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THE INFLUENCE OF WETTING AND DRYING CYCLES ON THE BIOGEOCHEMICAL DYNAMICS OF MERCURY IN SEDIMENTS

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ABSTRACT

Decommissioning hydro-dams increases sediment exposure to air, altering biogeochemical cycling of metals. Hg and MeHg mobility was studied in reservoir sediments from two Eastern Ontario lakes (Stump (SL) and Black Donald (BDL)) submitted to wet/dry cycles with artificial rainwater (pH~4.5) every two weeks. Leachate pH, sulphate, sulphide and MeHg, and sulphate-reducing bacteria (SRB) populations were monitored over 6 months. The sediments did buffer the rainwater at the start of the experiment, but leachate pH decreased over time for both lakes. MeHg release occurred during the first draining event (2-4 ppt), and decreased thereafter, with no relationship with pH, sulphide and sulphate. SRB populations remained constant over time. Over 70% of Hg was in the non-labile refractory organic and residual phases, where Hg moved to semi-mobile phases in SL, but shifted to more immobile phases in BDL over time. Decommissioning hydro-dam reservoirs may increase acid production and alter Hg partitioning in sediments, while MeHg release will be low.

Keywords: sediments, oxidation, Hg, MeHg, sequential extraction

RÉSUMÉ

Cette étude a pour but d'évaluer les changements biogéochimiques des sédiments exposés à l'air après le démantèlement de barrage hydro-électrique. Des carottes de sédiments ont été prises de deux lacs dans l'est de l'Ontario (Stump (SL) et Black Donald (BDL)) ayant des capacités tampons légèrement différentes. Ces sédiments ont été soumis à des épisodes de pluie et de sécheresse où la mobilité du mercure et du méthyle de mercure ainsi que les changements aux populations de bactéries sulpho-réductrices (BSR) ont été étudiés. Des mesures de la concentration du sulphate, du sulphure et du méthyle de mercure et du pH ont été faient sur le lexiviat des carottes. Les populations de BSR ont aussi été estimées. Les sédiments ont tamponné l'eau de pluie simulée acide, cependant le pH du lexiviat a diminué avec le temps pour les deux lacs. Le méthyle de mercure a pu être mesuré dans le lexiviat des premiers épisodes de pluies (2-4ppt) seulement. Aucune relation entre le méthyle de mercure et le sulphate ou les sulphures n'a été trouvée. Les populations de BSR ont demeurées constantes. Le mercure (~70%) était retrouvé dans la fraction organique réfractaire et résiduelle des sédiments. Une partie du mercure des sédiments de SL et de BDL a été redistribuée à la phase mobile et immobile, respectivement. Ces résultats indiquent que le démantèlement des barrages hydro-électriques pourrait augmenter la production d'acidité et la distribution et la mobilité de mercure dans les sédiments tandis que le relâchement du méthyle de mercure sera bas.

Mots clés: sédiments, oxidation, mercure (Hg), méthyle de mercure (MeHg), extraction séquentielle

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1.0 INTRODUCTION AND LITERATURE REVIEW

1.1 Introduction

Metallic elements enter aquatic systems as soluble inorganic or organic complexes or as suspended particulate material (Buykx et al., 2000). Ultimately, a large portion of metals accumulates in the sediment. Soluble metals may become sequestered in the sediment by complexation or adsorption to biogenic organic particles, including algae or biological debris, and inorganic particles, such as iron and manganese oxides, alumino-silicates (clays), and insoluble metal sulphides (Calmano et al., 1993, Chapman et al., 1998, Stumm and Morgan, 1996, and Preda and Cox, 2001). In sediments, metals will be partitioned between aqueous or solid phases.

Sequestration of metals, as a result of complexation and sorption reactions in sediments, depends on many physico-chemical factors including solubility, pH, redox potential, affinity for organic carbon, sediment grain size and sediment mineral composition (such as oxides of iron or manganese and acid volatile sulphides) (Ingersoll, 1995). The potential for metals to leach from sediment to the surface water column, ground water, or downstream, decreases with the degree of complexation (van den Berg, 1998, 2001a, and 2001b). Although metals may be sequestered, there are several factors that may increase their release from sediments and their bioavailability. Disturbance of the sediment by natural processes or by human activities may remobilize metals (Farrah and Pickering, 1993; Saeki et al., 1993). These may include water level variations, sediment dredging, decommissioning of hydro-reservoirs, water draw down, etc.

The exposure of natural sediments to air, as a result of natural and human activities, can change physico-chemical conditions of sediments and potentially affect the stability of certain metals. For example, exposing submerged anoxic sediment to air will cause them to dry and oxidize, altering redox conditions, and thereby affecting abiotic and biotic properties of the substrate. This will then change the solubility, bioavailability, and mobility of compounds within the substrate (Saeki et al., 1993, Baldwin et al., 2000 and Baldwin and Mitchell, 2000). Oxidation of sediments may increase or decrease the release of certain dissolved and weakly adsorbed metals if the substrate is flushed with water, in addition to changing the sediment chemical phase distribution of elements (Preda and Cox, 2000 and 2001 and Dollar et al., 2001). Activity of *in situ* microorganisms will be altered and anaerobic bacteria that thrive in oxygen free sediments will be replaced by aerobic microbes (Chapman et al., 1998 and Baldwin et al., 2000).

Many studies have shown that certain trace and heavy metals undergo changes in sediments following water fluctuation, although previous studies have not given much consideration to mercury (Saeki et al., 1993, Preda and Cox, 2000 and 2001, Dollar et al., 2001, Stephens et al, 2001a and 2001b). In order to predict mercury behavior in sediments that are submitted to water level fluctuations, such as in the case of decommissioning hydro-dam reservoirs, the present study investigates biogeochemical processes affecting mercury when natural sediments from such reservoirs are submitted to wetting and drying events. The focus of the study is on mercury and methylmercury cycling, in addition to the activity of sulphate-reducing bacteria, known methylators of mercury which thrive in anoxic environments (Compeau and Bartha, 1985 and King et al., 2002). Acid generation in the sediments and the cycling of sulphur, specifically sulphates and sulphides, in water leached from sediments, will also be assessed. The following section discusses the current literature on the topic.

1.2 Dams, their uses, environmental impacts, and the removal debate

Dams have been constructed in rivers for several reasons. Hydroelectric dams provide renewable, inexpensive and efficient power generation for communities (Pringle, 2001). Dams offer effective flood control during wet seasons and can be used to create a water supply for both irrigation of agricultural lands and drinking water (Poff and Hart, 2002). The creation of a reservoir also provides recreational opportunities such as fishing and water sports (Bednark, 2001).

Although there are many socio-economic benefits from dams, their presence may cause ecological impacts. The most apparent impact is the alteration of natural water flow through rivers and watersheds, which changes the biological and physical characteristics of the ecosystem. Dams fragment communities of rivers, inhibiting the exchange of sediment, nutrients and organisms between the bodies of water (Pringle, 2001). The migration of various organisms may be impeded or stopped completely (Kanehl et al., 1997). For example, upstream migration of Atlantic salmon to their preferred spawning beds is often blocked by dams (Lovett, 1999). Organisms that pass through turbines of dams may be harmed or killed, such as eels at the Cornwall dam between the St. Lawrence River and Lake Ontario (D. Lean, personal communication). River ecosystems are locally changed into reservoir ecosystems, causing shifts in species composition (Power et al., 1996). Over the past few years, much debate has addressed whether dams should be removed in order to restore the natural river ecosystem. Throughout North America, many dams have deteriorated over the years, and are no longer economically viable and have a high maintenance costs (Bednark, 2001).

Few studies have fully addressed socio-economic, ecological and environmental impacts of dam removal (Babbitt, 2002 and Bednark, 2001). As dams are removed, slow moving lake-like habitat (lentic) behind dams becomes free-flowing river habitats (lotic), which can drastically change the watershed (Babbit, 2002). The habitat of fish or birds that nest along the shores will be disturbed (Kanehl et al., 1997). Communities that have established homes or businesses that depend on the waterway may have to relocate (Tiner, 1998 and Babbit, 2002). Physical changes may include sediment transport, decreased water temperature, erosion, alterations in flow regimes, decreases in wetlands, and potential decreases in groundwater recharge (Hart et al., 2002). After years of storage of fine-grained sediments, dam removal will result in sediment re-suspension and transport, as river flow is returned. The re-suspended sediment will experience turbulence and abrasion in the water column, possibly releasing sediment-bound contaminants (Verneiu, 1997, Hart et al., 2002 and Poff and Hart, 2002). The extent of environmental damage will depend on the quality and quantity of sediment accumulated by the dam (Poff and Hart, 2002). Therefore, due to the many socio-economic and environmental factors associated with dam removal, the benefits and risks must be considered before dams are removed (Babbit, 2002). The present study focuses on impacts of exposing once submerged sediments to air if dams are removed.

1.3 Sediment exposure to air and geochemical impacts

When sediment is exposed to the atmosphere, two different physical effects occur. Firstly, oxygen content in sediment will increase, and secondly, the sediment moisture content will decrease (Baldwin and Mitchell, 2000). The top portion of anoxic sediments will become oxygenated very quickly, followed by oxygen diffusion to the deeper parts of the sediment, which is a drastic change from when it was covered by water and under anaerobic conditions (Saeki et al., 1993). Decreases in pH and alkalinity, increased nutrient content and increased material transport in runoff from dried sediment have been observed in several studies (Westling, 1991, Saeki et al., 1993, Baldwin, Mitchell and Rees, 2000 and Preda and Cox, 2001).

1.3.1 Effect of pH and redox changes

As sediment dries, speciation and mobility of toxic compounds, such as metals, may undergo several changes. Two main factors affect the mobilization of metals in sediments; pH and redox potential (Peverly and Kopka, 1991, Baldwin and Mitchell, 2000 and Perrin et. al., 2000). Both factors are greatly affected when sediment is exposed to air and consequently dried and oxidized. Sediment oxidation may increase the production of hydrogen ions, causing the pH to decrease (Calmano et al., 1993). As pH decreases, metals may be released into solution (Drever, 1997). Table 1.1 depicts common types of acid producing reactions that occur in aquatic system upon oxidation. The oxidation of inorganic and organic sulphur, iron and nitrogen species present in sediment or water systems are the most influential producers of hydrogen ions (Calmano et al., 1993 and Christensen, 1998). Approximate redox potential values for transformations of sulphur, iron and nitrogen species are - 75 to -150 mV, 120 mV and 250 mV, respectively (Mitsch and Gosselink, 1993).

The extent of acidification is modified by the buffering capacity or acid neutralizing

Table 1.1. Important acid producing oxidation reactions in aquatic systems (Calmano et al., 1993).

Elements		Reactions
Inorganic	S	$H_2S + 2\ O_2 = SO_4^{2-} + 2\ H^+$
	S	$S^o + 3/2\ O_2 + H_2O = SO_4^{2-} + 2\ H^+$
	S, Fe	$FeS + 9/4\ O_2 + 3/2\ H_2O = FeOOH + SO_4^{2-} + 2\ H^+$
	S, Fe	$FeS_2 + 15/4\ O_2 + 5/2\ H_2O = FeOOH + 2\ SO_4^{2-} + 4\ H^+$
	Fe	$Fe^{2+} + 1/4\ O_2 + 5/2\ H_2O = Fe(OH)_3 + 2\ H^+$
	N	$NH_4^+ + 2\ O_2 = NO_3^- + H_2O + 2\ H^+$
	N	$NO_x + 1/4(5-2x)\ O_2 + 1/2\ H_2O = NO_3^- + H^+$
Organic	N	$R\text{-}NH_2 + 2\ O_2 = R\text{-}OH + NO_3^- + H^+$
	S	$R\text{-}SH + H_2O + 2\ O_2 = R\text{-}OH + SO_4^{2-} + 2\ H^+$

capacity of the sediment. Presence of compounds such as $CaCO_3$, Al_2O_3 and Fe_2O_3 have the ability to neutralize, or buffer, the acidity in soils or sediment (Calmano et al., 1993). If the buffering capacity of the sediment does not neutralize the acidity generated by acid-producing reactions such as those in Table 1.1, the pH of the sediment will decrease, and metal dissolution and mobilization may occur (Baldwin and Mitchell, 2000).

Redox potential is the second main factor that affects the mobilization of metals in the sediment (Peverly and Kopka, 1991 and Baldwin and Mitchell, 2000). Redox potential, a measure of the electron concentration, or availability, in a solution, determines the tendency of soils or sediments to oxidize or reduce elements or compounds (Mitsch and Gosselink, 1993). Variations in oxygen supply and consumption in sediments will cause fluctuations in redox potential, or oxido-reduction potential (van den Berg et al., 1998). The oxic layer in sediment, referred as the oxidation zone, contains oxygen and is usually very thin, only a few millimeters thick (Stumm and Morgan, 1996). As the concentration of oxygen is fully depleted deeper in the sediment profile, sediment will become anoxic, known as the reduction zone (Mitsch and Gosselink, 1993).

The oxic/anoxic boundary in sediments control many chemical transformations. For example, S, Fe, Mn cycles are greatly affected by redox changes (Mitsch and Gosselink, 1993). The oxic/anoxic boundary also affects the speciation and bioavailability of metals (Farrah and Pickering, 1993; Saeki et al., 1993). For example, the oxidation of metal sulphides to metal sulphates releases metals bound to the surfaces of sulphides, increasing the mobility and bioavailability of the particular metals, in addition to decreasing the pH as hydrogen ions are produced (Astrom, 1998, Stephens et al, 2001a).

1.4 Chemical fractionation and speciation of metals

It is evident that variations in pH and redox potential can have a great impact on metal mobility, especially the speciation of metals. Total metal content of sediment may give insight to the potential toxic risk, but the speciation of elements will determine the toxicity, reactivity, mobility and bioavailability (Buykx et al., 2000). Metals sequestered in sediments or soils can be distributed in the following chemical phases; water soluble, exchangeable, carbonates, iron oxides, manganese oxides, sulphides, organic matter, pyrites, and lattices of primary and secondary minerals (Saeki et al., 1993). Some phases bind metals more strongly than others, depending on the binding capacity of the particles and their affinity for certain elements while other phases are affected to a greater extent by redox fluctuations or changes in pH (Dollar et al, 2001). Sequential chemical extraction is a procedure developed to determine the partitioning of metals among sediment or soil fractions/phases. This may provide insight into which phases will be released under different environmental conditions, such as changes in pH or redox potential (Tessier et al., 1979).

Various sequential chemical extraction procedures have been developed to measure the chemical phase distribution of metals. Most procedures use similar techniques whereby metals can be separated into 4 or 5 operationally defined geochemical phases (Tessier et al., 1979). The procedures involve applying chemical leachates of increasing strengths and measuring the quantity of each element extracted during each step (Buykx et al., 2000, Mulligan et al., 2001). It is widely held that the technique leaches metals in the order of decreasing bioavailability and mobility (Tessier et al., 1979). Modifications to the extraction procedures are necessary depending on the composition of the sediment or soil, as the partitioning of metals can differ between substrates with high organic matter versus high clay content, due to their different binding capacities (Buykx et al., 2000, Dollar et al., 2001, and Mulligan et al., 2001). Table 1.2 identifies the chemicals used in a typical Tessier-type sequential extraction procedure.

The exchangeable phase (Table 1.2) is thought to represent trace metals associated to clays, hydrated oxides of iron and manganese, or humic acids. Metals in this fraction are the most loosely adsorbed to the sediment and easily released during slight changes in water ionic composition (Tessier et al., 1979). The carbonate fraction contains metals present in carbonate minerals and metals bound to their surfaces. Metals in this fraction are largely affected by pH changes (Tessier et al., 1979). Metals bound to iron and manganese oxides (also known as the reducible phase) are affected by changes in redox potential. Iron and manganese oxides have high adsorption capacities and are strong scavengers for trace metals (Jenne, 1977 and Drever, 1997). They undergo reductive dissolution under anoxic conditions, releasing sorbed trace metals into solution (Fortin et al., 1993 and Tessier et al., 1996). The organic/sulphide phase includes metal sulphides and metals bound to organic matter and sulphide minerals under anoxic conditions. Increasing oxidizing conditions can release metals bound to this sediment fraction through degradation of organic matter and oxidation of metal sulphides (Tessier et al., 1979). Metals bound to the residual phase are mainly present in the lattice of primary and secondary minerals and are not released under natural environmental conditions. As such, these metals are unavailable to organisms. The residual fraction is the most stable sediment fraction (Tessier et al., 1979).

In terms of metal mobility, metals considered easily available are in the exchangeable phase and metal mobility decreases stepwise towards the residual phase

Table 1.2. Typical sequential extraction reagents and target extracted component (modified from Tessier et al., 1979).

Extracted Component	Reagents
Exchangeable	1 M $MgCl_2$, pH 7
Bound to carbonates	1 M NaOAc, pH 5
Bound to iron/manganese oxides	0.04 M $NH_2OH{\cdot}HCl$ in 25% (v/v) HOAc
Bound to organic matter /sulphides	0.02 M HNO_3, 30% H_2O_2, 3.2M NH_4OAc in 20% (v/v) HNO_3
Residual	5:1 concentrated HF – $HClO_4$ (Aqua Regia)

(Tessier et al., 1979, Saeki et al, 1993, Dollar et al., 2001, Stephens et al., 2001a and 2001b, and Hall and Pelchat, 2005). Concentrations of a metal within the various chemical phases will differ depending on its chemical form in the sediment, how it reacts under different redox conditions and under different pH ranges. The physico-chemical characteristics of a specific metal will determine how it will react during changes in environmental conditions within the sediment or water (Drever, 1997). As metals have different characteristics, the amount of metals bound to particular sediment chemical phases and how they react when environmental conditions change, will not always be the same.

Several studies have shown that sediment oxidation may cause some metals to mobilize during air exposure (Farrah and Pickering, 1993, Saeki et al., 1993, Buykx et al., 2000, Dollar et al., 2001, Stephens et al., 2001a and 2001b). As previously mentioned, iron and manganese oxides, organic matter and metal sulphides are highly affected by changes in redox potential (Tessier et al., 1979). It is expected that as sediments oxidize, metal concentrations associated with the organic/sulphidic phase will decrease as oxidized organic matter will degrade, causing the release of metals (Tessier et al., 1979). In contrast, metals associated with iron and manganese oxides will increase, due to their high adsorption capacity and strong ability to scavenge metals (Tessier et al., 1996 and Drever, 1997). Such trend was observed by Saeki et al. (1993), who studied the mobility of Cd, Cu, Pb, Zn, Fe and Mn in drying contaminated sediments. Concentrations of Cd, Cu, and Zn bound to iron and manganese oxides increased, whereas they decreased in the organic/sulphide phase. Saeki et al. (1993) also observed increases of these particular metals in the exchangeable phase, and decreases in the residual fraction. However, Fe and Mn concentrations increased in the organic/sulphide phase and decreased in the exchangeable and iron and manganese oxides phase (Saeki et al. 1993), indicating that not all metals react the same to changes in environmental conditions and redox potential. Other studies (Dollar et al., 2001 and Stephens et al. 2001b) have also shown metal mobility in oxidized sediments was a function of the chemical nature of the metals. In summary, changes in natural environmental conditions (pH, ionic composition, redox potential) will lead to some metal redistribution within sediment chemical phases. If there is a shift of metals to more available phases, such as the exchangeable phase, the once insoluble metals prior to oxidation will become more soluble and available, potentially mobilizing into the porewater, which could be detrimental to biota (Buykx et al., 2000, Dollar et al., 2001 and Stephens, 2001b).

1.5 Effects of "re-flooding" the sediment and metal mobilization

Dried sediments re-wetted by rain or floods can release metals into the water column. Preda and Cox (2001) showed sediments submitted to flood events after a dry period released substantial quantities of acid and Al, Fe, and Mn into lakes and rivers. Singh et al. (1998 and 2000) observed Cd, Cr, Cu, Ni, Pb, and Zn concentrations in runoff and in water percolated through sediments were higher for recently dredged and oxidized sediments, compared to surface soils. Tack et al. (1998) presented similar results, as porewater Cd, Cu, and Zn concentrations were higher in sediments submitted to alternating wet and dry regimes, compared to metal porewater concentrations in sediments that remained wet.

When dried sediment is flushed with water, a portion of the exchangeable metals will be released when sediments are rewetted (Saeki et al., 1993, Dmytriw et al., 1995, Dollar et al., 2001, Stephens et al., 2001a and 2001b). If sediments remain submerged, and anoxic conditions return, some fractions may stabilize or increase in metal content. The metals sequestered within the organic/sulphide phase are unlikely to be mobilized during rewetting events, and may be further stabilized (Dollar et al., 2001). Metals bound to the iron and manganese oxides (reducible phase) will dissolve under anoxic conditions, thereby releasing the bound metals (Drever, 1997). Whether the released metals will remain as soluble metals in the sediment or water column, or bind to organic or sulphide compounds, is difficult to predict. As most metals have an affinity to complex with organic matter or sulphide compounds under anoxic conditions, the metals will most likely bind to the organic/sulphide phase (Drever, 1997). Some metals can be leached from sediments if not adsorbed or complexed to sediment fractions, thereby increasing the metal load in the water column. The mineralogy of the sediment may also change, altering normal chemical and biological processes in the sediment, potentially affecting metal bioavailability (Baldwin et al., 2000).

The amount of metals leached out from rewetted sediment can vary over time (van den Berg et al., 1998 and Stephens et al. 2001b). Stephens et al. (2001b) used water leaches to observe the leachability of metals (Cd, Cr, Cu, Zn) from rewetted dried sediment over time. Initially, there was an increase in metal leachability followed by a decrease 4 to 5 weeks later. This was also observed in the runoff from dredged canal sediment from Singh et al (2000). Therefore, the concentrations of metals leaching out of sediments when they are re-flooded, will be high at first, but the return of anoxic conditions in the sediment will allow for metals to once again become sequestered and rendered unavailable. If the sediment remains flooded, it has been recorded that over time the sediment may regain the similar chemistry and microbiological properties as submerged sediment (Stephens et al., 2001b).

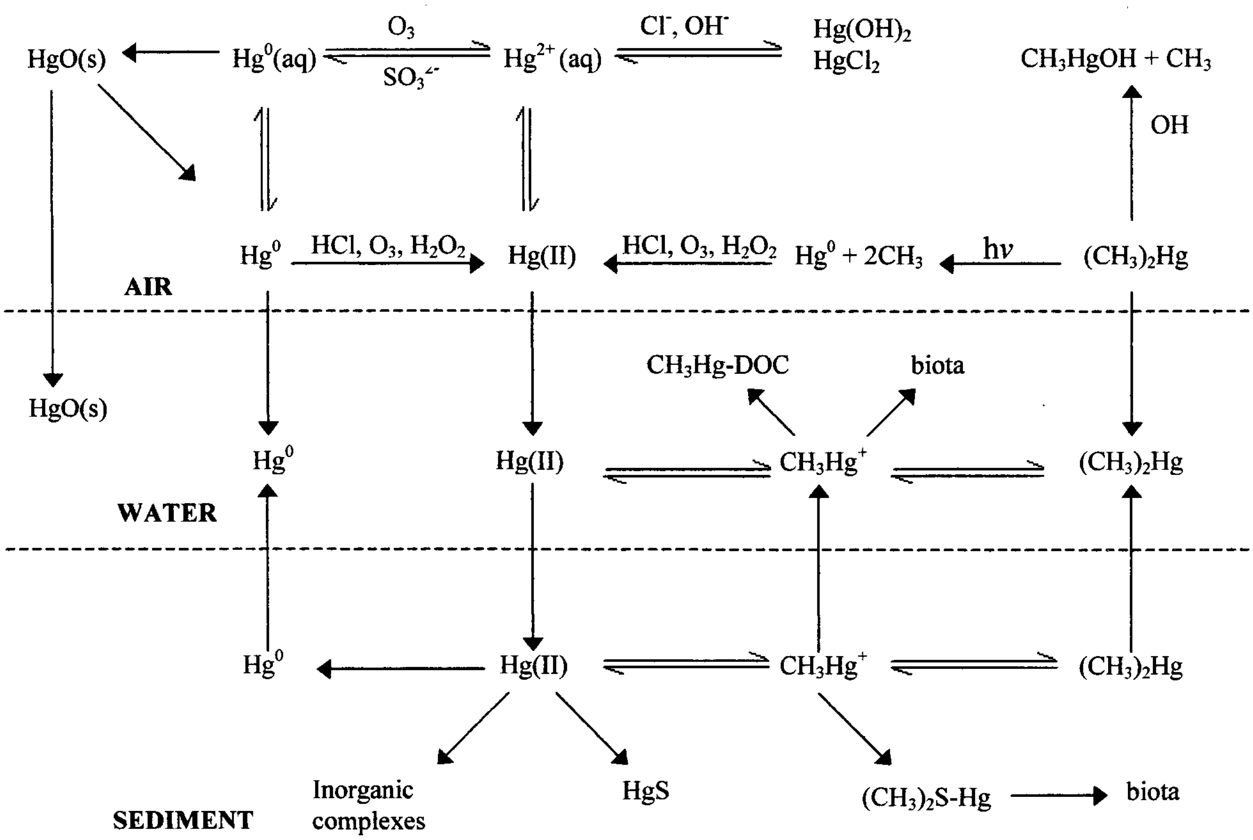
1.6 The effect of sediment wetting and draining cycles on mercury and methylmercury

1.6.1 Mercury in the environment

It has been well documented that newly created reservoirs have increased dissolved mercury concentrations in water and toxic levels of mercury within organisms that often exceed the amounts in natural freshwater systems by five to six times (Tremblay et al., 1993 and Verdon et al., 1991). Mercury, which is one of the most toxic metals, is highly toxic to all organisms and a pervasive pollutant within the environment (Morel, 1998 and Regnell et al., 2001). Mercury is naturally emitted into the environment by terrestrial and oceanic fluxes, although anthropogenic emissions have become very important (Mason et al., 1994 and Morel, 1998). Humans have tripled their emissions of mercury into the environment over the past century (Mason et al., 1994 and Morel, 1998). Due to the toxicity of the metal, the increased anthropogenic emissions and the elevated concentrations of mercury present in reservoirs, mercury has become a contaminant of great interest and importance.

Mercury may be in various chemical forms in the environment, although it is its speciation which determines its bioavailability and toxicity (Tremblay et al., 1993 and Stein et al., 1996). Mercury, which can be a gas or liquid at room temperature, occurs in three oxidation states: elemental mercury (Hg^0), mercurous mercury (Hg^{1+}) and mercuric mercury (Hg^{2+}) (Morel et al, 1998). Hg^0 (g) is the most common form of mercury in the atmosphere, while Hg^{1+} is unstable and not commonly found in the environment. Hg^{2+} is the form most commonly found in aquatic systems, and tends to form many stable organic and inorganic compounds (Tremblay et al., 1993). Mercury can also be in the organic forms of monomethylmercury, dimethylmercury, and ethylmercury. Figure 1.1 illustrates typical transformations of mercury in an aquatic system.

Figure 1.1. Mercury cycle in an aquatic system, illustrating the most important transformations and distributions pathways in air, water and sediment. Note: Dashed lines represent boundaries separating environmental compartments in the typical aquatic system. (modified from Stein et al., 1996)



$HgO(s)$
$Hg^0(aq)$
O_3
SO_3^{2-}
$Hg^{2+}(aq)$
Cl^-, OH^-
$Hg(OH)_2$
$HgCl_2$
$CH_3HgOH + CH_3$
OH
Hg^0
HCl, O_3, H_2O_2
Hg(II)
HCl, O_3, H_2O_2
$Hg^0 + 2CH_3$
hν
$(CH_3)_2Hg$
AIR
$HgO(s)$
CH_3Hg-DOC
biota
Hg^0
Hg(II)
CH_3Hg^+
$(CH_3)_2Hg$
WATER
Hg^0
Hg(II)
CH_3Hg^+
$(CH_3)_2Hg$
Inorganic complexes
HgS
$(CH_3)_2S$-Hg
biota
SEDIMENT

1.6.2 Mercury in sediment

When Hg enters a natural freshwater system, a substantial amount accumulates in sediments (Stein et al., 1996 and Remy et al., 2003). A majority of the particulate-bound Hg will settle to the bottom of a lake, while dissolved Hg may directly adsorb to the bottom sediments or be scavenged by microscopic organisms, phytoplankton (Regnell, 2001 and Remy et al., 2003). Most mercury in sediment (50-75%) is bound to organic matter, especially organic ligands containing sulphur (S) functional groups (Beldowski and Pempkowiak, 2003, Morel et al, 1998 and Schuster, 1991). Hintelmann et al. (1995) states that Hg may bind to organic matter exclusively by S-ligands. In anoxic sediment, Hg^{2+} has a high affinity for sulphide, the reduced form of S, forming insoluble HgS and polysulphides (Wallschlager et al., 1996, Morel et al, 1998, and Zhang et al., 2004). The remaining Hg may be bound to the inorganic fraction, such as clay minerals, metal oxides or sulphides. Once Hg is within the sediment, its speciation depends on the pH, redox, microbial activity and sediment composition (Stein et al., 1996)

1.6.3 Methylmercury

Of the organic forms of mercury, monomethylmercury is the most abundant and toxic form of organic Hg (Tremblay et al., 1994). Monomethylmercury, commonly referred as methylmercury (MeHg), biomagnifies in food chains and is a known neurotoxin (Pak and Bartha, 1998). The lipophilic toxin is produced from methylation of Hg^{2+}, by abiotic or microbial processes (Stein et al., 1996 and Morel et al., 1998). Although there are several ways MeHg can be produced, microbial transformation in anoxic sediments is considered the most significant means, which is shown in the following reaction (Berman and Bartha, 1986 and Gilmour et al., 1992).

$$CH_3Co\,[B12] + Hg^{2+} + H_20 \rightarrow (H_20Co\,[B12])^{+} + CH_3Hg^{+} \qquad (1)$$

where Co is Cobalamine, (an enzymatic catalyst) (Tremblay et al., 1994). Mercury methylation may occur in the water column, but at a much slower rate compared to in sediments (Matilainen et al., 1991).

Several microorganisms from several taxonomic groups can methylate mercury, although sulphate-reducing bacteria (SRB) are thought to be primary mercury methylators in freshwater and estuarine anoxic sediments (Compeau and Bartha, 1985 and Macalady et al., 2000). Various factors can change the rate of MeHg production in sediments. For example, sulphate concentrations up to 200-500 μM in both field observations and laboratory experiment increased methylation rates compared to typical background levels in lakes, while higher sulphate concentrations decrease methylation rates (Compeau and Bartha, 1985, Gilmour and Henry, 1991 and King et al., 2002). Sulphide concentrations above the micromolar levels are known to inhibit MeHg production, as sulphides will compete for inorganic mercury, forming strong aqueous complexes of HgS, resulting in less Hg^{2+} available for methylation (Berman and Bartha, 1986, Gilmour and Henry, 1991, Gilmour et al., 1998 and King et al., 1999).

1.6.4 Mercury, methylmercury and reservoirs

The creation of reservoirs, causing terrestrial soils and vegetation to be flooded, has been observed to increase levels of Hg and MeHg in the sediment and water column (Wright and Hamilton, 1982, Tremblay et al., 1993, Rudd, 1995, Kelly et al., 1997, Rosenberg et al., 1997 and Diamond et al., 2000). Many studies have observed that MeHg levels significantly increase in fish (Bodaly, R. A. et al., 1984, Johnston et al., 1991, Morrison and Therien, 1991, Verdon, et al. 1991, Kelly et al., 1997 and 1995) and zooplankton (Paterson et al., 1998) within newly created hydroelectric reservoirs in various parts of northern Canada. Several fisheries in northern Ontario have closed due to MeHg contamination (Bodaly, et al., 1984).

As reservoirs are created, low lying land is flooded, causing soil and vegetation to be submerged. As the flooded soil and plant matter decomposes, naturally present inorganic Hg can be released into the substrate or water (Schuster, 1991, Porvari et al., 1995 and Wallschlager et al., 1996). The simultaneous release of high amounts of organic matter and nutrients has been known to increase the rates of mercury methylation as it creates a favourable environment for increased bacterial growth and activity (Bodaly et al., 1984 and Morrison and Therien, 1991). Studies have found that Hg levels may remain above background Hg concentrations for several years after filling a reservoir (Abernathy and Cumbie 1977 and Cox et al., 1979), or for decades in the case of northern Canada (Bodaly et al., 1984). All of these factors easily facilitate the production of MeHg, and depending on factors such as the area flooded and the type of vegetation, concentrations of MeHg can be substantially higher than previous ambient levels of MeHg (Johnston et al., 1991 Rudd, 1995 and Verdon et al., 1991).

Drying and oxidation of reduced sediments may be unfavorable to MeHg formation because it reduces or halts SRB activity, as they require anoxic environments to function and facilitate the production of MeHg (Hao et al., 1996). Oxidation and desiccation of sediment may have a profound effect on many microbial communities in the sediments

(Baldwin and Mitchell, 2000 and Fierer et al., 2002). Anaerobic microbe activity will decrease, and over time they may undergo sporulation or die (Baldwin et al., 2000). Decreases in microbial activity may stop the cycling of nutrients and assimilation of trace metals that normally occur in sediments with oxic/anoxic zones. This may cause trace metals to remain mobile in sediments (Baldwin et al., 2000). It has also been observed that during times of high microbial mortality, many nutrients such as N and P, may be released and leached from lake sediments as they are re-wetted (Qui and McComb, 1996). In the case of MeHg, oxic conditions may stop the activity of bacterial methylators resulting in reduced MeHg production as the sediment dries.

Although MeHg production may be stopped or reduced, there may be the risk of MeHg, in addition to other Hg species, being flushed or leached from sediment as it is rewetted by precipitation or raised water levels. Westling (1991) showed different amounts of both total Hg and MeHg were present in runoff from drained and undrained wetlands. Drainage and oxidation of deep, organic soils in wetlands led to increased MeHg concentrations in water leached from drained wetland sediments compared to undrained wetland sediments (Westling, 1991). Oxidation and drying of organic soils may change wetlands from Hg sinks to Hg sources. Therefore drained wetlands are no longer receiving and storing Hg, but releasing it, often in the toxic form of MeHg. It is then possible that exposed reservoir sediments can release MeHg and Hg as the substrate is flushed with water (Westling, 1991).

