MERCURY IN ONTARIO WETLANDS: CONCENTRATIONS IN WATER, SEDIMENTS, AND A COMMON AQUATIC PLANT IN RELATION TO GEOCHEMICAL VARIABLES

by

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Thesis submitted to the
School of Graduate Studies and Research
University of Ottawa
in partial fulfilment of the requirements for the
M.Sc. degree in the

Ottawa-Carleton Institute of Biology

Thèse soumise à
l'École des études supérieures et de la recherche
Université d'Ottawa
en vue de l'obtention de la maîtrise ès sciences à

L'Institut de biologie d'Ottawa-Carleton

ISBN 0-612-16467-5

UNIVERSITÉ D'OTTAWA
UNIVERSITY OF OTTAWA

ACKNOWLEDGEMENTS

I am thankful to Dr. Pick for giving me the chance to prove myself, and for not asking too many times "Can I have just 1 section". I would never have been able to do this project if it hadn't been for her. She has been a great supervisor and friend, and the best roomie to travel to Miami and T.O with (except for her habit of getting up at the crack of dawn!!).

I am also grateful to Dr. Ron Hall and Tom Whitfield for introducing me to those beautiful wetlands in Temagami.

When it came to sampling, I had wonderful help from Christine Headon, who also allowed me to stay with her for 2 months, thank you Chris. In addition, when I sampled the St. Lawrence River, my friend Monique Richard came with me. We had so much fun it hardly seemed like work. Monique was great to sample with, she didn't mind getting dirty, and she didn't just sit in the boat and watch the wildlife.

A big part of this work was done at the Geological Survey of Canada. I would like to send a special thanks to Dr. Gwendy Hall, J.C. Pelchat, and Judy Vaive. Without the use of the ICP-MASS and all the expertise at the GSC, I would never have been able to determine the Hg in my samples, and thus no results. So thanks for the help, support and of course my results.

There are a few other people I would like to thank for giving me moral support throughout this long process and they are the clique of Ben and Andrew for their friendship, Robin for joining the third floor and making it fun and a little less masculine, Louise Cossette who was ever so efficient in ordering my acids and ensuring they arrived on time, and my hubby Paul who said on our wedding day that I smelled like "bog muck", but still married me. Funding for this project was provided by Dr. Pick's NSERC operating grant, and the St. Lawrence Ecosystem Recovery Tri-Council grant.

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ABSTRACT

Previous research on the bioaccumulation of mercury in aquatic lakes and rivers has identified pH, alkalinity, dissolved organic carbon, and the organic content of the sediments as the major environmental variables which predict corresponding Hg levels in fish and invertebrates. However, the factors that regulate bioaccumulation in wetland systems and aquatic plants in particular have yet to be identified.

Concentrations of total mercury were determined in water, sediments, and the yellow pond lily (*Nuphar variegatum* L.) for 22 wetlands from South-Central Ontario (Lat. 45^0 11', Long. 78^0 50'; Lat. 46^0 19', Long. 80^0 47') and for 23 wetlands from the St. Lawrence River (Lat. 45^0 02', Long. 74^0 44'). The South-Central wetlands are remote from any industrial sources of mercury, whereas the wetlands from the St. Lawrence River come from an area of the River where known Hg contamination exists.

Significant and positive correlations were found between the organic content of the sediments and the sediment Hg concentrations for both the South-Central and the St. Lawrence River wetlands. However, no relationships between water Hg and pH, alkalinity, and dissolved organic carbon (DOC) were found in either wetland group.

The yellow pond lily is a common floating leaved aquatic plant which is the nutrient and energy source for a variety of wildlife, ranging from nematodes to moose. Thus, the importance of determining if the yellow pond lily accumulates toxic levels

of mercury, which can be passed on to its consumers is evident. The results of this study indicated that *Nuphar variegatum* does not accumulate mercury in relation to pH, alkalinity, DOC, or the organic content of the sediment, despite a wide range in these variables from the two wetland groups. The results indicated that *Nuphar variegatum* is an "excluder" or "non-indicator" of mercury, where the concentration ratios from sediment and water to the pond lily, regardless of the sediment and water mercury concentrations, are low.

Remote South-Central wetlands had significantly higher concentrations of mercury in the sediments and the water compared to the St. Lawrence River wetlands. The South-Central wetlands also had significantly higher amounts of organic matter in the sediments and DOC in the water, both of which are known to bind mercury.

Résumé

Les recherches antérieures sur la bioaccumulation du mercure dans les lacs et les fleuves ont identifié le pH, l'alcalinité, le carbone organique dissout et le contenu organique des sédiments comme les variables environnementales qui prédisent le mieux le niveau de mercure chez les poissons et les invertébrés. Cependant, les facteurs qui règlent la bioaccumulation dans les marécages et chez les plantes aquatiques n'ont toujours pas été déterminés.

Les concentrations totales du mercure dans l'eau, dans les sédiments et chez le nénuphar jaune (*Nuphar variegatum* L.), ont été mesurées dans 22 marécages de la région centre-sud de l'Ontario (Lat. 45^0 11', Long. 78^0 50'; Lat. 46^0 19', Long. 80^0 47') et dans 23 marécages du fleuve St-Laurent (Lat. 45^0 02', Long. 74^0 44'). Les marécages du centre-sud sont éloignés de sources ponctuelles et industrielles de mercure, tandis que, les marécages du fleuve St-Laurent sont situés à proximité d'une source fluviale où il y a déjà eu une pollution directe par le mercure.

Une corrélation positive et significative a été révelée entre le contenu organique des sédiments et la concentration du mercure des sédiments dans les marécages de la région centre-sud et la région du fleuve St-Laurent. Cependant, aucune relation n'a été déterminée entre la concentration de mercure dans l'eau et le pH, l'alcalinité ou le carbone organique dissout dans l'un ou l'autre des deux groupes de marécages.

Le nénuphar jaune (*Nuphar variegatum*) est une plante aquatique commune, à feuille flottante, qui est une source des nutriments et d'énergie pour un grand nombre d'espèces fauniques. Les consommateurs de nénuphar jaune allant du nématode à l'élan. Donc, l'importance de déterminer si le nénuphar jaune peut accumuler un niveau toxique de mercure qui pourrait être passé aux consommateurs est évident. Les résultats de cette étude indiquent que *Nuphar variegatum* n'accumule pas le mercure en relation avec le pH, l'alcalinité, le carbone organique dissout ou le contenu organique des sédiments, et ceci en dépit d'une grande variation dans les variables provenant des deux groupes de marécages. Les résultats indiquent que *Nuphar variegatum* est un non-indicateur de mercure, où les rapports de la concentration des sédiments et de celle l'eau au nénuphar sont relativement bas, et ceci sans égard à la concentration de mercure dans les sédiments et dans l'eau.

Les marécages du centre-sud ont une concentration de mercure significativement plus élevée dans les sédiments et dans l'eau en comparaison avec les marécages du fleuve St-Laurent. Les marécages du centre-sud ont aussi un niveau significativement plus élevé de matière organique dans les sédiments et dissoute dans l'eau qui tous deux sont connus comme fixateurs du mercure.

Chapter 1.0

INTRODUCTION

1.1 General Overview of the Mercury Problem

Since the Minimata (Japan) incident of the 1950's, where mercury discharged from industry caused severe neurological disorders and death in the inhabitants, industrialized nations have been attempting to reduce the amounts of mercury released into the environment (Jenson and Jenson, 1991). Despite such efforts, mercury is still emitted in considerable amounts to the atmosphere from anthropogenic sources (Nriagu, 1990) and remains a human health hazard.

Mercury (Hg) is one of the rare elements in the Earth's crust (0.08%) (Huckabee et al., 1983), but it is so widely disseminated by natural processes that it can be found everywhere, and will always be present to some extent. The primary natural source of Hg is degassing of the Earth' s crust involving mainly elemental Hg vapour and subsequent distribution by aerial circulation and precipitation (Gerstenberger et al., 1993). Other natural sources of Hg include chemical weathering of rocks and soil leaching (Kabata-Pendias and Pendias, 1992), wind-borne soil particulates, volcanoes, sea salt spray and wild forest fires (Nriagu, 1989). However, it is currently believed that the majority of Hg in the environment (60% - 80%) is attributable to human activity (Nriagu, 1990; Watras et al., 1995a). The atmospheric flux of anthropogenically derived Hg is globally greater than the natural flux (Percy, 1983). Natural

sources only exceed anthropogenic sources at regional scales where geological formations containing Hg-bearing minerals such as cinnabar (HgS) exist.

The first recorded use of Hg dates back to the 1800's when pelts were preserved with it in the making of beaver hats, hence the expression "mad as a hatter" (Grant, 1969). More recently, Hg has been used in the chlor-alkali and pulp and paper mill industries, in agriculture and horticulture (seed protection, pesticides, fungicides and fertilizers), in the production of cement, in paints, and in commercial chlorinated bleaches and hand soaps (Fimrette, 1970; Cranston and Buckley, 1972; Jackson et al., 1990; Newton et al., 1993). In addition, Hg is used extensively in the mining of gold (Lacerda et al., 1991). Even though the concentration of Hg in fossil fuels/ores is low (Joensu, 1981), the major source of atmospheric Hg on a global scale is the combustion of fossil fuels in power plants, industrial and residential burners, and automobiles (Nriagu and Pacyna, 1988). Refuse incineration is estimated to be the second largest source of Hg emissions to the atmosphere (Pacyna et al., 1995). It is not surprising, with all these anthropogenic sources of Hg, that atmospheric deposition has been identified as the dominant means by which Hg enters surface waters and soils in the northern hemisphere. Hg depth profiles from the sediments of remote lakes indicate that atmospheric Hg deposition has increased by a factor of 3-5 since the industrial revolution (Watras et al., 1995a).

Metals and metalloids, unlike organic contaminants, are not biodegradable, but undergo a biochemical cycle during which transformations into more or less toxic species occur. Hg is a prime example of this. In aquatic systems, the chemical and physical forms of mercury can be controlled by pH, organic matter, and biological activity (Baker et al., 1983).

Volatile, elemental Hg (Hg^0) constitutes about 98% of the Hg in the atmosphere which is dispersed globally and delivered to aquatic systems (Jeffries and Snyder, 1981; Nriagu, 1994), the remainder is associated with particles (Lindquist, 1991). This elemental Hg may be oxidized to mercuric ion (Hg^{+2}) by a variety of photocatalytic reactions (Pfeiffer et al., 1983). Mercuric ion is soluble in water and scavenging by precipitation is thought to be the major process of Hg removal from the atmosphere (Winfrey and Rudd, 1990; Vandal et al., 1993). Hg deposited to aquatic systems via atmospheric deposition, runoff or direct discharge appears primarily in three forms, elemental Hg, mercuric Hg, and phenyl Hg (Gillis et al., 1993). The fate of Hg once released into the aquatic environment is dominated by rapid adsorption to soluble and most importantly, particulate carbon compounds (Bryan and Langston, 1992), as well as inorganic particles (Loring, 1975). As a general rule, due to its density, elemental Hg is deposited close to the site of entry, whereas mercuric Hg is deposited in association with organic material.

Hg in aquatic environments can be found in three main reservoirs: water; sediments; and biota. The biotic reservoir is

small compared tc the water, which in turn is much smaller than the sediment reservoir. It follows therefore, that virtually all of the Hg at any point in time resides in the sediments. Most of the Hg in aquatic systems is associated with the finely grained bottom sediments and resuspended particles (Jackson et al., 1993). Plants, fish and invertebrates contain only 0.2% of the total mercury (Czuba and Mortimer, 1980).

Hg in natural waters and sediments is mostly in the form of inorganic species. However, the harmful biological effects are chiefly due to the much less abundant organometallic compound, methyl mercury (CH_3HG^+). Organomercury compounds, such as monomethyl and dimethyl Hg, are formed by biochemically mediated or chemical conversions. Although methyl Hg usually represents only a small fraction (<1%) of the total Hg present in water or sediments, it bioaccumulates so that more than 90% of the Hg in biota is in the form of methyl Hg (Jernelov et al., 1975). This is mainly a result of the high affinity of methyl Hg for organic material and the efficiency at which it is retained by organisms.

Sediments contain various kinds of microorganisms which gradually transform inorganic Hg into methyl Hg by methylation of "available" or biochemically reactive free Hg (Jackson, 1989; Johnston et al., 1991). These microorganisms do not require or seek out Hg, but rather they deal with it when it is present (Förstner and Wittmann, 1981). In addition, humic substances, both in the sediments and as dissolved organic carbon in waters, can act as reagents (methyl donors) for abiotic methylation of Hg

(Nagase et al., 1982; Zillioux et al., 1993; Hamasaki et al., 1995). Once the Hg is methylated, it can escape from the bottom sediments to the water column (Loring, 1975), where it is taken up by microscopic plants and other aquatic organisms. This process of methylation is dependent not only upon the metabolic activity of bacteria and on the concentration of inorganic Hg, but also on pH, redox potential, organic substrate and temperature (Lindberg et al., 1987). Methylation can also occur in the water column (Winfrey and Rudd, 1990), however, the highest rates of methylation have been measured in sediments (Matilainen and Verta, 1995). Thus, the total amount of methyl Hg in an aquatic system is the result of its formation, and also the result of the rates of processes that alter the availability of inorganic Hg for methylation and rates of methyl Hg decomposition (Steffan et al., 1988; Winfrey and Rudd, 1990), as well as the presence of bacteria (Zhang and Planas, 1994).

Methyl Hg is water soluble and lipophilic (Weber, 1973), is rapidly absorbed by aquatic organisms from the water column (Gillis et al., 1993), and leads to toxicity (Baker et al., 1983). The selective retention of methyl Hg at each step in the food chain, relative to inorganic Hg, is related to its high lipid solubility and ease of transfer across membranes, long biological half life, and increased longevity of top predators. As a result, methyl Hg provides one of the rare examples of metal biomagnification in food chains. Methyl mercury can enter through the cell membrane and once inside can bind to proteins containing

sulfhydryl groups, where it is not further metabolized by most organisms (Suszcynsky and Shann, 1995). Pathways of bioaccumulation of methyl Hg include direct uptake from water, and through the food chain. The relative importance of either pathway depends on trophic level, duration and intensity of exposure, and environmental factors.

The magnitude of the Hg problem is exemplified in remote lakes. In Ontario, Hg is the main cause of lakes being closed to sport and commercial fishing (Evans, 1986). Most of the lakes in Southern and Northern Ontario have no known point sources of Hg (Evans, 1986), and these so called "Hg contaminated" lakes are quite distant from industries which could potentially release Hg into the atmosphere. Results frequently indicate that two variables, pH (Lodenius et al., 1983; McMurtry et al., 1989) and/or organic carbon concentrations (Fjeld and Rognerud, 1993), correlate with fish Hg concentrations. The findings relating to Hg in fish may have applications for studies of Hg in other aquatic organisms and systems, such as plants in wetlands.

1.2 The Importance of Wetlands

The term wetland identifies a broad range of habitats that contain, or periodically have standing water. These ecosystems are distinguished on the basis of vegetation, morphology, hydrology, or chemistry, and these ecosystems have complex hydrological and biochemical cycles. These cycles directly influence or modify parameters such as pH and dissolved oxygen,

which in turn causes specific responses in both vegetation and wildlife (Anderson, 1986).

With 14% of Canada and 33% of Ontario covered by wetlands (NWWG, 1987), there is little doubt that these areas are vital for the survival of a variety of plant and animal species. Wetlands produce biomass and create habitats for plants and animals. In addition, they perform major ecological functions including provision of nursery or stopping grounds for fish and wildlife, retention of nutrients, purification of aquifers, biogeochemical transformations of elements, and exchange of chemicals, nutrients and organic matter with associated ecosystems (Urban and Bayley, 1986; Catalla, 1993; Hook, 1993). Mathias and Moyle (1992) estimated that 35% of the rare and endangered animal species are in some way dependent on wetlands, since they are essential nesting, feeding, resting, and wintering habitats for a large number of bird species. In spite of their ecological importance, millions of hectares of wetlands in Canada have been drained or filled for agriculture, highways, housing, and industrial uses (Environment Canada, 1986). As wetland ecosystems are diminished in size or as the volume and/or toxicity of pollutants, such as Hg, increases, the ability of the system to accommodate and eliminate wastes can be impaired.

Despite the considerable research over the last two decades on the distribution and bioaccumulation of mercury in aquatic systems, very little work has focused on mercury cycling in wetlands (ie. Zillioux et al., 1993). Wetlands are often

perceived as sinks or storage areas for nutrients and metals, but dredging of these valuable ecosystems may lead to the release of mercury into the environment. Westling (1991) reported that drainage and oxidation of deep wetland organic soils increased the concentrations of methyl mercury, and recently, St. Louis et al. (1994) reported that a large fraction of the methyl Hg in lakes originates from surrounding wetland areas.

Wetlands are rich in organic matter, and mercury has been shown to be associated with organic matter (Zillioux et al., 1993). In addition, humic matter which is a suitable methyl donor for abiotic methylation of mercury (Nagase et al., 1982), is plentiful in wetlands. Seventy percent of the dissolved organic carbon (DOC) in wetland surface water is humic matter (Weber, 1973), and humic matter constitutes close to 90% of the organic matter in wetland sediments (Weber, 1973; Wood, 1989). Therefore, both the organic content of the sediments and the DOC of the water may play an important role in the concentrations of mercury found in wetlands and in their corresponding biota.

Depending on location, wetlands receive varying amounts of anthropogenic Hg. The major entry routes of mercury in wetlands is runoff from point and non-point sources, followed by fluvial transport to the wetlands (Catalla, 1993). Other routes include deposition of atmospheric particles, aerosols and oxidants, direct discharge of contaminated dredge materials, and direct introduction of wastes from transportation activities, pipelines, and landfills (Zillioux et al., 1993). Figure 1.1 illustrates the

major routes for mass Hg transport in wetlands.

