean of the first group, then dividing this value by the pooled standard deviation of both groups. If the two values have an equal number of samples then the pooled standard deviation is simply the average of the standard deviations of the two groups. The formula is as follows.

$$d = \frac{M_A - M_B}{SD_{pooled}}$$

·When *d* is ≥ |1.00| the strength of the effect is much larger than typical.

·When *d* is near |0.80| the strength of the effect is large or larger than typical.

·When *d* is near |0.50| the strength of the effect is medium or typical.

·When *d* is near |0.20| the strength of the effect is small or smaller than typical.