1.7 Thesis objectives and hypotheses

From the above literature review it is known that various biogeochemical changes can occur as sediments dry and oxidize. Such increases in redox can be caused by either dam removal or climate change induced drought. Changes in chemistry, mineralogy, and microbial community structure in the sediment will occur (Baldwin et al., 2000). Oxidation of sediment may decrease the pH of substrates, causing mineral dissolution and metal mobilization from the sediment (Chapman et al., 1998 and Christensen, 1998). Because reservoirs are known to accumulate metals, especially mercury, in the sediments it is important to be able to better predict biogeochemical processes regulating the cycling of metals in exposed sediments prior to a dam removal project being undertaken (Hart et al., 2002). Predicting changes in metal speciation and chemical phase distribution in sediment is also important to assess the ecotoxicological response to changes in metal bioavailability following wet and dry events caused by anthropogenic or natural events.

With the above considerations in mind, the main objectives of the present study are:

1. Determine chemical (pH, sulphate, and sulphide) and microbial (sulphate-reducing bacteria) changes that occur when sediments are dried and oxidized as a result of wetting and drying events, and if the natural buffering capacity of sediments (from 2 different lakes) impacts these chemical and microbial changes.
2. Assess if the chemical and microbial changes that occur during wetting and drying cycles affect methylmercury release from the sediments over time.
3. Measure changes in MeHg concentrations and the chemical fractionation of total mercury in sediments that are dried and re-wetted.

Assessment of these processes throughout this study will allow for the following hypotheses to be tested:

1. pH of water drained from oxidized cores will decrease over time, and to a greater extent in sediments with a lower buffering capacity.
2. Total mercury and methylmercury, in sediments submitted to wetting and drying cycles, will become more mobile and bioavailable by redistributing into more mobile chemical phases.
3. More MeHg will be released from Stump Lake sediments due to the high amount of decomposing vegetation in the lake.
4. Sulphate-reducing bacteria populations will decrease over time.

Material for this project, (i.e. sediment cores, porewater, etc.), was also used by Michelle Nugent, a Master's of Earth Science candidate at the University of Ottawa. Miss Nugent and I shared the workload and in consequence the data (pH, Eh, sulphide, sulphate, sediment moisture and organic matter content).

2.0 MATERIALS AND METHODS

2.1 Study sites

Two hydroelectric dams reservoirs, Stump and Black Donald Lake, were sampled for this study. The choice of study lakes/reservoir was based upon the age and size of the reservoir, bedrock composition (buffering capacity) and proximity to Ottawa. Both lakes are located approximately 150 km west of Ottawa, Ontario.

Stump Lake (44°95' N, 76°63' W), also known as Smiths and Thompsons Bay, is located along the Mississippi River, 35 km southwest of Perth, ON. The lake is shallow, with a water depth of less than 3 m in most areas. It was formed where the river valley was flooded when the dam at High Falls Generating Station was established in the 1920s. Because trees were not cut down and removed before the dam was installed, there are now stumps and logs which litter the lake floor. Due to the great amount of vegetative debris, the sediments are highly organic at all sites, often composed of partially decomposed wood. The surrounding bedrock is composed of "Lavant Gabbro Complex", with granite, gabbros and diorites, which contribute to the moderate buffering capacity of the lake (Pauk, 1989).

When the Mountain Chute Generating Station dam was installed in 1967, two adjacent lakes, Centennial and Black Donald Lake, were joined and called Black Donald Lake (45°14' N, 76°55' W). This lake is located along the Madawaska River, 35 km northeast of Renfrew, ON. Black Donald is much deeper than Stump Lake (~ 46 m) and the sediment has much less organic matter. The sediment which consists of fine to coarse sand grains which increases in silt with depth. The lake overlies a carbonate-rich metasediment bedrock, resulting in a well buffered system (Lumbers, 1982).

2.2 Field sampling

2.2.1 Sediment sampling

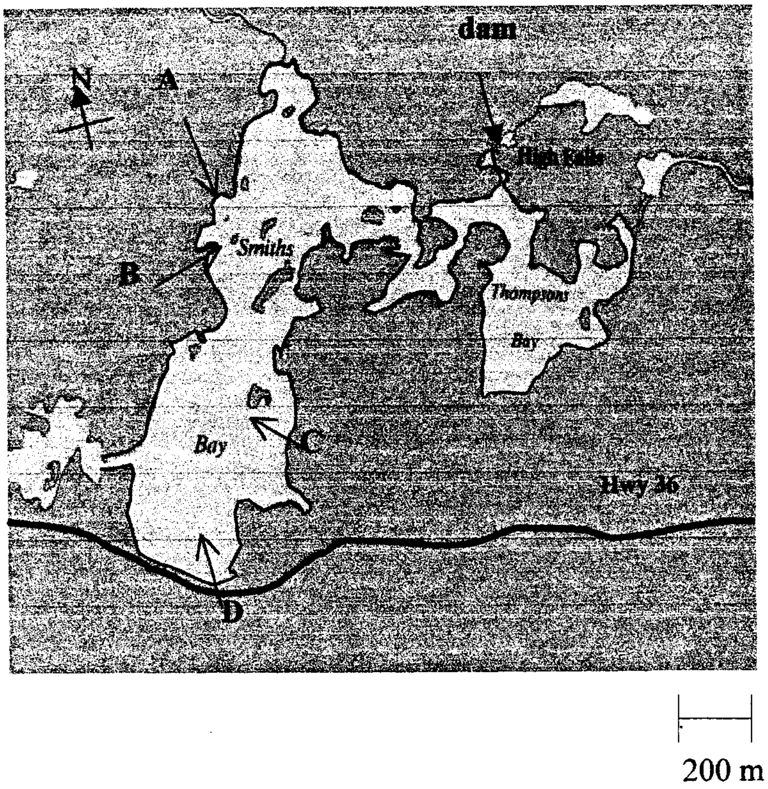
In August and September of 2002, four study sites were selected in each lake to obtain four replicate sediment cores for the wetting and drying experiment, which will be explained in detail in section 2.3 (Figure 2.1). The sediment cores were extracted at similar water depths (2-3 m). Visually, the study sites within each lake had similar shorelines, sediment type, and lake bottom vegetative properties for most sites, which are described in Table 2.1. Sampling was focused around the shoreline, as shallow sediments are most likely to be exposed after dam removal. All sediment collection was done from a boat that was anchored at both ends to reduce movement and stirring of the sediment.

The sediment cores were obtained with a modified Kayak-Brinkhurst® corer and stored in Lexan® polycarbonate plastic tubes (60 cm in length and 7 cm in diameter) with black rubber stoppers covered with a layer of Teflon® to prevent trace metal contamination. Of the four replicate sediment core samples from each site, two of the polycarbonate plastic tubes had 5 vertical sampling ports (1 cm in diameter), 2.5 cm and 90° apart along the core (Figure 2.2). The position of the sample ports are as follows; sample port a was located 2.5 cm above the sediment-water interface, port b at the sediment-water interface, and the remaining ports c-e located at 2.5 cm, 5 cm and 7.5 cm below the sediment-water interface respectively. The sample ports would facilitate water sampling during wetting events, which will be explained in more detail in section 2.3 (Figure 2.2). Silicone plugs covered the sampling ports, and were secured with duct tape during sampling.

Approximately 20 cm of sediment plus a similar amount of overlying water was collected in each core. The sediment core was positioned to assure the sediment-water

Figure 2.1. Geographical locations of sampling sites in Stump Lake (a) and Black Donald Lake (b).

a)


dam
N
A
High Falls
B
Smiths
Thompsons
Bay
Bay
C
Hwy 36
D
200 m

b)

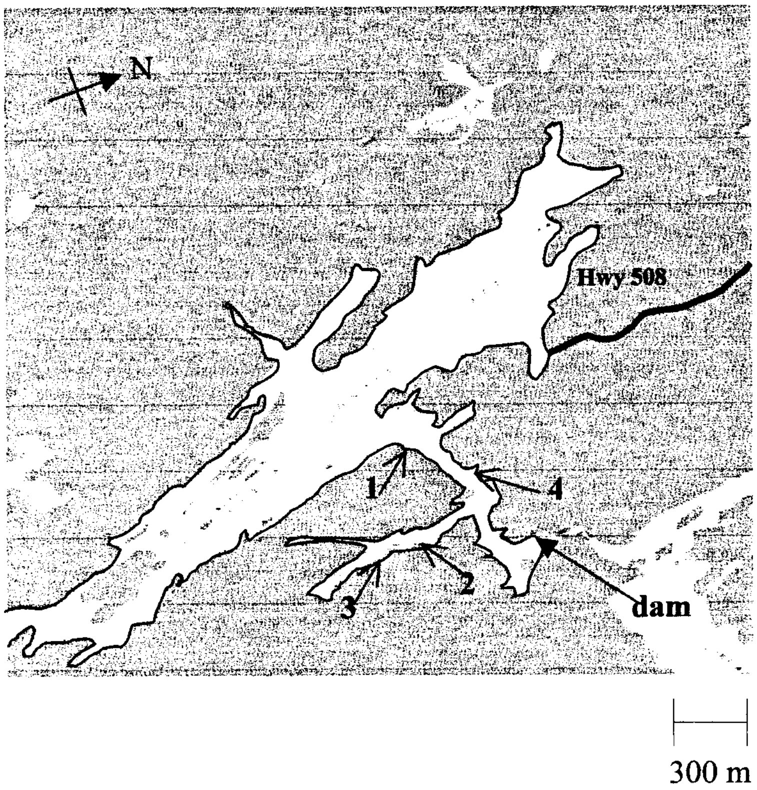
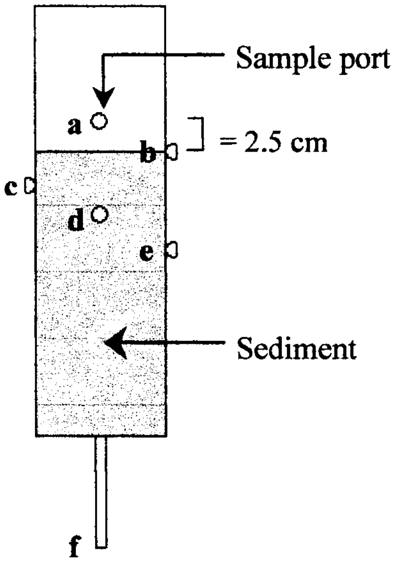

N
Hwy 508
1
4
2
3
dam
300 m

Table 2.1. Physical and vegetative characteristics of the sample sites in Stump and Black Donald Lakes (Note: these are only visual observation).

Lake	Site	Depth (m)	Position and site characteristics	Sediment visual characteristics
Stump	A	2	Set in a small bay away from the underlying river current	Highly organic sediment, large protruding tree stumps litter this site; abundant tree litter and submersed vegetation
	B	3	Just to the side of the river current	Sandy to clayey sediment with little to no tree litter
	C	2	In open area of lake; lightly affected by river current	Highly organic sediment with abundant tree litter and large tree stumps and some submersed vegetation
	D	2	In open area of lake nearest to boat launch; lightly affected by river current	Highly organic sediment with large abundant tree litter and submersed vegetation
Black Donald	1	2.5	In a small bay away from wind and river current	Fine to coarse grain sandy sediments with increasing silt composition with depth, lakebed littered with large rocks; minimal submersed vegetation
	2	2	Small indentation in small island just off the shore	Fine to coarse grain sandy sediments with increasing silt composition with depth, small to medium rocks litter lakebed; minimal submersed vegetation
	3	2	Very small bay	Fine to coarse grain sandy sediments with increasing silt composition with depth, few rocks; large tree stumps that were cut on shoreline, submersed vegetation
	4	3	In a small sheltered bay away from wind and river current, small gravel beach	Fine to coarse grain sandy sediments with increasing silt composition with depth, few rocks; submersed vegetation

Figure 2.2. Diagram of a sediment core and the position of the sampling ports a to f (in bold located on the left side of a sample port) along the core.


Sample port
a
= 2.5 cm
b
c
d
e
Sediment
f

interface would be located at the second sampling port, port b (Figure 2.2). The polycarbonate plastic tubes were then covered with Parafilm® and plastic caps to prevent water loss, and covered with aluminum foil to protect the samples from light. The sediment cores were kept cool (in coolers with ice) and positioned upright until they were transported by van to the laboratory, for subsequent 4°C storage.

2.2.2 Lake water sampling

Water samples were collected with a Van Dorn water sampler from each of the four sites from the two lakes to determine lake water chemistry (section 2.3). All samples were collected in high density polyethylene (HDPE) bottles which were previously cleaned by 10% concentrated HNO_3 rinse, followed by rinsing three times with deionized water, except for those used for methylmercury (MeHg) analysis. Bottles for lake water MeHg analysis were cleaned by rinsing three times with deionized water, followed by 125 ml of 1% HCl and 2.5 ml of mixed brominating reagent (0.05 M KBr: 0.1 M $KBrO_3$) (2%) until sampling event (Cai et al, 1996). Upon sample collection, 500 µl of hydroxylamine hydrochloride was added to the bottles to remove the bromine, followed by rinsing three times with deionized water. All bottles were rinsed three times with water from the sampling site before collection.

For MeHg analysis, one liter samples were collected in HDPE 1L bottles, and acidified with concentrated trace element grade HCl to 0.5%, to preserve mercury speciation. Water was collected for pH, Eh and total alkalinity were measured on site while waters for conductivity were measured in the lab (section 2.4). The preserved samples were kept at 4°C until analyzed.

2.2.3 Porewater

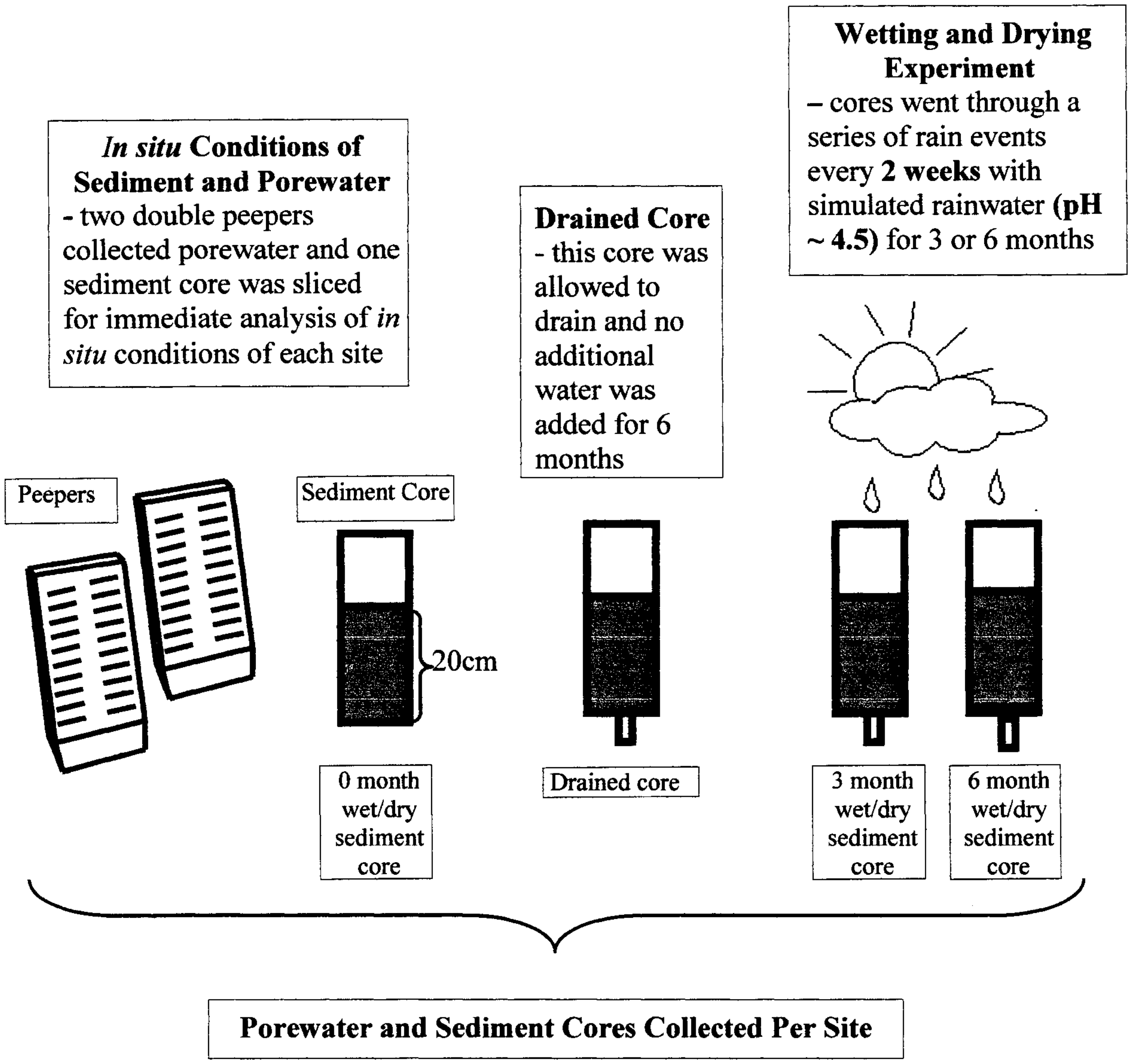
The collection of sediment porewater was facilitated with a pair of double peepers (composed of Lexan® with a Gelman HT 200 membrane 0.2 μm) installed at each sampling site (Carignan, 1984). The peepers, containing degassed ultra-pure deionized water, were installed by qualified divers, and positioned to collect porewater between an approximate depth of 20 cm below and 5 cm above the surface of the sediment. The peepers remained in the sediment for 3 weeks, at which point the porewater was quickly collected, with the use of sterile, purged syringes. On site, pH and Eh were measured, while another set of samples were preserved for sulphide measurements upon arrival at the laboratory (section 2.4). The remaining porewater samples were preserved with concentrated trace element grade HNO_3 to 0.1 ~ 1.0% for later sulphate analysis. The MeHg samples were preserved with concentrated trace element grade HCl to 0.5%. All preserved samples were kept at 4°C and away from light until analyzed.

2.3 Wetting and drying experiment

2.3.1 Experimental set-up

Once in the laboratory, from the four sediment cores collected per site, one core per site was sectioned in 2 cm increments (0-16 cm), to determine various characteristics of the original sediment cores and *in situ* conditions such as soil moisture, organic matter, sulphate-reducing bacteria populations, MeHg, and total Hg by sequential chemical extraction (see sections 2.5 and 2.6 for analytical methods) (Figure 2.3). Of the remaining three sediment

Figure 2.3. Diagram of experimental design for the sediment cores and porewater collected for each site.



***In situ* Conditions of Sediment and Porewater**
- two double peepers collected porewater and one sediment core was sliced for immediate analysis of *in situ* conditions of each site

Drained Core
- this core was allowed to drain and no additional water was added for 6 months

Wetting and Drying Experiment
– cores went through a series of rain events every **2 weeks** with simulated rainwater **(pH ~ 4.5)** for 3 or 6 months

cores per site, one core, referred as the "drained core", was allowed to drain from the bottom and remained in this state (i.e. no additions of water and left to dry under the normal/ambient lab conditions) for the entire six months study. The two remaining sediment cores were reserved for the wetting and drying experiment, where the sediment cores were submitted to a series of wetting and draining events every two weeks for 3 or 6 months (Figure 2.3), which will be further discussed in section 2.3.2.

Before the experiment began, the cores were attached to a lab-frame and exposed to ambient room temperature and light. The cores were covered with Parafilm® with approximately 15 holes (0.25 cm in diameter) pierced through the top to allow air circulation but to limit particle contamination from the ambient air. Aluminum foil covered the sides of the core to prevent the sediment from being exposed to the light. For the two cores per site used for the wet/dry cycles, modified 5 cm long stainless steel needles (length 1½ inches, size 18 gauge with the plastic tips removed) were inserted into the silicone plugs in the sampling ports (ports a-e), assuring that 1.5 cm of the end of the needle remained outside the core. The tips of the needles were covered with Teflon® tape to stop the flow of water (Figure 2.2). A vinyl tube (1 cm in diameter) was inserted into the bottom of the core plug, to facilitate sampling from the bottom of the sediment (port f). The tube was clamped in between sampling events, to prevent water flow from the cores and oxygen from penetrating the sediments.

Before the wet/dry cycles began, the cores were drained of their lake water from initial sampling (October 4, 2002 for Stump Lake and October 10, 2002 for Black Donald Lake). Water was siphoned off the surface of the cores while the remaining water was collected along the side sampling ports (ports a-e) and the bottom of the cores (port f) and preserved for sulphate and sulphide, and for MeHg from port f (section 2.4) (Figure 2.2). pH of the leachate (i.e., water collected along the side and at the bottom of the core) was measured immediately. The cores were drained down to 10 cm below the sediment-water interface to mimic the level the water table would be at during drying events in a lake. No additional water was added for two weeks, until the next wetting event.

2.3.2 Wetting and drying cycles and collection of water samples

The sediment cores went through subsequent wet/dry cycles with simulated rainwater at pH 4.5 every two weeks (start date of October 18, 2002 for Stump Lake and October 24, 2002 for Black Donald Lake). Of the two cores from the same site, one core went through 3 months of wet/dry cycles, while the remaining core underwent wet/dry cycles for 6 months. The simulated rainwater was prepared in a clean 30 L HDPE bottle according to daily precipitation chemistry values from Environment Canada's CAPMoN site in Chalk River, Ontario for 1995-2000. The composition of the rainwater can be found in Appendix A, and rain event dates are listed in Appendix B.

During a rain event, 250 ml of simulated rainwater was poured onto the top of the Parafilm® covered core which facilitated small droplet formation to simulate rainfall onto the sediment. The 250 ml of rainwater amounts to a rain event of approximately 50 mm, a value typical of 14 days of rain. Although this quantity of rain is slightly higher compared to average rainfall noted from Environment Canada rainfall data (85 mm per month), the higher amount was required for an adequate sample collection for the various analyses (http://www.climate.weatheroffice. ec.gc.ca). After four hours (residence time for the water), water samples were collected beginning at the top sampling ports and ending with the core's bottom vinyl tubing (Figure 2.2). Water samples were collected from all sample ports (a – f) and analyzed for pH, sulphate and sulphide concentrations. For MeHg measurement, 50 ml of drained water was collected only at the bottom of the sediment core (port f). After sample collection, the tips of the needles were covered with Teflon tape and the bottom tubing clamped. The collected water was analyzed immediately for pH and S^{2-}, while the remaining water samples were acidified to 0.1-1.0% with concentrated acid. Trace element grade HCl was used prior to 7 November 2002 and concentrated trace element grade HNO_3 after this date for later sulphate analysis. The reason for the change in the procedure was due to change in instrumentation requirements. MeHg samples were preserved to 0.5% with concentrated HCl. All preserved samples were kept at 4°C in the dark until analyzed.

After the sediment cores had gone through the wet/dry cycles (i.e., after 3 or 6 months), the cores were sliced in 2 cm increments (from 0-16 cm), and analyzed for soil moisture, organic matter (LOI), sulphate-reducing bacteria populations, MeHg, and the chemical fractionation of total Hg (sections 2.5 and 2.6). The final rain events occurred April 4, 2003 for Stump Lake and April 9, 2003 for Black Donald Lake. The "drained cores" (i.e. the sediment cores that were drained at the beginning of the experiment and did not have any rainwater additions for 6 months), were only analyzed for soil moisture, organic matter and sulphate-reducing bacteria populations.

2.4 Chemical analysis of water

2.4.1 pH and redox analysis

Both measurements were made with a VWR Symphony SP Series Waterproof Portable pH Meter Model SP21 (VWR Scientific Products) with a VWR SympHony 3-1 Gel pH electrode and a Corning Redox Platinum Comb electrode. For pH measurements, the meter was calibrated with pH 4, 7, and/or 10 buffers depending on the pH of the water sample. For Eh measurements, the meter was calibrated with Zobell solution (Nordstrom, 1977).

2.4.2 Conductivity

The conductivity was measured upon arrival in the laboratory with a Corning M-90 meter, calibrated with fresh standards (1413uS and 12.88mS).

2.4.3 Water alkalinity

Total alkalinity was measured on site, with a HACH Digital Titrator, following the HACH Water Analysis Handbook method (HACH, 1989). The acid titration cartridge containing 1.6 N $^{\pm}$0.005 H_2SO_4 was used for the titration.

2.4.4 Sulphide analysis

Sulphide concentrations were determined with the Cline method (Cline, 1969), with a detection limit of 0.05 μM (0.002 ppm). 1.5 ml of water sample was collected in a 3 ml syringe and transferred into 2 ml amber vials containing 120 μl of Cline reagent. The optical density was measured at 670 nm with a Beckman DU-65 spectrophotometer, with a single cuvette (until November 8, 2002), and the Molecular Devices SpectraMax Plus spectrophotometer, with a 96 micro-well plate (after November 8, 2002).

2.4.5 Sulphate analysis

Sulphate was measured following the turbidity method with a detection limit of 0.05 μM (0.005ppm) (Rodier, 1975). Modifications to the method were made due to concentration constraints of the many samples. The leachate samples were diluted 4 times (200 µl) due to high concentrations of sulphate⁻. Optical density was measured at 650nm with the Molecular Devices SpectraMax Plus spectrophotometer, with a 96 micro-well plate UV-spectrophotometer as soon as possible, by transferring 300 µl in with a 96 micro-well plate.

2.4.6 Methylmercury

For the lake water MeHg measurements, 1L samples were collected, while only 50 ml water samples were collected from the bottom of the sediment cores during the draining events. All water samples underwent a solid-phase extraction on sulphide columns, followed with an acidic-potassium bromide/copper sulphate elusion and dichloromethane extraction (Cai et al., 1996 and Lee and Mower, 1989). The extracted MeHg was measured by capillary gas chromatograph-atomic fluorescence spectrometry with a Hewlett Packard GC with PSA atomic fluorescent detector, with a detection limit of 20 pg.

2.5 Sediment geochemistry

2.5.1 Sediment moisture content and organic matter content

Approximately 1 g of wet sediment (or up to 3 g if sediment had a high water content) was weighed in aluminum weigh boats of known weight. The sediments were placed in a drying oven (Stabil-Therm® Laboratory Oven) at 100°C for 24 hours, and reweighed when removed to determine the moisture content. The sediments were then placed in a muffle furnace (Lindburg®) at 400°C for 8 hours for organic matter content by weight loss on ignition (LOI) measurements. The weight loss was attributed to the combustion of organic matter.

2.5.2 Sequential chemical extraction for total mercury and trace metals

The chemical fractionation of total Hg in sediment was performed on cores from Black Donald Lake (sites 2 and 4) and Stump Lake (sites B and D) only. All cores from these particular sites underwent the procedure outlined below, except for the six-month dry cores. Sediment samples were sliced in 2 cm increments, from 0 to 16 cm. The sediment was homogenized, and approximately 1.0g (dry weight) of sediment was used for the extraction. Only a few selected cores and months were analyzed by sequential chemical extraction due to the cost of the extraction.

The sequential chemical extraction (modified from Hall et al., 1996, Hall and Pelchat, 1997 and 1999, Hall et al., 2005 and Hall and Pelchat, 2005) included the following fractions (see Appendix C for the detailed extraction protocol):

1- Exchangeable phase (soluble / bioavailable metals) using 0.01 M $Ca(NO_3)_2$

2- Soluble organic phase (Hg bound to humic and fulvic complexes in sediments) with 0.1 M $Na_4P_2O_7$ at pH of 10 for two consecutive leaches

3- Refractory organic phase (Hg bound to refractory organic compounds or non-labile organic forms and/or with oxides, with no HgS) using 40% HNO_3

4- Residual phase (by Aqua Regia) to leach HgS or metal sulphides with 12M HCl and 16M HNO_3 (in a ratio of 3:1)

Blanks and sediment standards of (LKSD-5), 1 each per 8 samples, were prepared and extracted in an identical manner with the other sediment samples (Lynch, 1990). The extractions and leachate analysis were performed at the Analytical Method Development

Laboratory of the Geological Survey of Canada, Ottawa. Total Hg concentrations were determined by Vapour Gas Plasmaquad II+ with a hydride generator, with a detection limit between 0.3 and 1.4 ppb depending on the fraction analyzed. For Hg analysis, 1% $NaBH^4$, 0.1M NaOH was used as the reductant, and the samples are made to 4 M with concentrated HCl.

2.5.3 Sediment methylmercury

MeHg was determined in each core (except for the 6 month dry cores) by collecting 4 g of sediment from each 2 cm slice, and fully homogenizing it. To extract the MeHg, the sediment underwent a sodium thiosulphate wash, isolating the organomercury species as bromide derivatives by acidic-potassium bromide and copper sulphate, followed by the final extraction into dichloromethane (Cai et al., 1997). The extracted MeHg was measured by capillary gas chromatograph-atomic fluorescence spectrometry with a Hewlett Packard GC with PSA atomic fluorescent detector, with a detection limit of 20 pg.

2.6 Sulphate-reducing bacteria (SRB) enumeration

The most-probable-number (MPN) method (Cochran, 1950) was used to estimate the number of SRB in all the sediment cores throughout the experiment. SRB enumeration was performed on the fresh sediments collected from both lakes, on the sediments submitted to wet/dry cycles for 3 and 6 months and the sediment dried for 6 months. For the fresh sediments, the samples were taken every cm within 0-6 cm and every 2 cm from 6-16 cm. For the 3- and 6-month old cores, samples were taken every 2 cm, down to 16 cm, due to the compression of the soil throughout the wet/dry events.

The growth medium used for the MPN enumeration was a modified version of

Postgate's medium B (Postgate, 1984) (10 g/L bacto-peptone (Difco), 5.9 ml/L Na-lactate (60%), 0.4101g/L Na-acetate, 0.3405 g/L Na-formate, 0.55 g/L Na-pyruvate, 2 g $MgSO_4 \cdot 7H_2O$, 0.5 g/L $FeSO_4 \cdot 7H_2O$, and 0.5 g/L Na_2SO_3) and a reducing agent (RAS) (7.5 g/L ascorbic acid and 7.5 ml/L thioglycollic acid). The solutions were adjusted to pH of 7.5 with 2 N NaOH. Growth medium (9 ml) was dispensed into 12 ml culture tubes and autoclaved along with the reducing agent (RAS). Before inoculation, 1 ml of sterile RAS was added to each culture tube containing the growth medium. In preparation for the inoculation, dilution series of 10^{-1} to 10^{-8} were made in 12 ml culture tubes containing 9 ml of autoclave sterilized reduced water (2.1 g/L NaCl and 100 ml/L RAS adjusted to pH 7.5 with 2 N NaOH), starting with approximately 1 g of wet sediment into 9 ml of reducing water. Each individual dilution was used to inoculate 5 vials of growth medium, by adding 1 ml of dilution water under sterile conditions. The culture tubes were then placed in a dark place at room temperature for 2 weeks. After the incubation period, the enumeration of SRB was done by counting the number of positive tubes (i.e. tubes that had a black Fe-sulphide precipitates). MPN values were calculated with the use of statistical tables (Cochran, 1950). The final bacterial counts were expressed as colony forming units per gram of sediment dry weight (CFU gdw^{-1}).

2.7 Statistical analysis

All statistical tests were preformed with statistical software SYSTAT® 10 for Windows®. All conditions outlined by the particular statistical tests for the data sets were met before the statistical tests were performed, and the null hypothesis was rejected when $p < 0.05$. The 3 and 6 month wet/dry cores for each site were compared with one-factor ANOVA throughout the study. Two-factor ANOVA without replication were used to determine the effect of depth and time on various parameters that were measured throughout the study, especially for the sediment water and organic matter content, drain data, total Hg in sediment and SRB population counts. Nested ANOVA tests and 3-factor ANOVA tests, by a General Linear Model, were conducted on the drain data and bacterial counts. Pearson correlations were performed on MeHg concentrations in water drained from the cores, to pH levels, and sulphide and sulphate concentrations.

3.0 RESULTS

3.1 Water chemistry of the lakes

The pH of Stump and Black Donald Lake water was slightly above neutral, Stump Lake being more alkaline (Tables 3.1 and 3.2). The redox potential of the surface waters of both lakes was higher compared to the water directly above the sediment. Overall, Stump Lake waters had higher redox values. Black Donald Lake waters exhibited a greater Eh decrease with depth compared to Stump Lake, although all redox measurements indicated oxic waters (Tables 3.1 and 3.2). The total alkalinity and conductivity of Stump Lake was higher than Black Donald, while methylmercury (MeHg) concentrations were slightly higher in Black Donald Lake.

3.2 Porewater chemistry of the fresh lake sediments

The pH of sediment porewaters of both lakes exhibited near neutral conditions (pH 6-8) (Figures 3.1a and 3.2a). Black Donald Lake porewaters were more acidic than Stump Lake, pH 5.6 to 7.3 compared to pH 6.5 to 7.6, respectively. More variation was observed between the pH profiles of Black Donald Lake sites. Porewater pH values decreased with depth in Black Donald Lake sediments, while generally remained the same in Stump Lake except for site C, which decreased with depth (Figure 3.1a).

Redox measurements in this section can only be viewed as general estimates as the measurements were conducted in the field under open atmosphere, where the potential for oxygen contamination was a factor. Nevertheless, any changes would be small. More variation was observed between porewater redox conditions for Black Donald Lake sites (Figure 3.2b) than Stump Lake (Figure 3.1b). Black Donald Lake porewaters were oxic to

Table 3.1. Physico-chemical conditions of Stump Lake waters.