In the present study, we examined Hg distribution in two types of wetlands. A set of "remote" wetlands was chosen in South-Central Ontario, these wetlands are removed from point sources of anthropogenic Hg, yet would receive atmospheric inputs over and above the natural levels of Hg (Winfrey and Rudd, 1990). The second set of wetlands, along the shores of the St. Lawrence River, has been exposed to point sources in an area with a known mercury problem. Mercury contamination of the St. Lawrence River ecosystem between Cornwall, Ontario, and Valleyfield, Quebec, has been a concern since the late 1960's because it is a potential health risk for people who eat fish caught in this area (Loring, 1975; St. Lawrence RAP team and PAC, 1994). A number of Cornwall industries release Hg directly into the River through discharge pipes or to the air through stacks. These industries have been targeted for reduction in their discharge and effluent (Metcalfe-Smith, 1994; St. Lawrence RAP team and PAC, 1994). There are no significant United States point sources of mercury in this area (St. Lawrence RAP Team and PAC, 1994). In both these types of wetlands we examined the factors regulating bioaccumulation of Hg in a common wetland plant.

1.3 Mercury in Aquatic Plants and the Choice of *Nuphar variegatum*.

The accumulation of mercury in biota other than fish has received relatively little attention, but for a full understanding of the fate of this element it is also necessary to study its occurrence in other organisms. There are few studies on Hg bioaccumulation in plants despite the fact that the majority of wetland organisms depend either directly or indirectly on plant tissue for energy and nutrients and diet is considered the major source of Hg to wildlife (Ahmed et al., 1987; Scheuhammer, 1991). Plants are often perceived to be less of a concern than fish with respect to human health and there is little public interest in most plant species.

Several authors suggest that macrophytes can be useful as monitors of the contaminant levels of aquatic systems by toxic metals (Mortimer, 1985; Campbell et al., 1988; Coquery and Stokes, 1989). In addition, plant material is more abundant and tends to be easier to sample than animal material. Furthermore, metals in plant materials are at higher concentrations than in the water and thus, detection limits are less restrictive (Chigbo et al., 1982; Sager and Puscko, 1991). Bryophytes are recognized as conventional environmental monitors of mercury (Huckabee et al., 1983; Baldi et al., 1992), but few studies have examined higher aquatic plants (Eriksson and Mortimer, 1975; Lodenius, 1980; Mortimer, 1985).

Rooted aquatic plants can absorb mercury from water,

sediments, or air (Campbell et al., 1988; Crowder, 1991; Coquery and Welbourn, 1994). The ability of aquatic macrophytes to accumulate metals from the sediment and water compartments suggests that aquatic plants can play a role in the cycling of mercury in lakes and wetlands by transferring mercury to herbivores, by releasing it during decomposition, and by secreting metals into the water column.

The aquatic macrophyte chosen for this study is the yellow pond lily, *Nuphar variegatum*. *Nuphar variegatum* belongs to the Class Dicotyledones, the Order Ranales, and the Family Nymphaeacea. It is a common floating leaved aquatic plant that is widespread throughout eastern and central North America (Niering, 1989), and it meets the criteria set forth by Franzin and McFarlane (1980) for use as a biological indicator of metal contamination. The yellow pond lily is representative of the area, ubiquitous and easily collected, easily and unequivocally identified, and it has a high tolerance of metals. Furthermore, its leaves and petioles die back each year and contribute to the organic build up in lakes and wetlands.

The yellow pond lily supplies nutrients and energy to muskrats (Everett and Anthony, 1976), porcupines (Angier, 1974), beavers and white tailed deer (Johnson, 1985), and moose (Fraser et al., 1984). In addition, mallards, ring necks, wood ducks, cranes and rails eat the seeds of this plant to some extent (Angier, 1974). Moreover, invertebrates also use the yellow pond lily as a food substance. Frequent invertebrate consumers of

Nuphar include water lily aphids, water lily leaf beetles (both adults and larva), brown china moths, mosquito and chironomid larva, caddis flies, and snails (Wallace and O'Hop, 1985; Stapely Water Gardens, 1989; Velde Van Der and Brock, 1991). Furthermore, once in the detrital food web, decomposing *Nuphar* is further consumed by isopods (Mann, 1988). *Nuphar* may be sought out by this large array of consumers because of its high sodium (Fraser et al., 1984) and protein content (Smock and Harlowe, 1983). There is little doubt that *Nuphar variegatum* plays an important role in wetland food chains and is a necessary plant for the survival of many animal species thus making it an ideal plant for study. Hg concentrations in muskrat (Everett and Anthony, 1976), beaver (Wren et al., 1980), and animals in detritus based food chains (Bryan and Langston, 1992) have been reported to be high. Since these animals receive part or all of their food from aquatic plants, part of the mercury which they accumulate may be derived from the yellow pond lily.

In addition to its importance to animals, *Nuphar variegatum* has been shown to accumulate Cu and Zn (Hutchinson, 1975; Campbell et al., 1985), Cd (Thompson et al., 1996), and Hg (Lodenius, 1980; Siegel et al., 1985).

The key factors that regulate Hg bioaccumulation in plants may be similar to those described for fish. Studies dealing with Hg accumulation by biota have identified pH, alkalinity, dissolved organic carbon, and organic matter of aquatic systems as the major variables predicting corresponding Hg levels in

biota. In the case of pH, it is believed that low pH leads to higher rates of microbial mediated methyl mercury production (Baker et al., 1983; Richman et al., 1988), which in turns leads to higher bioavailability to plants, fish and other organisms. In fact, pH has been shown to be negatively correlated with fish Hg levels (McMurtry et al., 1989). Similarly, the alkalinity of water is negatively correlated with fish Hg levels (Akielaszek and Haines, 1981). DOC may also be a major determinant of Hg availability to *Nuphar*, with positive correlations between Hg in fish and water DOC being noted (Grieb et al., 1990). Finally, the organic content of the wetland sediment may also be important in regulating Hg accumulation in *Nuphar*. Nelson and Campbell (1991) found sediments with the highest organic content to have the highest Hg content both in the sediments and in the associated biota.

Plants are selective and take up only the biologically available forms of a metal (Stokes et al., 1983). In the case of mercury, this is methyl mercury. There is much evidence to suggest that plants growing on toxic sediments can not prevent metal uptake, but only restrict it, and hence accumulate metals in their tissues to varying degrees (Antonovics et al., 1971; Baker, 1981). The strategy of survival is thus tolerance and not avoidance of metal toxicity. In the sediment, Hg is transformed to organometallic compounds that have increased bioavailability and toxicity. These compounds may concentrate in, accumulate in or be excluded by the yellow pond lily. Thus the total Hg

concentrations in the yellow pond lily in relation to the sediment Hg and water Hg concentrations will indicate whether the yellow pond lily is an accumulator, indicator, or an excluder of Hg (Ernst, 1975; Baker, 1981; Lenka et al., 1992).

Plants in which uptake and translocation of heavy metals to above sediment parts are regulated so that plant concentrations correspond with sediment concentrations, are known as "indicators". Plants in which metals are concentrated from low or high sediment levels are known as "accumulators". While plants in which concentrations of Hg in the petioles and leaves remain low or constant over a wide range of sediment concentrations until the mechanism breaks down and unrestricted transport occurs which is usually deleterious to the plants are known as "excluders", also termed "non-indicators" (Baker, 1981, Siegel et al., 1985, Lenka et al., 1992).

Thus, in determining the chemical factors in wetlands that account for high Hg concentrations in aquatic plants we can provide information on the risk to wetland consumers. Given the fact that the yellow pond lily is found in a wide range of environmental conditions, and because of its importance for wildlife species, it has the potential to serve as a biomonitor of Hg availability under a range of conditions.

1.4 Objectives

1. To relate *Nuphar variegatum* mercury concentrations to major chemical characteristics (pH, alkalinity, DOC, organic content of the sediment) for 22 wetlands from South-Central Ontario and 23 wetlands from the St. Lawrence River.

2. To relate *Nuphar* mercury concentrations to the concentrations of mercury in the water and the sediments for the 22 wetlands from South-Central Ontario and the 23 wetlands from the St. Lawrence River.

3. To determine if *Nuphar* is an accumulator, indicator, or excluder of mercury, and to calculate the Hg bioconcentration factors for sediments and water to the pond lily.

4. To determine if differences in the mercury concentrations in the sediments, water, and *Nuphar* exist between the 22 "remote" South-Central Ontario wetlands and the 23 St. Lawrence River wetlands, where known point sources of mercury occur.

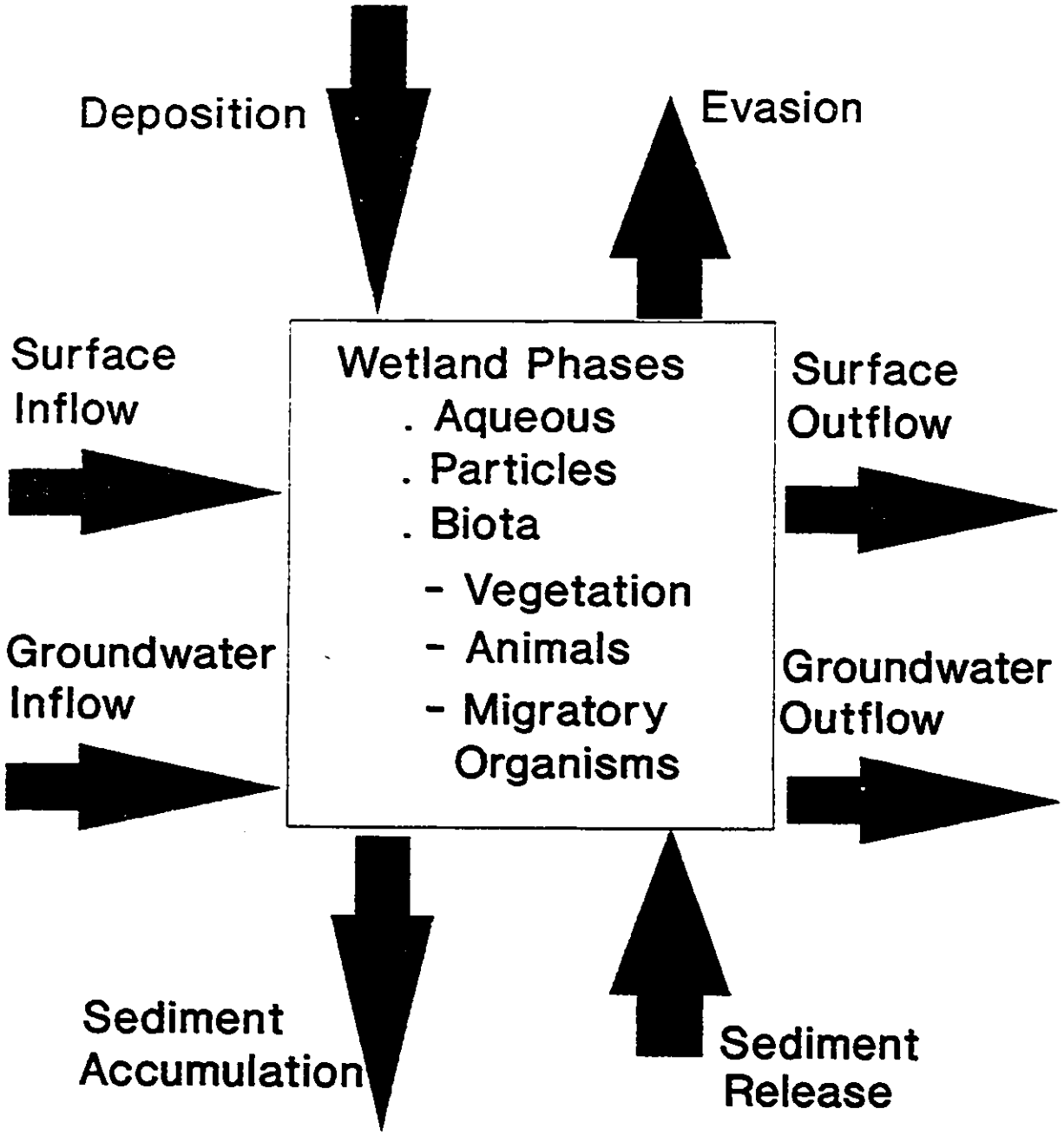

Figure 1.1 Major routes for mass Hg transport in wetlands. Adapted from Zillioux et al., 1993.

Chapter 2.0

Materials and Methods

2.1 Study Area

Twelve wetlands from the Muskoka-Haliburton highlands of Ontario were chosen to represent gradients in alkalinity and organic matter. General water and sediment characteristics of these twelve wetlands are described in Bendell-Young et al. (1994). These wetlands are on the Canadian Shield, which is almost entirely underlain by Precambrian metamorphic (granite and gneiss), plutonic and volcanic silicate rocks and overlain by a variety of thin Pleistocene glacial till (Guillet, 1969; Jeffries and Snyder, 1981; Hornbrook et al., 1988). In addition, ten wetlands from the Temagami-North Bay area of Ontario were sampled and their vegetation has been characterized (pers. comm. R. Hall-OMOEE, Dorset, Ontario, and T. Whitfield- Env. Consultant, North Bay, Ontario). These wetlands are underlain by a metamorphic complex of granites and gneissic rocks (mostly metasediments derived from siliceous sandstones and siltstone that accumulated considerable thickness during the middle Precambrian time) (Lumbers, 1971; Bennett, 1978). The twelve wetlands from the Muskoka-Haliburton highlands and the ten wetlands from the Temagami-North Bay area ranged from acidic *Sphagnum* dominated bogs to high alkaline fens dominated by sedges and grasses and are grouped together in the remainder of the text, and referred to as the wetlands from South-Central Ontario. The second set of wetlands were twenty three riverine wetlands along the shores of

the St. Lawrence River in and around the municipality of Cornwall, Ontario. The wetlands in this area overlie sedimentary rocks consisting mainly of Cambrian sandstone and Ordovician dolomite, limestone, and shale, with subsurface conditions generally consisting of silt and clay soils (Wilson, 1946; Hewitt, 1978; and Gartner Lee, 1982). The location of the three wetland sampling areas are shown in Figure 2.1, and the latitudes and longitudes of the individual South-Central and St. Lawrence River wetlands are located in Appendices 7.1 and 7.2, respectively.

The South-Central Ontario wetlands were sampled in July and August of 1993, and the St. Lawrence River wetlands were sampled in July and August of 1994.

2.2 Sampling of Wetlands

2.2.1 Sampling of Water

At the time of plant sampling, water samples were taken for pH, alkalinity, dissolved organic carbon (DOC), and Hg analyses. Triplicate water samples were collected in rigorously cleaned 1 L Nalgene bottles from the centre of the wetland at a depth of 0.5 m below the surface. Measurements of pH and alkalinities were determined the same day of collection. The alkalinity measurements followed the methods of Wetzel and Likens (1991). Triplicate water samples for Hg analyses (50mL) were preserved with 0.1 % "Ultrex" nitric acid and stored in clean acid washed Falcon tubes. A blank consisting of double distilled water,

transported from Ottawa, was preserved with the 0.1 % "Ultrex" nitric acid every fifth sample as a quality control. Triplicate water samples were collected, filtered through 0.45 µm Millipore HA filters, and stored in clean distilled water rinsed Falcon tubes for DOC. The DOC was determined by gas chromatography at the Geological Survey of Canada (GSC), Ottawa, Ontario, following the methods of Wetzel and Likens (1991). All water samples were stored at 4^0C until time of analyses.

2.2.2 Sampling of Sediments

At the time of plant sampling, sediments were collected for organic content and Hg analyses. Nine sediment samples were collected for Hg determination and six sediment samples were collected for organic contents of the sediments in each South-Central Ontario wetland. In the St. Lawrence River, three sediment samples were collected for Hg determination and three sediment samples were collected for organic contents of the sediments at each wetland. The sediments were collected using a Kaja-Brinkhurst gravity corer. The upper 2.5 cm of the sediment was removed from the coring tube and transferred to clean acid washed 50 ml Falcon tubes. The water was decanted, as the sediments settled, and the tubes were filled with more sediment to minimize head space. The tubes were sealed and frozen within 5 hours of collection until time of analyses.

The decision to collect only the top 2.5 cm of the sediment was based on the results of Lodenius et al. (1983), and Nelson

and Campbell (1991) in which they found elevated Hg concentrations in the peat column to be in the upper most 3 cm layer. In addition, the rhizomes of *Nuphar* do not extend much past this zone, and below 3 cm depth in most of the St. Lawrence River wetlands, hard clay full of tiny white mollusc dominated the sediment.

The organic content of the sediments was estimated by loss on ignition (LOI) at 500^{0}C for 2.5 hr (Wetzel and Likens 1991).

2.2.3 Sampling of Plants

Fifteen *Nuphar variegatum* plants (above sediment parts) per wetland were collected for Hg analyses in the South-Central wetlands. Five rhizomes were collected from twelve randomly selected South-Central wetlands. In the wetlands from the St. Lawrence River, only five plants (above sediment parts) per wetland were collected, and no rhizomes were collected. Individual plants were stored in Ziploc Bags and frozen within 5 h of collection until time of analyses.

2.3 Mercury Analyses

Hg analyses for water, sediments and plants were performed on an ICP Mass Spectrometer (Varian model VG Plasma Quad 2+) at the GSC in Ottawa, Ontario. Detection limits were 1 ng/L for water, and 1 ng/kg for the plants and sediments. The water, sediment and plant samples were run separately on the ICP Mass Spectrometer due to the different acids used in their digestion.

Plants and sediments were digested using a microwave unit (CEM model 2000). Methods were developed for the digestion procedures using trial and error, following the CEM guidelines (CEM Corporation, 1992) and those reported by Van Delft and Vos (1988).

2.3.1 Water Hg Analyses

Total Hg concentrations in the water were determined on the acidified samples using an ICP Mass Spectrometer at the GSC. The blanks which were prepared at the same time as the wetland waters were included with the runs on the ICP Mass Spectrometer. No suitable water standards existed at the time of these analyses, however, the GSC prepared solutions of mercuric chloride to calibrate and measure the accuracy of the results.

2.3.2 Plant Hg Analyses

Preparation for Hg analyses in the plant tissues began with a cleaning process using distilled and deionized water to remove extraneous material (ie. epiphytes, small crustaceans, and sediment debris). Following this cleaning, the plants were separated into two components: the leaves and the petioles. For wetlands where rhizomes were collected, rhizomes were separated from the rootlets. Leaf, petiole and rootlet tissues were dried separately in a drying oven at 40^0C for approximately 48-72 h. Rhizomes were dried at 40^0C for approximately 72-96 h. Temperatures higher than 60^0C may lead to loss of Hg species. The

tissues were ground to homogenize the samples and 0.5 g of sample was placed in a clean acid washed teflon bomb. Fifteen ml of nitric acid (Merck "pro analysi", with <0.0000005 % Hg; VWR) were added to the samples. A standard of Apple Leaves (Standard Reference Material #1515) from the National Institute of Standards and Technology (NIST, Gaithersburg, Maryland) was used to ensure quality and accuracy of results. In addition, blanks consisting solely of nitric acid were digested. Twelve samples (10 plant samples; 1 blank; and 1 standard) were digested at once in the microwave unit following the steps outlined in Appendix 7.3.