Tested parameter	**Site**				**Mean**	**Std Error**
	Site A	**Site B**	**Site C**	**Site D**		
GPS coordinates (18T-)	370248	370772	370855	370199	n/a	n/a
UTM	4977333	4978467	4877830	4977384	n/a	n/a
Altitude (m.a.s.l)	195	195	195	195	n/a	n/a
Depth (cm)	183	310	178	145	204	36
Temperature (^{o}C)	26.7	26.5	26.9	26.4	26.6	0.1
pH surface waters	8.48	8.36	8.55	8.73	8.5	0.08
bottom waters	8.52	8.42	8.51	8.68	8.5	0.05
Eh (mV) surface waters	412.5	390	390.5	384	394.3	6.3
bottom waters	377	376.9	377	371.9	375.7	1.3
Total Alkalinity (mg CaCO$_3$/L)						
surface waters	51.0	51.0	52.5	53.0	51.9	0.5
bottom waters	66.3	57.5	52.0	47.0	55.7	4.1
Conductivity (μS)	144.9	140.5	141.1	138.1	141.2	1.4
MeHg (ppt)	0.083	0.076	0.090	0.093	0.085	0.004

Table 3.2. Physico-chemical conditions of Black Donald Lake waters.

Tested parameter	**Site**				**Mean**	**Std Error**
	Site 1	**Site 2**	**Site 3**	**Site 4**		
GPS coordinates (18T-)	348364	034 8349	347518	349367	n/a	n/a
UTM	5007642	5006159	5005710	5007503	n/a	n/a
Altitude (m.a.s.l)	257	251	263	256	n/a	n/a
Depth (cm)	197	208	202	295	226	23
Temperature (^{o}C)	25.2	25.2	25	25.4	25	0
pH surface waters	8.11	8.13	8.23	8.24	8.2	0.03
bottom waters	8.08	8.08	8.21	8.06	8.1	0.03
Eh (mV) surface waters	387.7	361.7	411.7	362.7	381.0	11.9
bottom waters	328.7	340.1	355.5	329.7	338.5	6.2
Total Alkalinity (mg CaCO$_3$/L)						
surface waters	29.5	33.0	30.0	28.5	30.3	1.0
bottom waters	32.0	30.0	31.0	42.0	33.8	2.8
Conductivity (μS)	102.3	102.4	102.8	101.8	102.3	0.2
MeHg (ppt)	0.070	0.055	0.049	0.043	0.054	0.006

Figure 3.1. Porewater chemistry values for a) pH, b) Eh, c) sulphide and d) sulphate concentrations with depth for all sites from Stump Lake.

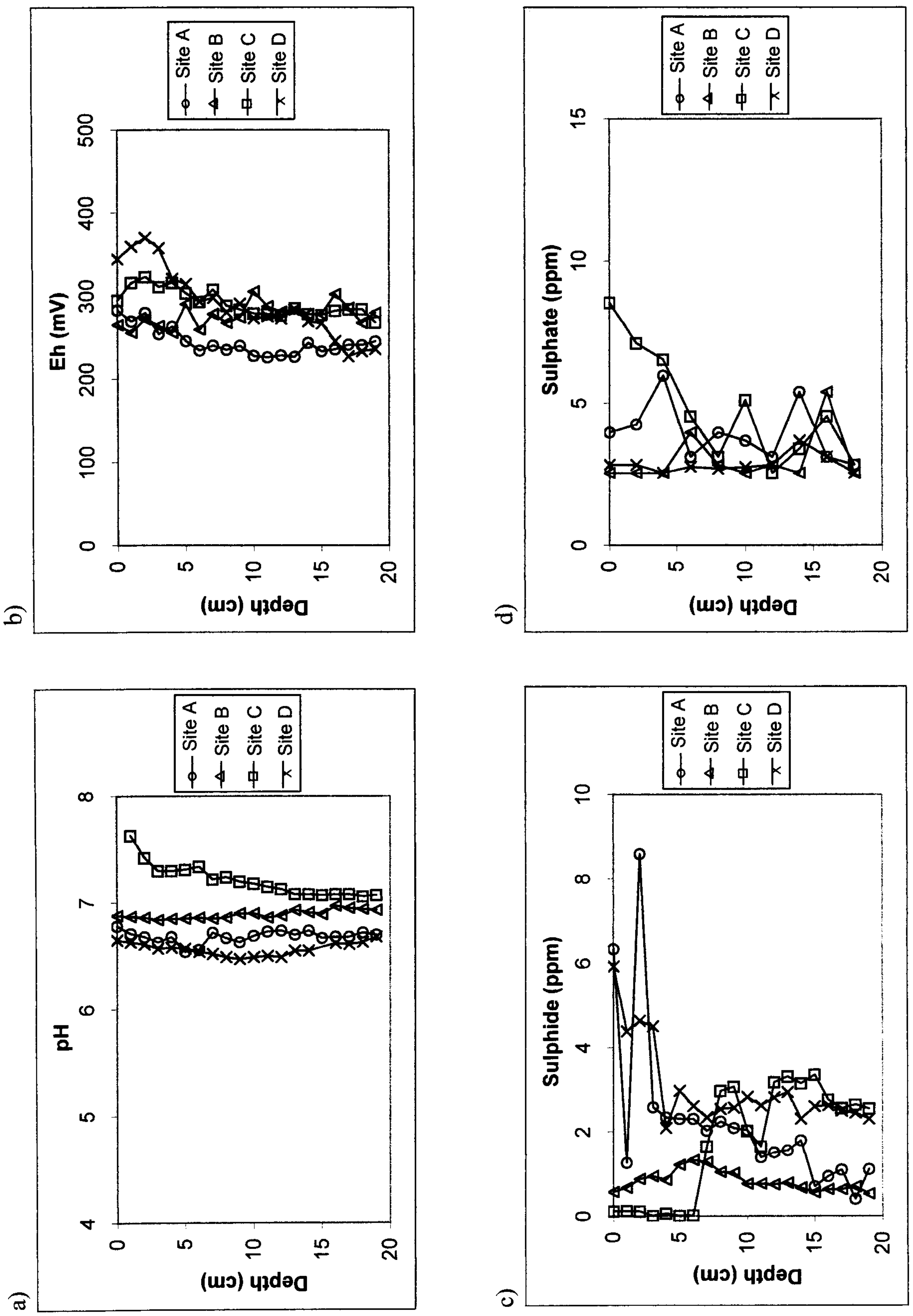
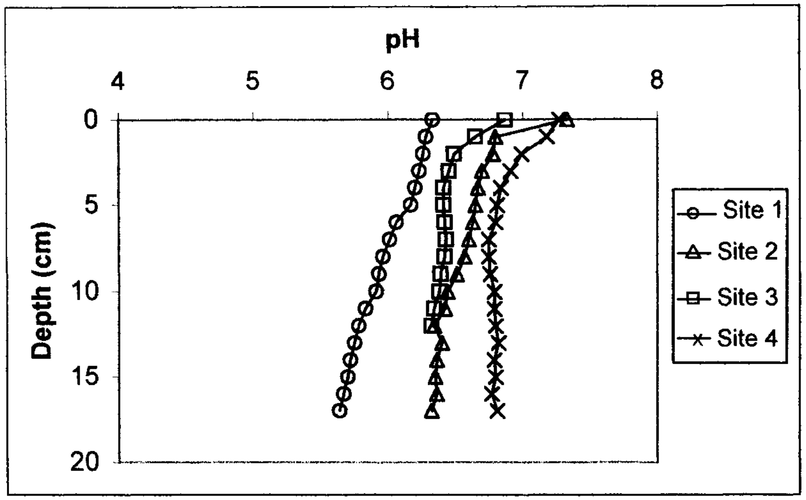
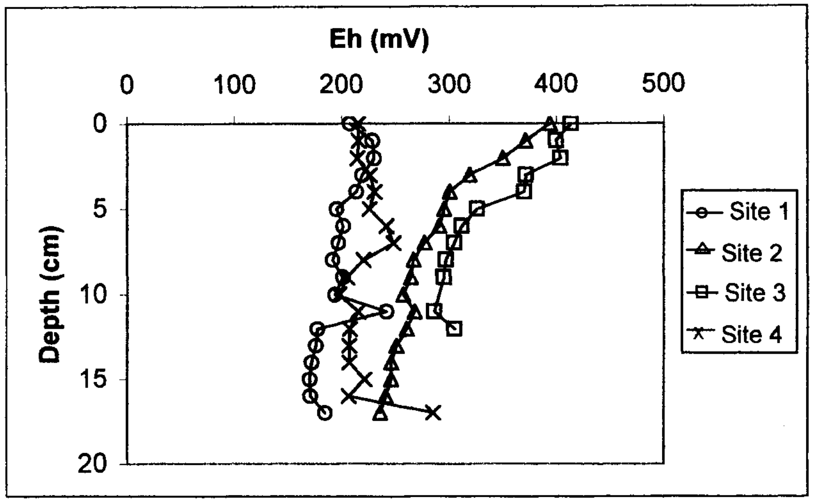

a)
pH
Depth (cm)
4
5
6
7
8
0
5
10
15
20
Site A
Site B
Site C
Site D
b)
Eh (mV)
Depth (cm)
0
100
200
300
400
500
0
5
10
15
20
Site A
Site B
Site C
Site D
c)
Sulphide (ppm)
Depth (cm)
0
2
4
6
8
10
0
5
10
15
20
Site A
Site B
Site C
Site D
d)
Sulphate (ppm)
Depth (cm)
0
5
10
15
0
5
10
15
20
Site A
Site B
Site C
Site D

Figure 3.2. Porewater chemistry values for a) pH, b) Eh, c) sulphide and d) sulphate concentrations with depth for all sites from Black Donald Lake.

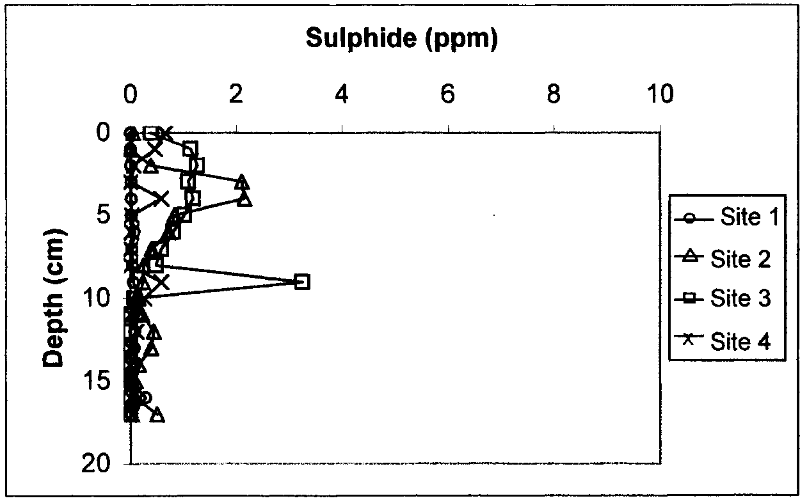
a)


pH
4
5
6
7
8
0
5
10
15
20
Depth (cm)
Site 1
Site 2
Site 3
Site 4

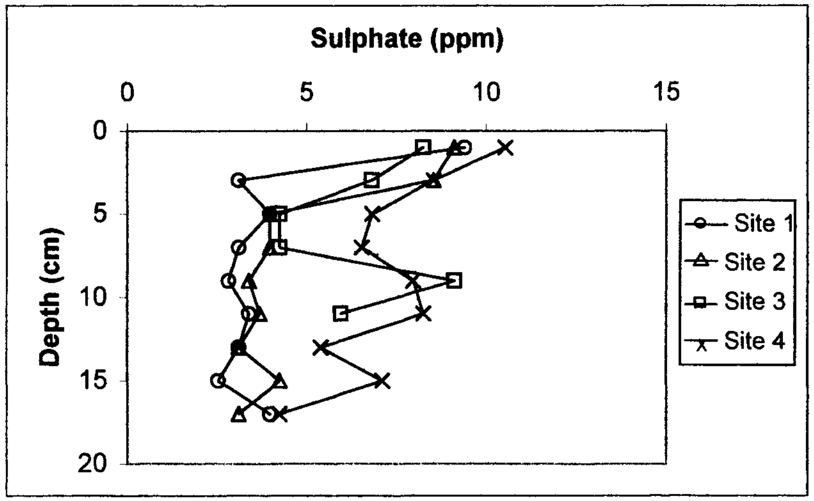
b)


Eh (mV)
0
100
200
300
400
500
0
5
10
15
20
Depth (cm)
Site 1
Site 2
Site 3
Site 4

c)


Sulphide (ppm)
0
2
4
6
8
10
0
5
10
15
20
Depth (cm)
Site 1
Site 2
Site 3
Site 4

d)


Sulphate (ppm)
0
5
10
15
0
5
10
15
20
Depth (cm)
Site 1
Site 2
Site 3
Site 4

slightly anoxic (170 to 410 mV) (Figure 3.2b) with slight decreases in Eh with depth for most sites, indicating the porewaters had less oxygen with depth. The redox conditions for all sites for Stump Lake indicated the porewaters were relatively oxic (227 to 370 mV), with little change with depth (Figure 3.1b).

Porewater sulphide concentrations were higher in Stump Lake sediments (0 - 8.6 ppm) (Figure 3.1c), compared to Black Donald Lake (0 to 3.2 ppm) (Figure 3.2c). More variation was apparent with depth and between sites for Stump Lake porewaters compared to Black Donald Lake. Most of the sulphides were measured in the surface sediments of sites A and D of Stump Lake, and their concentrations decreased with increasing depth (Figure 3.1c). Site B sulphide levels remained stable or constant with depth while site C had no sulphides in the first 6 cm of sediment, but increased thereafter. For Black Donald Lake, sulphide levels fluctuated with depth for sites 3 and 4, remained very low for site 1, while site 2 exhibited a peak in sulphides at 4 and 5 cm (Figure 3.2c).

Porewater sulphate levels were slightly higher in Black Donald Lake, ranging between 3.1 to 10.5 ppm (Figure 3.2d). Stump Lake levels ranged between 2.5 to 8.5 ppm (Figure 3.1d). Black Donald Lake had more differences between sites, in addition to greater variation with depth compared to Stump Lake (Figure 3.2d). Porewater sulphate levels decreased with depth for both lakes.

MeHg in porewater consisted of only one measurement for the entire sediment profile, as the sample volume (50mL) required for the measurement was considerably more than the water sample available at each depth (4mL). MeHg in Black Donald Lake porewaters averaged 1.5 +/- 0.6 ppt, while Stump Lake MeHg concentrations were below detection limit (0.1 ppb per 100 mg sample).

3.3 Sediment moisture and organic matter content of the sediment cores submitted to wetting and drying events

3.3.1 Sediment moisture

Stump Lake sediment water content was higher than Black Donald Lake sediment for most cores (Figure 3.3). Sediment water content for all Stump Lake cores within each site were fairly similar, ranging between 64 to 95%, where the drained cores (cores that were drained and air-dried for 6 months) displayed the lowest moisture content (Figure 3.3). The drained core from site B was an exception, as the sediment moisture content was lower compared to the typical moisture content of the other cores, ranging from 26 to 60% (Figure 3.3b). The sediment moisture content also remained stable or constant with depth for all sites except for site B (Figure 3.3).

The moisture content of sediment cores from Black Donald Lake ranged from 4 to 78% (Figure 3.3), with more variation with depth and between cores compared to Stump Lake. Most cores showed a decrease in moisture content with depth, decreasing by up to 55 % from surface sediments. Variations were apparent between the sediment water content of all cores within the particular sites. The drained cores generally had the lowest water content, between 4 to 50% (Figure 3.3).

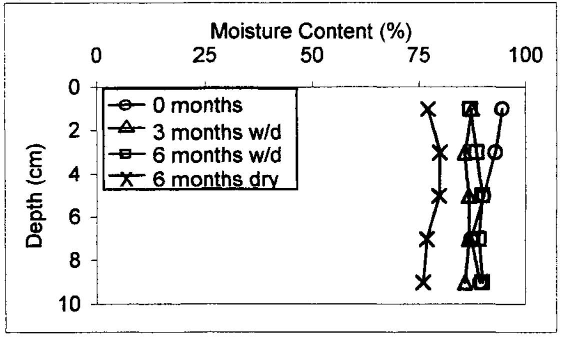
3.3.2 Sediment organic matter content

The total organic matter content, estimated by LOI (Loss On Ignition), of Stump Lake sediment was higher than Black Donald Lake (Figure 3.4). Stump Lake sediment was approximately 55 to 84% organic matter, with the exception of site B, which had a much lower organic matter content, between 9 to 39 % (Figure 3.4). Organic matter content of the 0 month wet/dry sediment cores was not measured due to sample contamination. The

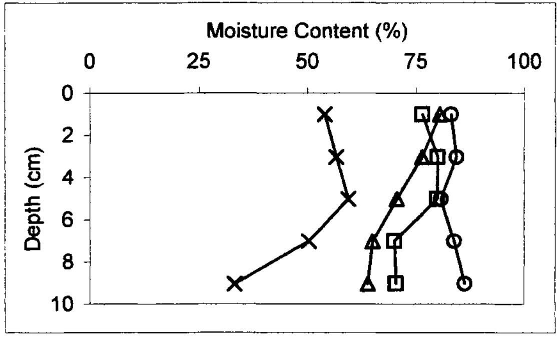
Figure 3.3. Gravimetric moisture content of sediment from Stump Lake sites a) A, b) B, c) C and d) D) and Black Donald Lake (sites i)1, ii) 2, iii) 3 and iv) 4) after 0, 3 or 6 months of wet/dry events or remaining dry for 6 months.

Stump Lake

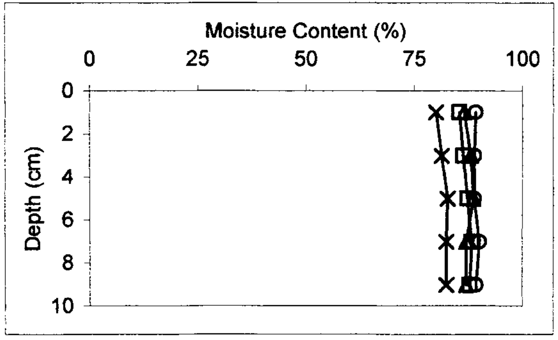
a)



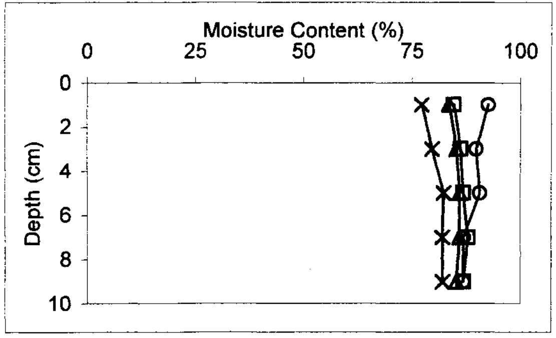
b)



c)

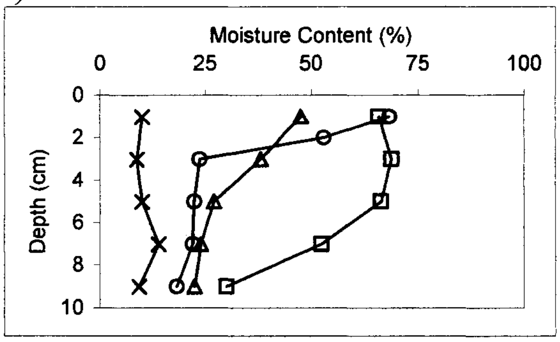


d)

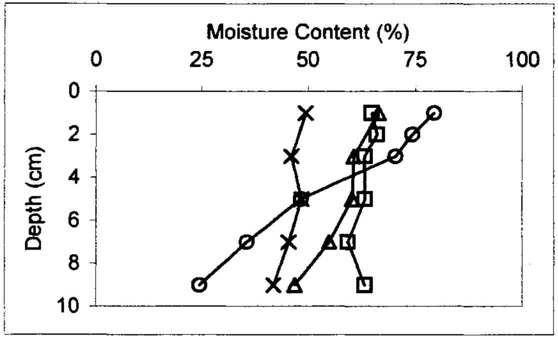


Black Donald Lake

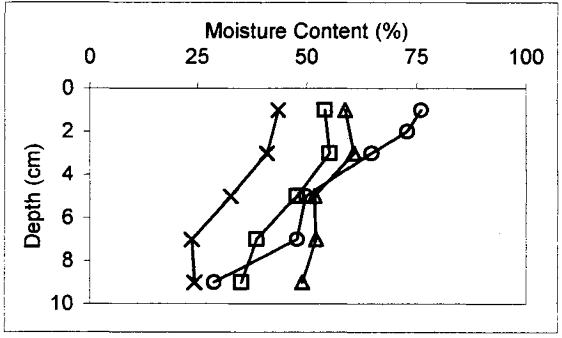
i)



ii)



iii)



iv)

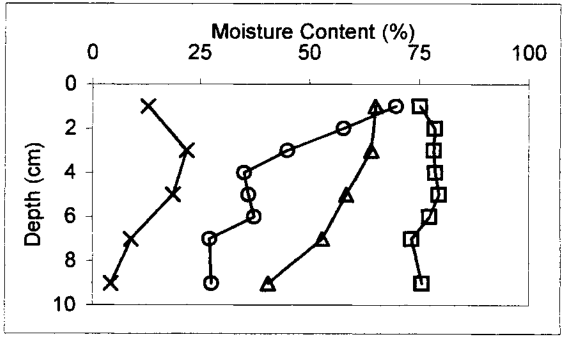
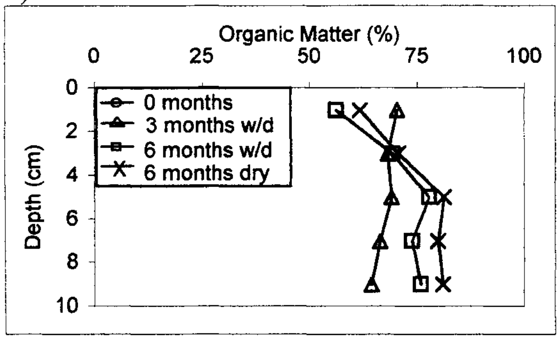


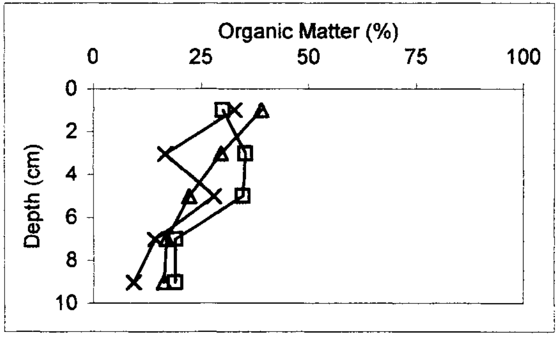
Figure 3.4. Percent organic matter content of sediment determined by loss on ignition (LOI) from Stump Lake (sites a) A, b) B, c) C and d) D) and Black Donald Lake sites i) 1, ii) 2, iii) 3 and iv) 4) after 0, 3 or 6 months of wet/dry events or remaining dry for 6 months

Stump Lake

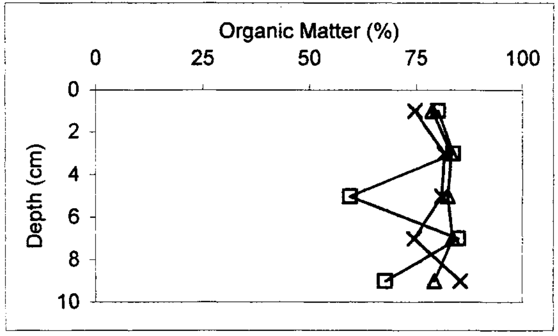
a)



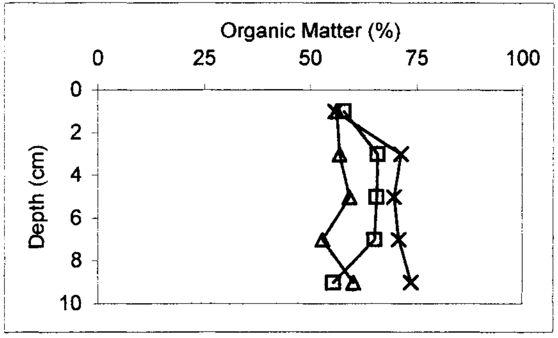
b)



c)

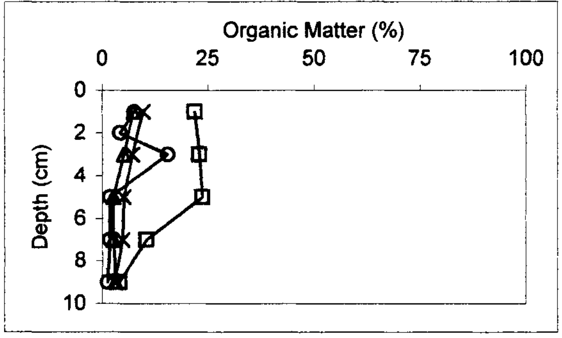


d)

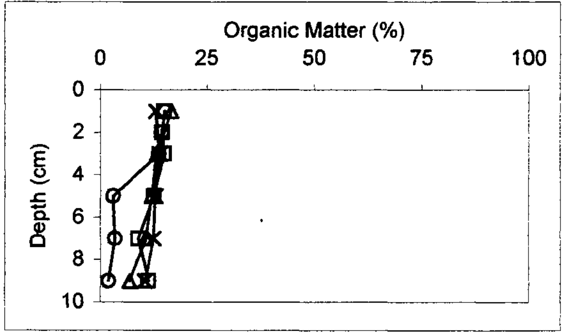


Black Donald Lake

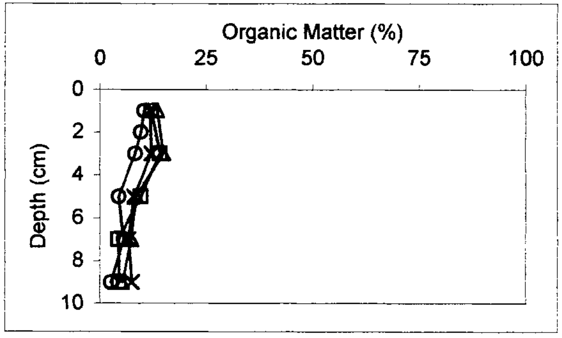
i)



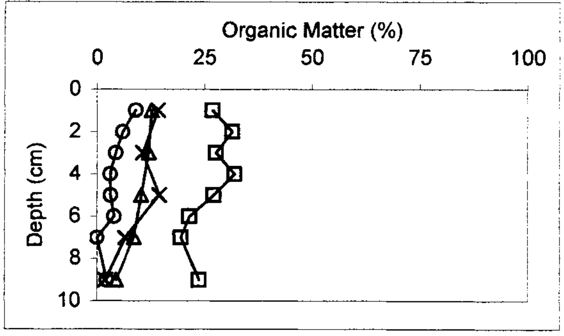
ii)



iii)



iv)



organic matter content of sediment from most cores in sites A and D increased with depth, site B decreased with depth, while site C remained stable or constant with depth (Figure 3.4).

The organic matter content of Black Donald Lake sediment was similar between the sites, ranging from 0 to 32%, which was considerably lower than Stump Lake sediment (Figure 3.4). Less variation was observed with depth and between the different cores within the sites for Black Donald Lake. The organic matter content decreased slightly with depth (Figure 3.4).

3.4 Physico-chemical changes in water drained from the sediment cores submitted to wetting and drying events

The following section presents the changes in pH, sulphide, sulphate and MeHg concentrations in water drained from the sediment cores during the wetting and drying experiment. Water samples collected from ports a to f refer to the sample depth along the core, which is explained in detail in sections 2.2 – 2.3 and Figure 2.2, while sampling dates are listed in Appendix B. No significant differences in pH, sulphate, sulphides and MeHg concentrations were found between 80% of the two cores submitted to 3 or 6 months of wetting and drying cycles from each site for all depths ($p > 0.05$) (Appendices D and E). As most cores showed no differences, only results from cores submitted to 6 months of wetting and drying events are discussed in this section, while the results for the 3 month cores are presented in Appendices F to I. 3.4.5

Overall, from the various statistical tests, there were definite differences in leachate pH, sulphide and sulphate concentrations between the two lakes ($p < 2.81 \times 10^{-5}$) (Appendix J). The lake the sediments came from caused more variation between leachate pH, sulphide and sulphate concentrations from the individual depths than by the sites the cores were extracted from (Appendices K - M).

3.4.1 pH

The pH of water drained from both Stump and Black Donald Lake cores after a wetting cycle was higher compared to the acidic rainwater (pH ~ 4.5) added during the wetting events (Figures 3.5 and 3.6). Leachate pH was slightly higher for Black Donald Lake sediment (pH 4.60 - 7.35) compared to Stump Lake sediment (pH 4.5 to 7.1) (Figures 3.5 and 3.6).

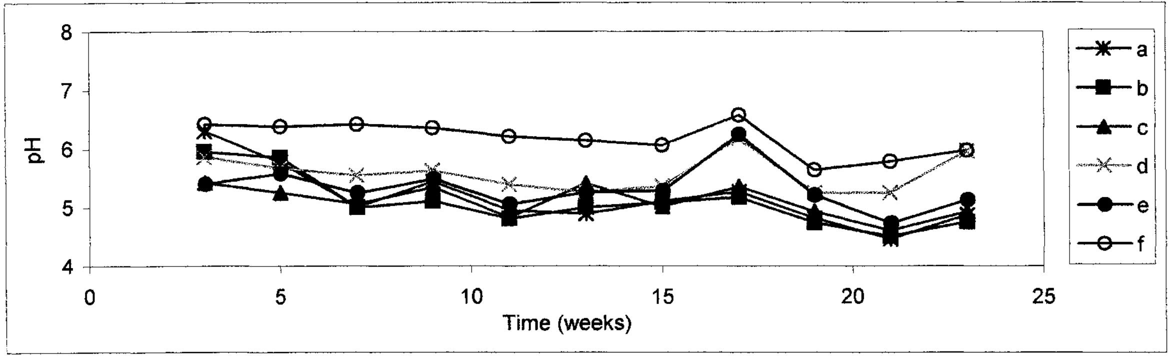
Leachate pH varied with depth in most sites for both lakes, where it generally increased with depth ($p < 0.002$) (Appendices N and O). The pH was commonly highest in water collected from the bottom of the cores (port f) compared to the surface sediment portion (ports a – e) (Figures 3.5 and 3.6). Significant changes in leachate pH were observed over time for all sites of both lakes ($p < 0.0002$), except for Stump Lake site C (Appendices N and O). The pH tended to decrease over time in the surface sediment of most cores in both lakes. However, the pH of the deeper sediment remained the same or increased in Black Donald Lake sediment cores (Figure 3.6).

3.4.2 Sulphide

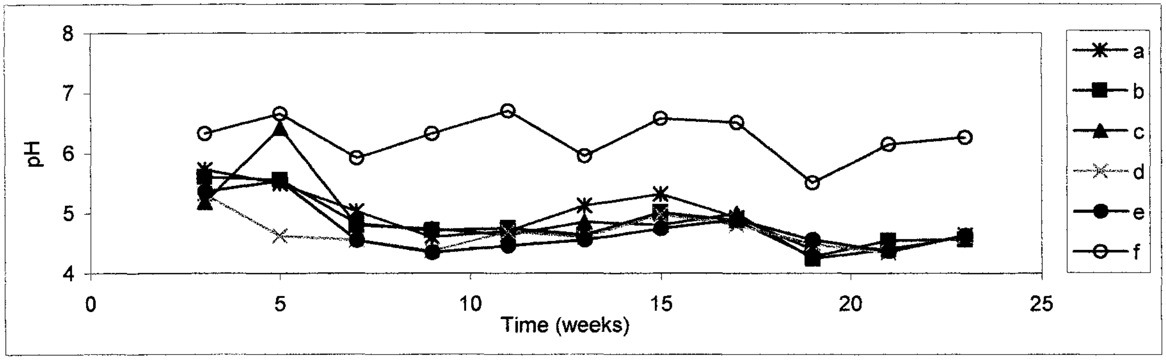
Sulphide concentrations in drained water after wetting events from Stump Lake sediments (0 to 11.2 ppm) (Figure 3.7) were higher compared to Black Donald Lake leachate (0 to 2.4 ppm) (Figure 3.8). Stump Lake sulphide levels were considerably higher in water drained from the bottom of the cores (port f) (Figure 3.7). Sulphides drained from the upper portion of the sediment (ports a – e) were very low (< 0.05 ppm), except for some

Figure 3.5. pH of drained water from sediment cores with depth and over time from sediment submitted to 6 months of wet/dry events from the 4 study sites, a) A, b) B, c) C and d) D for Stump Lake. The sample ports refer to the sampling depths (a = + 2.5 cm, b = 0 cm (sediment-water interface), c = - 2.5 cm, d = - 5 cm, e = - 7.5 cm, f = approximately - 20 cm).