The complete digestion procedure in the microwave unit took approximately 2.5 h. When the digests cooled down and the pressure reached less than 20 psi within the teflon bombs, the digests were added to clean acid washed polypropylene Falcon tubes, sealed with parafilm wax, and stored at 4^0C until analysis on the ICP Mass Spec. After each digestion run, the teflon bombs and lids were rinsed with distilled water, soaked in 5 % nitric acid overnight, rinsed four times with distilled water, and allowed to air dry upside down. After every fifth digestion day, a cleaning procedure using 5 ml of nitric acid in the microwave oven for 10 min at a pressure of 45 psi was performed.

2.3.3 Sediment Hg Analyses

Preparation for the South-Central Ontario sediments was similar to the plants. Stones, large wood chips, and plant

material were removed from the sediments, and the sediments were dried in a drying oven at 40^{0}C for 72-96 h. The sediments were ground and 0.5 g of sediment was placed in a clean acid washed teflon bomb. Fifteen ml of nitric acid (same as above), 10 ml of distilled deionized water, and 6 ml of hydrochloric acid (Merck Suprapur, with less than .005 ppm Hg; VWR) were added to each sample. A standard of Till 2 sediment (CANMET standard) provided by the GSC and blanks (nitric acid, water, and hydrochloric acid) were used to ensure quality and accuracy of results. As with the plants, 12 digests (10 sediments, 1 blank and 1 standard) were run at once in the microwave digestion unit following the steps outlined in Appendix 7.4. The digestion procedure took approximately 3 hours. After the cool down period and pressure drop to below 20 psi the digests were stored in the exact manner as the plants mentioned above.

The St. Lawrence River sediments were dried at 40^{0}C for 72-96 h similar to the Temagami and Haliburton sediments, followed by removal of tiny white mollusc along with stones, wood chips, and large plant material. However, due to the large quantity of sands present in the St. Lawrence River wetland sediments, and the fact that digestion following the procedure outlined for the South-Central Ontario sediments had little or no effect on the extraction of mercury from the St. Lawrence sediments, an additional step involving hydrofluoric acid was necessary for complete digestion (Appendix 7.5). This step involved using 5 ml of hydrofluoric acid (Anal-R, with less than .005 ppm Hg; BDH)

with 0.5 g of sediment. After the samples were allowed to cool down and upon reaching a pressure of 10 psi, the teflon bombs were opened and 15 ml of nitric acid, 10 ml of water, and 6 ml of hydrochloric acid were added to each sample and put through the original digestion procedure outlined in Appendix 7.5. In addition, the standard used with the St. Lawrence River wetlands sediments was Buffalo River Sediment (Standard Reference Material # 2704) from NIST. This standard was chosen due to its similarity in LOI and texture to the St. Lawrence River sediments and high concentration of anthropogenic mercury. Blanks involving the first step with hydrofluoric acid, and the second step with nitric acid, water, and hydrochloric acid were also digested. Twelve digests (10 sediment samples; 1 standard; and 1 blank) were performed simultaneously. Once again when the digests cooled and a drop in pressure below 20 psi was achieved the digests were stored in the same manner as the plants and the other sediment digests. Cleaning the teflon bombs and lids occurred in the same way as with the plants mentioned above. The procedure for the St. Lawrence River sediments was tested with both the Till 2 standard and the Buffalo River Sediment standard prior to the digestion of the wetland sediments. This ensured that the methods were appropriate and that the sediments were digested completely and the "true" amount of total sediment mercury was measured.

2.4 Standards and Recoveries

All but 8 of the 98 Apple Leaves measured values fell within the NIST concentration ranges. Similarly, all but 1 of the 7 Buffalo River Sediment measured values fell within the NIST concentration ranges, and all but 4 of the 17 Till 2 measured values fell within the CANMET concentration ranges. The observed and the expected Hg values of the Apple Leaves, Buffalo River Sediment, and Till 2 standards are presented in Appendix 7.6.

Randomly picked duplicates of water, plants and sediment samples were analyzed at a frequency of approximately 5 %. Recoveries for these duplicates were 94 $\pm$ 7 % S.D..

2.5 Statistical Analyses

Statistical analyses were performed using Sigma Stat Statistical Analysis System (1992) for the personal computer. Data used in the regression analyses were tested for normality and homoscedastic residual variances (ZAR, 1989). Transformations of some of the variables to common logarithms were done prior to statistical analyses to satisfy assumptions of normality and homoscedasticity.

To compare the South-Central wetlands and the St. Lawrence River wetlands, t-Tests were performed when the data met the normality and variance assumptions. The t-Test was used to determine differences between the concentrations of Hg in *Nuphar* leaves and petioles from the South-Central wetlands; between the concentrations of Hg in the leaves of *Nuphar* from the South-

Central wetlands and the St. Lawrence River wetlands; between the concentrations of Hg in the sediments from the South-Central wetlands and the St. Lawrence River wetlands; and between the concentration ratios(leaf or petiole Hg/water Hg) for the St. Lawrence River wetlands. When the normality and variance assumptions were violated, the Mann-Whitney Rank Sum Test was employed as the nonparametric test to compare the two groups. The Mann-Whitney Rank Sum Test was used to determine differences between alkalinity, DOC, organic content of the sediments and concentrations of Hg in the water from the South-Central wetlands and the St. Lawrence River wetlands; between the concentrations of Hg in the petioles of *Nuphar* from the South-Central wetlands and the St. Lawrence River wetlands; between the concentrations of Hg in the leaves and petioles from the St. Lawrence River wetlands; between the concentration ratios (*Nuphar* leaf or petiole Hg/sediment or water Hg from the South-Central wetlands; between the concentration ratios (*Nuphar* leaf or petiole Hg/sediment Hg) from the St. Lawrence River wetlands; and between the concentration ratios(*Nuphar* leaf or petiole Hg/sediment or water Hg) from the two groups of wetlands.

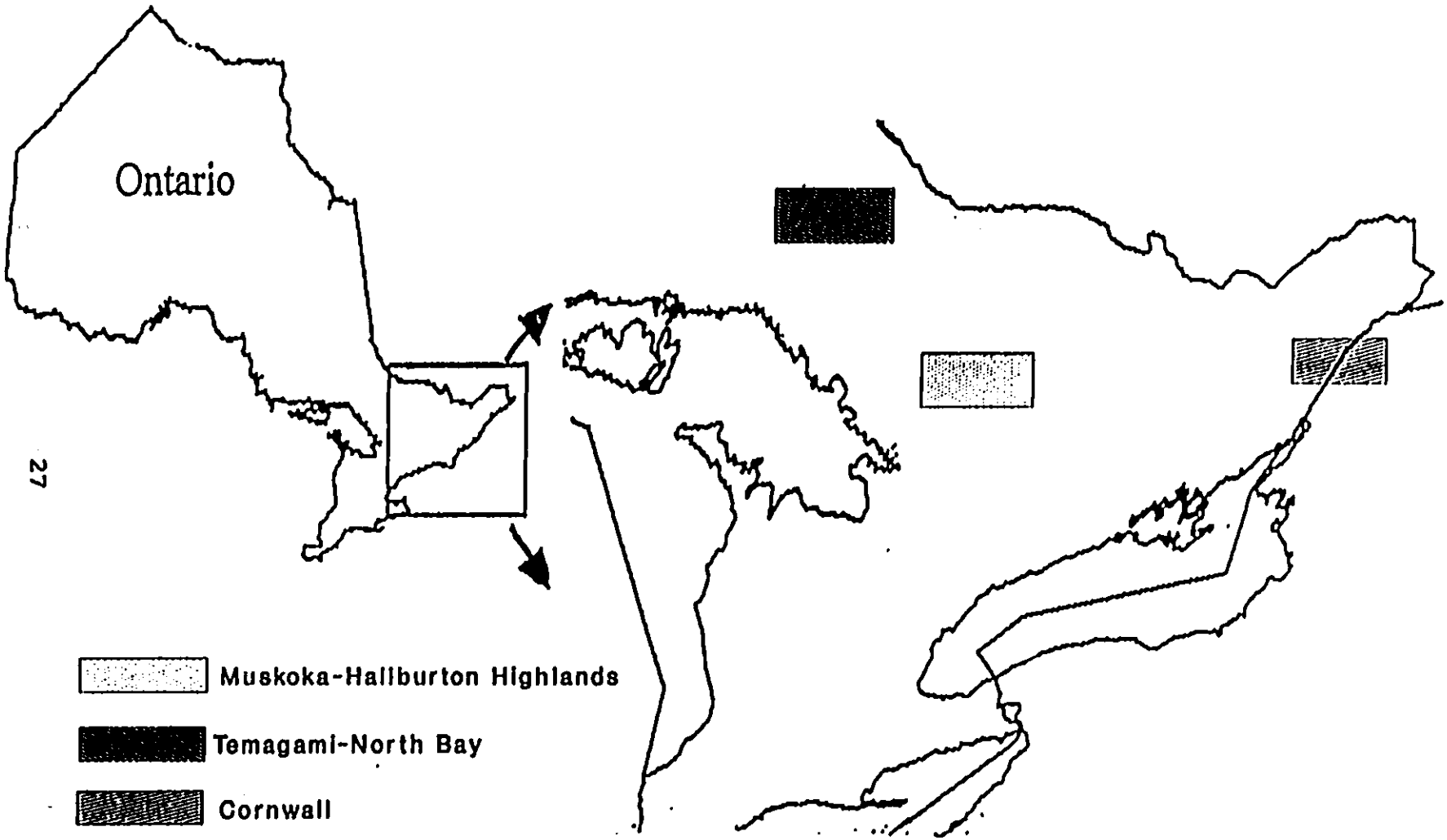

Figure 2.1 Map of Ontario showing the 3 wetland sampling areas.

Chapter 3.0

Results

3.1 South-Central Ontario Wetlands

3.1.1 Site Chemistry

The pH and the alkalinity of the 22 wetlands from South-Central Ontario are shown in Figure 3.1.1. The pH ranged from 4.4 to 7.7, and the alkalinity ranged from 0 to 1088.4 µeq/L Ca (mean = 354.32 ; S.D. = 331.37). Dissolved organic carbon content (DOC) ranged from 6.13 to 20.14 mg/L (mean = 13.07 ; S.D. = 3.6) (Figure 3.1.2). No significant relationships were obtained between DOC and alkalinity or between DOC and pH for these wetlands (App. 7.7).

Total Hg concentrations in the wetland waters ranged from 3 to 34 ng/L (mean = 17.18 ; S.D. = 9.04). Figures 3.1.3 and 3.1.4 show the concentrations of total Hg in the water against the pH of the water, and the concentrations of total Hg in the sediments, respectively. There were no significant relationships observed between total Hg in water and pH, alkalinity, DOC, or total sediment Hg (App. 7.8).

Total Hg concentrations in the wetland sediments ranged from 80.67 to 316 µg/kg (mean = 215.3 ; S.D. = 65.6) and the organic carbon contents of the sediments, estimated as the percent loss on ignition, ranged from 17.67 to 84.33 % (mean = 51.92 ; S.D. = 20.78). A positive and significant relationship ($p<0.001$; $r^2=$ 0.65) between these two variables was determined (Figure 3.1.5). The equation of the regression line is Total Sediment Hg= 83.73 +

2.53 LOI (App. 7.7).

3.1.2 Mercury Concentrations in *Nuphar variegatum*

Concentrations of total Hg in *Nuphar variegatum* leaves ranged from 13.2 to 28.94 ng/g (mean = 18.3 ; S.D. = 4.8), and in *Nuphar* petioles from 14.34 to 30.55 ng/g (mean = 21.64 ; S.D. = 5.2). Figures 3.1.6 and 3.1.7 illustrate the total Hg concentrations in *Nuphar* leaves and petioles, respectively, against the total Hg in the wetland waters. In addition, Figures 3.1.8 and 3.1.9 illustrate the total Hg concentrations in *Nuphar* leaves and petioles, respectively, against the total Hg in the sediment. No significant relationships were obtained between the total Hg concentrations in *Nuphar* leaves (App. 7.9) and petioles (App. 7.10) for pH, alkalinity, DOC, total Hg in water, LOI, total Hg in sediments, or petiole/leaf Hg. However, the relationship between total Hg in *Nuphar* leaves and water was close to being significant (p= 0.053). For the leaf regressions, transformations to common logarithms of *Nuphar* leaf Hg were used in the pH, alkalinity, LOI, and total Hg in sediments regressions to meet the normality assumption.

There was more variation in the mean *Nuphar* petiole concentrations of Hg than the leaves, as illustrated by the larger standard errors from each wetland (compare Figures 3.1.6 and 3.1.7). Despite the fact that the petioles of *Nuphar variegatum* contain significantly more Hg than the leaves (App. 7.19), it is clear that this species maintains a low and fairly

constant concentration of Hg in both it's leaves and petioles despite a wide range in water and sediment Hg concentrations (Figures 3.1.6 to 3.1.9). This finding is further illustrated in Appendix 7.11, where the concentration ratios (*Nuphar* leaf Hg/sediment Hg or *Nuphar* petiole Hg/sediment Hg) ranged from 0.05 to 0.23 (mean = 0.095 ; S.D. = 0.05) for leaf Hg to sediment Hg and from 0.06 to 0.24 (mean = 0.11 ; S.D. = 0.05) for the petioles. The concentration ratios from water (*Nuphar* leaf or petiole Hg/water Hg) ranged from 424 to 9647 (mean = 1892.3 ; S.D. = 2256) in the leaves, and from 473 to 9807 (mean = 2211.6 ; S.D. = 2496) in the petioles (App. 7.12).

Finally, Figure 3.1.10 illustrates concentrations of total Hg in *Nuphar* leaves, petioles, rhizomes, and rootlets from the 12 randomly selected South-Central Ontario wetlands. It is evident that the petioles contained the highest amount of mercury, followed by the leaves, the rootlets, and finally the rhizomes which contained the least mercury.

Results of a Kruskal-Wallis one way analysis of variance indicated significant differences ($p<0.001$) between the median Hg concentrations in the leaves and the petioles of *Nuphar* among the 22 South-Central wetlands.

3.2 St. Lawrence River Wetlands

3.2.1 Site Chemistry

The pH and the alkalinity of the 23 wetlands from the St. Lawrence River are depicted in Figure 3.2.1. The pH ranged from

7.6 to 8.4, and the alkalinity ranged from 1301.2 to 3127.9 µeq/L Ca (mean = 1657.98 ; S.D. = 416.35). The dissolved organic carbon content of these wetlands are plotted against the alkalinities in Figure 3.2.2. DOC values ranged from 2.8 to 11.85 mg/L (mean = 3.98 ; S.D. = 2.1). The DOC concentration was log transformed prior to statistical analysis to meet the assumptions of normality. A positive and significant relationship was obtained (App. 7.13); however, this relationship is due to the one high DOC value and this result should be viewed with caution. No significant relationship between DOC and pH was observed (App. 7.13).

Total Hg concentrations in the wetland waters ranged from 3 to 19 ng/L (mean = 8.22 ; S.D. = 4.0). Figures 3.2.3 and 3.2.4 show the concentrations of total Hg in the water against the pH of the water and the concentrations of total Hg in the sediments, respectively. There were no significant relationships obtained between total Hg in water and pH; alkalinity; or DOC (App. 7.14). However, a positive and significant relationship ($p= 0.007$; $r^2= 0.295$) between total Hg in water and total Hg in sediments was obtained, and the equation of this regression line is Total Hg in Water= 4.53 + 0.03 Total Sediment Hg (Figure 3.2.4; App. 7.14).

Total Hg concentrations in the sediments ranged from 14.71 to 373.1 µg/kg (mean = 143.88 ; S.D. = 85), and the organic carbon contents of the sediments ranged from 0.92 to 48.71 % (mean = 19.72 ; S.D. = 10.81). A positive and significant relationship ($p< 0.001$; $r^2= 0.57$) between these two variables was

obtained (Figure 3.2.5), and the equation of the regression line is Total Sediment Hg= 27.17 + 5.92 LOI (App. 7.13).

3.2.2 Mercury Concentrations in *Nuphar variegatum*

Concentrations of total Hg in *Nuphar variegatum* leaves ranged from 7.93 to 21.42 ng/g (mean = 14.72 ; S.D = 3.6), and in *Nuphar* petioles from 6.39 to 36.48 ng/g (mean = 19.14 ; S.D. = 8.8). There was no significant difference between the leaf Hg and petiole Hg concentrations (App. 7.20). Figures 3.2.6 and 3.2.7 illustrate the total Hg concentrations in *Nuphar* leaves and petioles, respectively, against the total Hg concentrations in water. In addition, Figures 3.2.8 and 3.2.9 illustrate the total Hg concentrations in *Nuphar* leaves and petioles, respectively, against the total Hg concentrations in the sediments. No significant relationships were obtained for concentrations of total Hg in *Nuphar* leaves (App.7.15) and petioles (App. 7.16) and chemical variables (pH; alkalinity; DOC; total Hg in water; LOI; or total Hg in sediments). However, a positive and significant relationship between the concentration of Hg in *Nuphar* leaves and *Nuphar* petioles was obtained for these 23 wetlands (App. 7.15 and 7.16).

Once again as with the South-Central wetlands, there was more variation in the mean Hg petiole concentrations than in the leaf concentrations for each wetland. Despite the fact that the petiole concentration of Hg has a larger range in values than the leaves, and that the petioles contain ,on average, more Hg than

the leaves, it appears that *Nuphar variegatum* from the St. Lawrence River wetlands, like their counterparts from the South-Central wetlands, maintain a low and fairly constant Hg concentration in both the leaves and petioles (Figures 3.2.6 to 3.2.9). Appendix 7.17 contains the sediment based concentration ratios (plant Hg/sediment Hg) of *Nuphar* leaves and petioles. It is interesting to note that the concentration ratios in all but one wetland are at or below 0.4 for both leaves and petioles. Furthermore, the wetland with the lowest sediment Hg concentration contained the plant with the highest concentration ratio in both the leaves and the petioles. Concentration ratios of leaf to sediment Hg ranged from 0.04 to 1.08 (mean = 0.167 ± 0.212), and in the petioles from 0.02 to 0.98 (mean = 0.204 ± 0.202). The water based concentration ratios (plant Hg/water Hg) for both the leaves and petioles of *Nuphar* from these 23 wetlands are located in Appendix 7.18. These concentration ratios of leaf to water Hg ranged from 610 to 4086 (mean = 2215.1 ± 1083) and from 426 to 8900 (mean = 2987 ± 2202) in the petioles.

Results of a Kruskal Wallis one way analysis of variance indicated that significant differences ($p<0.001$) in the median values of Hg in the leaves and petioles of *Nuphar* among the 23 St. Lawrence River wetlands occurred.

3.3 Differences Between the Two Wetland Groups

There were significant differences in the alkalinity, DOC, total Hg in the water, LOI, and total Hg in the sediments between

the South-Central wetlands and the St. Lawrence River wetlands (App. 7.20). The South-Central Ontario wetlands contained significantly higher amounts of DOC, total Hg in water, LOI, and total Hg in sediment. The St. Lawrence River wetlands contained significantly higher alkalinities.

The concentrations of Hg in the water from the South-Central wetlands reached a maximum value of 34 ng/L, while the maximum value obtained in the St. Lawrence River wetlands was 19 ng/L (compare Figures 3.1.4 and 3.2.4). In addition, there was a positive and significant relationship between the Hg concentrations in the water and the Hg concentration in the sediments from the St. Lawrence River (App. 7.14). Meanwhile, no similar relationship was obtained in the South-Central wetlands (App. 7.8).

The positive and significant relationship between total Hg in the sediments and the organic content of the sediments was slightly stronger for the South-Central wetlands (r^2= 0.65; Figure 3.1.5) than for the St. Lawrence River wetlands (r^2= 0.57; Figure 3.2.5).

There was a significant difference between the total Hg concentrations in *Nuphar* leaves from the two wetland groups (App. 7.19). The *Nuphar* leaves from the South-Central wetlands contained significantly more Hg than the *Nuphar* leaves from the St. Lawrence River. There was no significant difference between the Hg content of the *Nuphar* petioles from the South-Central wetlands and the St. Lawrence River wetlands (App. 7.20). In

addition, a positive and significant relationship between the total Hg in *Nuphar* leaves and *Nuphar* petioles from the St. Lawrence River existed (App. 7.15), while no similar relationship existed in the South-Central wetlands (App. 7.9).

Finally, despite the larger variation in the sediment based concentration ratios from the St. Lawrence River wetlands, there were no significant differences between the concentration ratios of *Nuphar* leaves and petioles from the South-Central wetlands and the St. Lawrence River wetlands (compare Appendices 7.11 and 7.17; App. 7.20). One interesting finding is that the *Nuphar* leaves and petioles exhibited concentration ratios of ≤ 0.24 in the South-Central wetlands, and ≤ 0.4 for all but one wetland in the St. Lawrence River. In the one wetland exceeding this intermediate value, the concentration ratio is 1.08 for the leaves and 0.98 for the petioles, indicating that accumulation of Hg in *Nuphar* is taking place in this wetland compared to the other 44 wetlands. Even though the water based concentration ratios for *Nuphar* are quite a bit higher than the sediment based concentration ratios, there are still no differences occurring between the leaf and petiole water based concentration ratios in either wetland group (App. 7.19; App 7.20). However, the water based Hg concentration ratios for *Nuphar* were significantly different between the two wetland groups (compare Appendices 7.12 and 7.18; App. 7.20), with the St. Lawrence River concentration ratios being higher for both the leaves and the petioles.

Multiple regressions between leaf Hg or petiole Hg and

variables (pH; alkalinity; DOC; LOI; water Hg; sediment Hg; and location (South-Central Ontario or St. Lawrence River); for all 45 wetlands were performed. Results of these regressions indicated that leaf Hg (App. 7.21) and petiole Hg (App. 7.22) can not be predicted from a linear combination of these independent variables.

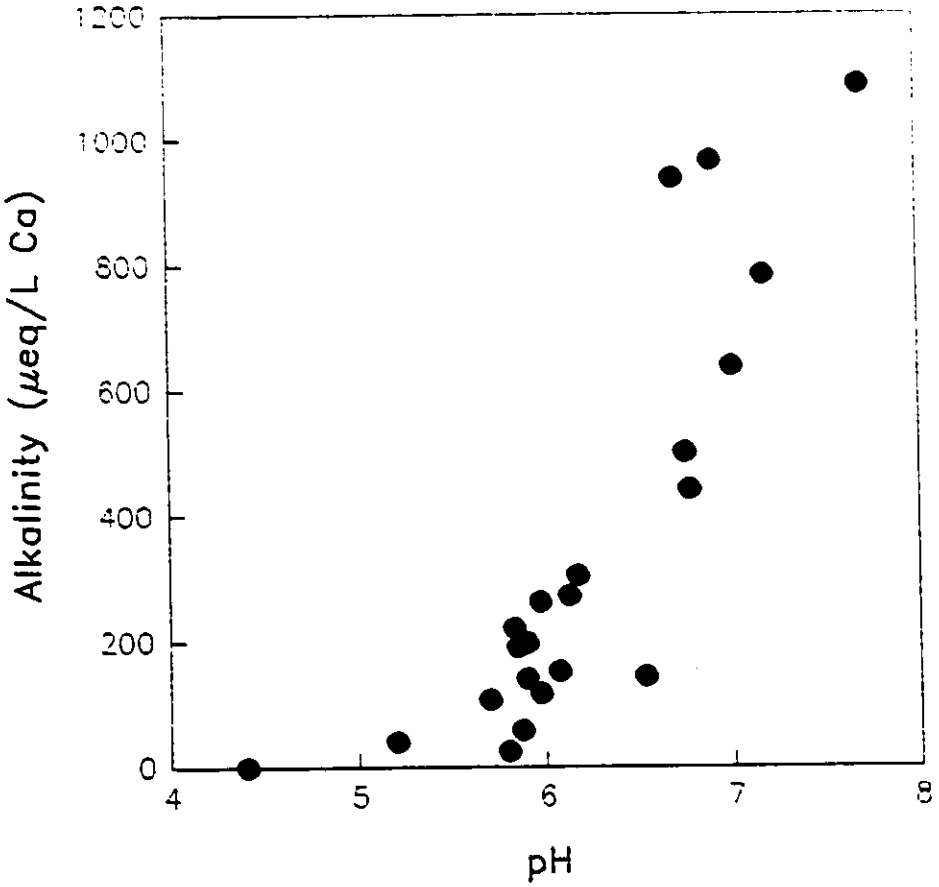

Figure 3.1.1 pH and alkalinity for the 22 wetlands from South—Central Ontario. Values represent mean of 3 measurements.

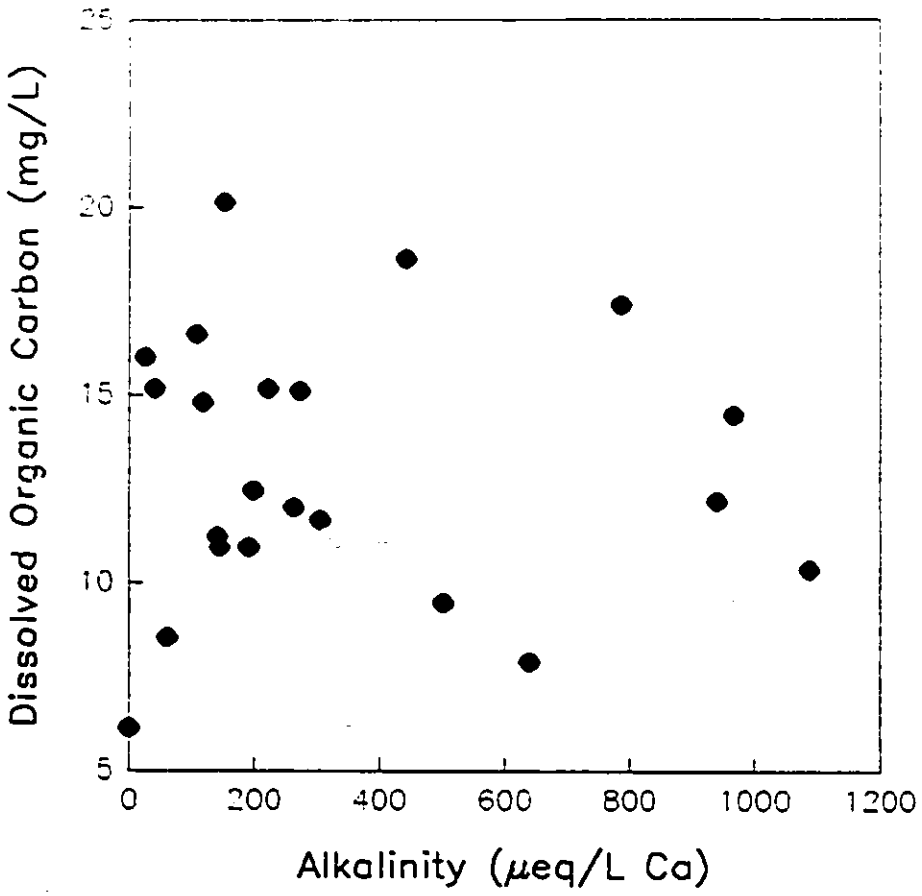

Figure 3.1.2 Alkalinity and DOC for the 22 wetlands from South–Centrai Ontario. Values represent mean of 3 measurements.

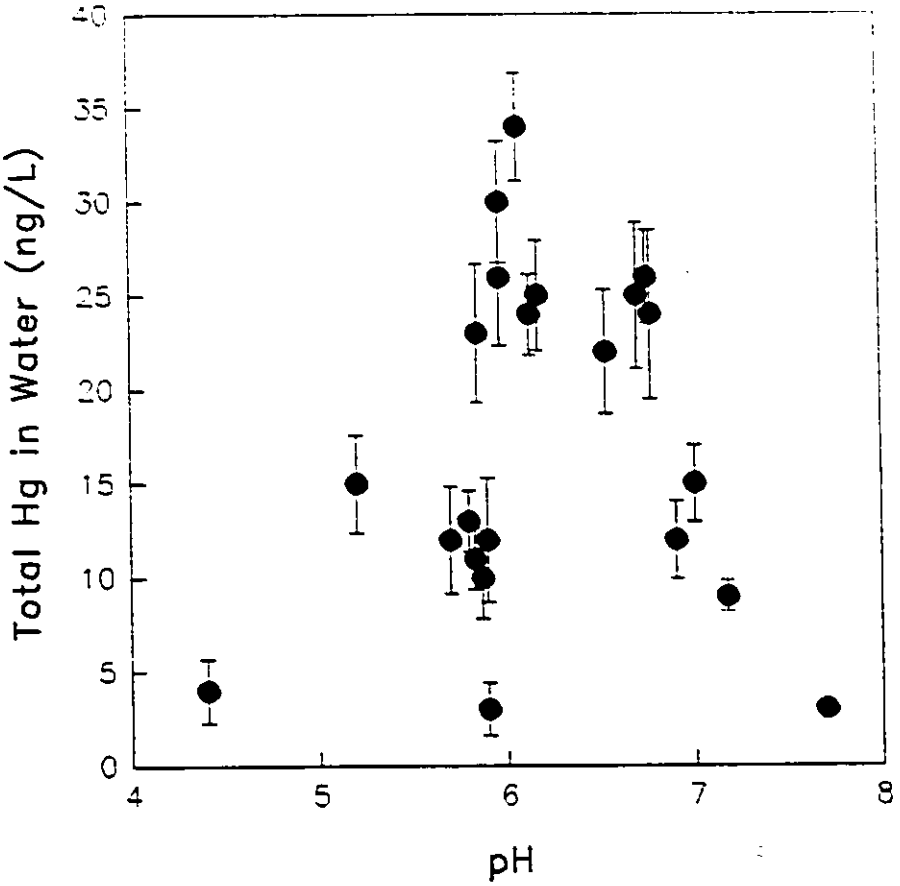

Figure 3.1.3 pH and concentrations of
Total Hg in the water for the 22 wetlands
from South–Central Ontario.
Values represent mean ± S.D. of 3 measurements.

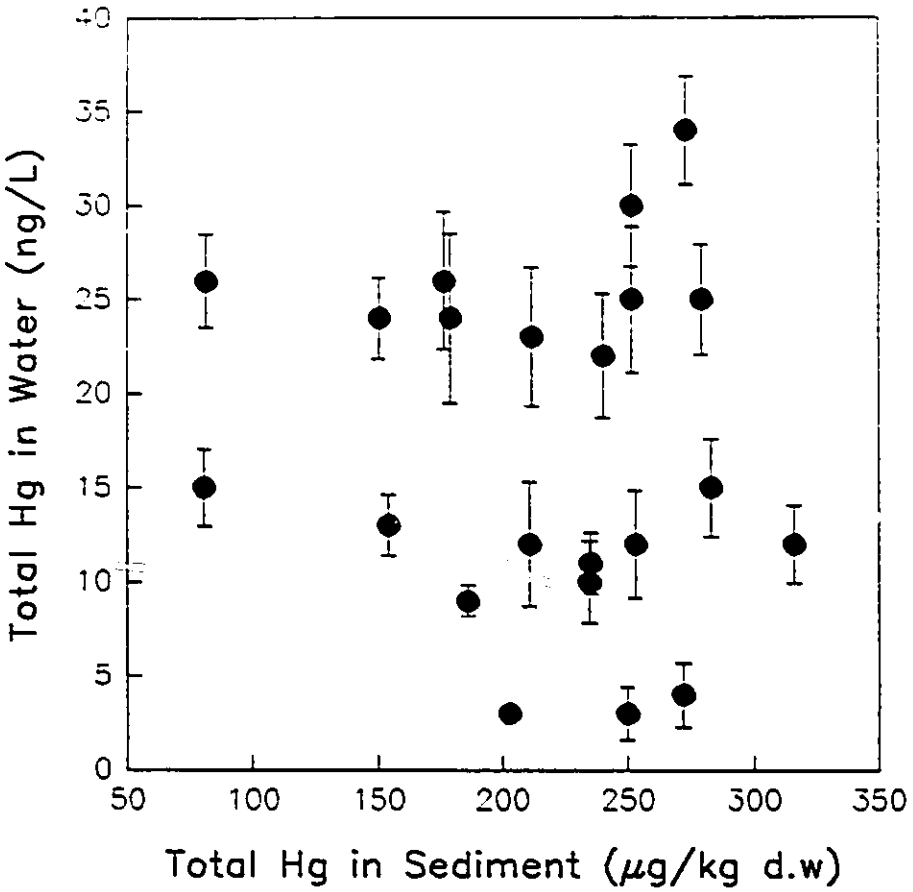

Figure 3.1.4 Concentrations of Total Hg in the sediments and the water for the 22 wetlands from South–Central Ontario. Values represent mean ± S.D. of 3 measurements.

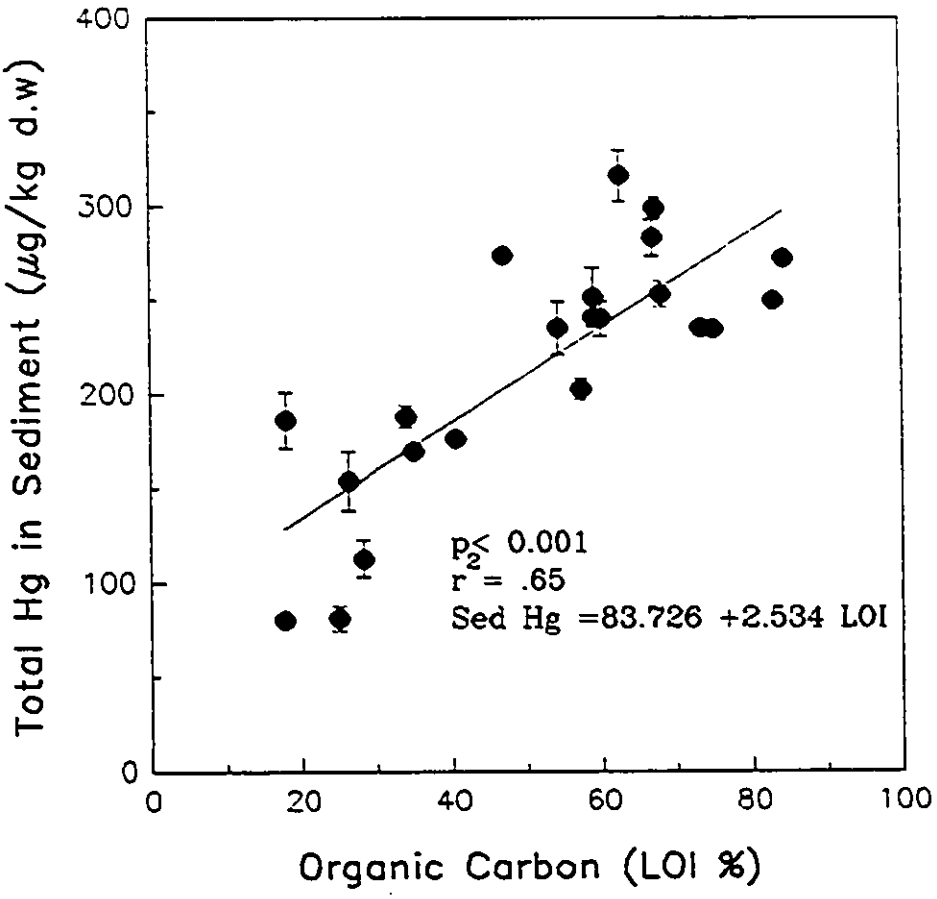

Figure 3.1.5 Percent loss on ignition of the sediments and Total Hg concentrations in the sediments for the 22 wetlands from South–Central Ontario. Values represent mean ± S.D of 9 measurements.