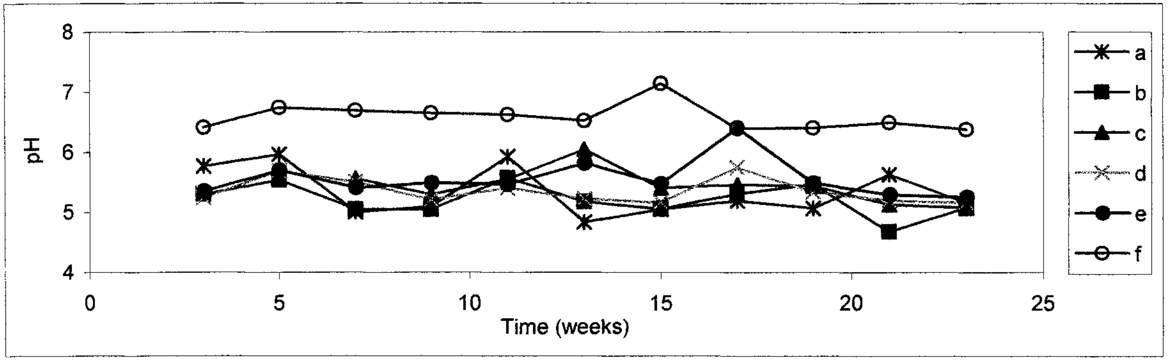
a)


8
7
6
5
4
pH
0
5
10
15
20
25
Time (weeks)
a
b
c
d
e
f

b)


8
7
6
5
4
pH
0
5
10
15
20
25
Time (weeks)
a
b
c
d
e
f

c)


8
7
6
5
4
pH
0
5
10
15
20
25
Time (weeks)
a
b
c
d
e
f

d)

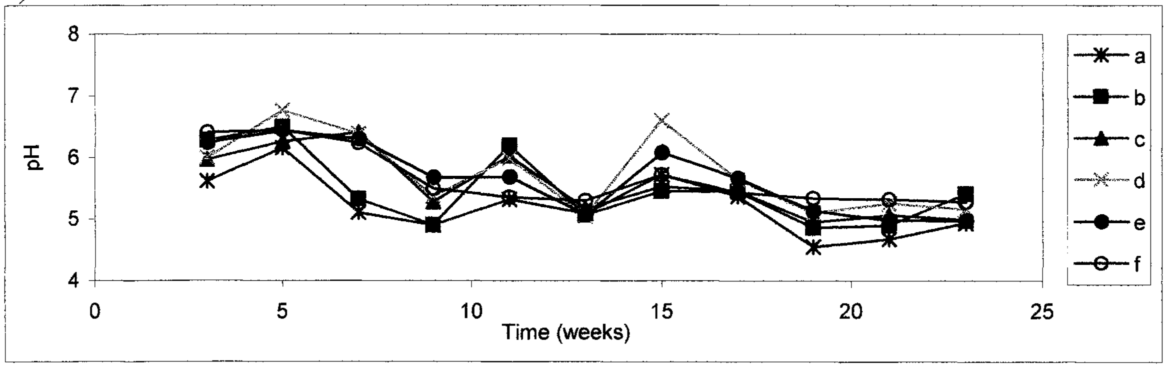
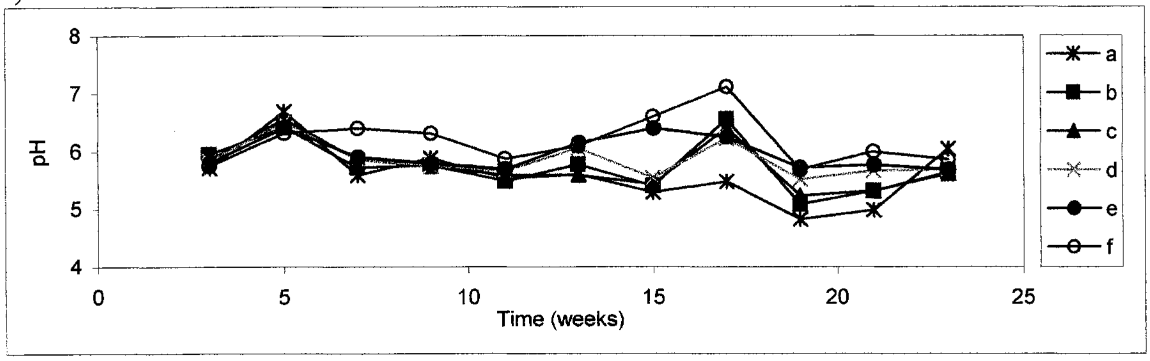
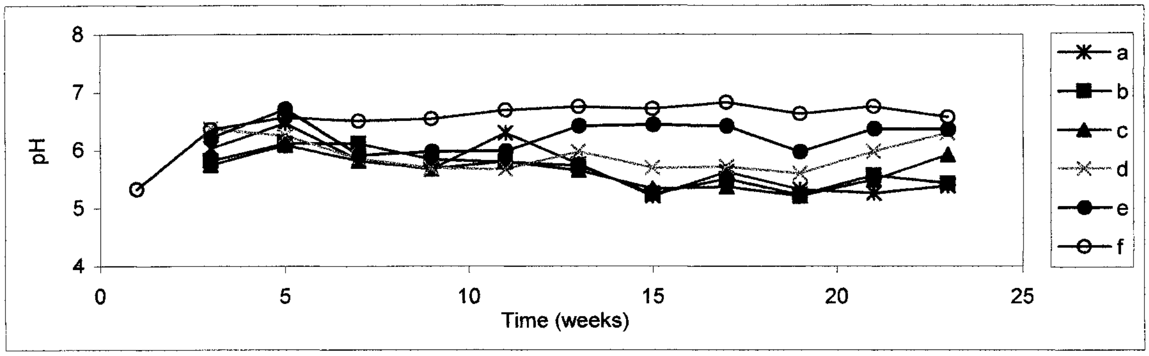

8
7
6
5
4
pH
0
5
10
15
20
25
Time (weeks)
a
b
c
d
e
f

Figure 3.6. pH of drained water from sediment cores with depth and over time from sediment submitted to 6 months of wet/dry events from the 4 study sites, a) 1, b) 2, c) 3 and d) 4 for Black Donald Lake. The sample ports refer to the sampling depths (a = + 2.5 cm, b = 0 cm (sediment-water interface), c = - 2.5 cm, d = - 5 cm, e = - 7.5 cm, f = approximately - 20 cm).

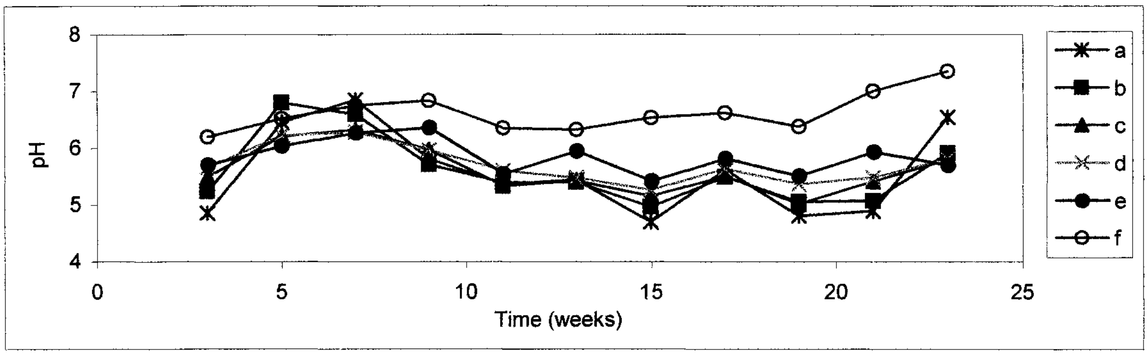
a)


8
7
6
5
4
pH
0
5
10
15
20
25
Time (weeks)
a
b
c
d
e
f

b)


8
7
6
5
4
pH
0
5
10
15
20
25
Time (weeks)
a
b
c
d
e
f

c)


8
7
6
5
4
pH
0
5
10
15
20
25
Time (weeks)
a
b
c
d
e
f

d)

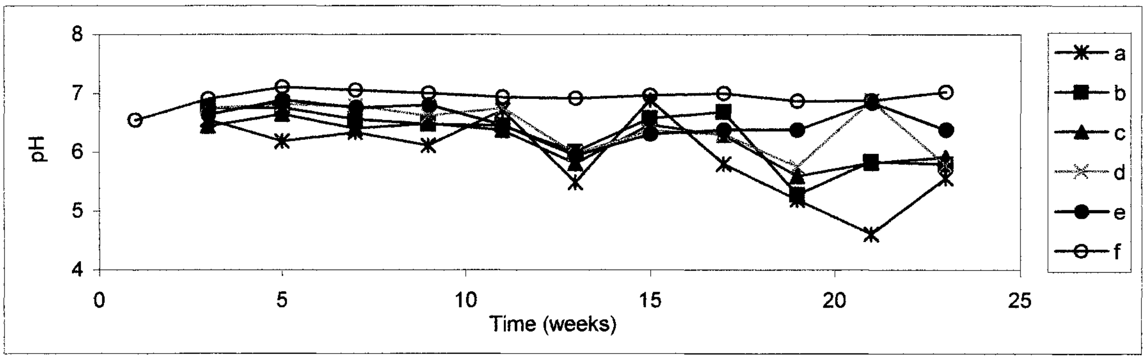
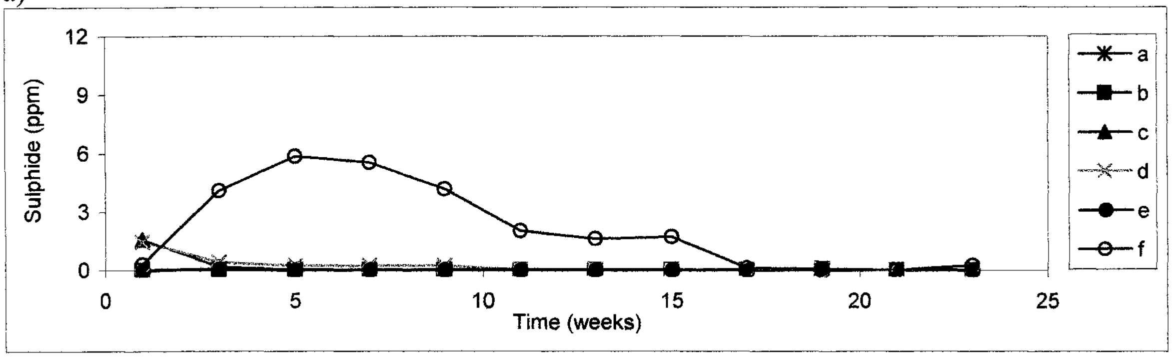
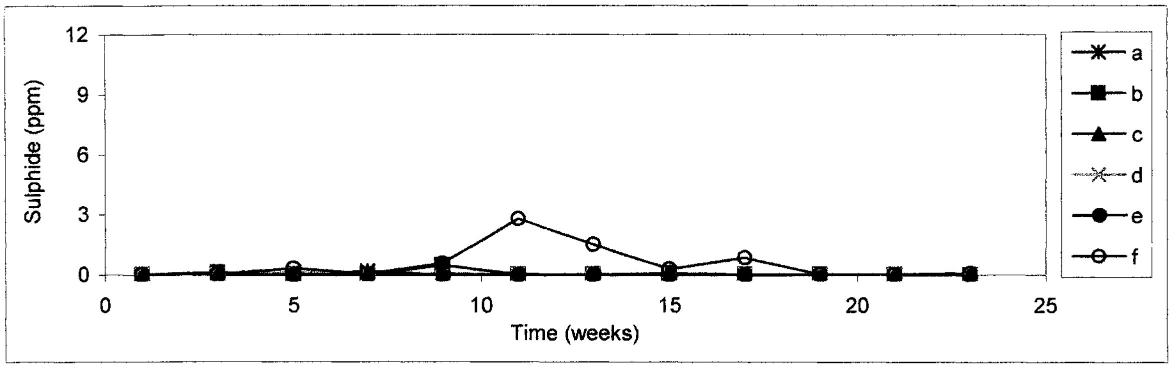

8
7
6
5
4
pH
0
5
10
15
20
25
Time (weeks)
a
b
c
d
e
f

Figure 3.7. Sulphide concentrations (ppm) in drained water from sediment cores with depth and over time from sediment submitted to 6 months of wet/dry events from the 4 study sites, a) A, b) B, c) C and d) D for Stump Lake. The sample ports refer to the sampling depths (a = + 2.5 cm, b = 0 cm (sediment-water interface), c = - 2.5 cm, d = - 5 cm, e = - 7.5 cm, f = approximately - 20 cm).

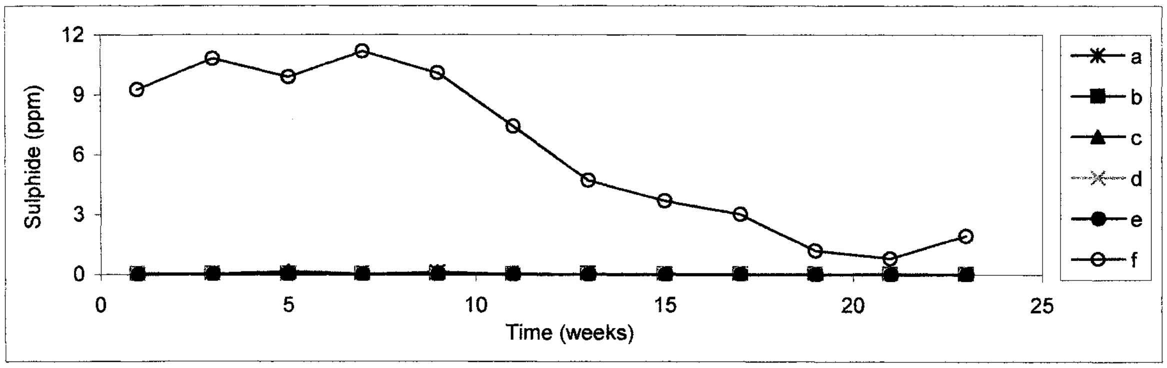
a)


Sulphide (ppm)
12
9
6
3
0
0
5
10
15
20
25
Time (weeks)
a
b
c
d
e
f

b)


Sulphide (ppm)
12
9
6
3
0
0
5
10
15
20
25
Time (weeks)
a
b
c
d
e
f

c)


Sulphide (ppm)
12
9
6
3
0
0
5
10
15
20
25
Time (weeks)
a
b
c
d
e
f

d)

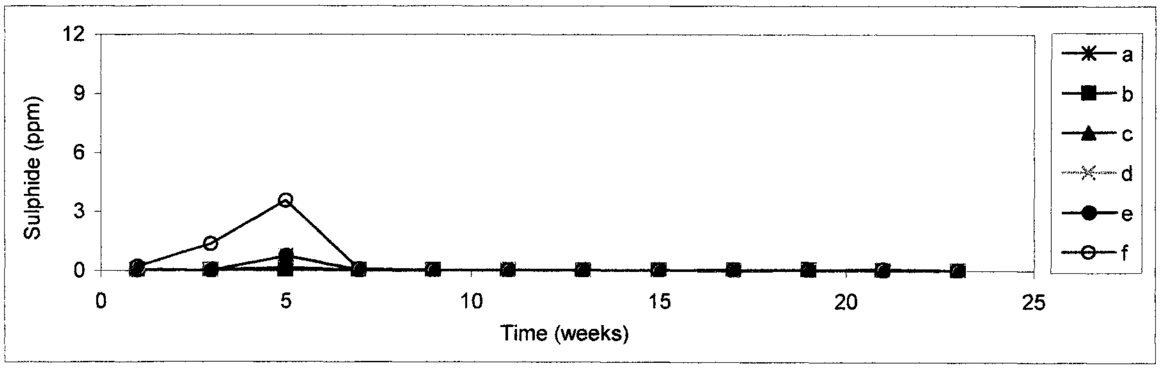
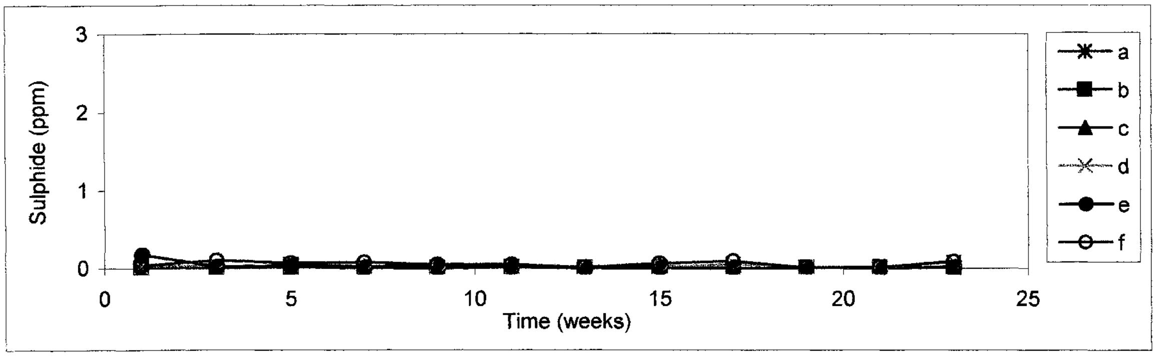
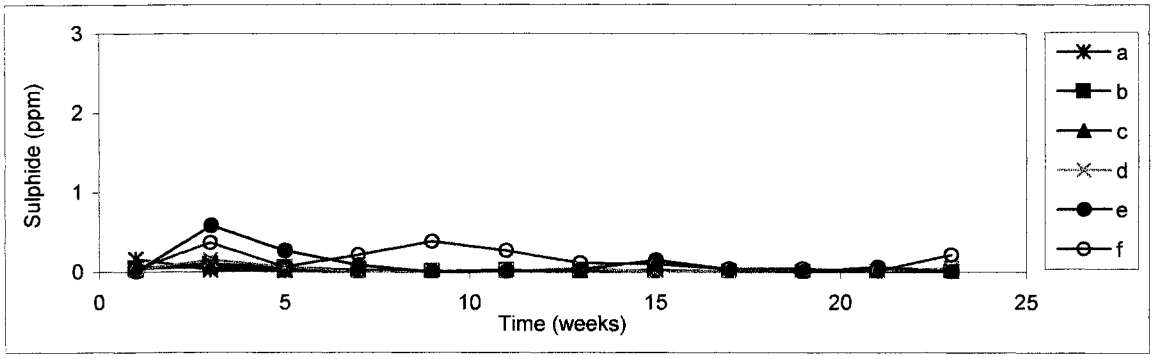

Sulphide (ppm)
12
9
6
3
0
0
5
10
15
20
25
Time (weeks)
a
b
c
d
e
f

Figure 3.8. Sulphide concentrations (ppm) in drained water from sediment cores with depth and over time from sediment submitted to 6 months of wet/dry event from the 4 study sites, a) 1, b) 2, c) 3 and d) 4 for Black Donald Lake. The sample ports refer to the sampling depths (a = + 2.5 cm, b = 0 cm (sediment-water interface), c = - 2.5 cm, d = - 5 cm, e = - 7.5 cm, f = approximately - 20 cm.

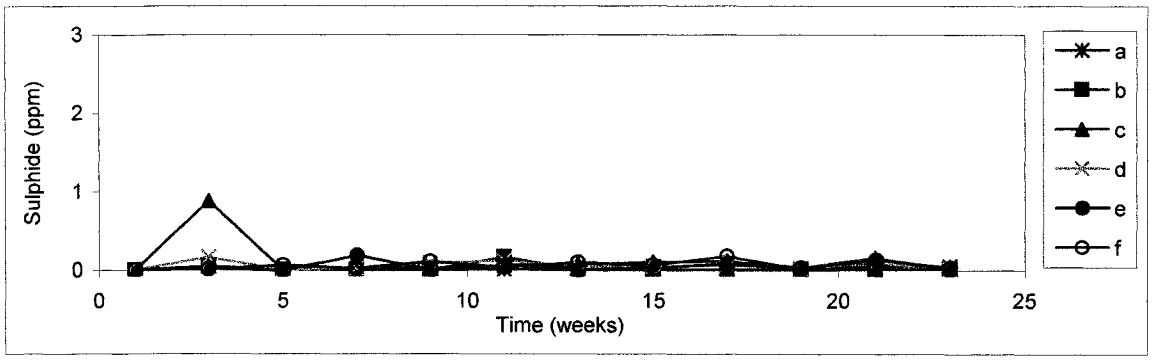
a)


Sulphide (ppm)
3
2
1
0
0
5
10
15
20
25
Time (weeks)
a
b
c
d
e
f

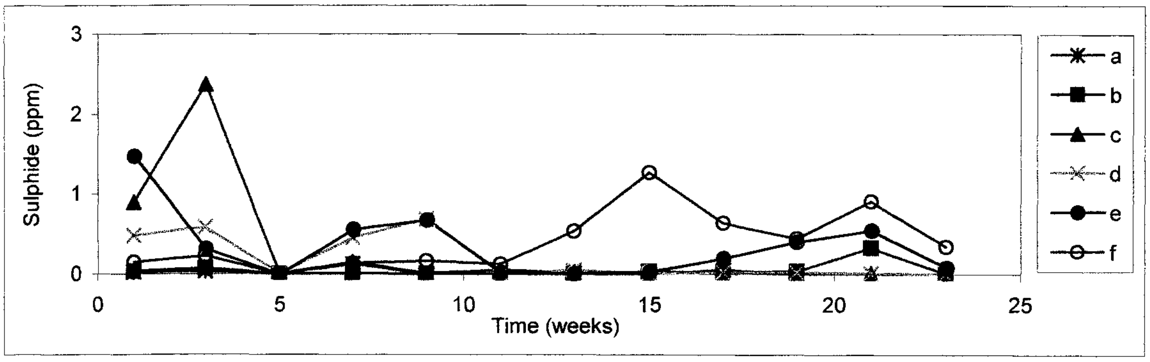
b)


Sulphide (ppm)
3
2
1
0
0
5
10
15
20
25
Time (weeks)
a
b
c
d
e
f

c)


Sulphide (ppm)
3
2
1
0
0
5
10
15
20
25
Time (weeks)
a
b
c
d
e
f

d)


Sulphide (ppm)
3
2
1
0
0
5
10
15
20
25
Time (weeks)
a
b
c
d
e
f

samples for which concentrations were no greater than 1.5 ppm from sites A and D during the first weeks of the experiment. Sulphide levels varied with depth for most cores ($p < 1.0 \times 10^{-6}$), except for site D (Appendix N). Over time, sulphides appeared to vary only from water drained from sample port f, where concentrations decreased over time for cores from sites A, C and D, while site B exhibited sulphide peaks only during the middle of the 6 month wetting and drying experiment (Figure 3.7). However statistically, only sites B and D showed a significant difference in sulphide concentrations drained over time during the weeks of wetting and drying cycles ($p < 0.027$) (Appendix N).

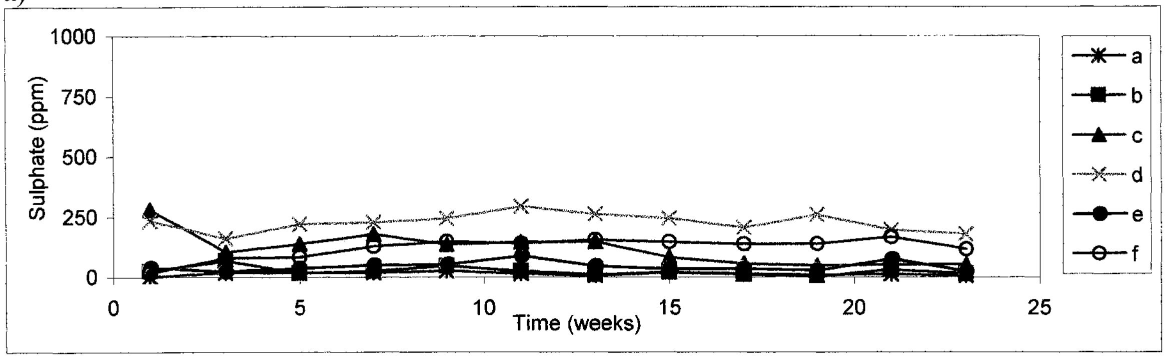
Black Donald Lake sediments also exhibited higher sulphide concentrations drained from the lower portion of the cores (port f), while water from sample ports a-d had very low sulphide levels for most cores (< 0.06 ppm) (Figure 3.8). Sulphides in leachate significantly varied with depth for most cores ($p < 0.042$), except for the core from site 3 ($p > 0.158$) (Appendix O). No significant differences were also observed in sulphides over time for most sites, except for the core from site 2 which appeared to decrease over time ($p < 0.012$) (Appendix O).

3.4.3 Sulphate

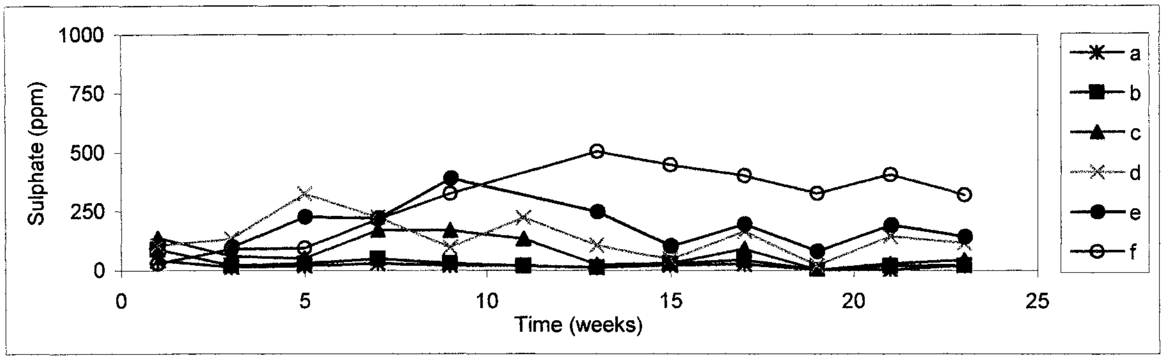
Stump Lake sulphate concentrations (0 to 941ppm) in water drained from the sediments were much higher than Black Donald Lake concentrations (0 to 175 ppm), during the rain events. (Figures 3.9 and 10). This is considerably higher compared to the *in situ* porewater concentrations for both lakes which did not exceed 10.5 ppm (Figure 3.1). Overall the sediment released a substantial amount of sulphate into the water drained through the sediment over time (Table 3.3).

Figure 3.9. Sulphate concentrations (ppm) in drained water from sediment cores with depth and over time from sediment submitted to 6 months of wet/dry events from the 4 study sites, a) A, b) B, c) C and d) D for Stump Lake. The sample ports refer to the sampling depths (a = + 2.5 cm, b = 0 cm (sediment-water interface), c = - 2.5 cm, d = - 5 cm, e = - 7.5 cm, f = approximately - 20 cm).

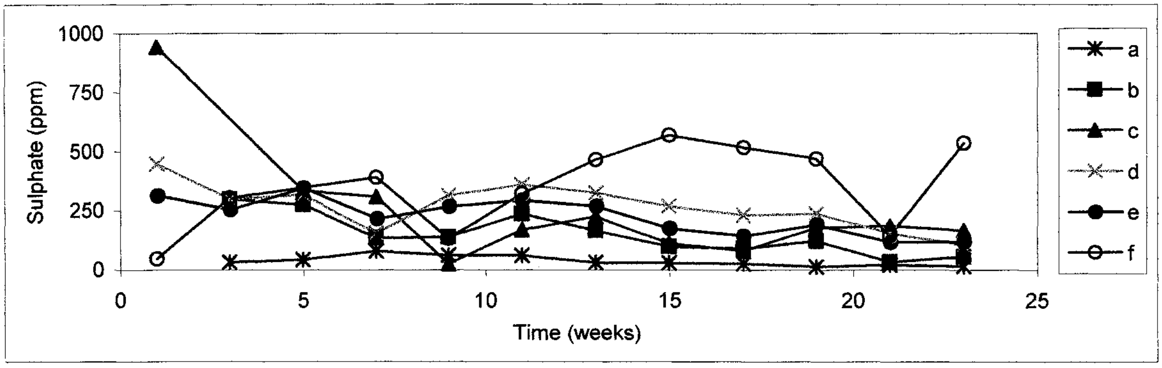
a)


Sulphate (ppm)
1000
750
500
250
0
0
5
10
15
20
25
Time (weeks)
a
b
c
d
e
f

b)


Sulphate (ppm)
1000
750
500
250
0
0
5
10
15
20
25
Time (weeks)
a
b
c
d
e
f

c)


Sulphate (ppm)
1000
750
500
250
0
0
5
10
15
20
25
Time (weeks)
a
b
c
d
e
f

d)

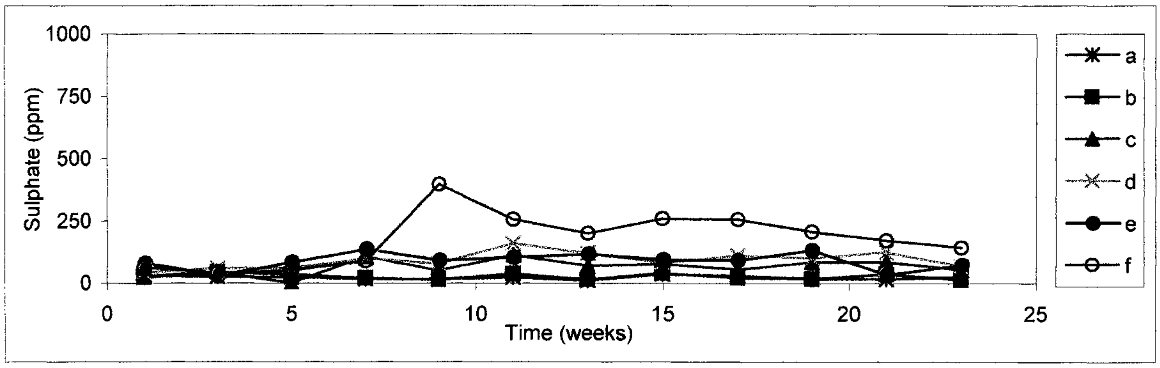
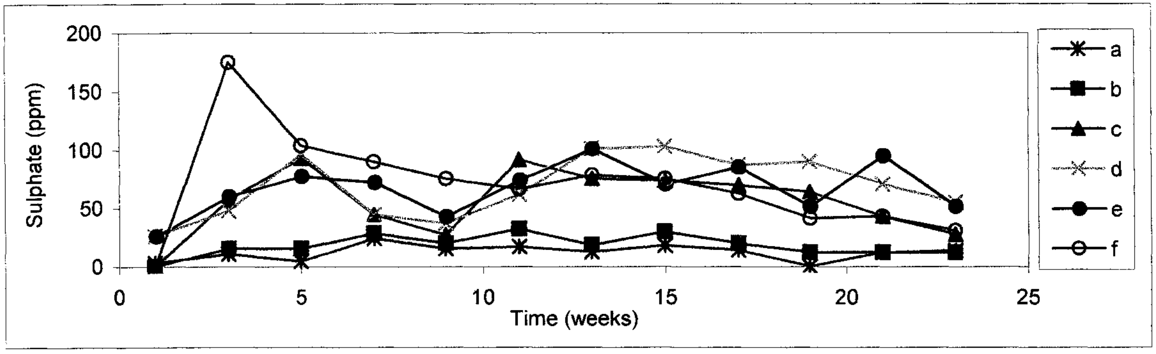
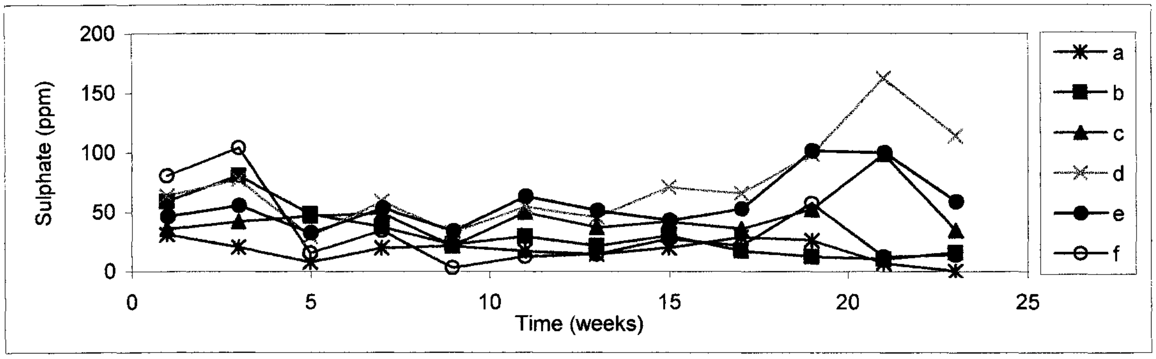

Sulphate (ppm)
1000
750
500
250
0
0
5
10
15
20
25
Time (weeks)
a
b
c
d
e
f

Figure 3.10. Sulphate concentrations (ppm) in drained water from sediment cores with depth and over time from sediment submitted to 6 months of wet/dry event from the 4 study sites, a) 1, b) 2, c) 3 and d) 4 for Black Donald Lake. The sample ports refer to the sampling depths (a = + 2.5 cm, b = 0 cm (sediment-water interface), c = - 2.5 cm, d = - 5 cm, e = - 7.5 cm, f = approximately - 20 cm)

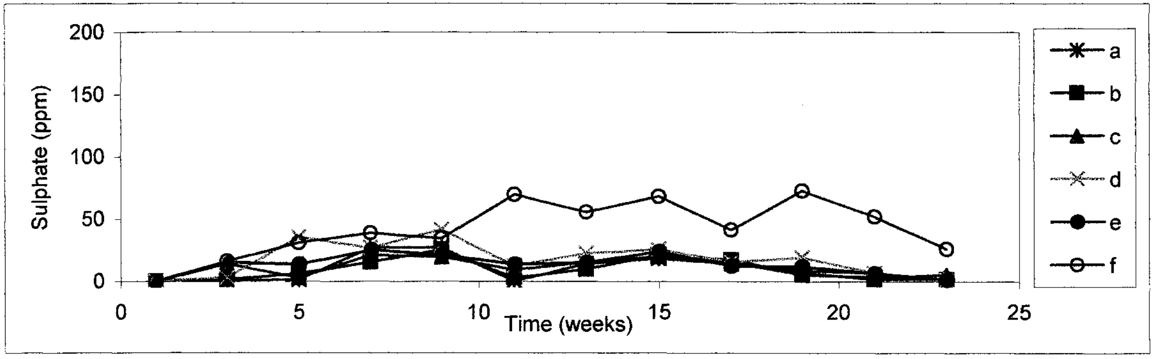
a)


Sulphate (ppm)
200
150
100
50
0
0
5
10
15
20
25
Time (weeks)
a
b
c
d
e
f

b)


Sulphate (ppm)
200
150
100
50
0
0
5
10
15
20
25
Time (weeks)
a
b
c
d
e
f

c)


Sulphate (ppm)
200
150
100
50
0
0
5
10
15
20
25
Time (weeks)
a
b
c
d
e
f

d)

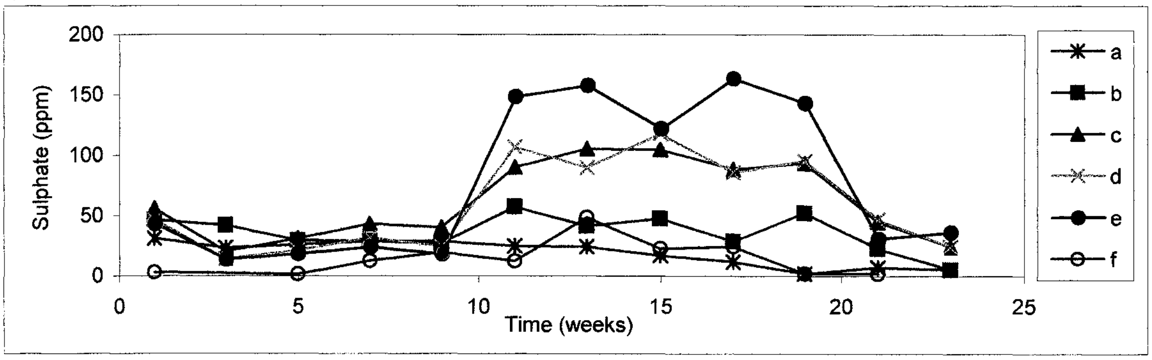

Sulphate (ppm)
200
150
100
50
0
0
5
10
15
20
25
Time (weeks)
a
b
c
d
e
f

Table 3.3. Sulphate concentration (ppm) of water drained at various depths and over time (24 ml) from cores for all sites from both lakes after 6 months of wetting and drying cycles (every two weeks with artificial rainwater, pH ~ 4.5). The sample ports refer to the sampling depths (a = + 2.5 cm, b = 0 cm (sediment-water interface), c = - 2.5 cm, d = - 5 cm, e = - 7.5 cm, f = approximately - 20 cm).