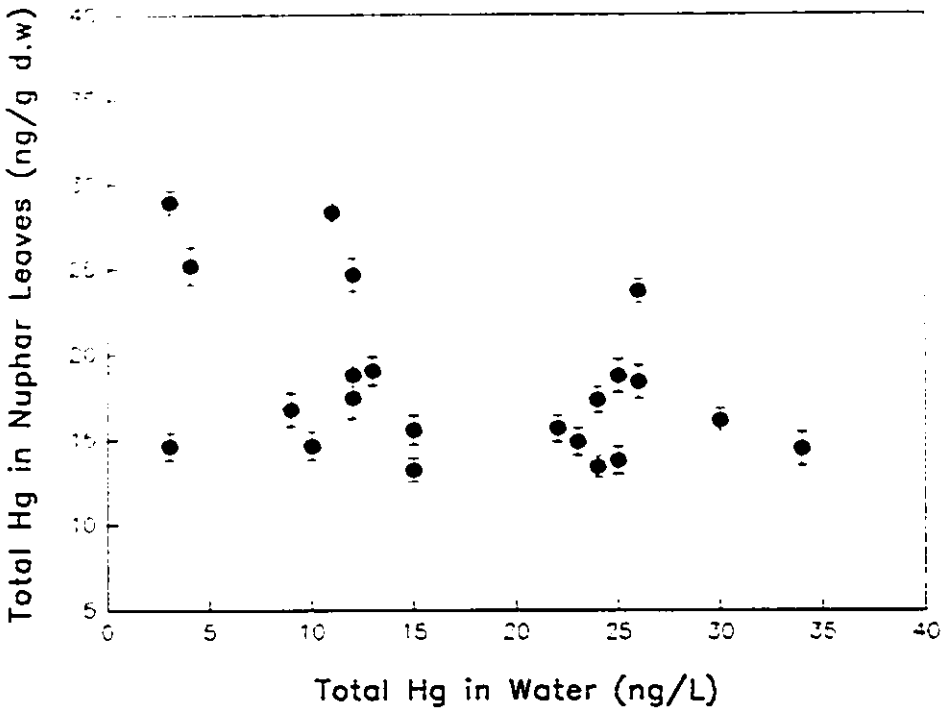

Figure 3.1.6 Total Hg in water and Nuphar leaves for the 22 wetlands from South–Central Ontario. Values represent mean ± S.E. of 15 samples.

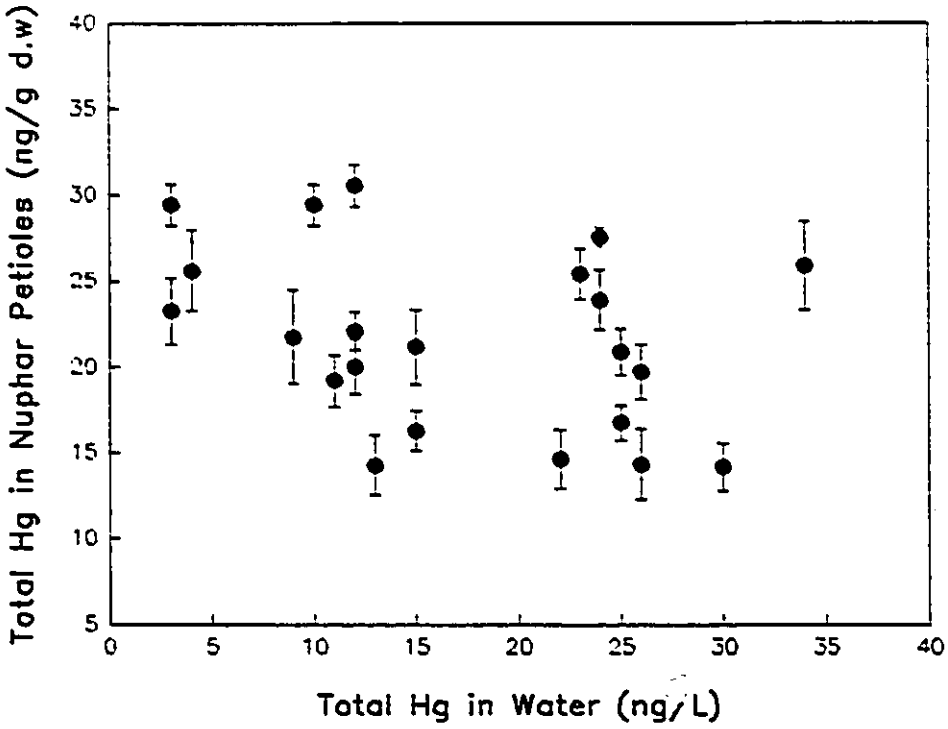

Figure 3.1.7 Total Hg in water and Nuphar petioles for the 22 wetlands from South–Central Ontario. Values represent mean ± S.E. of 15 samples.

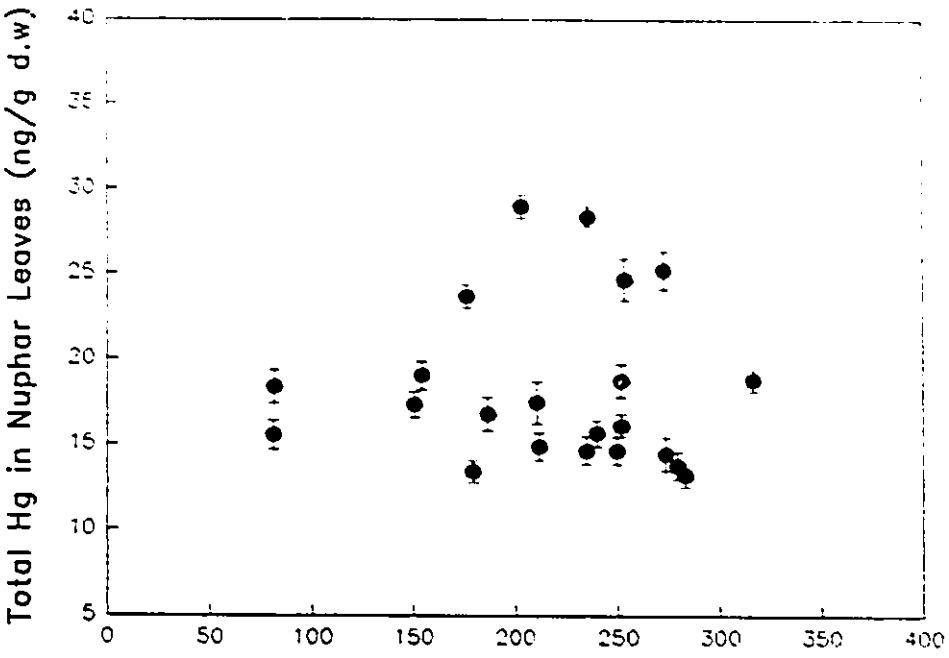

Figure 3.1.8 Total Hg in sediments and Nuphar leaves for the 22 wetlands from South–Central Ontario. Values represent mean ± S.E. of 15 samples.

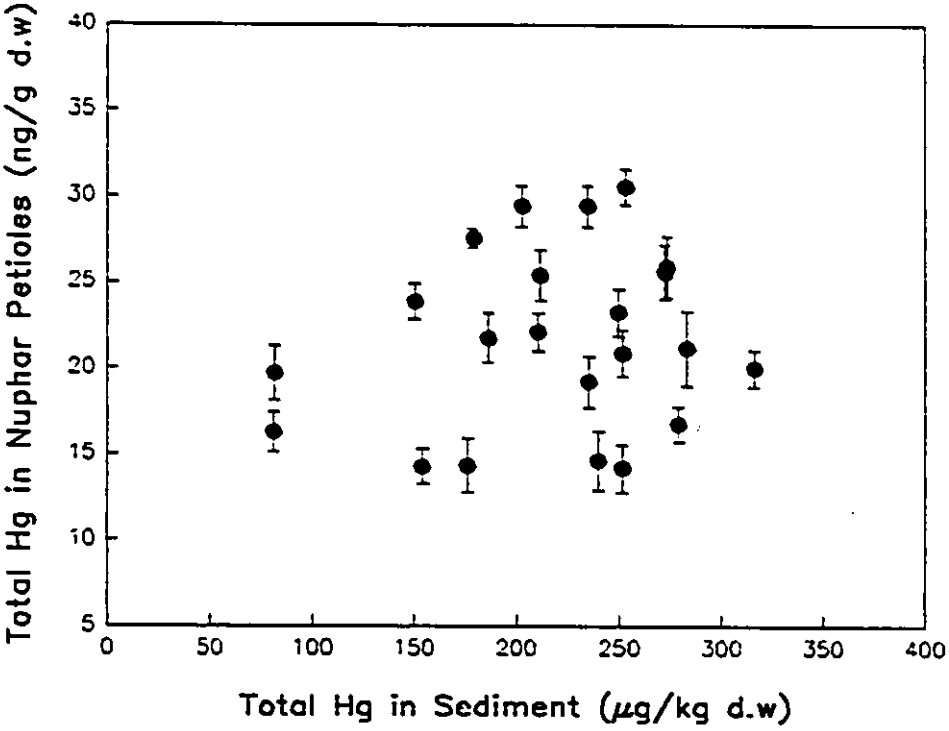

Figure 3.1.9 Total Hg in sediments and Nuphar petioles for the 22 wetlands from South–Central Ontario. Values represent mean ± S.E. of 15 samples.

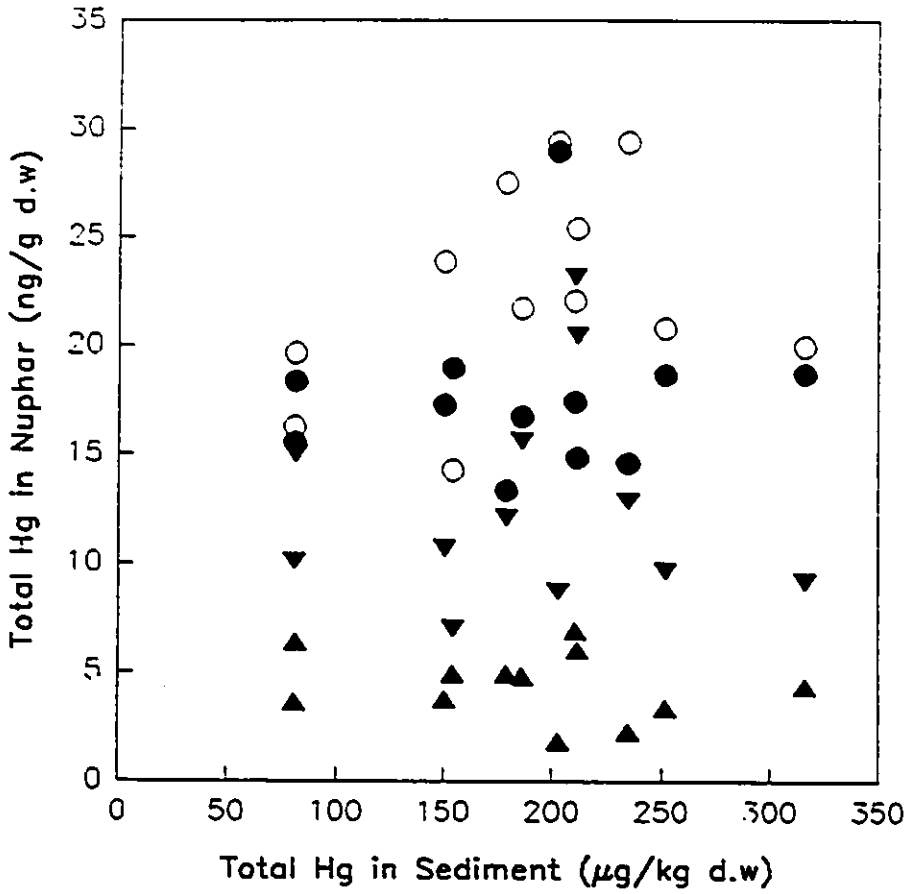

Figure 3.1.10 Total Hg in sediments and various Nuphar parts for 12 wetlands in South–Central Ontario.

- ○ petioles values represent mean of 15 samples
- ● leaves values represent mean of 15 samples
- ▲ rhizomes values represent mean of 5 samples
- ▼ rootlets values represent mean of 5 samples

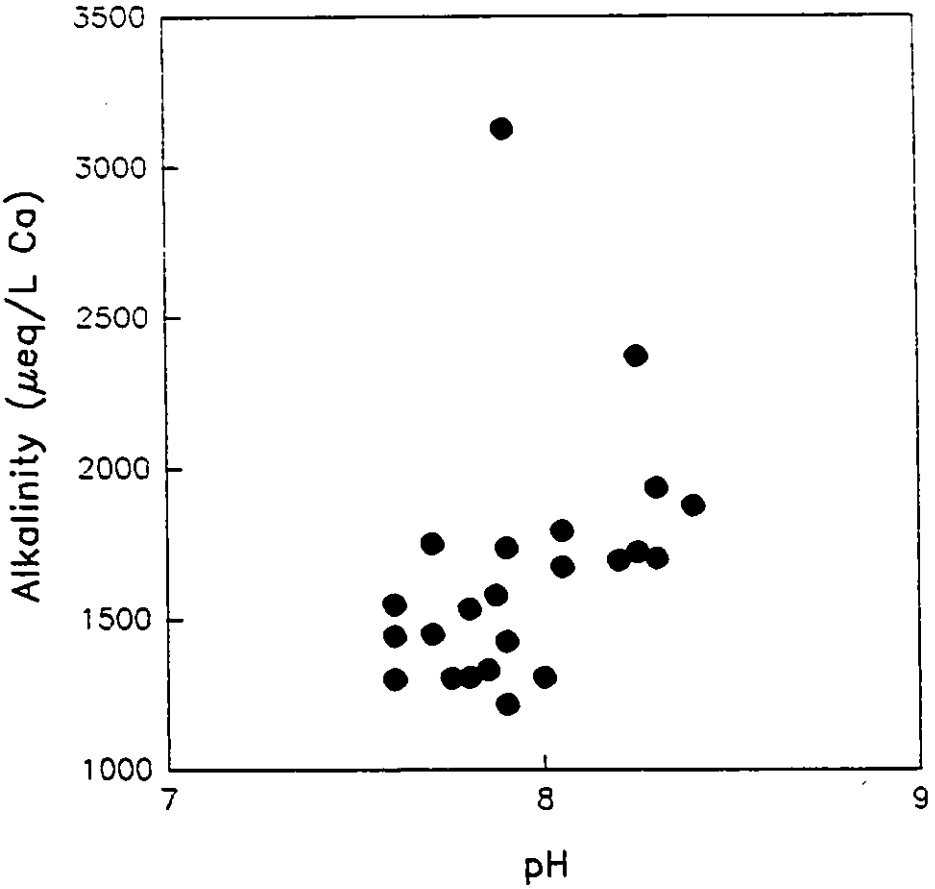

Figure 3.2.1 pH and the alkalinity for the 23 wetlands from the St. Lawrence River. Values represent mean of 3 measurements.

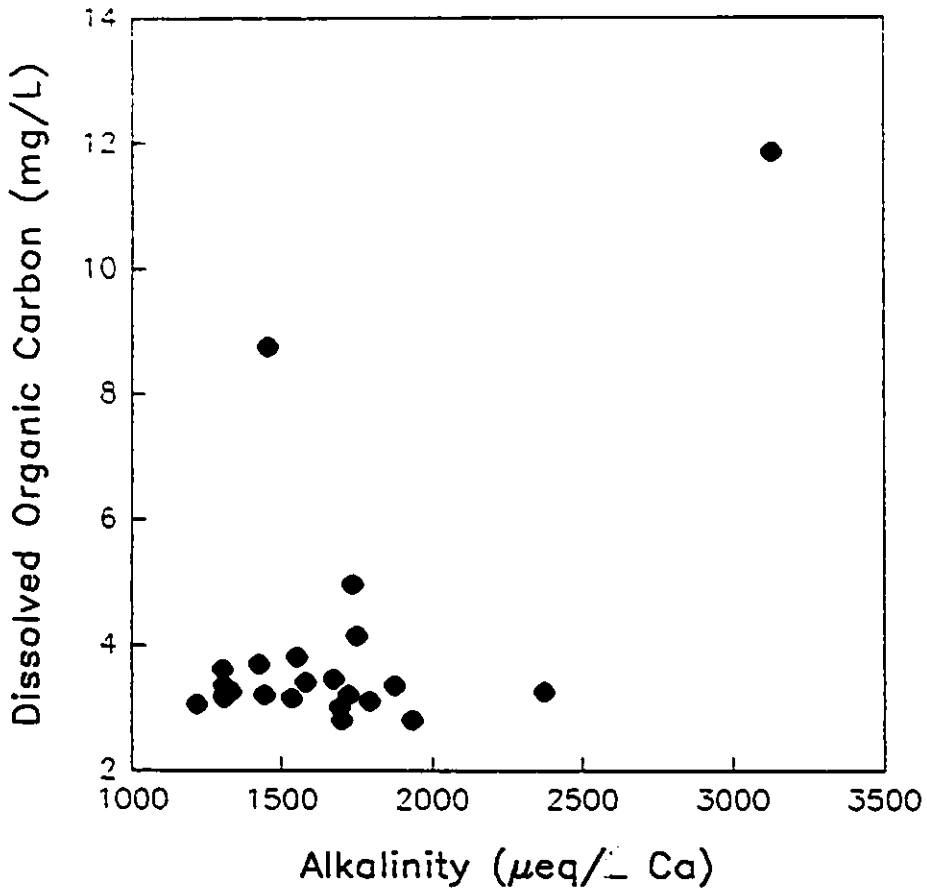

Figure 3.2.2 Alkalinity and DOC for the 23 wetlands from the St. Lawrence River. Values represent mean of 3 measurements.

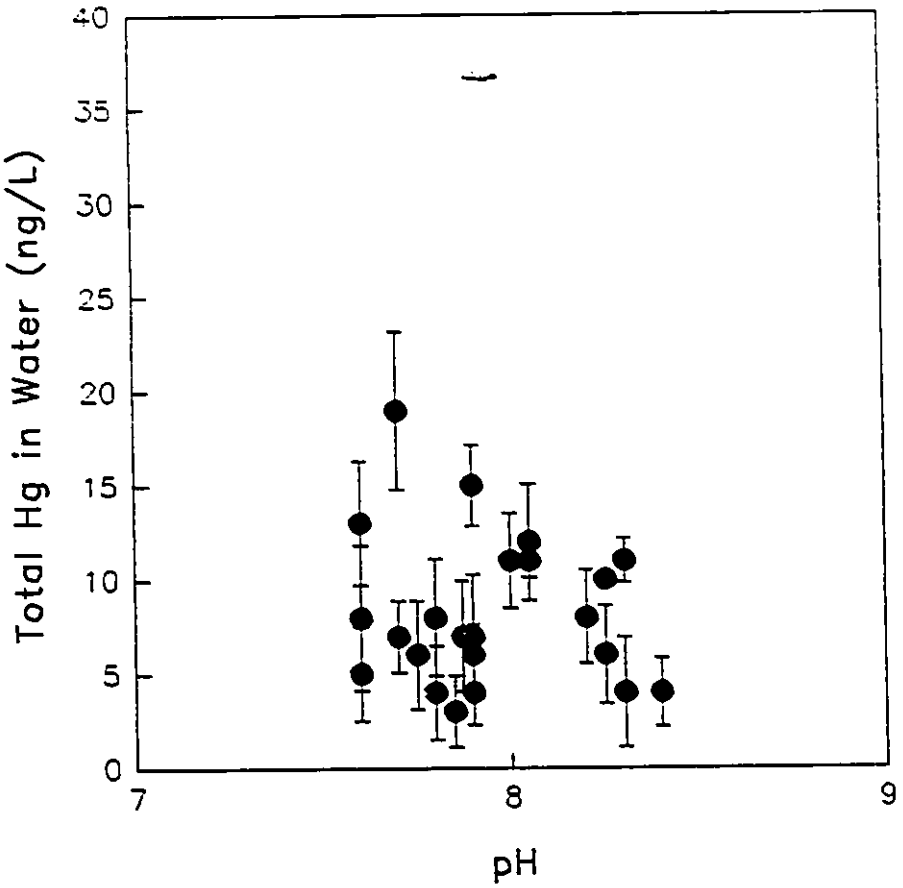

Figure 3.2.3 pH and the concentrations of Total Hg in water for the 23 wetlands from the St. Lawrence River.
Values represent mean ± S.D. of 3 measurements.

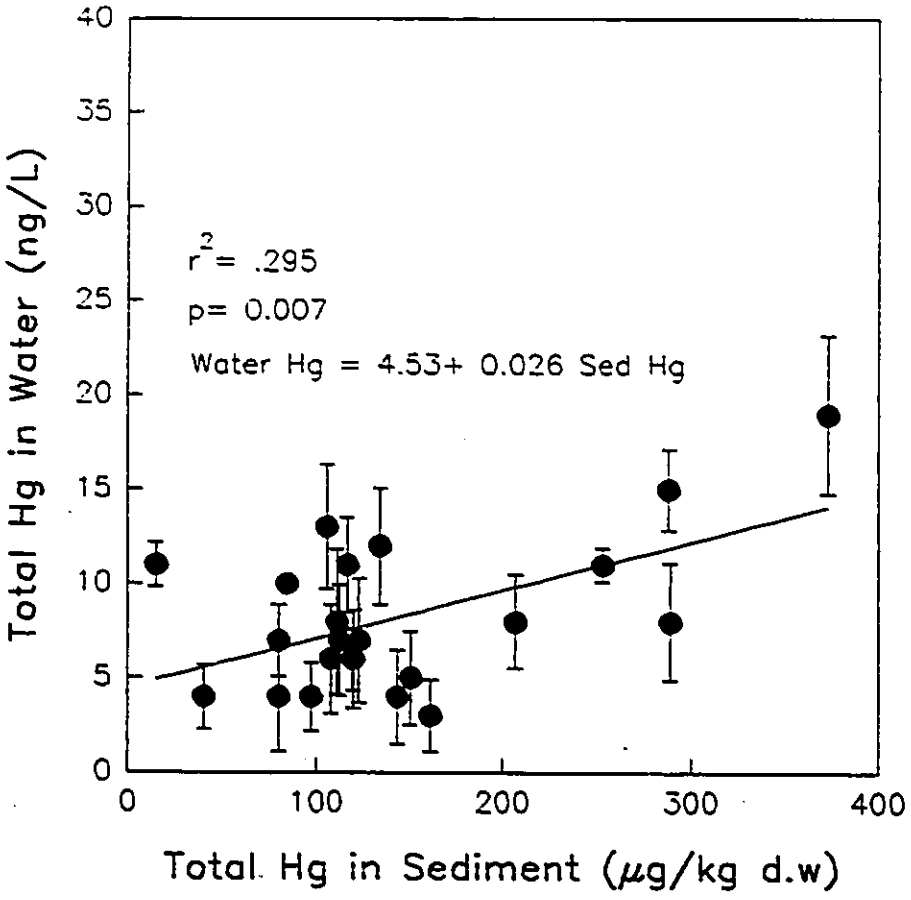

Figure 3.2.4 Concentrations of Total Hg in the sediments and the water for the 23 wetlands from the St. Lawrence River. Values represent mean ± S.D. of 3 measurements.

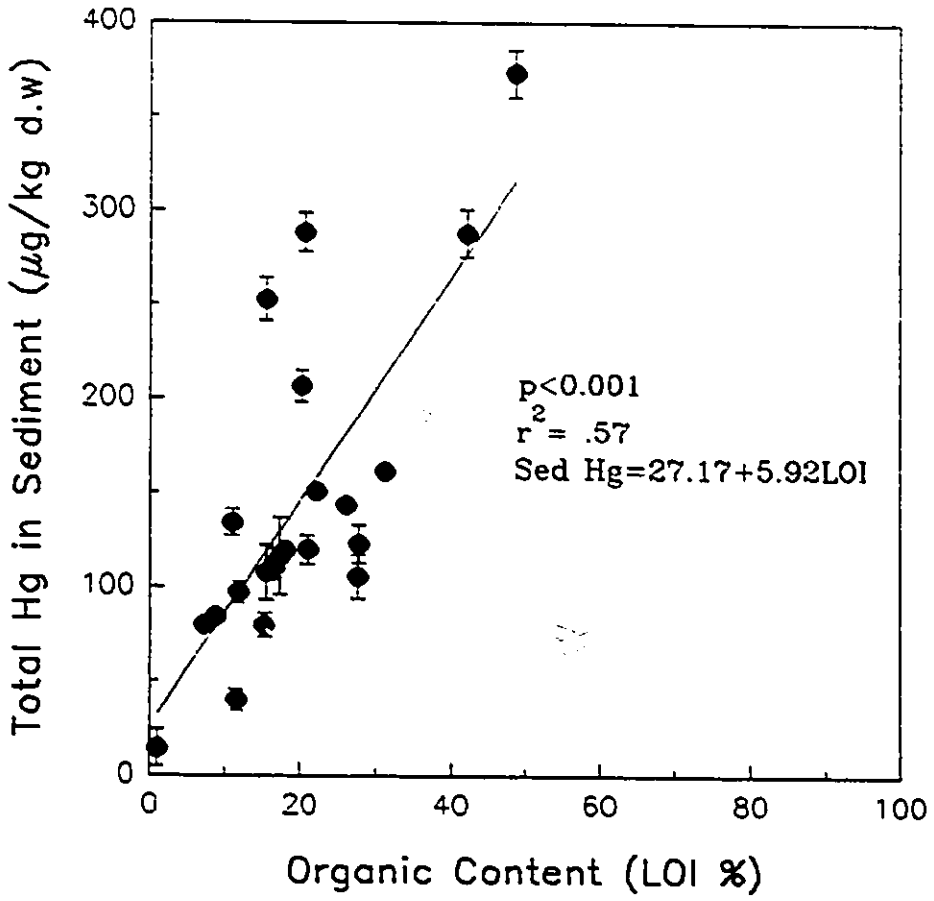

Figure 3.2.5 Percent loss on ignition of the sediments and total Hg concentrations in the sediments for the 23 wetlands from the St. Lawrence River. Values represent mean ± S.D. of 9 measurements.

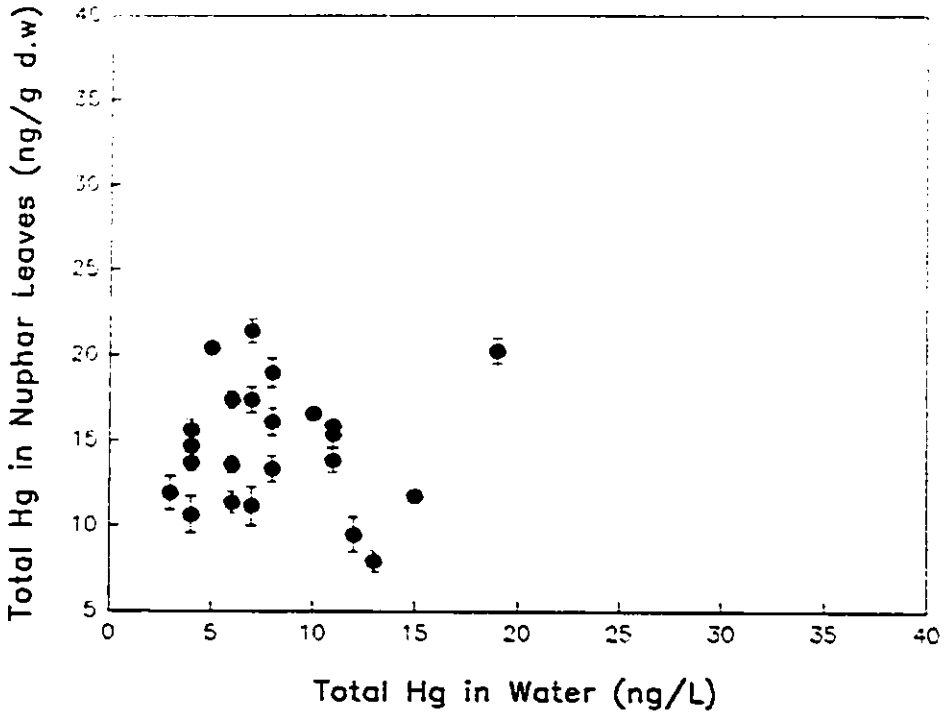

Figure 3.2.6 Total Hg in water and Nuphar leaves for the 23 wetlands from the St. Lawrence River. Values represent mean ± S.E. of 5 samples.

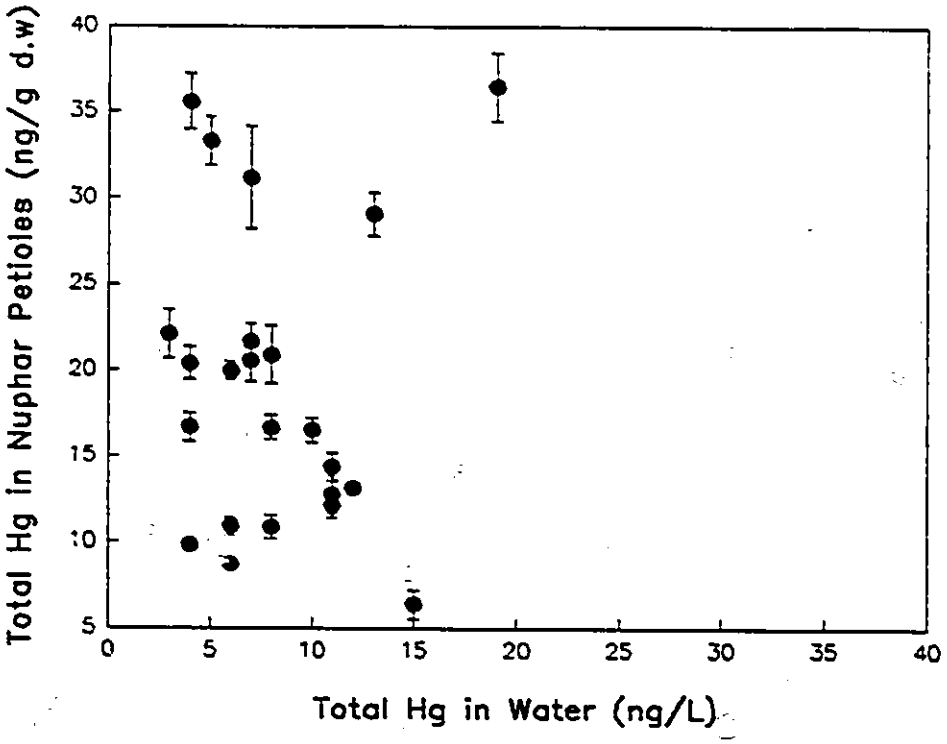

Figure 3.2.7 Total Hg in water and Nuphar petioles for the 23 wetlands from the St. Lawrence River. Values represent mean ± S.E. of 5 samples.

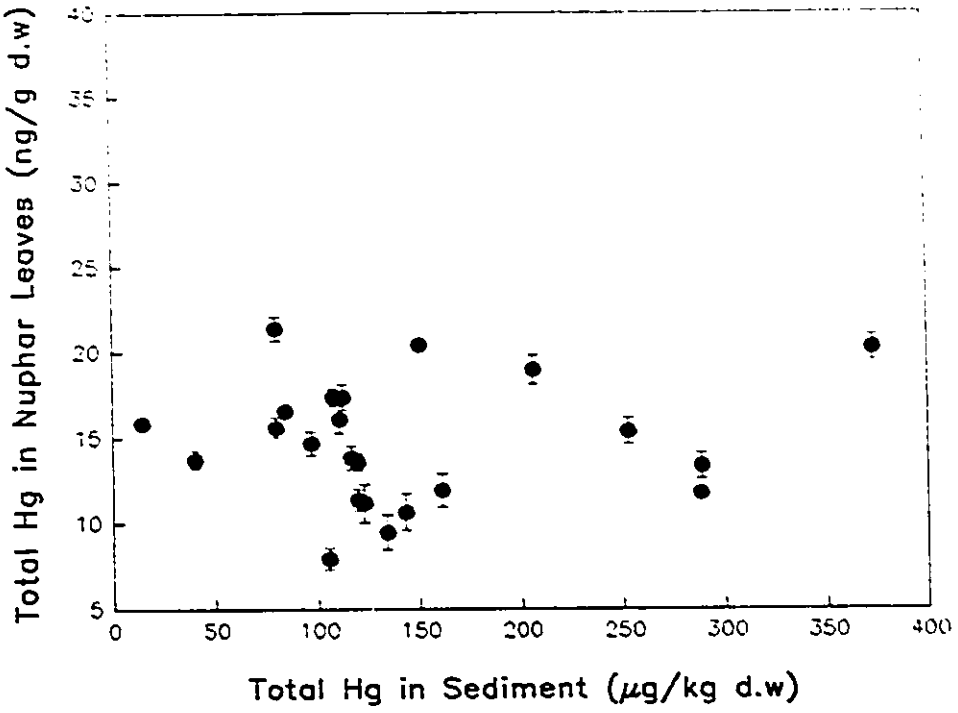

Figure 3.2.8 Total Hg in sediments and Nuphar leaves for the 23 wetlands from the St. Lawrence River. Values represent mean ± S.E. of 5 samples.

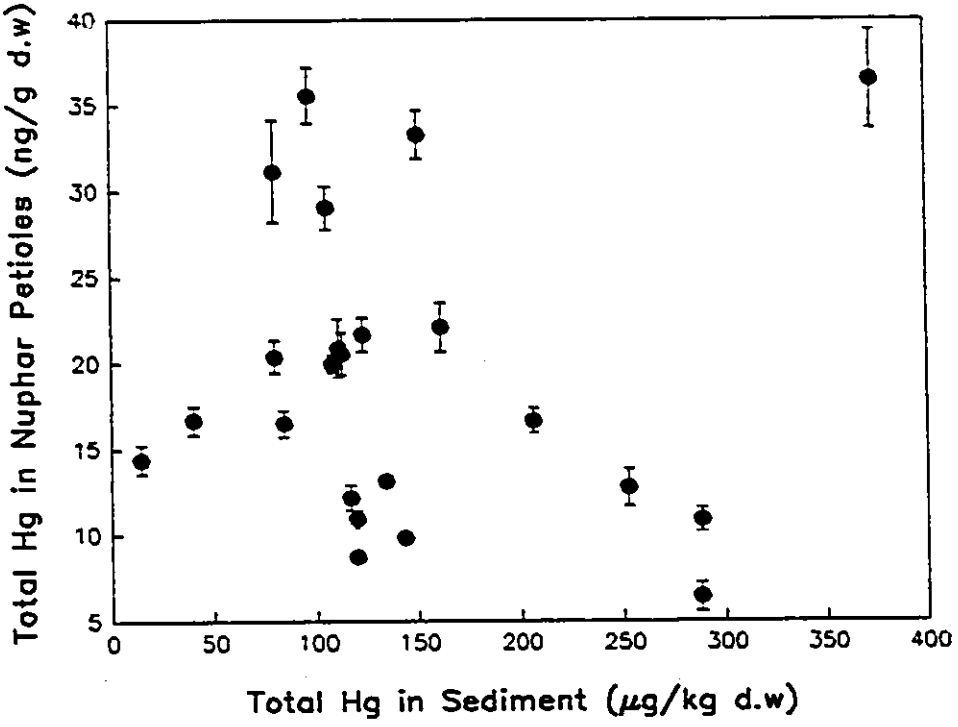

Figure 3.2.9 Total Hg in sediments and Nuphar petioles for the 23 wetlands from the St. Lawrence River. Values represent mean ± S.E. of 5 samples.

Chapter 4.0

DISCUSSION

4.1 Site Chemistry

Despite the great interest in mercury in aquatic systems, there are relatively few reliable measurements of Hg concentrations in surface waters. This fact is largely attributed to the very low Hg concentrations in natural waters, and the difficulties of analyzing water samples.

The total Hg concentration in waterbodies is regulated in Canada at the level of 200 ng/L (OME, 1984). The levels obtained in the 22 wetlands from South-Central Ontario are well below this permissible level, with a maximum concentration of 34 ng/L being observed (Figure 3.1.3.). Similarly, the levels obtained in the 23 wetlands from the St. Lawrence River are also well below this level, with a maximum concentration of 19 ng/L being measured in these wetlands (Figure 3.2.3). The values obtained are similar to those observed by other researchers and are not uncommon for uncontaminated lake and river waters: Ottawa River: 6.6 ng/L (Kudo et al., 1982), 15-20 ng/L (Mortimer, 1985); and Ontario lakes: 10-30 ng/L (Stokes et al., 1983), 5-20 ng/L (Mierle, 1990). However, these values are higher than levels obtained in Wisconsin lakes: 1-5 ng/L (Watras et al., 1995b), and from an earlier study in the St. Lawrence River: 2-4 ng/L (Richman, 1994). "Background" total Hg concentrations can range anywhere from 1 to 20 ng/L in freshwater ecosystems (Kudo et al., 1982; Bloom, 1989; and Mierle, 1990), and values exceeding these would

not necessarily indicate anthropogenic contamination (pers. comm. C.J. Watras).