Lake	Site	Sulphate (ppm) from sample ports					
		a	**b**	**c**	**d**	**e**	**f**
Stump	A	144	281	1407	2723	536	1440
	B	214	347	929	1698	1901	3149
	C	401	1635	2715	3206	2700	3885
	D	263	310	762	1105	1077	2124
Black Donald	1	145	215	666	819	808	841
	2	211	382	541	871	692	394
	3	119	109	131	210	162	503
	4	201	426	739	702	920	147

In Stump Lake, sites B and C had almost twice as much sulphate draining from the cores compared to sites A and D (up to 750 – 950 ppm compared to only 200 – 300 ppm). Significant differences in sulphate levels drained from different depths were observed for all sites ($p < 1.1 \times 10^{-5}$), where concentrations increased with depth for most sites (Appendix N) (Figures 3.9b, c and d). During the wetting and drying experiment, the sulphate released into the leachate did not significantly vary over time for all cores ($p > 0.105$) (Appendix N).

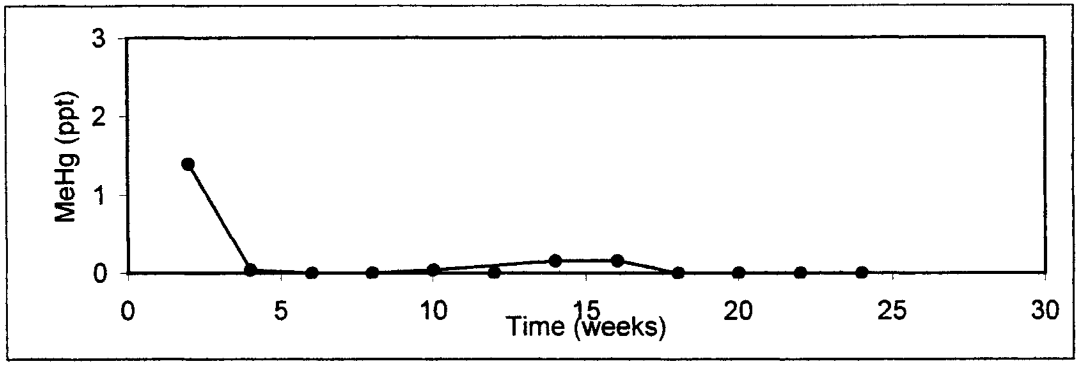
From Black Donald Lake sediments, significant differences in sulphate levels with depth were observed for all cores ($p < 2.7 \times 10^{-6}$) except for site 4 ($p > 0.521$) (Figure 3.9) (Appendix O). Sulphate appeared to increase with depth, although leachate collected from the bottom of the core (sample port f) did not always have the highest concentrations, as seen for most cores from Stump Lake (Figures 3.9 and 3.10). In sites 1, 2 and 4, sediment in the upper portion of the cores (i.e., 2.5 – 7.5 cm below the sediment-water interface; ports c – e), released the highest levels of sulphate into the water. The wetting and drying events affected sulphate drained from the cores from site 2 and 3 over time, where site 2 appeared to increase over time ($p < 0.033$), while sites 1 and 4 were not affected by time ($p > 0.1$) (Appendix O).

3.5 Methylmercury concentrations in leachate from the bottom sediments submitted to wetting and drying events and its relationship with sulphide and sulphate concentrations

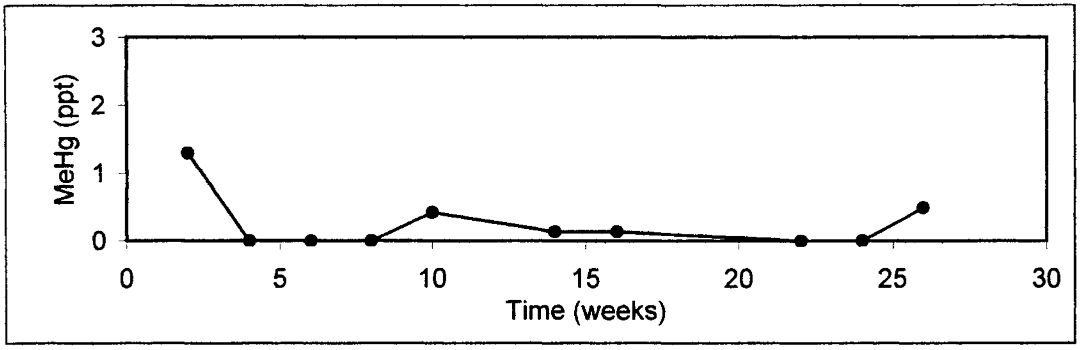
The concentrations of MeHg collected from the bottom of the cores (port f) at the first drain event for Stump Lake ranged between 1 – 2 ppt and decreased with time (Figure 3.11). In general, the concentrations of MeHg were less than 0.25 ppt for the entire experiment. Similarly to Stump Lake, the MeHg that drained from Black Donald Lake

Figure 3.11. MeHg (ppt) concentrations in water drained from the bottom of sediment cores (port f) during 6 months of wet/dry events for sites a) A, b) B, c) C and d) D for Stump Lake.

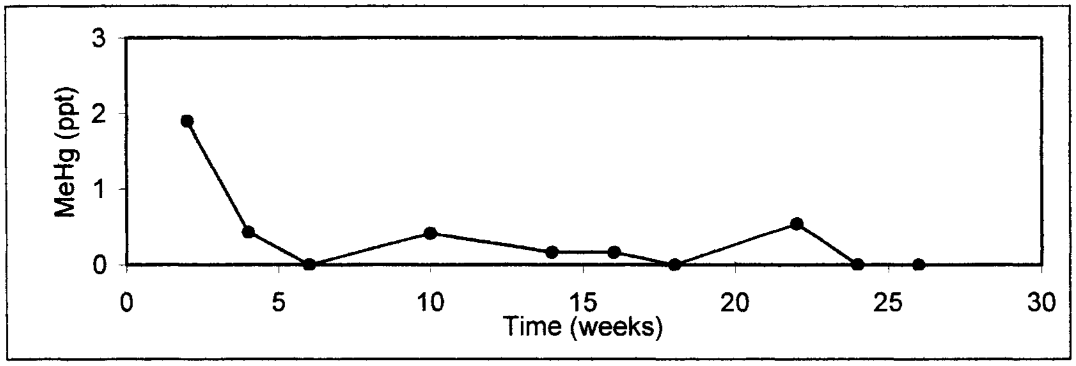
a)


MeHg (ppt)
0
1
2
3
Time (weeks)
0
5
10
15
20
25
30

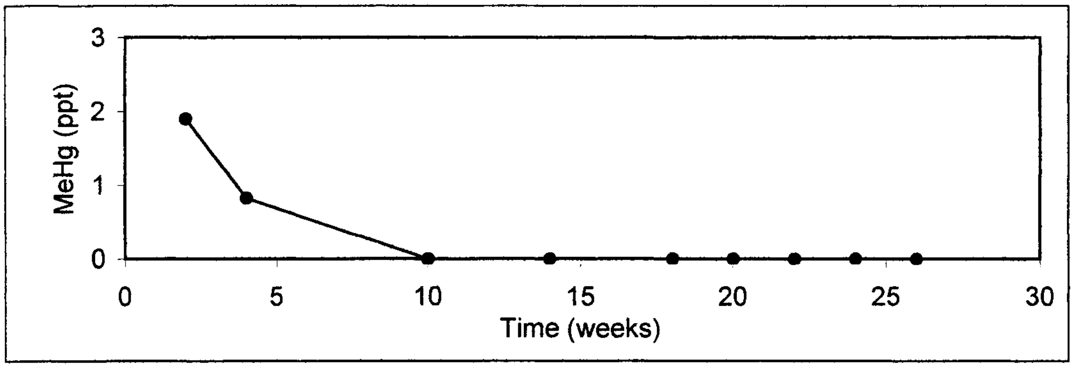
b)


MeHg (ppt)
0
1
2
3
Time (weeks)
0
5
10
15
20
25
30

c)


MeHg (ppt)
0
1
2
3
Time (weeks)
0
5
10
15
20
25
30

d)


MeHg (ppt)
0
1
2
3
Time (weeks)
0
5
10
15
20
25
30

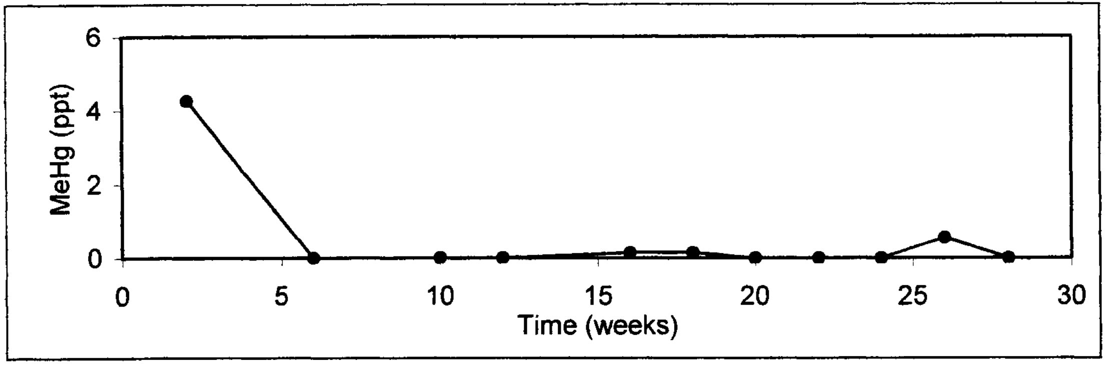
sediments during the first drain were higher than the following drain events (Figure 3.11). Black Donald Lake MeHg concentrations (2.5 – 4.5 ppt) were almost twice as high as Stump Lake at the first drain event, decreasing to less than 0.5 ppt for the remainder of the experiment (Figures 3.11 and 3.12). There were no clear relationships or trends for the correlations between MeHg and pH, sulphide or sulphate concentrations in water drained from the bottom of the sediment cores. Most correlations were low for all parameters ($r < 0.5$ or $r > - 0.5$, $p < 0.05$) (Appendix P).

3.6 Total mercury in sediment

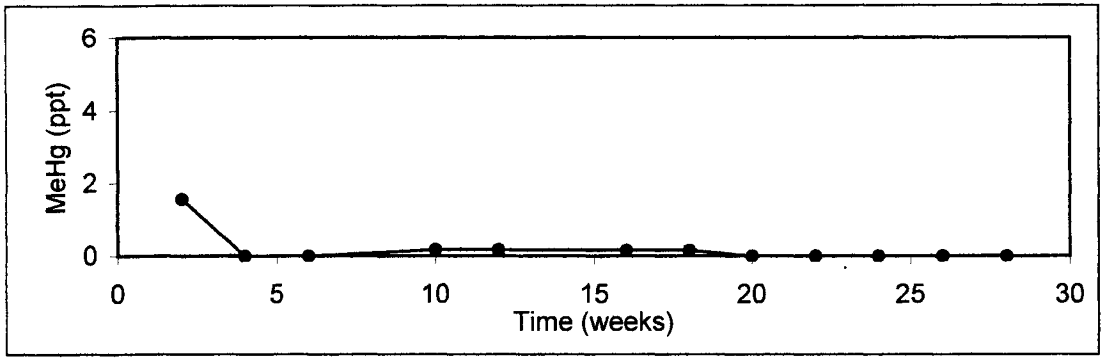
Total mercury (Hg) levels in sediment were higher in Stump Lake (46.2 to 226 ppb (dry weight)) than Black Donald Lake (14.1 to 135 ppb (dry weight)) (Figure 3.13). Hg tended to be higher in the top portion of the sediment profile, and decreased with depth in both lakes, where most cores exhibited a moderate to strong correlation with the organic matter in the sediment ($r > 0.63$, $p < 0.05$) (Appendix Q). All values are low compared with contaminated sites with values of 2000 ppb or more (Lean, personal correspondence). No significant differences with depth were found for site B for Stump Lake and site 4 for Black Donald Lake ($p < 0.021$) (Appendix R). No trends were evident with the amount of total Hg in the 0 month core compared to the cores that underwent three and six months of wetting and drying events, although significant differences were observed over time for both lakes ($p < 0.017$) (Appendix R). For some sites, the 3 or 6 month cores had more Hg than the 0 month core (Figure 3.13).

Figure 3.12. MeHg (ppt) concentrations in water drained from the bottom of sediment cores (port f) during 6 months of wet/dry events for sites a) 1, b) 2, c) 3 and d) 4 for Black Donald Lake.

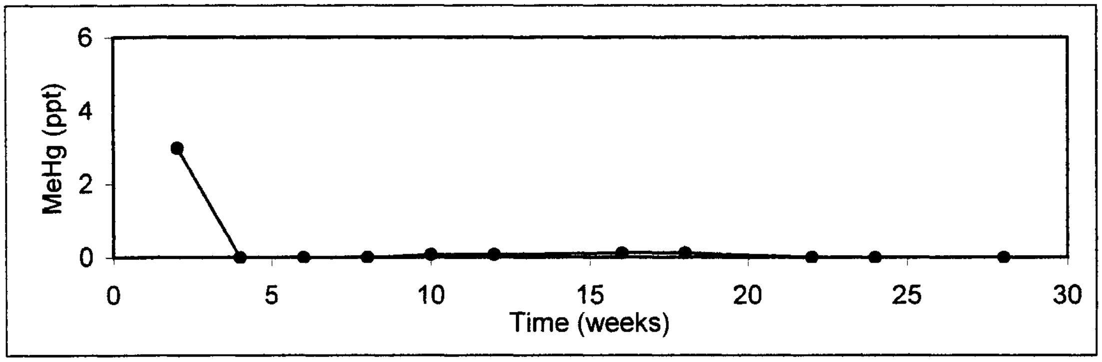
a)


MeHg (ppt)
6
4
2
0
0
5
10
15
20
25
30
Time (weeks)

b)


MeHg (ppt)
6
4
2
0
0
5
10
15
20
25
30
Time (weeks)

c)


MeHg (ppt)
6
4
2
0
0
5
10
15
20
25
30
Time (weeks)

d)

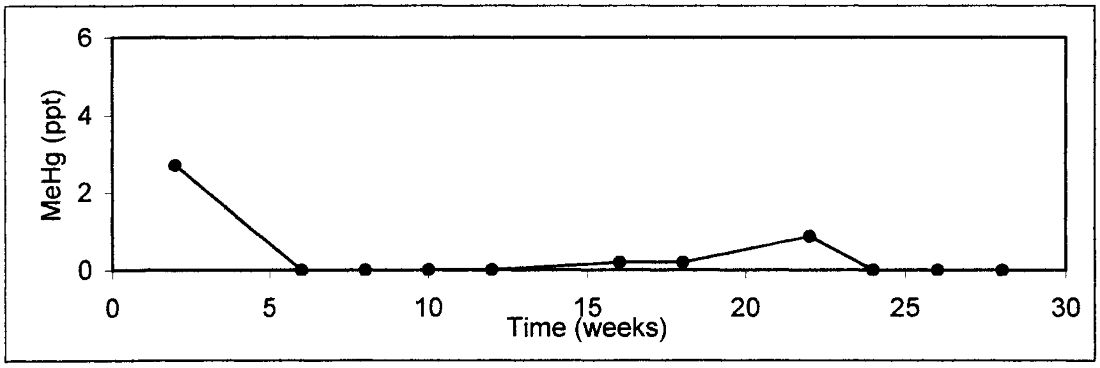
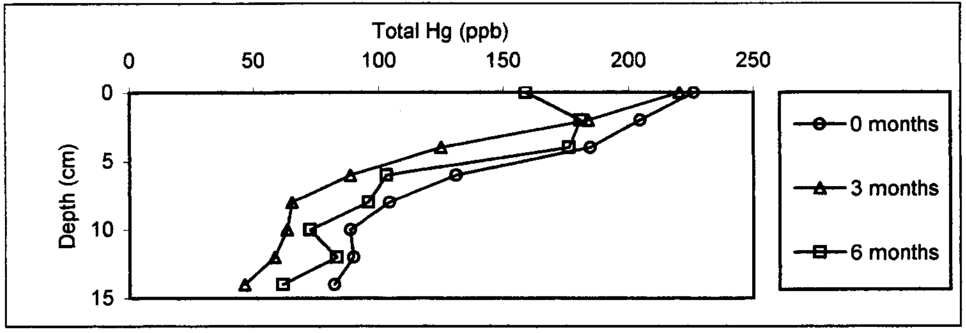

MeHg (ppt)
6
4
2
0
0
5
10
15
20
25
30
Time (weeks)

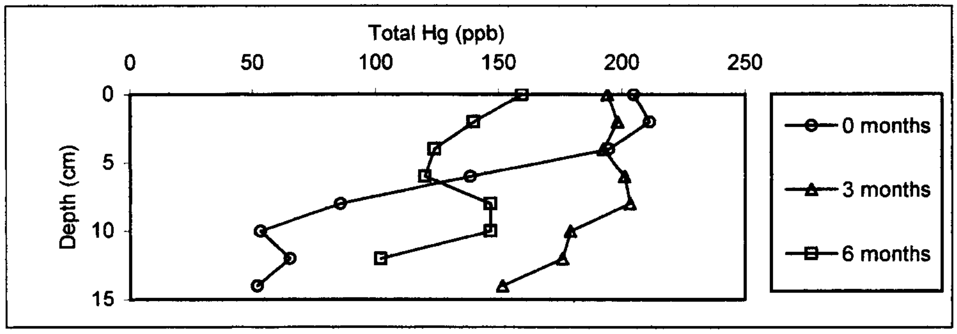
Figure 3.13. Total Hg (ppb) in sediment from Stump (sites a) B and b) D) and Black Donald Lake (sites a) 2 and b) 4) after 0, 3 and 6 months of wet/dry events.

Stump Lake

a)

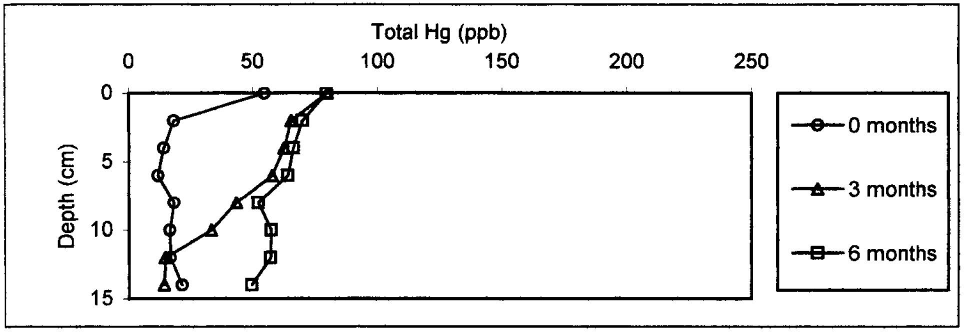


b)

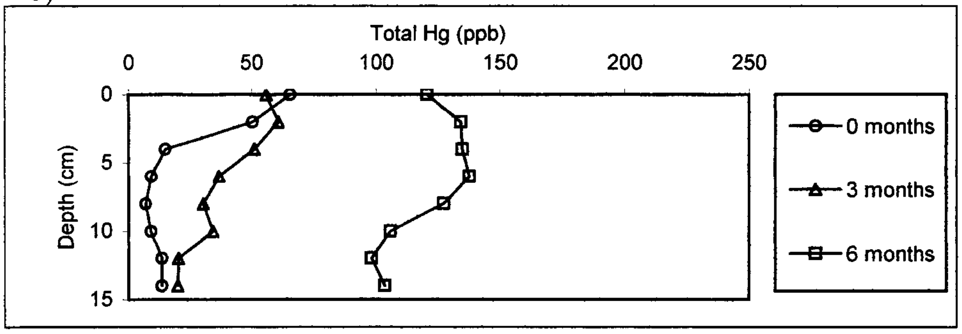


Black Donald Lake

a)



b)



3.7 Partitioning of Hg in the sediment cores submitted to wetting and drying events

The sequential extraction results indicated most of the total Hg (between 50-70 %) was bound to the refractory organic phase (i.e., bound to refractory organic compounds or non-labile organic forms and/or with oxides) of the sediment from both lakes (Figures 3.14 and 3.15). The only exception was the sediment core for the *in situ* (0 month core) from site 2 for Black Donald Lake. Hg bound to the refractory organic phase was lower in this core, ranging between (36.5 – 54 %), and more Hg was bound to the residual fraction at some of the lower depths (Figure 3.15a). The residual fraction, the next most abundant fraction, contained between 20 – 40 % of the total Hg found in the sediments. The remaining Hg was within the soluble organic phase (bound to the humic and fulvic complexes) (less than 20 %), except for site 2 from Black Donald Lake, which was higher (20 – 32%) (Figures 3.14 and 3.15). Total Hg bound to the exchangeable or bioavailable phase was below detection limit (< 1%) for most sites (Figures 3.14 and 3.15).

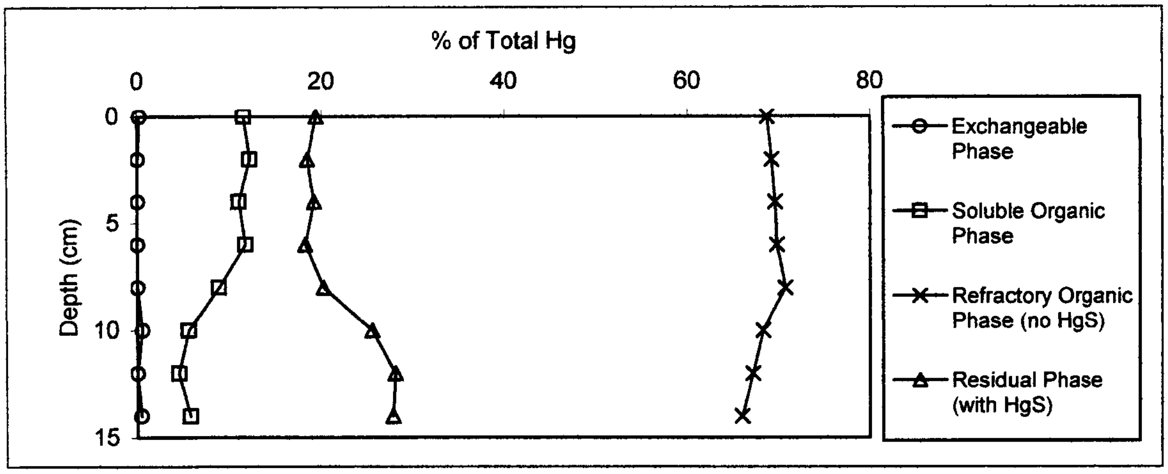
Hg within the sediment appeared to be evenly distributed with depth for both lakes, with differences of less than 10 % for most sediment fractions (Figures 3.14 and 3.15). The exception was the Hg bound to the refractory organic and residual phases for site 2 from Black Donald Lake, where the proportion of Hg noticeably decreased and increased, respectively, with depth (Figure 3.15).

The distribution of total Hg within the sediment chemical phases in Stump Lake showed some changes after 3 and 6 months of wetting and drying cycles (Figure 3.17). Differences were observed for Hg in the soluble organic phase over time ($p < 0.013$), where values increased with time for site B, from between 4.7-12.1 % at time zero to between 12.7-19.8 % (Figure 3.14a) (Appendix S). Decreases were also observed in the refractory organic and residual phases for site B over time (Figures 3.14a). Both phases appeared to

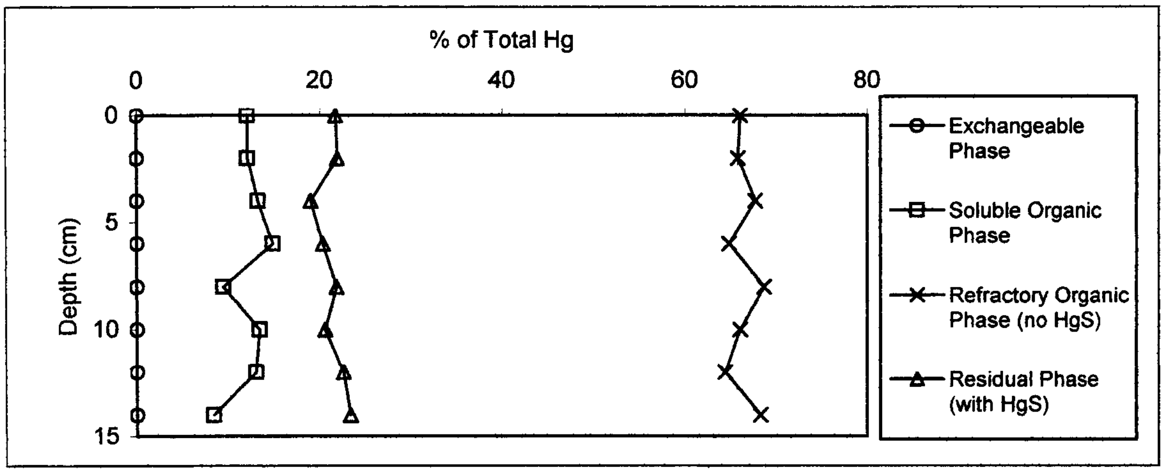
Figure 3.14. Extractable phase distribution of total Hg in sediment, shown as % of total Hg, from sites a) B and b) D from Stump Lake, after i) 0, ii) 3 and iii) 6 months of wet/dry events.

a)

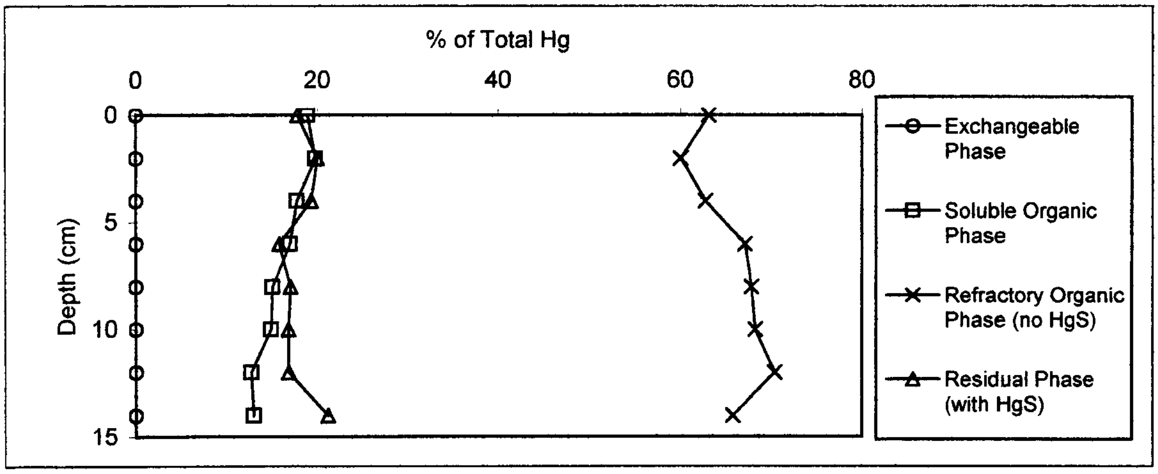
i)


% of Total Hg
0
20
40
60
80
Depth (cm)
0
5
10
15
Exchangeable Phase
Soluble Organic Phase
Refractory Organic Phase (no HgS)
Residual Phase (with HgS)

ii)

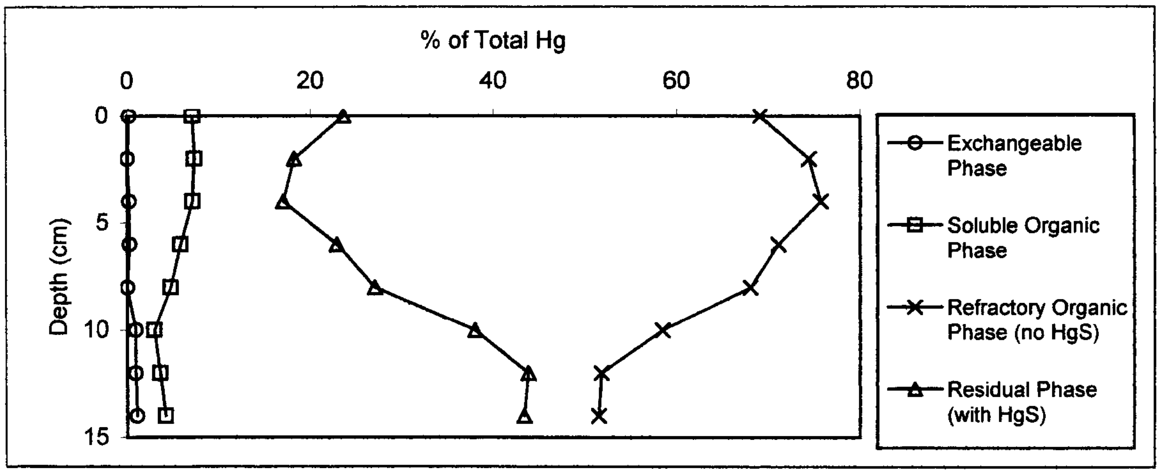

% of Total Hg
0
20
40
60
80
Depth (cm)
0
5
10
15
Exchangeable Phase
Soluble Organic Phase
Refractory Organic Phase (no HgS)
Residual Phase (with HgS)

iii)

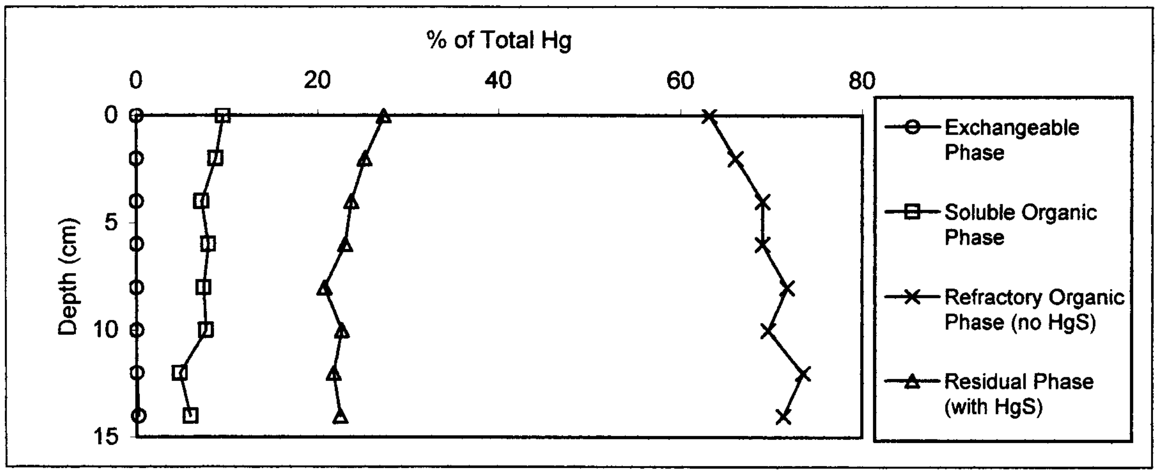

% of Total Hg
0
20
40
60
80
Depth (cm)
0
5
10
15
Exchangeable Phase
Soluble Organic Phase
Refractory Organic Phase (no HgS)
Residual Phase (with HgS)

b)

i)


% of Total Hg
0
20
40
60
80
Depth (cm)
0
5
10
15
Exchangeable Phase
Soluble Organic Phase
Refractory Organic Phase (no HgS)
Residual Phase (with HgS)

ii)


% of Total Hg
0
20
40
60
80
Depth (cm)
0
5
10
15
Exchangeable Phase
Soluble Organic Phase
Refractory Organic Phase (no HgS)
Residual Phase (with HgS)

iii)