The wetland waters from the South-Central wetlands contained significantly more Hg than the waters from the St. Lawrence River wetlands (App. 7.20). This may be primarily due to the tremendous flow of the St. Lawrence River, the exchange of wetland water with the main channel, and the River's dilution capacity as noted previously by Richman (1994), whereas in the South-Central wetlands the water residence time in the wetlands would be much longer. Furthermore, the South-Central wetlands contained significantly higher amounts of DOC (App. 7.20). Since Hg has been shown to be associated with DOC in wetlands (Zillioux et al., 1993), this fact alone may account for the higher mercury concentrations in the waters from the South-Central wetlands.

Waterborne total Hg concentrations have been shown to be related to the pH (Gerstenberger et al., 1993; Watras et al., 1995b); alkalinity (Akielaszek and Haines, 1981); and dissolved organic carbon (Nelson and Campbell, 1991; Watras et al., 1995b) of the waters. Of these variables, DOC is considered to be the best predictor of total Hg in water. A study of 41 surface waters found a strong relationship between DOC and total Hg, with the highest Hg concentrations occurring in a brown water wetland having concentrations of 30 to 40 mg/L DOC (Zillioux et al., 1993). On the other hand, Hakanson (1974) found no relationship between DOC and water Hg. This author suggested that this lack of correlation was due to the fact that organic particles are always

in surplus and of different origin. In the present study, the total Hg concentrations in the wetland waters for the 22 South-Central wetlands (App. 7.8) and the 23 St. Lawrence River wetlands (App. 7.14) were not related to pH, alkalinity or DOC. In addition, the Hg concentrations in the water were not related to the sediment concentrations of Hg for the 22 South-Central wetlands (App. 7.7). However, the Hg concentrations in the waters of the 23 St. Lawrence River wetlands were positively related to the sediment Hg concentration (App. 7.14). This is a little surprising given the fact that the flow of the St. Lawrence River would cause downstream transport of Hg. On the other hand, several of the wetlands from the River are in sheltered areas where the current is minimal which may explain this relationship.

Sediments may be regarded as the ultimate sink for Hg compounds in the aquatic environment since sediment Hg levels are greater than water Hg levels (Prahalad and Seenayya, 1988; Bryan and Langston, 1992). Figure 3.1.4 for the South-Central wetlands and Figure 3.2.4 for the St. Lawrence River wetlands illustrate that the sediments contain more Hg than the waters. Sediments for uncontaminated areas usually contain less than 400 µg/kg total Hg (Förstner and Wittmann, 1981; Martinelli, 1988), with background concentrations estimated within a range of 50 to 300 µg/kg for most aquatic systems around the world (Kabata-Pendias and Pendias, 1992; Engstrom et al., 1994). Thus Hg concentrations exceeding these values should be considered as contaminated from anthropogenic or natural sources according to Kabata-Pendias and

Pendias (1992). The total Hg values obtained for the South-Central wetland sediments ranged from 80.7 to 316 µg/kg total Hg, and from 14.7 to 373.1 µg/kg in the St. Lawrence River wetlands. These values are all below the acceptable level for uncontaminated areas. However, upon comparing the sediment Hg values from both the South-Central wetlands and the St. Lawrence River wetlands with the Provincial Sediment Quality Guidelines for Ontario (Persaud et al., 1992), some of these values exceed the lowest effect level (LEL), which is 200 µg/kg for Hg. The LEL is the level of sediment contamination that can be tolerated by the majority of benthic organisms. Concentrations greater than this 200 µg/kg level may impair benthic organism communities in these areas. Sixty-eight percent of the wetland sediments from South-Central Ontario exceed this LEL, while only 22 % of the wetland sediments from the St. Lawrence River exceed this LEL.

The values of the total Hg in sediments obtained in this study are similar to those reported by researchers of various lake and river sediments in Ontario: Ottawa River: 300 µg/kg (Förstner and Wittmann, 1981); Lake Superior: 90 - 356 µg/kg (Kemp et al., 1978); and Lake Erie: 30 - 210 µg/kg (Thomas and Jaquet, 1976). However, the values obtained are lower than those reported for the Gulf of St. Lawrence (Loring, 1975), and South-Central Ontario lakes (Evans, 1986). Similarly, Richman (1994) studied 17 stations from the Cornwall area of the St. Lawrence River in 1991 and found the concentrations of total mercury to range from 60 to 3260 µg/kg. Three of those seventeen sites are

the same as in this study and almost identical results were found. This suggests not only that the findings in this study are accurate, but also that the concentrations of Hg in the sediments of the St. Lawrence River may have neither increased r. r decreased from 1991 till the time of this sampling in 1994.

The sediments from the South-Central wetlands contained significantly more Hg than the sediments from the St. Lawrence River wetlands (App. 7.20). In addition, the sediments from the South-Central wetlands also possessed significantly higher organic carbon contents (App. 7.20), and since Hg in the sediments is primarily bound to organic complexes (Nelson and Campbell, 1991), this explains the higher Hg concentrations in the sediments from the South-Central wetlands. The relationship between sediment organic content and sediment Hg is illustrated by the high positive relationships achieved for both the South-Central wetlands (Figure 3.1.5) and the St. Lawrence River wetlands (Figure 3.2.5). Similar relationships have been reported by Thomas and Jaquet (1976) and Nelson and Campbell (1991) for lakes and Loring (1975) for the Gulf of St. Lawrence. On the other hand, Hakanson (1974) and Wren et al. (1983) found no obvious positive relationships between Hg concentrations in the sediments and the organic contents of sediments in lakes. Furthermore, Blume and Brummer (1991) stress the importance of the organic carbon content in determining the sensitivity of sediments to Hg pollution. Generally, the smaller the metal binding capacity of the sediment is, the more sensitive it is to

Hg pollution. In wetlands, these metal binding complexes are humic substances (Weber, 1973). Therefore, higher organic contents in the sediments supposedly result in less Hg available for biota to uptake. However, increases in organic matter in sediments have been shown to enhance both methylation rates (Campbell et al., 1988; Furutani and Rudd, 1988) and bacterial activity (Lee et al., 1985; Gilmour and Henry, 1991), which results in more biologically available Hg for biota to uptake.

The two positive and significant relationships obtained for the total Hg in the sediments and the LOI of the wetlands for the South-Central wetlands and the St. Lawrence River wetlands (Figures 3.1.5 and 3.2.5, respectively), reflect two obvious differences. Firstly, the slope is significantly steeper for the St. Lawrence River wetlands and the y-intercept is significantly lower ($p<0.001$), indicating that more Hg/unit LOI exists in these sediments, and secondly, although the South-Central wetlands contain significantly more Hg in the sediments on average, the wetland with the highest Hg content comes from the St. Lawrence River. In addition, 65 % of the variation in the concentration of Hg in the sediments from the South-Central wetlands was explained by the organic carbon content of the sediments, whereas, only 57 % of the variation in the concentration of Hg in the sediments from the St. Lawrence River wetlands was explained by the organic carbon content of the sediments.

4.2 Mercury Concentrations in *Nuphar variegatum*

The uptake of an environmental contaminant, such as Hg, into plants is the first step for accumulation of such pollutants in the aquatic food chain. Plants are sedentary organisms and their need to obtain the full spectrum of nutrients from their local environment has led to a maximizing of their surface area to absorb raw materials.

Rooted vascular aquatic plants can take up compounds from their environment by one of two routes, either from the water column through submerged shoots, or from interstitial water of the sediments through the roots. The relative importance of the two routes for nutrient and metal uptake is not clear, although it is accepted that both routes may operate in the same plant (Denny, 1972; Coquery and Welbourn, 1994). The route of metal uptake depends upon a number of factors including plant species, type of metal and its chemical form, and relative metal concentrations in the root and petiole environments. It is generally accepted that not all forms of a trace metal are equally available to uptake by biota. In the sediment, Hg is transformed by microorganisms and by abiotic methylation into its chemically available form, methyl Hg. This methyl Hg is the form which plants take up, so not all the Hg in the sediments is assimilated by plants (Gilbert, 1990). Correlations between sediment Hg and plant Hg (Mortimer, 1985; Siegel et al., 1985) and between water Hg and plant Hg (Campbell et al., 1988; Crowder, 1991) have been found. On the other hand, no

relationship between sediment Hg and plant Hg was found in a study of 7 Canadian lakes (Franzin and McFarlane, 1980) or from a Brazilian River (Lacerda et al., 1991). The results of this present study agree with the latter two examples, where no relationships between sediment Hg and *Nuphar* leaf or petiole Hg for the 22 South-Central wetlands (Figure 3.1.8; Figure 3.1.9) and for the 23 St. Lawrence River wetlands (Figure 3.2.8; Figure 3.2.9) were found. Similarly, there were no relationships between water Hg and *Nuphar* leaf or petiole Hg for the plants from the South-Central wetlands (Figure 3.1.6; Figure 3.1.7) or for the plants from the St. Lawrence River wetlands (Figure 3.2.6; 3.2.7).

Vegetation in uncontaminated areas contains < 100 ng/g Hg and usually less than 10 ng/g (Cocking et al., 1991; Siegel et al., 1985). The concentrations of Hg obtained for both the leaves and petioles of *Nuphar variegatum* from both wetland groups are well below this value of contamination. Water hyacinth, a floating leaved aquatic plant has been shown to accumulate two times more Hg in the leaves than in the petioles (Chigbo et al., 1982). However, in this study, concentrations of total Hg in *Nuphar* leaves (mean = 18.3 ng/g ; S.D. = 4.8) were significantly lower than those in the petioles (mean = 21.64 ng/g ; S.D. = 5.2) for the South-Central wetlands (App. 7.19), indicating that the leaves are not absorbing Hg as effectively as the petioles and this may result from a physiological uptake mechanism in the outer layers of the plant petioles as suggested by Eriksson and

Mortimer (1975). However, concentrations of total Hg for the *Nuphar* leaves (mean = 14.72 ng/g ; S.D. = 3.6) and petioles (mean = 19.14 ng/g ; S.D. = 8.8) from the St. Lawrence River wetlands showed no significant differences (App. 7.20). Furthermore, a significant difference between the Hg in the leaves of *Nuphar* from the South-Central wetlands and the St. Lawrence River was found (App. 7.19), yet no difference between the petiole concentrations of Hg from the South-Central wetlands and the St. Lawrence River wetlands was achieved (App. 7.20).

Table 4.1 lists the concentrations of total Hg in various waterlily species from previous studies around the world. The values obtained in this study are similar to those reported elsewhere for non-contaminated areas. In studies where known Hg contamination exists, the values in *Nuphar* are much higher than the present study.

Studies dealing with the accumulation of Hg by aquatic biota, mainly fish, have identified pH, alkalinity, DOC, and organic content of the sediments as the major environmental variables which predict corresponding Hg levels in these biota. In this study, pH, alkalinity, DOC, and organic content of the sediments showed no relationships to the Hg concentrations in *Nuphar* leaves and petioles (Appendices 7.9 and 7.10) from the South-Central wetlands. In addition, these variables did not predict corresponding Hg concentrations in the leaves and petioles of *Nuphar* (Appendices 7.15 and 7.16) from the St. Lawrence River wetlands.

Fish have been shown to have higher Hg concentrations from waters of low pH (Eriksson et al., 1989; Bloom and Effler, 1990). A decrease in the pH of the water appears to enhance bioaccumulation due to an increase in the methylation rate of Hg (Bryan and Langston, 1992; Matilainen and Verta, 1995), affect the speciation of Hg, favouring the production of monomethyl mercury which is accumulated by organisms, as opposed to dimethyl Hg which is volatilized to the atmosphere (Gilmour and Henry, 1991; Cope and Rada, 1992), and change the partitioning of Hg between water and sediments (Fagerstrom and Jernelov, 1972; Johnston et al., 1991). In addition, a low water pH affects the affinity of Hg for different types of materials (Hakanson, 1974), thus increasing the persistence of Hg within the water column. On the other hand, Lodenius and Autro (1989) and Nelson and Campbell (1991) reported that the sorption of Hg to organic matter increased with decreasing pH, which makes it less available to biota.

Water pH strongly affects the bioavailability of metals and these effects include alterations in the metal levels of aquatic macrophytes. Toxic metals can be released from sediments in ionic form due to changes in acidity. This change in acidity induces the release of normally insoluble metal ions and allows for their uptake by plants. This has been shown for *Nuphar lutea* with Cu and Zn (Jackson et al., 1993), as well as with Cd for *Nuphar variegatum* (Thompson et al., 1996) and *Nuphar lutea* (Lehtonen, 1989). However, when it comes to Hg accumulation in relation to

pH for aquatic plants, there are few studies. The accumulation of Hg in marsh plants has been shown to be affected by pH (Gambrell et al., 1976 cited in Jackson et al., 1993), while in *Nuphar lutea* (Lehtonen, 1989) and duckweed (Mo et al., 1989), pH had no effect on the accumulation of Hg.

Similarly, a reduction in the alkalinity of waters has been shown to increase concentrations of Hg in fish (Akielaszek and Haines, 1981; Cope and Rada, 1992). This negative relationship has been suggested to occur for a number of reasons such as an increase in the uptake from water by organisms due to increased permeability of fish gill membranes (Wiener et al., 1990), competition between Hg and Ca for cell binding sites (McFarlane and Franzin, 1980), or because of a decreased depuration rate (Gilmour and Henry, 1991). The study of the affects of alkalinity on Hg in aquatic plants is not very abundant. In fact, I could find only one reference dealing with alkalinity and plant Hg. In this sole study, Lehtonen (1989) found that alkalinity had no effect on the Hg accumulation in *Nuphar lutea*. Thus, based on the available fish data and the fact that *Nuphar variegatum* accumulated Cd in relation to a decreasing alkalinity (Thompson et al., 1996), it was expected that accumulation of Hg in *Nuphar variegatum* would be similar. However, as previously mentioned, no relationship between Hg concentrations in *Nuphar* and alkalinity was observed for either the South-Central wetlands or the St. Lawrence River wetlands, therefore, agreeing with the result found by Lehtonen (1989).

Positive correlations between DOC and fish Hg have been noted by several authors (eg. Lee et al., 1985; Grieb et al., 1990; and Wren et al., 1991). This relationship may be the result of DOC acting as a substrate for methylating microorganisms (Gill and Bruland, 1990), scavenging Hg (Krumgalz and Fainshtein, 1991), and binding methyl Hg (Lee and Hultberg, 1990) Furthermore, DOC is the primary carrier of Hg from terrestrial to aquatic ecosystems (Lodenius and Autro, 1989; Mierle, 1990). However, Matilainen and Verta (1995) and Winfrey and Rudd (1990) found decreasing methylation rates with increasing levels of DOC despite an increase in overall bacterial activity. Similarly, Akielaszek and Haines (1981) found that the biotic accumulation of Hg is high in waters where DOC compounds are not present in high concentrations because the organic complexes that normally decrease the availability are lacking. Once again, as with alkalinity studies, there is little information available on the effect of DOC and Hg accumulation in aquatic plants. Lacerda et al. (1991), however, suggested that the low Hg concentrations they found in aquatic plants indicated that the Hg is strongly bound to organic complexes in the water column making it unavailable. In this study, no significant relationships between DOC and Hg concentrations in *Nuphar* leaves and petioles (Appendices 7.9; 7.10; 7.15; and 7.16) were obtained indicating that despite the wide range in DOC (2.8 to 20 mg/L) it had no effect on the total Hg concentrations in *Nuphar*.

Jackson (1991) found that the Hg in fish from lakes and

reservoirs is primarily due to the stimulation of Hg methylating microorganisms by organic matter. Alternately, increases in the concentrations of sedimentary organic matter have been shown to decrease the availability of Hg in plankton (Jackson, 1988) because of the tendency of organic matter to bind Hg strongly. The organic matter in sediments plays an important role in the availability of trace elements to plants (Airey, 1982), and Hg is not an exception to this rule. Much organically bound Hg is resistant to all but the strongest chemical attacks, and is therefore, unlikely to be bioavailable to plants (Karanthanasis and Thompson, 1993). Thus, wetlands where the organic matter is highest and consequently, the Hg is tightly bound in the sediments, might therefore contain the *Nuphar* with the least amount of Hg. However, no relationships between the organic content of the sediments and the total Hg concentrations in *Nuphar* leaves and petioles were obtained in this study for either the South-Central wetlands (Appendices 7.9 and 7.10) or the St. Lawrence River wetlands (Appendices 7.15 and 7.16).

4.3 *Nuphar variegatum* as a Bioindicator of Mercury Accumulation

Aquatic macrophytes are known to concentrate metals in their tissues (eg. Franzin and McFarlane, 1980; Kabata-Pendias and Pendias, 1992). Mercury accumulation by aquatic organisms is governed by a variety of interconnected variables. Even if the availability of Hg from water and sediments is similar for different species, the uptake rates may vary. The concentrations

of Hg obtained in the leaves and petioles of *Nuphar variegatum* are lower than those obtained for other aquatic plants: *Myriophyllum* (Mortimer, 1985); *Sphagnum* sp. (Percy, 1983); *Saggiteria* and *Scirpus* (Eriksson and Mortimer, 1975) and *Vallisneria americana* (Manny et al., 1989 cited in Kabata-Pendias and Pendias, 1992); and *Eichhornia crassipes* (Panda et al., 1988). It appears that the rhizomes of *Nuphar variegatum* act to a certain degree as a barrier to Hg uptake, as found for other aquatic plants (Siegel and Siegel, 1979; Alberts et al., 1990). In the present study, the rhizomes contained less Hg than the rootlets, which in turn contained less Hg than the leaves and petioles (Figure 3.1.10). Lovett Doust et al. (1993) noticed a similar pattern with organochlorine contaminants, where these did not accumulate in the roots of aquatic macrophytes relative to other plant parts. It has been suggested that there is very little transport of Hg from the roots to the leaves in aquatic plants (Coquery and Welbourn, 1994), and downward transport has been noted in aquatic plants (Mortimer, 1985). If it is true that upward transport is minimal, then this further strengthens the hypothesis that *Nuphar* rhizomes and rootlets act as a barrier for Hg uptake, suggesting that the higher concentrations of Hg seen in *Nuphar* leaves and petioles as compared to the rhizomes result from *Nuphar* obtaining relatively more Hg from the water than the sediment.