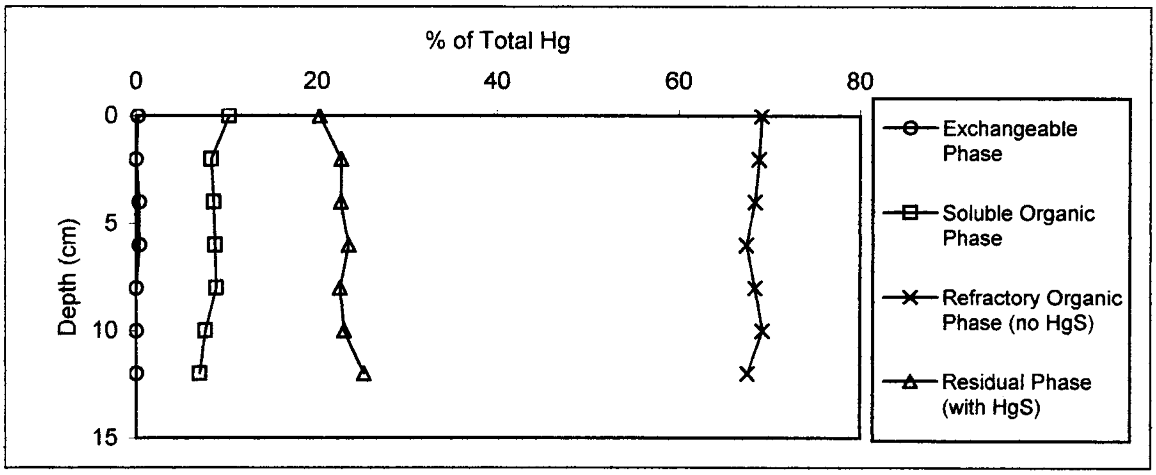
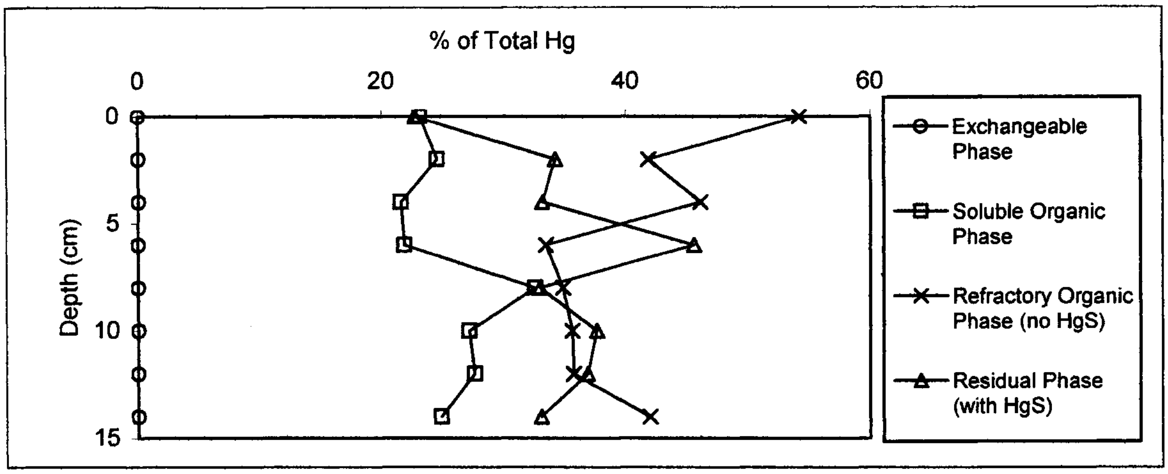

% of Total Hg
0
20
40
60
80
Depth (cm)
0
5
10
15
Exchangeable Phase
Soluble Organic Phase
Refractory Organic Phase (no HgS)
Residual Phase (with HgS)

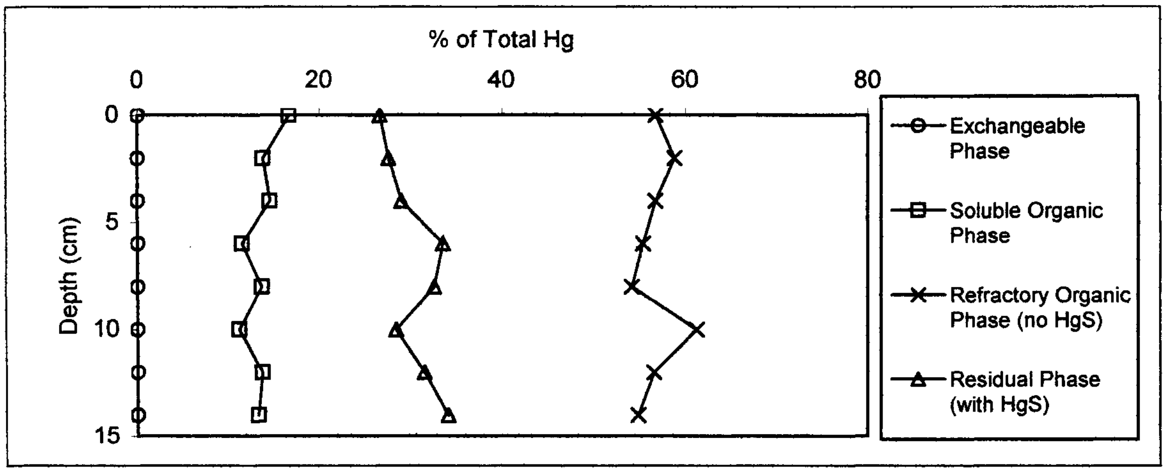
Figure 3.15. Extractable phase distribution of total Hg in sediment, shown as % of total Hg, from sites a) 2 and b) 4 from Black Donald Lake, after i) 0, ii) 3 and iii) 6 months of wet/dry events.

a)

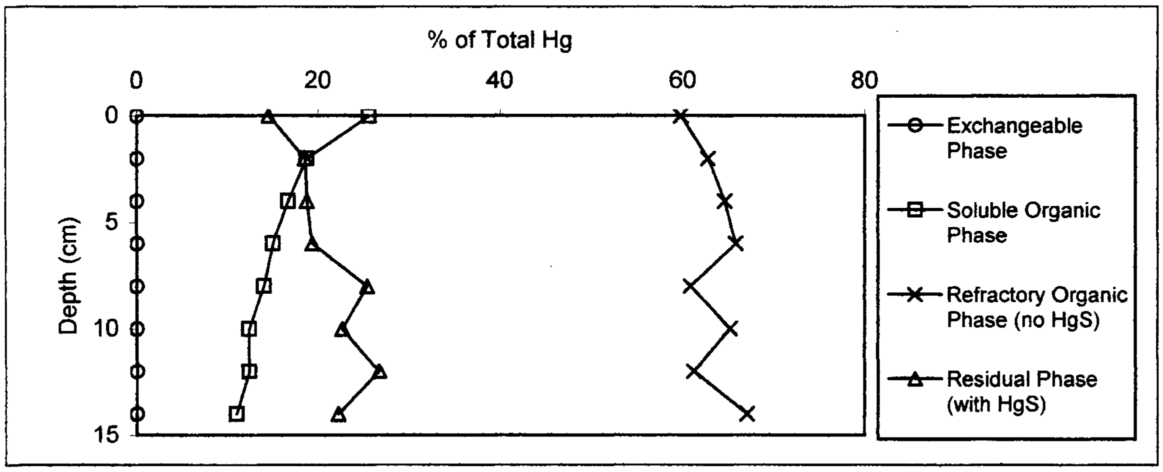
i)


% of Total Hg
0
20
40
60
Depth (cm)
0
5
10
15
Exchangeable Phase
Soluble Organic Phase
Refractory Organic Phase (no HgS)
Residual Phase (with HgS)

ii)

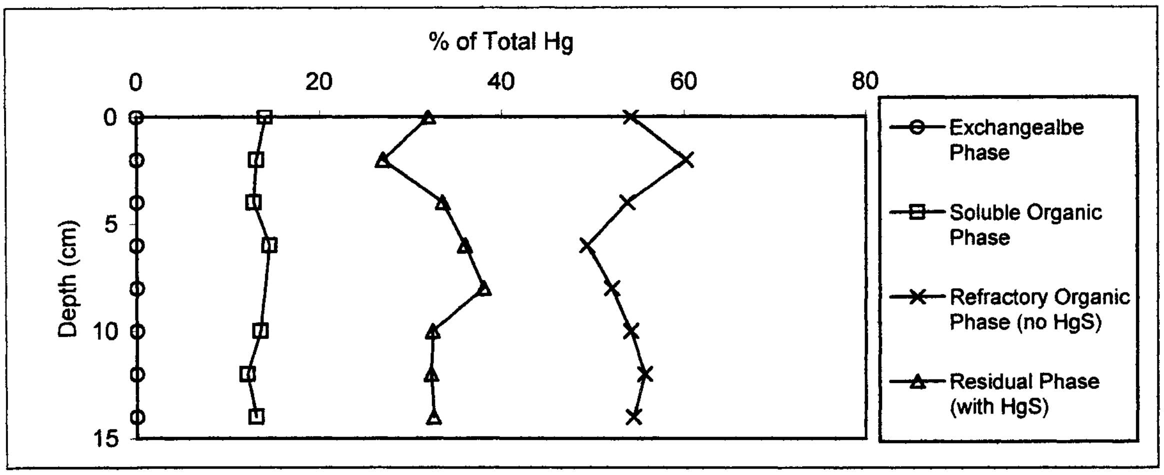

% of Total Hg
0
20
40
60
80
Depth (cm)
0
5
10
15
Exchangeable Phase
Soluble Organic Phase
Refractory Organic Phase (no HgS)
Residual Phase (with HgS)

iii)

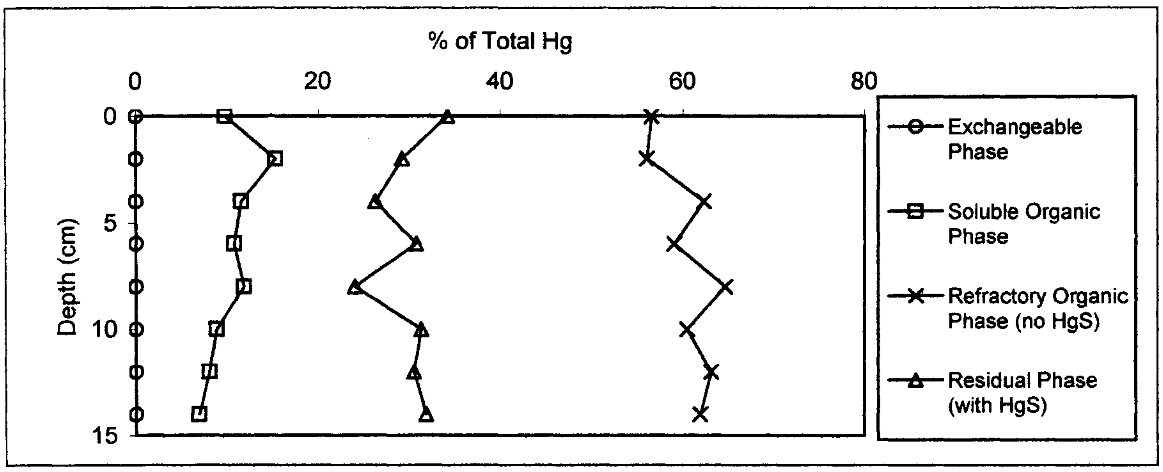

% of Total Hg
0
20
40
60
80
Depth (cm)
0
5
10
15
Exchangeable Phase
Soluble Organic Phase
Refractory Organic Phase (no HgS)
Residual Phase (with HgS)

b)

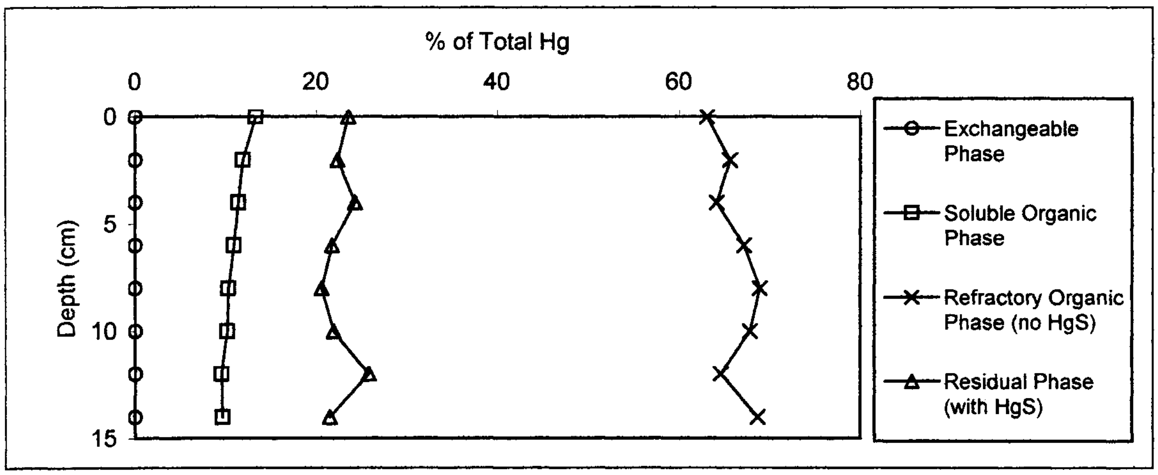
i)


% of Total Hg
0
20
40
60
80
Depth (cm)
0
5
10
15
Exchangealbe Phase
Soluble Organic Phase
Refractory Organic Phase (no HgS)
Residual Phase (with HgS)

ii)


% of Total Hg
0
20
40
60
80
Depth (cm)
0
5
10
15
Exchangeable Phase
Soluble Organic Phase
Refractory Organic Phase (no HgS)
Residual Phase (with HgS)

iii)


% of Total Hg
0
20
40
60
80
Depth (cm)
0
5
10
15
Exchangeable Phase
Soluble Organic Phase
Refractory Organic Phase (no HgS)
Residual Phase (with HgS)

lose between 3 to 4 % of total Hg throughout the sediment profile over time, although only site B had a significant change over time ($p < 0.014$) for the residual phase (Appendix S). No changes in the distribution of Hg with depth occurred within the various phases over time, except within the soluble organic fraction ($p < 7.9 \times 10^{-3}$) (Appendix S).

After 3 and 6 months of wetting and draining events, there were some definite changes in the distribution of Hg in the sediment chemical phases for Black Donald Lake ($p < 0.013$) (Appendix S) (Figure 3.15). Total Hg in the refractory organic phase increased, but slightly decreased in residual phase over time (Figure 3.15). A change in the distribution of Hg in the soluble organic phase was also observed for site 2 in Black Donald Lake (Figure 3.15a). After 3 months of wetting and draining events, total Hg decreased from between 21.9 – 32.6 % to between 11.2 – 16.7 %, with no change after 6 months of wetting and draining events. Hg levels in most chemical phases remained stable with depth over time ($p > 0.05$), except for Hg bound to the residual phase in site 2 ($p < 0.032$) (Figure 3.15) (Appendix S).

3.8 Methylmercury concentrations in sediment cores submitted to wetting and drying events

Stump Lake had higher concentrations of MeHg in sediment than Black Donald Lake (Table 3.4). Stump Lake MeHg levels ranged between 313 to 2705 ppt, compared to Black Donald Lake where concentrations varied from 141 to 1010 ppt (Table 3.4). There was no clear increase or decrease of MeHg concentrations over time, during the wet/dry experiment. For sites A, B and D for Stump Lake and sites 1 and 2 for Black Donald Lake, the 3 month core had the lowest concentration of MeHg, (Table 3.4). For these sites, it varied as to whether the 0 and 6 month cores were higher or lower than the

Table 3.4. Mean MeHg (ppt) (+/- Standard Error (SE)) in dry sediment of entire core after 0, 3 or 6 months of wet/dry events for all sites in Stump and Black Donald Lakes.

Lake	**Site**	**Month**	**Average MeHg (ppt) in dry sed**	**SE**
Stump	A	0	2705	419
	A	3	1381	98
	A	6	1455	190
	B	0	1317	300
	B	3	313	108
	B	6	717	123
	C	0	1662	414
	C	3	2052	67
	C	6	1473	270
	D	0	1689	327
	D	3	1579	331
	D	6	1812	460
Black Donald	1	0	312	46
	1	3	141	12
	1	6	323	32
	2	0	769	91
	2	3	395	84
	2	6	680	89
	3	0	416	46
	3	3	503	89
	3	6	428	64
	4	0	890	61
	4	3	N/A	N/A
	4	6	1010	129

MeHg concentration within the 3 month. The other sites behaved in a similar manner, except the 3 month core was higher than the other cores (site C for Stump Lake and site 3 for Black Donald Lake) (Table 3.4).

3.9 Sulphate-reducing bacteria population in sediment cores submitted to wetting and drying events

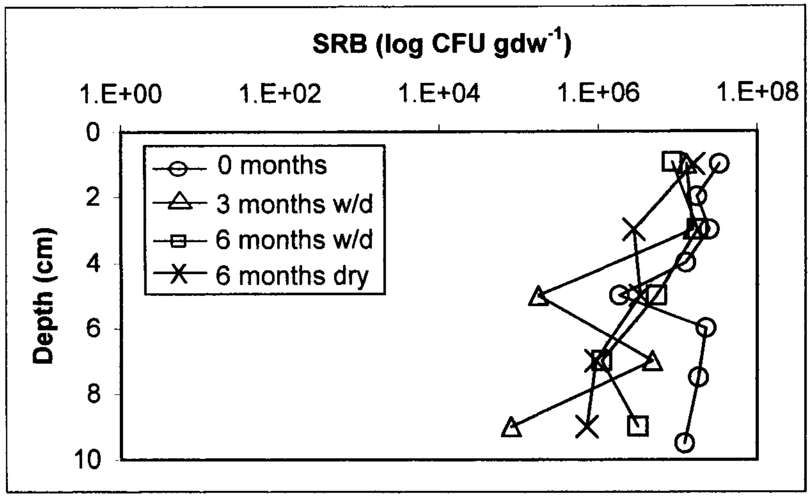
Sulphate-reducing bacteria (SRB) populations were present throughout the sediment cores of both lakes (Figures 3.16 and 3.17). *In situ* SRB populations were similar in both lakes, ranging between 10^5 to 10^8 CFU gdw^{-1}. Overall, Stump Lake SRB populations tended to be slightly higher (Figures 3.16 and 3.17) than Black Donald Lake. *In situ* SRB populations at time 0 appeared to vary slightly with depth and site, and tended to decrease with depth for most sites.

After 3 and 6 months of wetting and drying cycles, or remaining dry for 6 months, SRB populations generally did not change from the initial population counts within the sites for both lakes (Figures 3.16 and 3.17). Slight variations were observed in the distribution of the populations with depth for some sites from both lakes, although statistically there were few differences over time ($p < 0.05$) (Appendix T). Only site B in Stump Lake had a significant difference with depth ($p < 0.036$), while site A in Stump Lake ($p < 0.028$) and site 4 in Black Donald Lake ($p < 0.012$) had a significant difference over time.

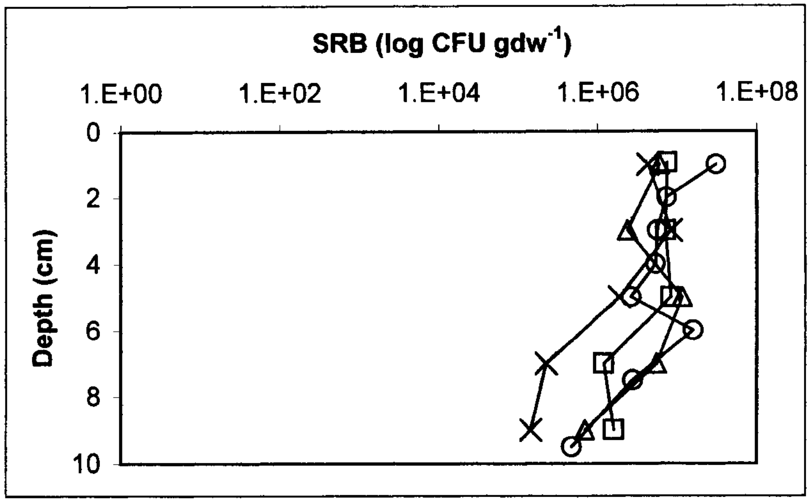
The lake, sediment depth and time of sampling had a significant affect on the overall SRB population count ($p < 0.0074$) (Appendix U). SRB counts at different depths and times depended on the lake while time affected by depth, and vice versa ($p < 0.027$). Looking at the individual depths, only the lake the sediment was collected from created variability in the amount of SRB at 0 – 2 cm and 6 – 8 cm ($p < 0.028$) (Appendix V).

Figure 3.16. Sulphate-reducing bacteria (SRB) populations in sediment with depth and after 0, 3 or 6 months of wet/dry (w/d) events or remaining dry for 6 months of sites a) A, b) B, c) C or d) D from Stump Lake.

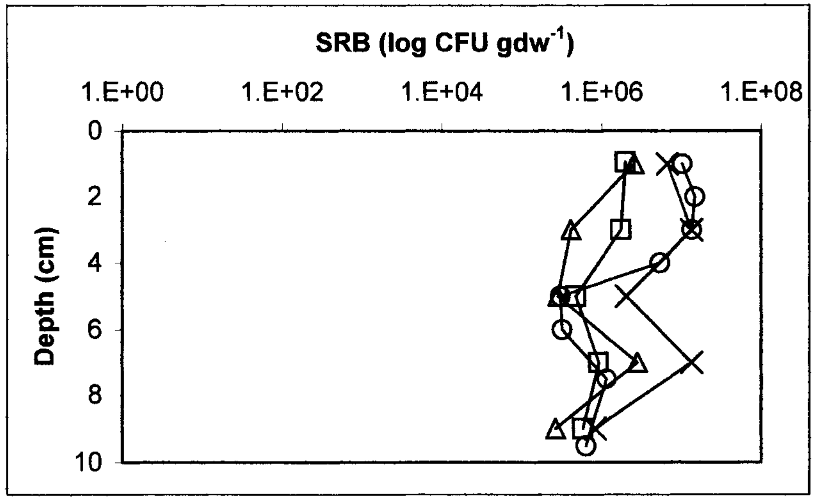
a)


SRB (log CFU gdw^{-1})
1.E+00
1.E+02
1.E+04
1.E+06
1.E+08
Depth (cm)
0
2
4
6
8
10
0 months
3 months w/d
6 months w/d
6 months dry

b)


SRB (log CFU gdw^{-1})
1.E+00
1.E+02
1.E+04
1.E+06
1.E+08
Depth (cm)
0
2
4
6
8
10

c)


SRB (log CFU gdw^{-1})
1.E+00
1.E+02
1.E+04
1.E+06
1.E+08
Depth (cm)
0
2
4
6
8
10

d)

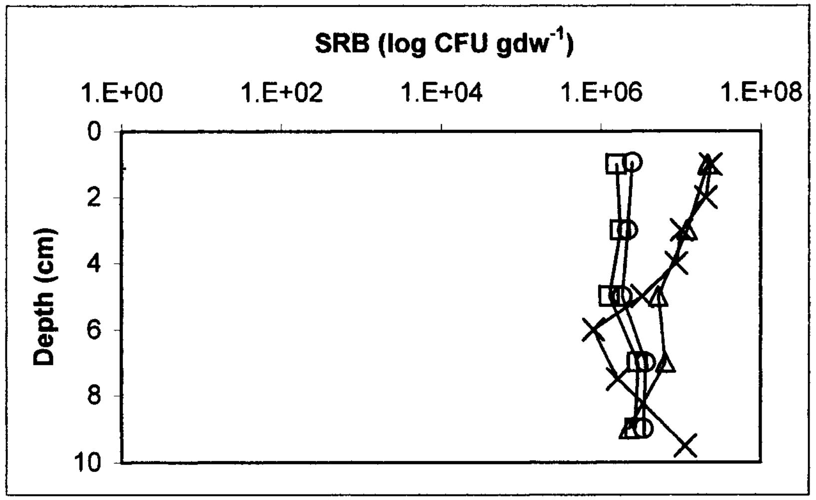
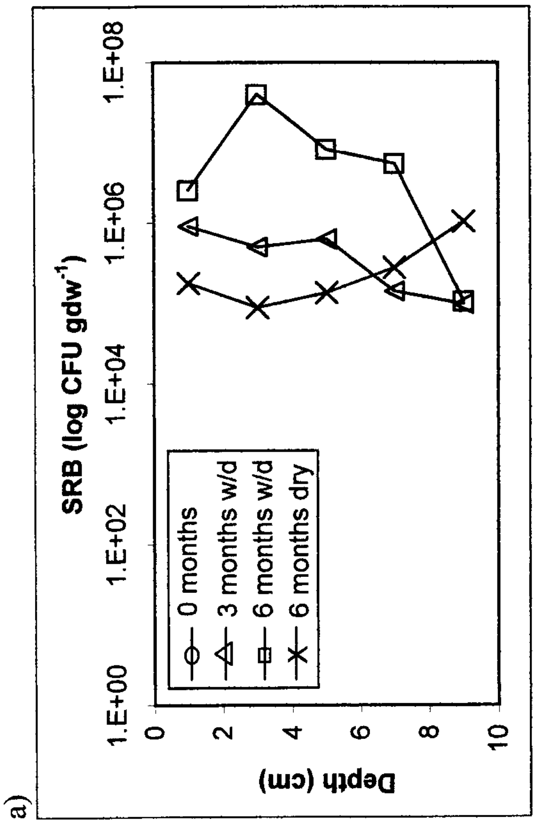
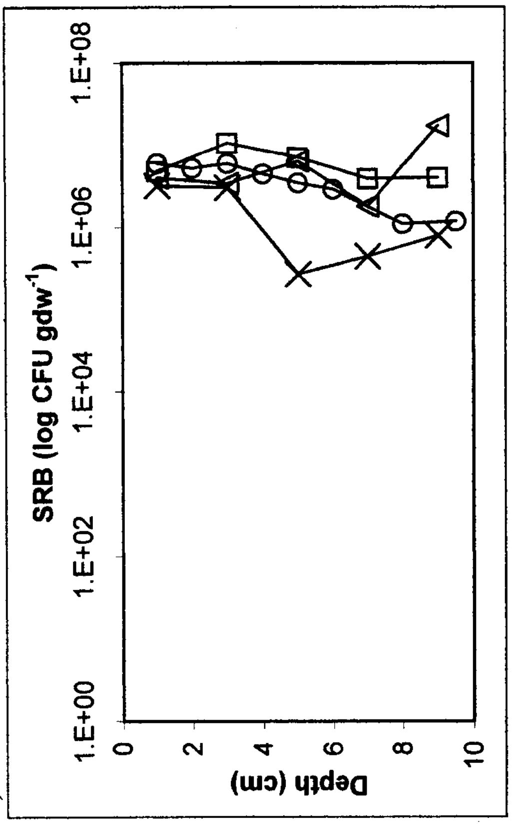

SRB (log CFU gdw^{-1})
1.E+00
1.E+02
1.E+04
1.E+06
1.E+08
Depth (cm)
0
2
4
6
8
10

Figure 3.17. Sulphate-reducing bacteria (SRB) populations in sediment with depth and after 0, 3 or 6 months of wet/dry (w/d) events or remaining dry for 6 months of sites a) 1, b) 2, c) 3 or d) 4 from Black Donald Lake.

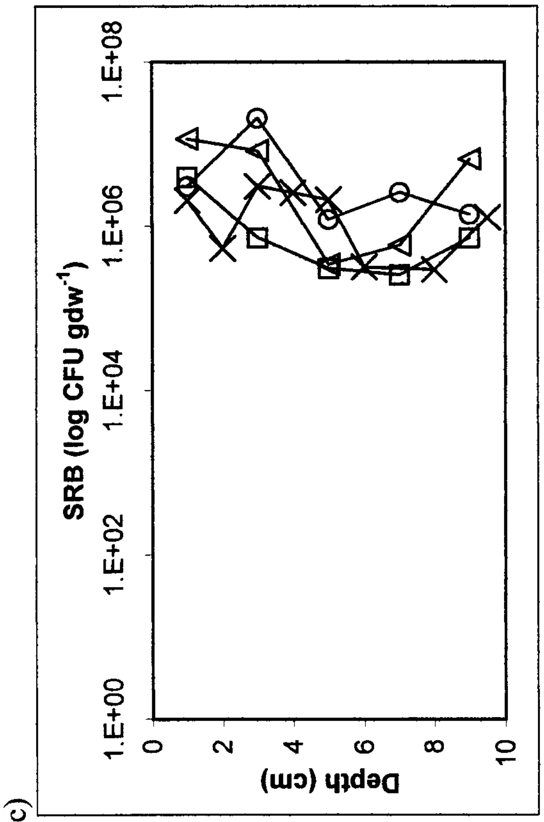
a)


SRB (log CFU gdw^{-1})
1.E+00
1.E+02
1.E+04
1.E+06
1.E+08
Depth (cm)
0
2
4
6
8
10
0 months
3 months w/d
6 months w/d
6 months dry

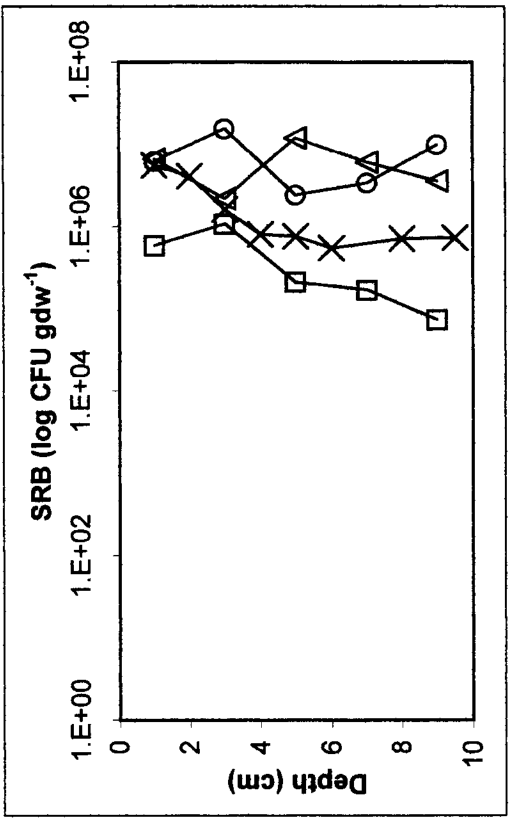
b)


SRB (log CFU gdw^{-1})
1.E+00
1.E+02
1.E+04
1.E+06
1.E+08
Depth (cm)
0
2
4
6
8
10

c)


SRB (log CFU gdw^{-1})
1.E+00
1.E+02
1.E+04
1.E+06
1.E+08
Depth (cm)
0
2
4
6
8
10

d)


SRB (log CFU gdw^{-1})
1.E+00
1.E+02
1.E+04
1.E+06
1.E+08
Depth (cm)
0
2
4
6
8
10

4.0 DISCUSSION

4.1 Physico-chemical conditions of the study lakes

One of the purposes of this study was to determine how two lakes with apparent differing buffering capacities and physico-chemical characteristics would behave during wetting and drying cycles. The buffering capacity of the two lakes was assumed to differ based on the different bedrock composition. Although the lake water and porewaters from both lakes exhibited neutral pH levels, where Stump Lake was more alkaline. It was assumed Stump Lake would have a lower buffering capacity, due to the presence of granitic bedrock in the surrounding area (Pauk, 1989). However, the high organic matter content of Stump Lake sediments due to the large amount of decomposing trees and plant matter in the lake, provided some alkalinity to the lake water. Organic matter has the ability to buffer acidity, by increasing the alkalinity or acid neutralizing capacity of substrates (Cai et al., 1998 and Stumm and Morgan, 1996). Functional carboxyl and/or hydroxyl functional groups in humic compounds from organic matter can bind protons and metals that enter a system, therefore increasing the acid neutralizing capacity (Cai et al., 1998; Tipping, 1998). In contrast, the buffering capacity of Black Donald Lake waters and sediments was primarily from a mineral source, the carbonate-rich bedrock in the area (Lumbers, 1982).

Conductivity and alkalinity levels of the two studied lakes were similar and comparable to those of other lakes in Ontario (Lean, unpublished data). Slightly higher alkalinity and conductivity levels in Stump Lake waters may be attributed to the highly organic nature of the sediments (Stumm and Morgan, 1996). Decreased porewater sulphate concentrations with sediment depth in both lakes, and the presence of sulphide around the same depths result from microbial sulphate reduction (Mitsch and Gosselink, 1993). Higher sulphide levels in Stump Lake sediment may be attributed to more sulphate being reduced, as seen by lower sulphate levels in the cores compared to Black Donald Lake, which consequently had lower porewater sulphides. The presence of Fe(II) in porewaters of both lakes also indicates that the sub-surface sediments were anoxic and that iron-oxides underwent reductive dissolution (Fortin et al., 1993). Evidence of Fe(III) reduction during the wetting and drying events was indeed reported during the column experiment (Nugent, 2005).

Sediment composition is one aspect where the lakes greatly differed. Higher sediment water content in Stump Lake was associated with higher organic matter content in the lake substrates. Organic matter naturally increases the water-holding capacity of soils and sediments (Mitch and Gosselink, 1993 and Gerrard, 2000). In contrast, the sandier, low organic matter sediments in Black Donald Lake allowed the water to drain quickly, resulting in drier sediments with lower moisture content (Gerrard, 2000) (Figures 3.3 and 3.4). Therefore, this may explain the highly variable range in sediment moisture of Black Donald Lake sediments over time, compared to Stump Lake sediments (Gerrard, 2000). The differences observed between sediment water or organic matter content for cores within the same site is due to the heterogeneity of the sediments within the sites, rather than wetting and drying events (Figures 3.3 and 3.4) (Mulligan, Yong and Gibbs, 2001). Despite the fact that all cores within a particular site were sampled within the same area, usually within 50 to 100 cm of each other, it does not guarantee similar sediment properties.

4.2 pH changes in water drained from cores submitted to wetting and drying cycles

pH of water drained from the sediments was affected by time (6 months wetting and drying experiment) and more importantly with depth (Figures 3.5 and 3.6). It is difficult to predict whether the acidic rainwater additions or biogeochemical reactions in the sediments, such as oxidation of inorganic and organic sulphur, iron and nitrogen species, which release H^+ ions, had the greater impact on the pH of the leachate (Calmano et al., 1993 and Christensen, 1998). However, the presence of high amounts of sulphate and low sulphides in water from sediments of both lakes does indicate that biogeochemical reactions did contributed to acid generation in the sediment, and to the subsequent decrease of pH (Figures 3.5 and 3.6). It is also clear that the sediment from both lakes buffered some of the acidic rainwater (pH ~ 4.5) and acidity generated from the biogeochemical reactions in the upper sediments, because the pH of the leachate collected from the bottom of the cores ranged between 4.50 and 7.35. The biogeochemical reactions occurring in the deeper sediments are described in greater detail in section 4.3.

The results indicate the sediments' buffering capacity was sustained over time (6 months) in the deeper portion of Black Donald Lake sediments, although this was not so in the upper sediment portion for both lakes (Figures 3.5 and 3.6). This is in partial agreement with hypothesis 1 and the studies of Saeki et al. (1993) and Peverly and Kopka (1991), who observed acid generation in sediments exposed to air. The buffering capacity of deeper Black Donald Lake sediments was most likely due to presence of sandy sediments rich in carbonate minerals from the surrounding bedrock and from microbial sulphate-reduction. This suggests that Black Donald Lake sediments had more buffering capacity than the organic-rich sediments of Stump Lake, which is consistent with hypothesis 1. The results only apply to the 6-month experiment and any extrapolation beyond 6 months would be speculative. Longer wetting and draining events might eventually lower the pH of the water flushed from the Black Donald sediments and negatively affect the biota and potentially increase the bioavailability of certain metals, as seen in several studies (Preda and Cox, 2000; 2001).

4.3 Leachate sulphide, sulphate and MeHg concentrations, the effect of wetting and draining events, and the relationship between pH, sulphide, sulphate vs. MeHg

4.3.1 Effect of wetting and drying cycles on sulphide and sulphate concentrations in leachate over time

Another one of the main objectives of the project was to determine if MeHg, a known neurotoxin that can be high in reservoirs (Verdon et al., 1991 and Tremblay et al., 1993), would be released from rewetted dried/oxidized sediments. As pH, sulphate and sulphide have an effect on the production and release of MeHg, as stated in section 1.6.3, another objective was to determine whether submitting sediments to wetting and drying events effect sulphide and sulphate concentrations over time.

For both lakes, high levels of sulphate were present in the leachate from the middle of the core for most cores (< 941 ppm from Stump Lake and < 175 ppm from Black Donald Lake) (Figures 3.9 and 3.10), while low levels of sulphides were present (Figures 3.7 and 3.8). Under oxic conditions caused by the drying events, sulphide would be oxidized to sulphate, decreasing the levels of sulphides and increasing the levels of sulphate in drained waters. The formation of insoluble metal sulphide species under reducing conditions in the deeper sediments of both lakes, may also have reduced the amount of soluble sulphides in the drained water (Drever, 1997).

When high concentrations of Fe(II) are present in porewaters, precipitation of FeS will likely occur, therefore limiting the concentration of sulphide in the porewater. Although sulphides were present in leachate from the bottom sediments (port f) of some sites, the low overall levels of sulphide in leachate may be related to the levels of Fe(II) in the porewater of the substrate (Nugent, 2005). Low levels of Fe(II) will lead to an excess of soluble sulphide. Therefore the concentrations of Fe(II) in the sediments porewaters from both lakes may have been sufficient for the precipitation of FeS, therefore reducing the levels of soluble sulphide in the leachate (Drever, 1997).

Evidence of microbial sulphate reduction in the sediments of both lakes over time is shown first by the presence of SRB populations (Figures 3.16 and 3.17) and secondly by the production of sulphide in the leachate, especially from port f in both lakes (Figures 3.7 and 3.8). In low temperature environments, sulphide can only be produced as a result of microbial sulphate reduction (Berner, 1985). Microbial sulphate reduction generates alkalinity as well (Berner, 1985), which could have consumed some of the acidity produced in the upper portion of the cores from both lakes during the wetting and drying events. Low levels of sulphides in the top portion of the sediment (ports b-e) indicate that microbial sulphate reduction may not have been a major acid neutralizing process at those depths.

Higher soluble sulphide and sulphate concentrations were present in Stump Lake leachate compared to Black Donald Lake. Because sulphides in porewater and leachate were much less than sulphate drained from the Stump Lake sediment cores, sulphate was most likely produced from decomposition of reduced organic sulphur compounds or the oxidation of pyrite (FeS_2), a more stable iron sulphide, rather than by sulphide oxidation (Brady, 1990 and Schippers and Jorgensen, 1998). Higher sulphate concentrations in leachate from

Stump Lake sediments can be the result of high amounts of sediment organic matter, which naturally release sulphur compounds during decomposition (Brady, 1990 and King et al., 2002). With the oxidation of mostly reduced sulphur compounds and sulphides, sulphate would naturally be produced. Therefore, it may be the reason for the high amounts of sulphate released from sediments from both lakes, in particular from the highly organic Stump Lake (Drever, 1997).

4.3.2 Methylmercury drained from the sediments submitted to wetting and drying events and the relationship with sulphide, sulphate and pH of the leachate

The wet/dry cycles did not increase the mobility of MeHg in the sediment. Although both lakes were expected to leach MeHg during wetting events, Stump Lake more so than Black Donald Lake, it was evident that the available MeHg in sediment from both lakes was leached at the start of the experiment, followed by minimal to below detection limit levels of MeHg leached afterwards (Figures 3.11 and 3.12). MeHg levels in water drained from the sediment during the first weeks of wet/dry cycles were similar to studies that measured the MeHg leached from flooded soils (Povari and Verta, 1995) and from a flooded boreal forest wetland (Kelly et al., 1997). The lower MeHg concentrations after the initial wet/dry cycles were similar to the low concentrations of MeHg in runoff from a study of drained and undrained wetlands (Westling, 1991).

After the initial release of MeHg from sediment during the first or second rain event, MeHg remained bound within the sediments, as there was still considerable amounts of MeHg left in the sediment cores (Table 3.4). MeHg in the sediment was most likely bound to organic matter, as Hg has a strong association with organic matter in both soils and sediments (Dmytiw et al., 1995, Hintelmann et al., 1995, Regnell 2001 and Remy et al., 2003). This was observed in the sequential extraction results, where approximately 55 – 75 % of total Hg was bound to soluble and refractory organic phases (Figures 3.14 and 3.15). Organic matter strongly binds Hg, reducing leaching, volatilization, or plant uptake of Hg (Stein et al., 1996). Given the high amounts of organic matter in Stump Lake sediments, this can explain why Stump Lake sediments released lower levels of MeHg compared to Black Donald Lake, even though it had much higher concentrations of MeHg in the sediment (Figures 3.4, 3.11 and 3.12).

It was hypothesized that MeHg drained from highly vegetative Stump Lake sediments would be higher, as flooded vegetation naturally releases Hg which can be methylated into MeHg (Kelly et al., 1997) (see section 1.6.3). Sediments high in organic matter are also known to enhance growth and activity of microbes, such as sulphate-reducing bacteria (SRB), which are known to methylate mercury under anoxic conditions (see section 1.6.3). (Bodaly et al., 1984 and Morrison and Therien, 1991). Although MeHg may have been produced under anoxic conditions as high amounts of flooded vegetation decomposed back when it was first flooded in the 1920s, some studies have shown MeHg levels in newly flooded reservoirs can return to baseline concentrations after some time (between 20 – 30 years) (Johnston, Bodaly and Mathias, 1991 and Verdon, 1991). Therefore, MeHg produced in the 1920s when the dam was installed, may have already returned to ambient levels.

Although it is known that pH, sulphate and sulphide concentrations affect mobility and production of MeHg, lack of a general patterns in the correlations between these measured parameters may be due to sampling technique in general and the below detection limit concentrations of MeHg after the first few weeks (Ramlal et al., 1985 and Benoit et al., 1999). Concentrations of sulphide and sulphate themselves may have been another reason for variations in correlations. High levels of sulphate (> 500 µM) and concentrations of sulphide above micromolar levels may reduce the production of MeHg (Compeau and Bartha, 1985, Gilmour and Henry, 1991 and King et al., 2002). Although *in situ* production of MeHg was not tested in this study, high levels of sulphate and sulphide, which were above these reported values for many of the sites could have resulted in lower levels of MeHg drained from the sediment cores, if any was being produced (Figures 3.5 - 3.8). MeHg production is known to mainly occur in anoxic conditions where SRB thrive and mediate mercury methylation, and degrades in oxic condition, which could have reduced any MeHg production and leaching (Bodaly et al., 1984, Povari and Verta, 1995 and Morel et al., 1998). The near neutral pH of water drained from the lower sediment portion may have also resulted in minimal levels of MeHg released as MeHg mobility is known to increase with a decrease in pH (Hintelmann et al, 1995).

4.4 Sulphate-reducing bacteria populations and methylmercury production and the effect of wet/dry cycles

Another objective of this project was to assess the presence of SRB and evaluate the effect of wetting and drying events on the populations, as they are known methylators of Hg. SRB were found at all sites and depths for both lakes, which can be related to near-neutral pH conditions of the sediment, preferred by these microbes (Widdle, 1988 and Hao et al., 1996). Throughout the wet/dry cycles over the weeks, it was hypothesized that increased oxic conditions would decrease the activity and number of SRB altogether, as SRB tend to prefer anoxic conditions (Widdle, 1988). Periods of drying throughout the experiment showed no effect on SRB populations present over 3 or 6 months of wetting and drying cycles, in addition to drying for 6 months in most sites (site A for Stump Lake and site 4 for

Black Donald Lake) (Figure 3.16 and 3.17). The results can be attributed to SRB's ability to survive in low levels of oxygen and to the ability of some species to sporulate when conditions are not favourable (Dilling and Cypionka, 1990 and Baldwin et al., 2000). The culture-based technique used to enumerate SRB might have caused a bias in the actual number of SRB present at all sites and sampling dates (Fortin et al., 2000). Several studies indicate that the Most Probable Number (MPN) method tends to underestimate total populations, compared to other microbiological and molecular techniques (Teske et al., 1996 and Vester et al., 1998). However, the SRB population trends obtained by MPN can still be used for comparative analysis (Fortin et al., 2000). Values may not give actual population counts, but the technique definitely indicated SRB populations typically did not decrease throughout the sediment cores during wetting and drying cycles, or drying for 6 months. *In situ* sulphate reduction rates would have been better indicators of microbial sulphate-reduction, as recently shown by Praharaj and Fortin (2004). The results from this study showed that SRB could be recovered from both oxic and anoxic regions of the sediment cores, as observed in other studies (Fortin et al., 2000; 2002 and Fortin and Praharaj, 2004). However, the MPN technique cannot be used to infer SRB activity and it is unknown if SRB were indeed active under oxic conditions in the sediment cores of both lakes.

The ability of the SRB to methylate Hg also could not be quantified by the MPN experimental method. The presence of very low MeHg concentrations in drain water suggests that the activity of the SRB may have been inhibited in the sediment cores, assuming that the main methylation pathway in the sediment was biotic. Oxic conditions caused by the drying events may have also resulted in reduced Hg methylation over time, if Hg methylation was occurring in the sediment (Compeau and Bartha, 1985 and Macalady et al., 2000). Therefore, low MeHg in drained water from the sediment may have resulted from wet/dry cycles, as conditions were not conducive to microbial Hg methylation.

4.5 Mercury and methylmercury in sediment and the effect of wet/dry cycles

Another objective of the project was to determine if wetting and drying cycles had an effect on the distribution of total Hg in sediments. Higher Hg concentration in Stump Lake sediment compared to Black Donald Lake sediment is most likely from the amount of flooded vegetation, which naturally released Hg as it decomposed, as previously mentioned (Schuster, 1991, Porvari et al., 1995 and Wallschlager et al., 1996). Hg and MeHg levels are commonly higher in sediments with more organic matter content (Stein et al., 1996).

Higher concentrations of total Hg within cores submitted to 3 or 6 months of wetting and drying events compared to Hg in the core at time zero, contradicted the initial hypothesis that Hg would leach from sediments over time. Higher levels of Hg in the 3 or 6 month cores, compared to the *in situ* Hg concentrations (0 month core that did not undergo wet/dry events) could be due to sediment heterogeneity within the particular sites, as explained in section 4.1. It is also necessary to relate the values to dry weight basis, as drier cores appear to have more mercury on a wet weight basis. Although all cores from one site were sampled within a small area, usually within 50 – 100 cm of each other, there may have been slightly different concentrations of Hg within the sediment, which attributed to the apparent gain of Hg over 3 and 6 months of wetting and draining for certain sites for both lakes, while the simulated rainwater had no Hg (Appendix A). This can also explain the variability of MeHg in sediment, where MeHg seemed to increase, while MeHg was most likely not being produced, as mentioned in section 4.3.5 (Table 3.4). Although the results may not infer if Hg was lost from sediments, it still gives a good account of Hg concentrations in sediments from both lakes at time of slicing and analysis. Mixing the sediment at the start of the experiment to make sediments homogeneous was not an option, as it would have disrupted the normal distribution of the sediment profile, which was required for the experiment.

As noted in section 2.5.2, distributions of Hg in sediment geochemical fractions or phases were determined by sequential chemical extraction (modified from Hall et al., 1996, Hall and Pelchat, 1997; 1999, Hall et al., 2005 and Hall and Pelchat, 2005). Hg is most mobile in the soluble/bioavailable phase, referred to as the exchangeable phase (0.01 M $Ca(NO_3)_2$), where Hg is easily removed under normal environmental conditions (i.e. water flushing through the sediment). Mercury associated to the soluble organic phase (0.1 M $Na_4P_2O_7$) is moderately mobile. The refractory organic phase includes all Hg bound to refractory organic compounds, as Hg bound in non-labile organic forms and/or with oxides, but does not extract HgS. Hg bound to this phase is relatively stable and immobile, since it was subjected to a 40% HNO_3 leach. The residual phase includes all metals released by an Aqua Regia leach (12M HCl and 16M HNO_3 in a ratio of 3:1), which removed all remaining Hg in the sediment, including HgS. The Hg bound to this phase is considered immobile (Hall and Pelchat, 2005).

As previously mentioned, Hg has a high affinity for organic matter and sulphur ligands, which coincides with most Hg being associated with refractory organic and residual phases (Dmytriw, 1995, Bilali et al., 2002 and Beldowski and Pempkoiak, 2003). Both phases are considered immobile, as metals remain strongly bound under normal environmental conditions (Beldowski and Pempkowiak, 2003 and Han et al., 2003). Therefore Hg bound to these phases, which is over 70% for both phases in most sites, cannot be easily flushed from sediments during wetting events under normal environmental conditions, resulting in the minimal MeHg drained from sediments throughout the wetting and drying experiment. Undetectable concentrations of Hg in the exchangeable phase, the most bioavailable phase for Hg mobility, further emphasizes that Hg will likely remain bound within the sediment for both lakes.

Definite changes occurred with the distribution of Hg over time for both lakes during the wetting and drying experiment (Figure 3.14 and 3.15). For Black Donald Lake in particular, increases of Hg into more immobile phases, such as the refractory organic phase, means Hg is less likely to be leached from sediments under normal environmental conditions (Beldowski and Pempkowiak, 2003, Han et al., 2003 and Sahuquillo et al., 2003). Sediment heterogeneity may have caused the difference between month 0 compared to the 3 and 6 month cores over time, and with depth for site 2 from Black Donald Lake as well (Fig 3.15). Distribution of Hg in the sediment chemical phases in the 0 month core was very different from the two other cores which went through 3 and 6 months of wetting and draining events, with no significant difference between cores ($p > 0.05$). Therefore, differences in the distribution of Hg may have been due to wet/dry cycles, although it was most likely due to sediment heterogeneity for this particular site.

The increase of Hg from relatively immobile phases to more mobile phases, such as increases of Hg in the soluble organic phase over time for Stump Lake sites, is of some concern (Figure 3.14). Hg may be leached from these sediments as water flushes through them (Saeki et al., 1993, Grondin et al, 1995 and Buykx et al., 2000). Although site B and D experienced a redistribution of Hg to the more bioavailable soluble organic phase, the two sites from Black Donald Lake indicated Hg may have become more stable, with a general increase of Hg in the refractory organic phase. Therefore, the potential of Hg leaching from Black Donald Lake sediments is low, while Stump Lake sediment may release Hg. Seeing that Stump Lake had higher total Hg concentrations in the sediment overall, this is also of some concern (Figure 3.14). Although because low concentrations of MeHg were leached from sediments over time, this illustrates Hg may remain bound in the sediment. However, if Hg continues to redistribute to the more mobile soluble organic phase, bioavailability of Hg can increase over time.

4.6 Future Work

As both study lakes had neutral waters and good buffering capacities, it would be important to study lakes with poor buffering capacities as well. This would allow one to determine if metals would be mobilized as wet and dry cycles progress, since metals are known to mobilize in acidic conditions (Preda and Cox, 2000; 2001). Measuring the mobilization of other metals, in addition to testing sediments that were heavily contaminated with Hg or other metals, would also be another aspect of the project that would be beneficial to study.

Sulphate in water drained from the sediments was very high. Therefore, it would be interesting to determine how the sulphate is being produced in the sediments.

Sulphate-reducing bacteria (SRB) are known methylators of Hg in certain conditions (King et al, 2002), therefore it would be worthwhile to determine if the activity of SRB is affected by the wetting and drying events, by measuring *in situ* sulphate reduction rates. Measuring the type of SRB present and whether the types of SRB change over time could also be done.

5.0 CONCLUSIONS

This study has shown that wetting and drying cycles in sediments from lakes can alter certain chemical aspects of water drained through the sediments. Although the wetting and drying cycles had no apparent effect on the estimates of sulphate-reducing bacteria populations in Stump or Black Donald Lake sediments throughout the experiment, there were several other parameters that changed over time. The buffering capacity of the sediments definitely affected the pH variations in the leachate, as simulated acidic rainwater was buffered to a neutral pH for both lakes. The pH of water drained through the sediments did decrease over time in the sediments from Stump Lake, indicating that the sediments' buffering capacity declined over time. However, the pH of waters drained from the bottom of most Black Donald Lake sediments remained the same or increased over the 6 month wetting and drying experiment. The decreasing pH of the leachate for all other cores, may be of some concern as the pH of water flushed through the sediments may continue to decrease, creating acidifying conditions, which is known to mobilize certain metals (Preda and Cox, 2000; 2001).

The wetting and drying cycles did not affect the sulphide and sulphate in drained water from sediment for most sites of both lakes. Sulphide concentrations were low (<11 ppm) for both lakes, while sulphate levels were considerably higher (<941 ppm). The oxidation of reduced sulphur compounds and pyrite in the sediment is the likely source of the sulphate flushed from the sediments, especially from the highly organic sediments of Stump Lake which had approximately five time more sulphate in the leachate compared to Black Donald Lake. The concentrations of sulphate can be a factor in Hg methylation at certain concentrations and conditions, although no relationships were found.

The wetting and drying events appeared to decrease the amount of MeHg drained from the sediments from both lakes. The oxidizing conditions during the drying events may have reduced the methylation of mercury as the process mainly occurs in anoxic condition (King et al, 2002). The oxic conditions may have also reduced the activity (although not quantified in this experiment) of anaerobic sulphate-reducing bacteria, which are known methylators of Hg. Therefore, the unfavourable conditions for Hg methylation in addition to the minimal MeHg drained from the sediment after the first week of rain events, suggest that MeHg was not being produced and the MeHg present in the sediment was remaining bound to the substrate, most likely the organic matter. The further binding of most of the Hg to immobile or non-labile fractions in the sediment (refractory organic phase), and the increase of Hg in these fractions over time for Black Donald Lake suggests the wetting and drying cycles further stabilized the Hg. Although the increase of Hg in the semi-mobile fraction (soluble organic phase) in Stump Lake sediments may be of concern, as it may flush out of the sediments. However, it must be noted that Hg may be easily mobilized in acidic waters, and if the pH decreases, the amount of Hg, in addition to MeHg, in water flushed through the sediment might increase over time, which may be of concern in poorly buffered aquatic systems (Hintelmann et al, 1995).

It is evident from the study that the physico-chemical conditions of lake water and sediments should be assessed, in addition to the biogeochemical changes that may occur, before and after water levels are altered by such events as drought induced by climate change, reservoir draw down, or the removal of dams altogether. Although the changes in Stump and Black Donald Lake sediments were not severe, as most of the Hg accumulated in immobile sediment fractions and minimal amounts of MeHg were released from the sediments from both lakes, more may have been released if the acidic rainwater was not buffered by the sediments of the two lakes. Therefore, if the buffering capacity of the sediments continued to decrease, the effect of wetting and drying events may also increase the mobility of metals from the sediment. In the future, the biogeochemical processes in lakes must be assessed to fully determine the implications of altering water levels, to reduce the influx of metals and acidity to the aquatic system, which may ultimately affect the overall health of aquatic systems.

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Appendix A. Simulated Rainwater Recipe

The simulated rainwater recipe was prepared according to daily precipitation chemistry values from Environment Canada's CAPMoN site in Chalk River, Ontario for 1995-2000. The average ion concentrations of the various elements found in the daily precipitation were converted to their respective molar concentration (Table A-1). Specific chemicals were selected to assure only these particular ions, and no others, would be added to the simulated rainwater. The appropriate weight needed to produce the desired ion concentrations similar to the daily precipitation were calculated (Table A-2).

The simulated rainwater was prepared in a clean 30 L HDPE barrel. The chemicals, listed in Table A-2, were added to the 30 L barrel containing 25L of deionized water. Once the solution was thoroughly mixed, the pH of the simulated rainwater was adjusted to approximately pH 4.5 with 0.1M HNO_3 and 0.1M NaOH. The simulated rainwater was stored at 4°C away from light. The required rainwater was removed from cold storage approximately 16 hours before a rain event and left in the laboratory to acclimate to room temperature.

Table A-1. Major ions of average daily precipitation (DP) and simulated rainwater (RW) and their respective concentrations.

Major ions	Concentration of major DP ions (mg/L)	Concentration of major RW ions (mg/L)
H^+ [b]	0.05	0.029
SO_4^{2-} [a]	2.14	2.14
NO_3^-	2.9	4.54
Cl^-	0.14	0.14
NH_4^+ [a]	0.54	0.96
Na^+	0.06	0.06
Ca^{2+}	0.24	0.24
Mg^{2+}	0.04	0.04
K^+	0.04	0.04
pH	4.45	~ 4.5

[a]- The concentration of these chemicals differs from what was necessary to match the DP because of a calculation error made during the preparation of the first simulated rainwater batch. This mistake was purposefully perpetuated to avoid introducing a bias.

[b]- The concentration of protons [H^+] could not be increased because all other element concentration requirements had been met or exceed.

Appendix B. Dates of rain events and sample collection for coth lakes during the 6 month wetting and drying experiment.

Week of sample collection	Date of rain event/sample collection	
	Stump Lake	Black Donald Lake
0	October 18/02	October 24/02
2	November 1/02	November 7/02
4	November 15/02	November 21/02
6	November 29/02	December 5/02
8	December 13/02	December 17/02
10	January 10/03	January 15/03
12	January 24/03	January 29/03
14	February 7/03	February 12/03
16	February 21/03	February 26/03
18	March 7/03	March 12/03
20	March 21/03	March 26/03
22	April 4/03	April 9/03

Appendix C. Chemical Sequential Extraction Protocol

Sample preparation

Approximately 1 g (dry weight) of sediment was added to clean labeled 50 ml polypropylene centrifuge tubes.
Blanks and sediment check standards (LKSD-4...to add reference), 1 each per 8 samples, were included in the entire procedure, identically to the sediment samples.

Sequential Chemical Extraction Procedure

Set A: SOLUBLE / BIOAVAILABLE PHASE

35 ml of 0.01M $Ca(NO_3)_2$ was added to each sample and capped tightly.
The samples were shaken by hand and vortexed with a Vortex Genie® to suspend the sample.

The samples were shaken horizontally on mechanical shaker for 1 hour (160 shakes per minute).
The samples were centrifuged for 10 minutes at 2800 rpm.
The supernatant was decanted into new 50 ml polypropylene centrifuge tubes.
The residues were rinsed with 10 ml of 0.01M $Ca(NO_3)_2$, capped, vortexed to suspend sample and centrifuged for 10 minutes at 2800 rpm.
The rinse was decanted into centrifuge tubes from step 5 and hand shaken to mix well.
Immediately after the extraction was finished, dilutions were made for analysis. 5 ml of a 2.5x dilution with the supernatant and distilled de-ionized (DDI) H_2O were dispensed directly into a 15 ml polypropylene test tube containing 2.5 ml concentrated HCl and 0.05 ml BrCl reagent. The dilutions were vortexed to mix.
The dilutions were stored in the fridge, and analyzed as soon as possible by Vapour Generation ICP-MS.
The residues were refrigerated until the next extraction.
The remaining supernatant was also refrigerated until analysis was completed.

Set B: SOLUBLE ORGANIC PHASE (1st leach)

Once the reagent of 0.1 M $Na_4P_2O_7$ was made (44.606 g $Na_4P_2O_7.10\ H_2O$ and 60 μL 16 M HNO_3 (high purity) in 1 L of DDI H_2O) the pH of the solution was measured and adjusted to 10.0 +/- 0.1 with additional 16 M HNO_3 if necessary.
35 ml of 0.1 M $Na_4P_2O_7$ was added to each tube containing the residues from the previous extraction and capped tightly.
The samples were shaken by hand and vortexed to suspend the sample.
The samples were shaken horizontally on mechanical shaker for 1 hour (160 shakes per

minute).

The samples were centrifuged for 10 minutes at 2800 rpm.

The supernatant was decanted into new 50 ml polypropylene centrifuge tubes.

Immediately after the extraction was finished, dilutions were made for analysis. 5 ml of a 5x dilution with the supernatant and DDI H_2O were dispensed directly into a 15 ml polypropylene test tube containing 2.5 ml concentrated HCl and 0.05 ml BrCl reagent. The dilutions were vortexed to mix.

The dilutions were stored in the fridge, and analyzed within two weeks by Vapour Generation ICP-MS.

The residues were refrigerated until the next extraction.

The remaining supernatant was also refrigerated until analysis was completed.

Set C: SOLUBLE ORGANIC PHASE (2nd leach)

(Note: this leach was repeated for both lakes due to the high organic content in the sediment from Stump Lake, to assure all Hg of metals in the soluble organic phase were removed)

35 ml of 0.1 M $Na_4P_2O_7$ was added to each sample and capped tightly.

The samples were shaken by hand and vortexed to suspend the sample.

The samples were shaken horizontally on mechanical shaker for 1 hour (160 shakes per minute).

The samples were centrifuged for 10 minutes at 2800 rpm.

The supernatant was decanted into new 50 ml polypropylene centrifuge tubes.

The residues were rinsed with 10 ml of 0.1 M $Na_4P_2O_7$, capped and vortexed to suspend and centrifuged for 10 minutes at 2800 rpm.

The rinse was decanted into centrifuge tubes from step 26 and hand shaken to mix well.

Immediately after the extraction was finished, dilutions were made for analysis. 5 ml of a 5x dilution with the supernatant and DDI H_2O were dispensed directly into a 15 ml polypropylene test tube containing 2.5 ml concentrated HCl and 0.05 ml BrCl reagent. The dilutions were vortexed to mix.

The dilutions were stored in the fridge, and analyzed within two weeks by Vapour Generation ICP-MS.

The residues were refrigerated until the next extraction.

The remaining supernatant was also refrigerated until analysis was completed.

Set D: RESIDUALS (without HgS or metal sulfides)

35 ml of 40 % HNO_3 (high purity) was added to each sample and capped tightly.

The samples were shaken by hand and vortexed to suspend the sample.

The samples were shaken horizontally on mechanical shaker for 2 hour (160 shakes per minute).

The samples were centrifuged for 10 minutes at 2800 rpm.

The supernatant was decanted into new 50 ml polypropylene centrifuge tubes.

The residues were rinsed with 10 ml of 40% HNO_3 capped and vortexed to suspend and centrifuged for 10 minutes at 2800 rpm.

The rinse was decanted into centrifuge tubes from step 37 and hand shaken to mix well.

Immediately after the extraction was finished, dilutions were made for analysis, or the leachate was refrigerated until ready. 5 ml of a 20x dilution with the supernatant and DDI H_2O were dispensed directly into a 15 ml polypropylene test tube containing 2.5 ml concentrated HCl. The dilutions were vortexed to mix.

The dilutions were stored in the fridge until analysis by Vapour Generation ICP-MS.

The residues were refrigerated until the next extraction.

The remaining supernatant was also refrigerated until analysis was completed.

Set F: HgS PHASE (Aqua regia)

6 ml of 12M HCl and 2 ml of 16M HNO_3 were added to the residue from previous extraction and cap tightly.

The samples were vortexed to suspend.

The caps were loosened and the samples were left in a fume hood overnight.

In the morning, the caps were tightened and the sample vortexed to re-suspend.

The caps were loosened and the samples were heated samples in a water bath (90 °C for approximately 3 hours.

The samples (capped tightly) were vortexed every 30 minutes. The caps were loosened before being replaced in the water bath.

When color of content of blank sample solution changed from bright orange to pale yellow, the extraction was complete, and the samples were removed from the water bath.

To decrease the acid strength before centrifuging, 20 ml DDI H_2O was added to each tube.

The tubes were vortexed to suspend the samples.

The samples were centrifuged for 10 minutes at 2800 rpm.

The supernatant was decanted into new 50 ml polypropylene centrifuge tubes.

The residues were rinsed with 10 ml of DDI H_2O capped and vortexed to suspend and centrifuged for 10 minutes at 2800 rpm.

The rinse was decanted into centrifuge tubes from step 53 and hand shaken to mix well.

The final volume of tubes was adjusted to 50 ml with DDI H_2O and mixed thoroughly.

The residues and leach solution did not need to be refrigerated after this point.

Dilutions were made by adding 5 ml of a 5x dilution with the supernatant and DDI H_2O were dispensed directly into a 15 ml polypropylene test tube containing 2.5 ml concentrated HCl. The dilutions were vortexed to mix and refrigerated until analysis.

Appendix D. Single factor ANOVA results used to determine a significant difference between the 3 and 6 month wet/dry cores for the measured parameters for all sites in Stump Lake.

Measured Parameter	Site	Sample Port	df	F	P-value	F critical
pH	A	a	1	0.727	0.419	5.318
		b	1	0.030	0.867	5.318
		c	1	2.103	0.185	5.318
		d	1	3.612	0.094	5.318
		e	1	2.987	0.122	5.318
		f	1	10.251	0.013	5.318
	B	a	1	1.675	0.232	5.318
		b	1	0.620	0.454	5.318
		c	1	0.042	0.842	5.318
		d	1	1.881	0.207	5.318
		e	1	0.312	0.592	5.318
		f	1	0.002	0.963	5.318
	C	a	1	0.873	0.377	5.318
		b	1	0.000	0.992	5.318
		c	n/a	n/a	n/a	n/a
		d	1	0.978	0.352	5.318
		e	1	0.308	0.594	5.318
		f	1	5.214	0.063	5.987
	D	a	1	1.322	0.284	5.318
		b	1	0.138	0.720	5.318
		c	1	4.906	0.058	5.318
		d	1	7.622	0.025	5.318
		e	1	0.051	0.827	5.318
		f	1	6.683	0.032	5.318
Sulphide	A	a	1	0.127	0.731	5.318
		b	1	0.305	0.596	5.318
		c	1	0.088	0.774	5.318
		d	1	10.669	0.011	5.318
		e	1	0.084	0.780	5.318
		f	1	14.405	0.005	5.318
	B	a	1	0.685	0.432	5.318
		b	1	0.945	0.354	4.965
		c	1	0.310	0.590	4.965
		d	1	0.796	0.393	4.965
		e	1	3.644	0.085	4.965
		f	1	4.025	0.080	5.318
	C	a	1	1.466	0.265	5.591
		b	1	0.000	1.000	5.318
		c	1	0.669	0.437	5.318
		d	1	1.997	0.195	5.318
		e	1	1.016	0.343	5.318
		f	1	110.477	0.000	5.318
	D	a	1	1.869	0.202	4.965
		b	1	2.304	0.160	4.965

		c	1	3.288	0.100	4.965
		d	1	0.691	0.425	4.965
		e	1	4.171	0.068	4.965
		f	1	0.998	0.341	4.965
Sulphate	A	a	1	0.089	0.774	5.318
		b	1	0.143	0.715	5.318
		c	1	0.816	0.393	5.318
		d	1	0.057	0.817	5.318
		e	1	18.384	0.003	5.318
		f	1	6.433	0.035	5.318
	B	a	1	0.069	0.798	4.965
		b	1	1.844	0.204	4.965
		c	1	5.039	0.049	4.965
		d	1	8.185	0.017	4.965
		e	1	3.899	0.096	5.987
		f	1	30.257	0.001	5.318
	C	a	1	21.464	0.002	5.318
		b	1	13.883	0.006	5.318
		c	1	0.085	0.778	5.318
		d	1	1.590	0.243	5.318
		e	1	1.273	0.292	5.318
		f	1	4.627	0.064	5.318
	D	a	1	1.051	0.330	4.965
		b	1	0.700	0.422	4.965
		c	1	14.464	0.005	5.318
		d	1	18.329	0.002	4.965
		e	1	41.631	0.000	4.965
		f	1	0.561	0.471	4.965
MeHg	A	f	1	2.350	0.674	5.318
	B	f	1	0.089	0.479	4.965
	C	f	1	19.010	0.015	5.318
	D	f	1	1.565	0.120	4.965

Appendix E. Single factor ANOVA results used to determine a significant difference between the 3 and 6 month wet/dry cores for the measured parameters for all sites in Black Donald Lake.

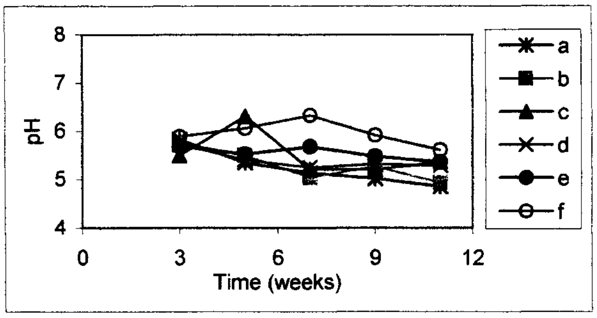
Measured Parameter	Site	Sample Port	df	F	P-value	F critical
pH	1	a	1	0.547	0.481	5.318
		b	1	0.881	0.375	5.318
		c	1	0.245	0.634	5.318
		d	1	1.276	0.291	5.318
		e	1	0.829	0.389	5.318
		f	1	5.802	0.043	5.318
	2	a	1	1.685	0.231	5.318
		b	1	0.939	0.361	5.318
		c	1	0.998	0.347	5.318
		d	1	1.372	0.275	2.318
		e	1	2.555	0.149	5.318
		f	1	0.002	0.969	5.318
	3	a	1	0.302	0.598	5.318
		b	1	1.702	0.228	5.318
		c	1	2.356	0.163	5.318
		d	1	0.758	0.409	5.318
		e	1	0.079	0.786	5.318
		f	1	0.270	0.617	5.318
	4	a	1	2.542	0.150	5.318
		b	1	4.201	0.075	5.318
		c	1	11.067	0.010	5.318
		d	1	13.411	0.006	5.318
		e	1	22.778	0.001	5.318
		f	1	2.959	0.124	5.318
Sulphide	1	a	1	0.220	0.652	5.318
		b	1	0.986	0.350	5.318
		c	1	2.456	0.156	5.318
		d	1	1.333	0.282	5.318
		e	1	0.642	0.446	5.318
		f	1	0.731	0.417	5.318
	2	a	1	0.434	0.529	5.318
		b	1	0.273	0.616	5.318
		c	1	1.188	0.308	5.318
		d	1	4.931	0.057	5.318
		e	1	1.292	0.289	5.318
		f	1	4.863	0.059	5.318
	3	a	1	1.606	0.234	4.965
		b	1	0.998	0.341	4.965
		c	1	0.506	0.493	4.965
		d	1	1.853	0.203	4.965
		e	1	0.197	0.667	4.965
		f	1	5.949	0.035	4.965
	4	a	1	0.017	0.899	4.965
		b	1	0.037	0.851	4.965

		c	1	3.710	0.083	4.965
		d	1	15.963	0.003	4.965
		e	1	7.443	0.021	4.965
		f	1	21.644	0.001	4.965
Sulphate	1	a	1	1.391	0.272	5.318
		b	1	1.849	0.211	5.318
		c	1	12.582	0.008	5.318
		d	1	13.137	0.007	5.318
		e	1	66.740	0.000	5.318
		f	1	22.548	0.001	5.318
	2	a	1	0.207	0.661	5.318
		b	1	0.298	0.600	5.318
		c	1	1.373	0.275	5.318
		d	1	1.049	0.336	5.318
		e	1	0.240	0.638	5.318
		f	1	8.286	0.021	5.318
	3	a	1	3.310	0.099	4.965
		b	1	3.080	0.110	4.965
		c	1	0.589	0.461	4.965
		d	1	5.136	0.047	4.965
		e	1	0.016	0.902	4.965
		f	1	0.198	0.666	4.965
	4	a	1	1.051	0.329	4.965
		b	1	1.379	0.267	4.965
		c	1	5.502	0.041	4.965
		d	1	4.736	0.055	4.965
		e	1	5.911	0.035	4.965
		f	1	3.793	0.080	4.965
MeHg	1	f	1	1.100	0.351	5.318
	2	f	1	2.642	0.741	4.965
	3	f	1	5.612	0.055	5.318
	4	f	1	2.643	0.241	4.965

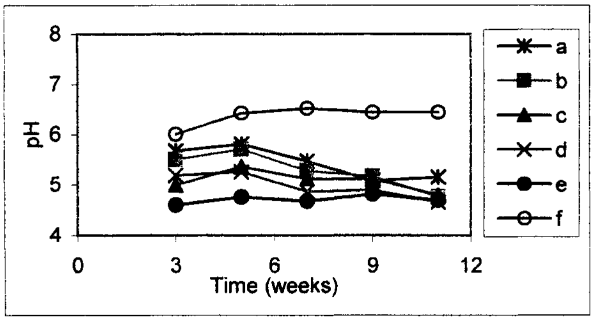
Appendix F: pH of leachate drained from sediment cores submitted to 3 months of wetting and draining events for all sites in both lakes.

Stump Lake

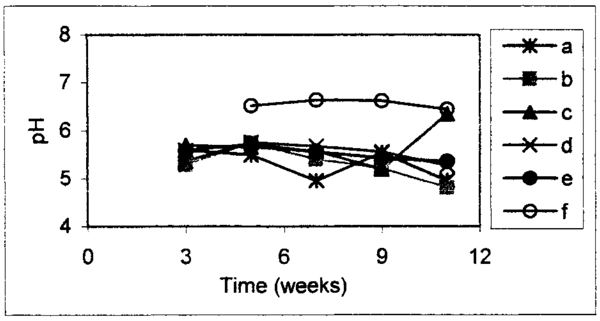
Site A



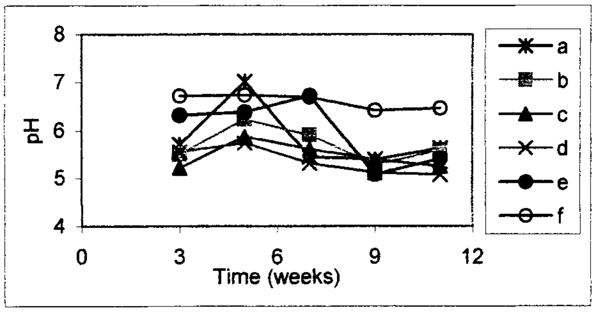
Site B



Site C

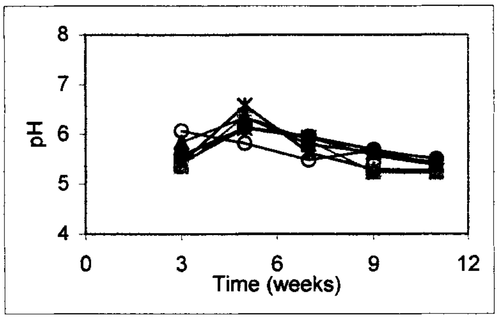


Site D

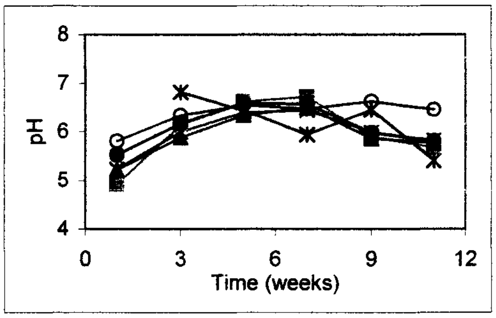


Black Donald Lake

Site 1



Site 2



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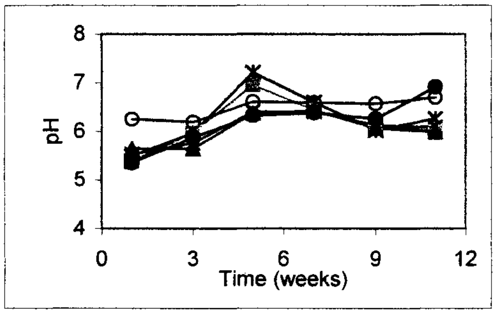

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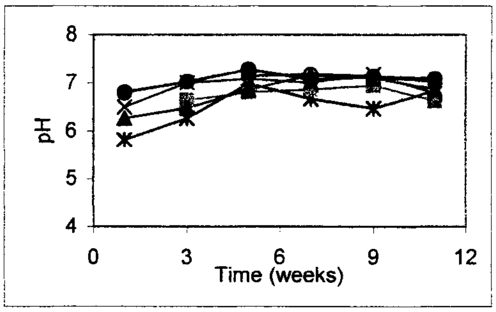
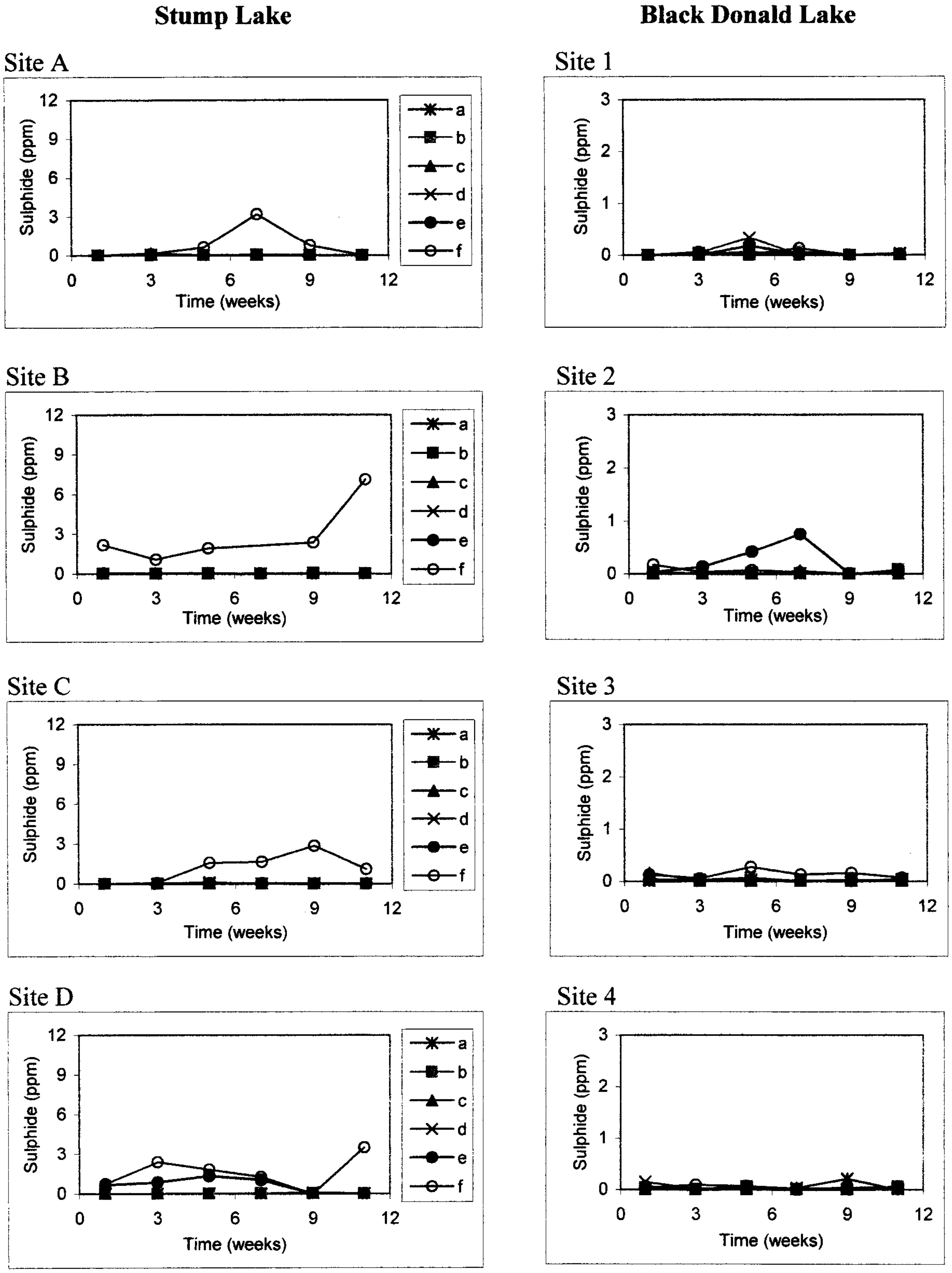


Appendix G. Sulphide (ppm) in leachate drained from sediment cores submitted to 3 months of wetting and draining events for all sites in both lakes.



Stump Lake

Black Donald Lake

Site A

Site 1

Site B

Site 2

Site C

Site 3

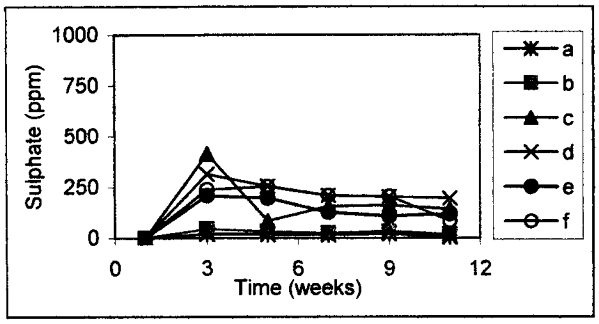
Site D

Site 4

Appendix H: Sulphide (ppm) in leachate drained from sediment cores submitted to 3 months of wetting and draining events for all sites in both lakes.

Stump Lake

Site A



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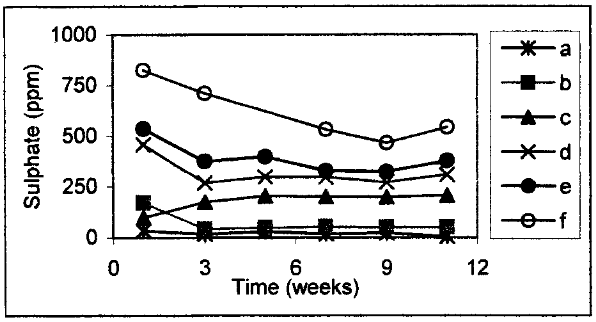
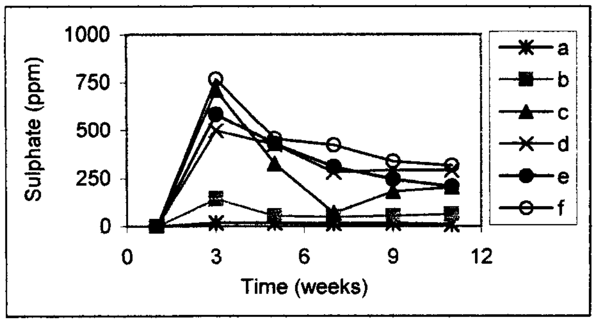
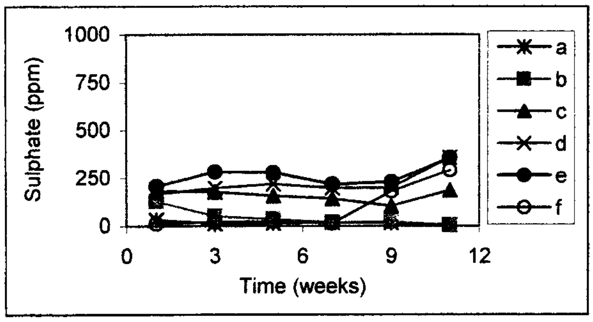


Site C

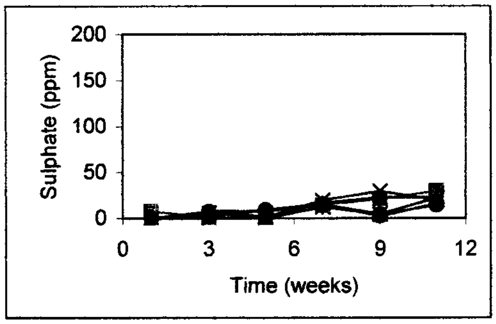


Site D

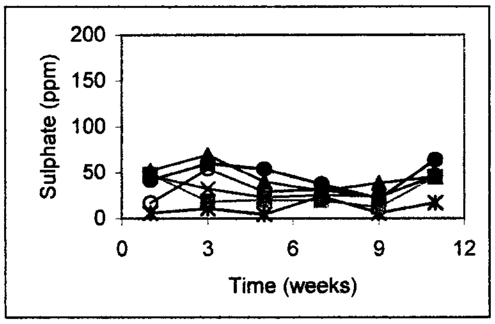


Black Donald Lake

Site 1



Site 2



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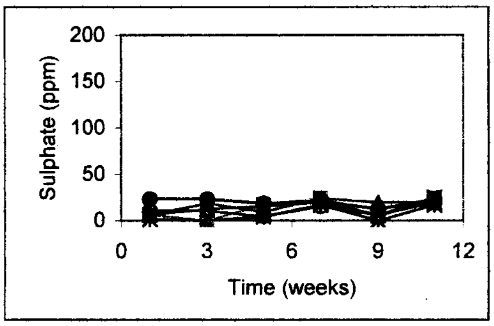

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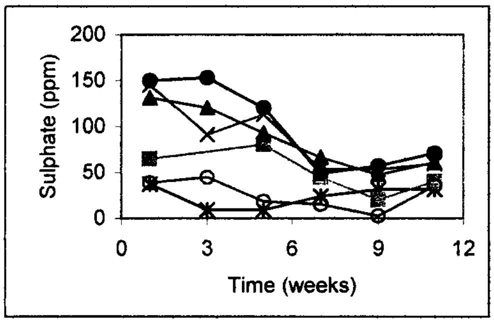
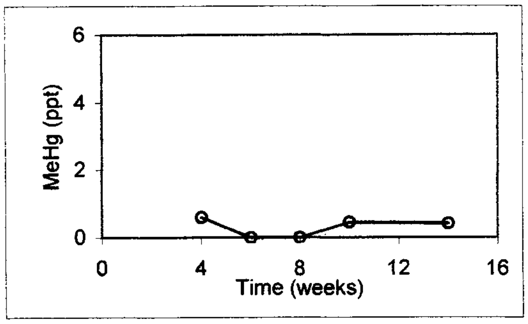


Appendix I. MeHg (ppt) in leachate drained from sediment cores submitted to 3 months of wetting and draining events for all sites in both lakes.

Stump Lake

Site A



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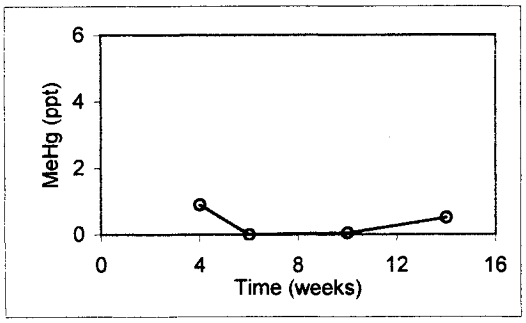

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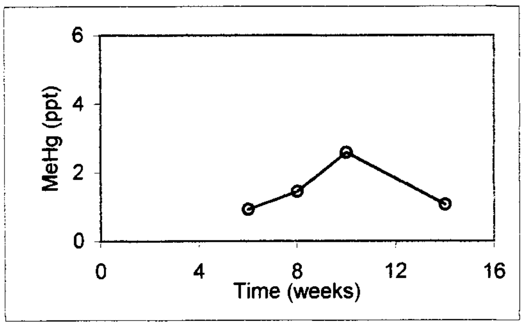

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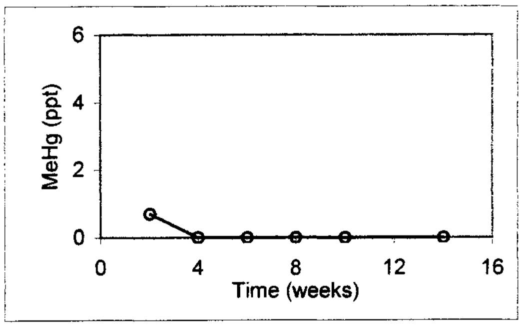
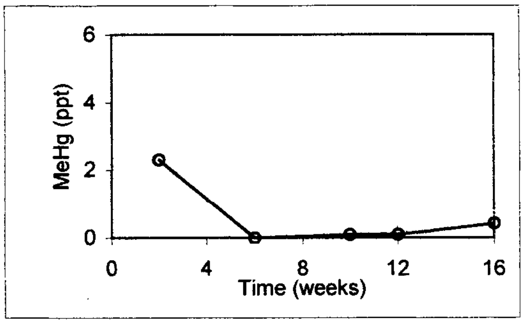


Black Donald Lake

Site 1



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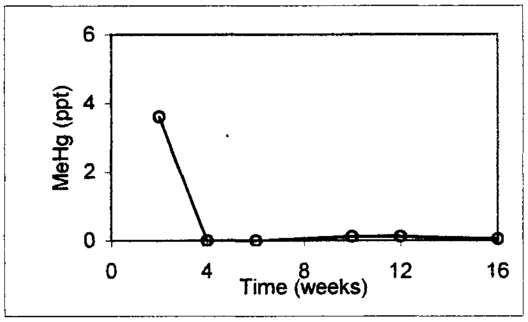

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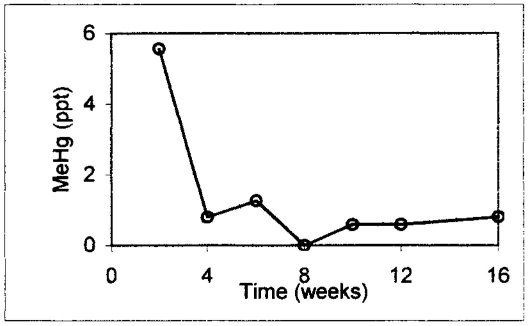
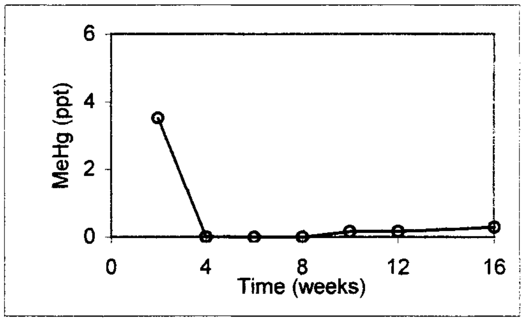


Site 4



Appendix J. Three-factor ANOVA results (with replication) for pH, sulphide and sulphate concentrations comparing the effect of lake, time and depth and their interactions.

Measured Parameter	Tested Factor or Interaction	df	F-ratio	p
pH	Lake	1	349.5	1.71E-11
	Week	10	22.801	1.01E-11
	Depth	5	66.779	1.06E-11
	Lake x Week	10	2.605	0.004
	Lake x Depth	5	3.525	0.004
	Week x Depth	50	1.036	0.41
	Lake x Week x Depth	50	0.658	0.967
Sulphide	Lake	1	17.783	2.81E-05
	Week	11	1.056	0.395
	Depth	5	26.666	1.36E-11
	Lake x Week	11	0.869	0.571
	Lake x Depth	55	0.67	0.968
	Week x Depth	5	21.423	1.46E-11
	Lake x Week x Depth	55	0.831	0.802
Sulphate	Lake	1	215.21	1.86E-11
	Week	5	36.071	1.25E-11
	Depth	11	1.571	0.103
	Lake x Week	11	1.464	0.14
	Lake x Depth	5	24.491	1.40E-11
	Week x Depth	55	0.649	0.977
	Lake x Week x Depth	55	0.756	0.902

Appendix K. Nested ANOVA results for pH of drained water from sediment at various depths (sample port) for both lakes.

Depth	Source of Variation	df	F-ratio	p
a	Lakes	1	17.743	0.006
	Sites (within Lakes)	6	0.796	0.599
	Cores (within Sites (within Lakes))	8	7.392	7.03E-08
b	Lakes	1	11.001	0.016
	Sites (within Lakes)	6	2.594	0.106
	Cores (within Sites (within Lakes))	8	5.167	1.64E-05
c	Lakes	1	8.563	0.026
	Sites (within Lakes)	6	2.935	0.081
	Cores (within Sites (within Lakes))	8	4.529	8.54E-05
d	Lakes	1	7.954	0.030
	Sites (within Lakes)	6	5.922	0.012
	Cores (within Sites (within Lakes))	8	3.392	0.002
e	Lakes	1	7.346	0.035
	Sites (within Lakes)	6	9.705	0.003
	Cores (within Sites (within Lakes))	8	2.794	0.007
f	Lakes	1	0.966	0.364
	Sites (within Lakes)	6	3.202	0.066
	Cores (within Sites (within Lakes))	8	5.186	1.58E-05

Appendix L. Nested ANOVA results for sulphide concentrations in drained water from sediment at various depths (sample port) and both lakes.

Depth	Source of Variation	df	F-ratio	p
a	Lakes	1	0.595	0.470
	Sites (within Lakes)	6	2.227	0.146
	Cores (within Sites (within Lakes))	8	0.001	1.000
b	Lakes	1	1.099	0.335
	Sites (within Lakes)	6	3.364	0.058
	Cores (within Sites (within Lakes))	8	0.001	1.000
c	Lakes	1	0.806	0.404
	Sites (within Lakes)	6	0.966	0.502
	Cores (within Sites (within Lakes))	8	0.122	0.998
d	Lakes	1	0.048	0.834
	Sites (within Lakes)	6	0.841	0.572
	Cores (within Sites (within Lakes))	8	0.096	0.999
e	Lakes	1	0.220	0.655
	Sites (within Lakes)	6	2.310	0.136
	Cores (within Sites (within Lakes))	8	0.137	0.997
f	Lakes	1	8.992	0.024
	Sites (within Lakes)	6	0.897	0.540
	Cores (within Sites (within Lakes))	8	22.742	5.27E-21

Appendix M. Nested ANOVA results for sulphate concentrations in drained water from sediment at various depths (sample port) and both lakes.

Depth	Source of Variation	df	F-ratio	p
a	Lakes	1	0.007	0.938
	Sites (within Lakes)	6	1.750	0.227
	Cores (within Sites (within Lakes))	8	0.989	0.448
b	Lakes	1	2.439	0.169
	Sites (within Lakes)	6	3.218	0.065
	Cores (within Sites (within Lakes))	8	1.446	0.184
c	Lakes	1	11.166	0.016
	Sites (within Lakes)	6	2.976	0.078
	Cores (within Sites (within Lakes))	8	5.638	4.41E-06
d	Lakes	1	30.693	0.001
	Sites (within Lakes)	6	1.127	0.425
	Cores (within Sites (within Lakes))	8	12.202	9.76E-13
e	Lakes	1	11.476	0.015
	Sites (within Lakes)	6	1.459	0.303
	Cores (within Sites (within Lakes))	8	19.366	1.64E-18
f	Lakes	1	9.990	0.020
	Sites (within Lakes)	6	3.220	0.065
	Cores (within Sites (within Lakes))	8	24.293	7.80E-22

Appendix N. Two-factor ANOVA without replication results for the effect of depth or time on pH, sulphide or sulphate concentrations in water drained from sediments for the 3 or 6 month wet/dry cores for Stump Lake.

Measured Parameter	Site	Core	Tested Factor	df	F	F critical	P-value
pH	A	6 mths	depth	5	34.390	2.400	4.61E-15
			time	10	10.385	2.026	3.33E-09
	B	6 mths	depth	5	59.425	2.400	7.41E-20
			time	10	10.482	2.026	2.88E-09
	C	6 mths	depth	5	39.502	2.400	3.15E-16
			time	10	1.951	2.026	0.060
	D	6 mths	depth	5	5.870	2.400	2.42E-04
			time	10	21.142	2.026	1.16E-14
Sulphide	A	6 mths	depth	5	9.780	2.383	1.00E-06
			time	11	0.983	1.968	0.473
	B	6 mths	depth	5	59.425	2.400	7.41E-20
			time	10	10.482	2.026	2.88E-09
	C	6 mths	depth	5	28.352	2.383	4.33E-14
			time	11	1.017	1.968	0.444
	D	6 mths	depth	5	2.078	2.383	0.082
			time	11	2.208	1.968	0.027
Sulphate	A	6 mths	depth	5	55.193	2.383	3.28E-20
			time	11	1.083	1.968	0.391
	B	6 mths	depth	5	11.490	2.383	1.28E-07
			time	11	0.999	1.968	0.459
	C	6 mths	depth	5	7.951	2.383	1.08E-05
			time	11	0.966	1.968	0.487
	D	6 mths	depth	5	17.833	2.383	1.81E-10
			time	11	1.671	1.968	0.105

Appendix O. Two-factor ANOVA without replication results for the effect of depth or time on pH, sulphide or sulphate concentrations in water drained from sediments for the 3 or 6 month wet/dry cores for Black Donald Lake.

Measured Parameter	Site	Core	Tested Factor	df	F	F critical	P-value
pH	1	6 mths	depth	5	6.832	2.400	6.31E-05
			time	10	9.675	2.026	9.93E-09
	2	6 mths	depth	5	28.091	2.400	1.97E-13
			time	10	3.294	2.024	0.002
	3	6 mths	depth	5	19.362	2.400	1.13E-10
			time	10	10.717	2.026	2.03E-09
	4	6 mths	depth	5	12.622	2.400	6.04E-08
			time	10	5.747	2.026	1.10E-05
Sulphide	1	6 mths	depth	5	5.286	2.383	0.001
			time	11	1.254	1.968	0.275
	2	6 mths	depth	5	5.680	2.383	3.00E-04
			time	11	2.534	1.968	0.012
	3	6 mths	depth	5	1.673	2.400	0.158
			time	10	1.308	2.026	0.252
	4	6 mths	depth	5	2.498	2.383	0.042
			time	11	1.205	1.968	0.306
Sulphate	1	6 mths	depth	5	19.330	2.400	1.16E-10
			time	10	1.725	2.026	0.101
	2	6 mths	depth	5	9.004	2.383	2.69E-06
			time	11	2.129	1.968	0.033
	3	6 mths	depth	5	22.804	2.400	7.60E-12
			time	10	3.886	2.026	0.001
	4	6 mths	depth	5	0.849	2.383	0.521
			time	11	0.883	1.968	0.561

Appendix P. Pearson correlation results ($p < 0.05$) for MeHg vs pH, sulphide and sulphate concentrations in drained rain water from the bottom of cores (sample port "f") over time for the 3 and 6 month wet/dry cores of all sites from both Stump and Black Donald Lakes.

Lake	Site	Tested correlation and correlation coefficient (*r*)		
		MeHg/pH	MeHg/Sulphide	MeHg/Sulphate
Stump	A	0.095	-0.261	-0.752
	B	0.042	-0.162	-0.469
	C	0.549	0.354	-0.460
	D	0.526	0.039	-0.488
Black Donald	1	-0.220	-0.552	-0.298
	2	-0.080	0.593	0.645
	3	-0.247	0.218	0.272
	4	-0.250	-0.068	0.451

Appendix Q: Correlation results for total Hg vs organic matter in sediments from Stump and Black Donald Lake after 0, 3 and 6 months of wet/dry cycles.

Lake	Site	Core	Correlation MeHg vs Organic Matter
Stump	B	0 month	N/A
		3 month	0.206
		6 month	0.905
	D	0 month	N/A
		3 month	0.633
		6 month	0.670
Black Donald	2	0 month	0.512
		3 month	0.999
		6 month	0.911
	4	0 month	0.777
		3 month	0.939
		6 month	0.755

Appendix R. Two-factor ANOVA without replication results for total Hg in sediment comparing the effect of depth and time for two study sites from Stump Lake and Black Donald Lake.

Lake	Site	Tested parameter (depth or time)	df	F	p	F critical
Stump	B	depth	7	3.538	0.0211115	2.764
		time	2	113.015	2.296E-09	3.739
Stump	D	depth	7	1.764	0.1733327	2.764
		time	2	5.984	0.0132362	3.739
Black Donald	2	depth	7	2.445	0.0732005	2.764
		time	2	5.682	0.0156098	3.739
Black Donald	4	depth	7	3.538	0.0211115	2.764
		time	2	113.015	2.296E-09	3.739

Appendix S. Two-factor ANOVA without replication results for Hg sequential extractions and the effect of depth or time for Stump and Black Donald Lakes.

Extractable fraction	Lake	Site	Tested Factor	df	F	F critical	p
Soluble Organic	Stump	B	Depth	7	4.526	2.764	7.90E-03
			Time	2	32.092	3.739	5.90E-06
	Stump	D	Depth	7	7.247	2.764	8.88E-04
			Time	2	19.418	3.739	9.17E-05
	Black Donald	2	Depth	7	0.857	2.764	0.561
			Time	2	23.734	3.739	3.18E-05
	Black Donald	4	Depth	7	1.809	2.764	0.163
			Time	2	6.020	3.739	0.013
Refractory Organic	Stump	B	Depth	7	0.688	2.764	0.681
			Time	2	3.155	3.739	0.074
	Stump	D	Depth	7	0.780	2.764	0.615
			Time	2	0.876	3.739	0.438
	Black Donald	2	Depth	7	0.791	2.764	0.607
			Time	2	50.107	3.739	4.2E-07
	Black Donald	4	Depth	7	0.688	2.764	0.681
			Time	2	32.861	3.739	5.15E-06
Residual	Stump	B	Depth	7	1.823	2.764	0.161
			Time	2	5.900	3.739	0.014
	Stump	D	Depth	7	1.303	2.764	0.318
			Time	2	1.950	3.739	0.179
	Black Donald	2	Depth	7	3.143	2.764	0.032
			Time	2	30.584	3.739	7.78E-06
	Black Donald	4	Depth	7	0.467	2.764	0.842
			Time	2	22.316	3.739	4.43E-05

Appendix T. Two-factor ANOVA without replication results for the effect of depth or time for SRB MPN results for all sites for Stump (SL) and Black Donald Lake (BDL).

Lake	Site	Tested Factor	df	F	F critical	P-value
SL	A	depth	4	2.443	3.056	0.092
		time	3	3.926	3.239	0.028
	B	depth	4	3.408	3.056	0.036
		time	3	0.685	3.239	0.574
	C	depth	4	1.551	3.056	0.238
		time	3	2.483	3.239	0.098
	D	depth	4	1.454	3.056	0.265
		time	3	2.570	3.239	0.091
BDL	1	depth	4	0.767	3.056	0.563
		time	3	2.212	3.239	0.126
	2	depth	4	0.586	3.056	0.678
		time	3	2.142	3.239	0.135
	3	depth	4	1.768	3.056	0.188
		time	3	1.295	3.239	0.310
	4	depth	4	0.153	3.056	0.959
		time	3	5.034	3.239	0.012

Appendix U. Three-factor ANOVA results (with replication) for SRB (MPN data) in sediment comparing the effect of lake, time and depth and their interactions.

Tested Factor or Interaction	df	F-ratio	p
Lake	1	8.7301	3.80E-03
Depth	4	8.2954	6.09E-06
Time	3	4.1824	7.40E-03
Lake x Depth	4	2.8473	0.027
Time x Depth	12	2.3219	0.011
Lake x Time	3	10.5994	3.11E-06
Lake x Time x Depth	12	1.6435	0.088

Appendix V. Nested ANOVA results for SRB (MPN data) in sediment at varoius depths for both lakes.

Depth	Source of Variation	df	F-ratio	p
0-2 cm	Lakes	1	9.450	0.022
	Sites (within Lakes)	6	0.791	0.586
2-4 cm	Lakes	1	0.127	0.733
	Sites (within Lakes)	6	0.779	0.594
4-6 cm	Lakes	1	0.597	0.469
	Sites (within Lakes)	6	0.472	0.822
6-8 cm	Lakes	1	8.300	0.028
	Sites (within Lakes)	6	0.123	0.992
8-10 cm	Lakes	1	0.091	0.773
	Sites (within Lakes)	6	0.411	0.864