The low concentrations of Hg measured here in *Nuphar variegatum* compared to other aquatic plants may result from the

observations that rhizomes of aquatic plants can change their rhizospheric oxidation (Jaynes and Carpenter, 1986) and pH (Crowder, 1991) which may alter the uptake of Hg. In addition, Fe and Mn oxides can scavenge Hg, making it less available to plants (Crowder 1991). Finally, the low Hg concentrations seen in *Nuphar variegatum* may result from the inference that waterlilies reduce ionic Hg to elemental Hg and subsequently release it to the atmosphere (Siegel et al., 1987). Regardless of the mechanism, it is clear that *Nuphar* possesses low levels of mercury relative to other aquatic plants.

In Table 4.1 the Hg concentrations of various waterlily species show fairly constant and low levels, except in two studies, where known Hg contamination existed. Furthermore, by looking at Figures 3.1.6 to 3.1.9 for *Nuphar* leaves and petioles from the South-Central wetlands, it is quite clear that *Nuphar variegatum* maintains a low and fairly constant Hg level over a wide range of water and sediment values. Similarly, Figures 3.2.6 to 3.2.9 indicate that *Nuphar* leaves and petioles from the St. Lawrence River wetlands also maintain a low and fairly constant Hg level over varying water and sediment values.

A way to determine the potential usefulness of *Nuphar variegatum* as a biological indicator of Hg accumulation is by determining bioconcentration ratios (Hg in leaves or petioles versus Hg in sediments or water). A low concentration ratio is indicative of little storage of Hg by the plants, while a large concentration ratio indicates active uptake (Alberts et al.,

1990). Intermediate values imply that tissue concentrations are controlled by a steady state exchange which is strongly influenced by the magnitude of the sediment concentrations. When the concentration ratio is ≥ 1, the process is considered to be accumulation of Hg, either by active uptake or low turnover (Benes and Hawlik, 1979; Alberts et al., 1990). Few aquatic plants have been shown to have concentration ratios of ≥ 1 for Hg (eg. Furr et al., 1979; Gilbert, 1990).

The concentration ratios determined for Hg from the sediments in *Nuphar* leaves or petioles ranged from 0.05 to 0.24 for the South-Central wetlands. The sediment based concentration ratios in the leaves or petioles ranged from 0.02 to 1.08 in the *Nuphar* from the St. Lawrence River wetlands (Appendices 7.11 and 7.17). These values are all low, well below an intermediate value of ≤ .4, except in one wetland from the St. Lawrence River. In this wetland, the concentration ratio in the leaves in 1.08, while in the petioles it is 0.98. This wetland contains the lowest amount of Hg in the sediment and the lowest organic content (LOI%) and this high concentration ratio indicates that accumulation of Hg in the *Nuphar* from this wetland is occurring compared to the other 44 wetlands. This agrees with the finding of Lacerda et al. (1991), in which they found the highest concentration ratios in aquatic plants to be from areas where low environmental Hg concentrations are present.

There were no differences between the sediment based concentration ratios for *Nuphar* leaves and petioles in the South-

Central wetlands or in the St. Lawrence River wetlands (App. 7.20). This further indicates that *Nuphar variegatum* from both remote wetlands, and from wetlands where known Hg sources exist, are similar in the way they obtain Hg.

Aquatic plants have been shown to accumulate metals from the water by factors of 10 000 (Campbell et al., 1985), and as high as 10 000-60 000 for Hg (Lodenius, 1980). *Nuphar variegatum* is an example of this, where the water based concentration ratios ranged from 424 to 9647 in the leaves and from 473 to 9807 in the petioles of the plants from the South-Central wetlands (App. 7.12). Similarly, the water based concentration ratios ranged from 610 to 4086 for the leaves and from 426 to 8900 for the petioles of the *Nuphar* from the St. Lawrence River wetlands (App. 7.18). There were no significant differences between the water based concentration ratios for the leaves and petioles from either the South-Central wetlands (App. 7.20) or the St. Lawrence River wetlands (App. 7.19). However, significant differences existed between the two wetland groups (App. 7.20), with the *Nuphar* water based concentration ratios from the St. Lawrence River wetlands being significantly higher than those from the South-Central wetlands. Thus, it appears that despite the significantly lower Hg concentrations in the water, *Nuphar* in the St. Lawrence River are absorbing more Hg from the water column than their northern counterparts.

The results of this study indicated that *Nuphar variegatum* exhibited remarkable uniform behaviour in it's Hg concentrations

over a wide range of sediment and water Hg levels, and that *Nuphar* is an "excluder" or "non-indicator" of Hg. The Hg concentrations of *Nuphar* species show fairly low and constant concentrations, and only in two studies (Table 4.1), where a known Hg contamination source exists, does *Nuphar* exhibit concentrations of total Hg ≥ 30 ng/g. Although this "unresponsive" behaviour may be based on exclusion, the idea that *Nuphar* might be able to release volatile Hg to the atmosphere to keep tissue Hg low can not be ignored.

Location	Plant	Plant [Hg]	Sediment [Hg]	Reference
British Columbia wetland (mining area)	*Nuphar* spp	22	180	Siegel et al. 1985
Finland acid lake buffered lake	*Nuphar lutea*	 20 20	 260 260	Lehtonen 1989
Finland control lake .5 km downstream chlor-alkali plant .5 km upstream chlor-alkali plant	*Nuphar lutea*	20 - 30 3100 700	300 8300 15000	Lodenius 1980
India lake (Hg discharged into water prior to 1968)	*Nuphar lutea*	11	not determined	Särkkä et al. 1978
Brazil river (gold mining area)	*Victoria amazonica* giant water lily	910	300	Martinelli et al. 1988
Ontario South-Central wetlands	*Nuphar variegatum*	leaf 18 petiole 22	217	present study
Ontario St. Lawrence River wetlands	*Nuphar variegatum*	leaf 15 petiole 19	144	present study

Table 4.1 Concentrations of total Hg in waterlilies (ng/g) and sediments (µg/kg) from various studies around the world, including the present study.

Chapter 5.0

CONCLUSION

The sediment Hg concentrations in the 22 South-Central Ontario wetlands and the 23 St. Lawrence River wetlands were positively and significantly related to the sediment organic contents. Mercury concentrations in the wetland waters showed no relationships with water pH, alkalinity, or DOC for the 22 South-Central wetlands or for the 23 St. Lawrence River wetlands. However, water Hg concentrations were positively and significantly related to the sediment Hg concentrations for the St. Lawrence River wetlands, but not for the South-Central wetlands.

Despite the fact that correlations between aquatic plants and both sediment and water Hg have previously been found, concentrations of total Hg in the leaves and petioles of *Nuphar variegatum* showed no relationship to either the sediment or the water Hg concentrations for either the South-Central or the St. Lawrence River wetlands. Furthermore, total Hg in *Nuphar variegatum* leaves and petioles showed no relationships with the major chemical variables (pH, alkalinity, DOC, and organic content of the sediment) which predict corresponding Hg levels in other aquatic biota.

Based on the results of this study, indicated by the low and fairly constant concentration ratios, both from the sediment and from the water, for both the South-Central and the St. Lawrence River wetlands, it appears that *Nuphar variegatum* is an

"excluder" or "non-indicator" of Hg. *Nuphar variegatum* may possess some mechanism to biologically control the uptake of Hg, or it may be an efficient plant in the volatilization of elemental Hg from its leaves.

Furthermore, despite the fact that the St. Lawrence River possesses known sources of Hg, the water and sediment Hg concentrations from these riverine wetlands contained significantly lower amounts of Hg than the wetlands from remote areas of South-Central Ontario. This is partly due to the dilution capacity of the St. Lawrence River as well as to the fact that significantly higher amounts of organic matter, and therefore, higher organically bound Hg, occurred in the South-Central wetlands. In addition, the leaves of *Nuphar variegatum* from the South-Central wetlands contained significantly more Hg than the *Nuphar* leaves from the St. Lawrence River, while no significant differences appeared between the petiole concentrations.

In conclusion, this study indicated that *Nuphar variegatum* does not accumulate Hg and subsequently, the wildlife which rely on *Nuphar variegatum* for energy and nutrients are not substantially at risk with respect to Hg. This is true for *Nuphar variegatum* in wetlands from both South-Central Ontario and the St. Lawrence River.

Chapter 6.0

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APPENDIX 7.1

The latitudes and the longitudes for the 22 wetlands from the Temagami-North Bay and Muskoka-Haliburton areas of South-Central Ontario.

WETLAND	LATITUDE	LONGITUDE
1	46° 34' 30s	80° 53' 00s
2	46° 38' 51s	80° 57' 33s
3	46° 38' 04s	80° 56' 44s
4	46° 38' 31s	80° 58' 02s
5	46° 39' 26s	80° 56' 38s
6	46° 41' 49s	80° 05' 20s
7	46° 41' 33s	80° 04' 00s
8	46° 41' 05s	80° 05' 41s
9	46° 41' 30s	80° 06' 09s
10	46° 41' 28s	80° 05' 38s
11	45° 35' 17s	78° 31' 11s
12	45° 35 26s	78° 50' 53s
13	45° 34' 51s	78° 45' 12s
14	45° 05' 03s	78° 59' 22s
15	45° 36' 58s	78° 36' 38s
16	45° 06' 09s	78° 55' 44s
17	45° 06' 23s	78° 41' 52s
18	45° 36' 11s	78° 21' 22s
19	45° 35' 19s	78° 22' 36s
20	45° 02' 33s	78° 48' 00s
21	45° 06' 06s	79° 45' 55s
22	45° 07' 03s	79° 12' 21s

APPENDIX 7.2

The latitudes and longitudes for the 23 wetlands from the St. Lawrence River area of Ontario.

Wetland	LATITUDE	LONGITUDE
1	45° 05' 35s	74° 30' 51s
2	45° 04' 12s	74° 32' 22s
3	45° 01' 57s	74° 39' 56s
4	45° 03' 15s	74° 33' 49s
5	45° 02' 51s	74° 35' 00s
6	45° 07' 34s	74° 29' 33s
7	45° 01' 28s	74° 36' 26s
8	45° 01' 39s	74° 35' 57s
9	45° 01' 53s	74° 35' 12s
10	45° 04' 12s	74° 30' 24s
11	45° 03' 09s	74° 29' 17s
12	45° 01' 49s	74° 34' 18s
13	45° 02' 06s	74° 35' 08s
14	45° 00' 10s	74° 37' 53s
15	45° 00' 27s	74° 44' 46s
16	45° 00' 28s	74° 43' 06s
17	45° 00' 39s	74° 42' 00s
18	45° 00' 59s	74° 36' 24s
19	45° 02' 32s	74° 31' 38s
20	45° 03' 12s	74° 32' 28s
21	45° 01' 03s	74° 37' 38s
22	45° 02' 28s	74° 36' 30s
23	45° 03' 46s	74° 26' 58s

APPENDIX 7.3

The microwave digestion procedure for *Nuphar variegatum* leaves, petioles, rhizomes, and rootlets.

STAGE	1	2	3
POWER	100%	100%	100%
PSI	40	85	125
TIME	15 min	15 min	20 min
TAP	5 min	10 min	10 min
FAN	100%	100%	100%

This protocol is based on 12 vessels at a time, using a 650 Watt microwave oven (CEM model MDS 2000). If fewer than 12 vessels are used, the power and time need to be adjusted.

POWER = Percentage of the total 650 Watts needed to reach the designated pressure in the allotted time.
PSI = Pressure at which the plant parts are digested.
TIME = The amount of time needed for the 12 vessels to reach the designated pressure reading.
TAP = Time At Pressure. The amount of time that the digests are held at the corresponding pressure reading.
FAN = The internal fan system, always at 100%.

APPENDIX 7.4

The microwave digestion procedure for the sediments from the South-Central Ontario wetlands.

STAGE	1	2	3	4
POWER	100%	100%	100%	100%
PSI	40	80	120	160
TIME	15 min	10 min	20 min	30 min
TAP	3 min	3 min	10 min	10 min
FAN	100%	100%	100%	100%

This protocol is based on 12 vessels at a time, using a 650 Watt microwave oven (CEM model 2000). If fewer than 12 vessels are used, the power and time need to be adjusted.

POWER = Percentage of the total 650 Watts needed to reach the designated pressure in the allotted time.
PSI = Pressure at which the sediments are digested.
TIME = The amount of time needed for the 12 vessels to reach the designated pressure reading.
TAP = Time At Pressure. The amount of time that the digests are held at the corresponding pressure reading.
FAN = The internal fan system, always at 100%.

APPENDIX 7.5

Predigestion procedure for the sediments from the St. Lawrence River wetlands.

STAGE	1	2	3
POWER	100%	100%	100%
PSI	60	80	120
TIME	30 min	15 min	30 min
TAP	5 min	10 min	10 min
FAN	100%	100%	100%

The second microwave digestion procedure for the sediments from the St. Lawrence River wetlands.

STAGE	1	2	3	4
POWER	100%	100%	100%	100%
PSI	40	80	120	160
TIME	15 min	10 min	20 min	30 min
TAP	3 min	3 min	10 min	10 min
FAN	100%	100%	100%	100%

This protocol is based on 12 vessels at a time, using a 650 Watt microwave oven (CEM model 2000). If fewer than 12 vessels are used, the power and time need to be adjusted.

POWER = Percentage of the total 650 Watts needed to reach the designated pressure in the allotted time.
PSI = Pressure at which the sediments are digested.
TIME = The amount of time needed for the 12 vessels to reach the designated pressure reading.
TAP = Time At Pressure. The amount of time that the digests are held at the corresponding pressure reading.
FAN = The internal fan system, always at 100%.

APPENDIX 7.6

Observed and expected mercury values for Standard Reference Materials; Apple Leaves (NIST # 1515); Buffalo River Sediments (NIST # 2704); and a CANMET standard Till 2.

	APPLE LEAVES	BUFFALO RIVER SEDIMENT	TILL 2
Observed Values	45.39 ng/g	1490.33 µg/kg	74.35 µg/kg
(± S.D.)	3.41	47.86	7.41
n=	98	7	17
Expected Values	44.00 ng/g	1470.00 µg/kg	70.00 µg/kg
(± S.D.)	4.00	70.00	7.00

APPENDIX 7.7

Results of regressions: Total Hg concentrations in sediments versus the organic content of the sediment (LOI); dissolved organic carbon (DOC) versus Alkalinity (Alk.); and dissolved organic carbon (DOC) versus pH for the South-Central Ontario wetlands.

Regression	F Value	p value	r^2	slope
Sed Hg * LOI	36.293	<0.001	0.645	2.534
DOC * Alk.	0.024	0.879	0.0012	0.00038 n.s.
DOC * pH	0.210	0.652	0.010	0.514 n.s.

n.s. = not significant at p=0.05 level.

APPENDIX 7.8

Results of regressions: Total Hg concentrations in water versus pH; alkalinity; dissolved organic carbon; and the total Hg concentration in sediments for the South-Central Ontario wetlands.

Variable	F Value	p value	r^2	slope
pH	0.119	0.734	0.006	0.976 n.s.
alkalinity	0.225	0.641	0.011	0.003 n.s.
DOC	2.405	0.137	0.107	0.824 n.s.
[Hg] sediment	0.241	0.629	0.012	0.016 n.s.

n.s. = not significant at p=0.05 level.

APPENDIX 7.9

Results of regressions: Total Hg concentrations in *Nuphar variegatum* leaves versus pH: alkalinity; dissolved organic carbon; total Hg in water; % organic content of sediment; total Hg in sediment; and the total Hg concentration in *Nuphar* petioles for the South-Central Ontario wetlands.

Variable	F Value	p value	r^2	slope
pH **	0.006	0.939	0.000303	0.003 n.s.
alkalinity **	0.949	0.342	0.045	0.0000671 n.s.
DOC	0.511	0.483	0.025	0.210 n.s.
[Hg] water	4.249	0.053	0.175	0.222 n.s.
LOI **	0.019	0.892	0.000941	0.000154 n.s.
[Hg] sediment **	0.022	0.885	0.00108	0.0000557 n.s.
[Hg] petiole	0.426	0.521	0.021	0.133 n.s.

** = *Nuphar variegatum* leaf Hg concentration used in regression is the common log.
n.s. = not significant at p=0.05 level.

APPENDIX 7.10

Results of regressions: Total Hg concentrations in *Nuphar variegatum* petioles versus pH; alkalinity; dissolved organic carbon; total Hg in water; % organic content of sediment; total Hg in sediment,; and the total Hg concentration in *Nuphar* leaves for the South-Central Ontario wetlands.

Variable	F Value	p value	r^2	slope
pH	0.030	0.864	0.0015	0.284 n.s.
alkalinity	0.067	0.799	0.003	0.000905 n.s.
DOC	0.092	0.765	0.005	0.098 n.s.
[Hg] water	2.510	0.129	0.112	0.192 n.s.
LOI	2.002	0.173	0.091	0.076 n.s.
[Hg] sediment	0.941	0.344	0.045	0.017 n.s.
[Hg] leaf	0.426	0.521	0.021	0.157 n.s.

n.s. = not significant at p=0.05 level.

APPENDIX 7.11

The sediment based concentration ratios (*Nuphar* Hg/sediment Hg) for the leaves and petioles of *Nuphar variegatum* for the 22 South-Central Ontario wetlands. These values are arranged in order of increasing leaf concentration ratios with their corresponding petiole concentration ratios located in a separate column. Values represent the mean of 15 plant, and 9 sediment measurements.

Leaf Concentration Ratio	Petiole Concentration Ratio
0.05	0.07
0.05	0.06
0.05	0.10
0.06	0.09
0.06	0.06
0.06	0.13
0.06	0.06
0.07	0.06
0.07	0.12
0.07	0.08
0.08	0.15
0.08	0.11
0.09	0.12
0.09	0.09
0.10	0.12
0.12	0.16
0.12	0.08
0.12	0.09
0.13	0.08
0.14	0.15
0.19	0.20
0.23	0.24
mean = 0.095 ± .046	0.110 ± .047

APPENDIX 7.12

The water based concentration ratios (*Nuphar* Hg/water Hg) for the leaves and petioles of *Nuphar variegatum* for the 22 South-Central Ontario wetlands. These values are arranged in order of increasing leaf concentration ratios with their corresponding petiole concentration ratios located in a separate column. Values represent the mean of 15 plant, and 3 water measurements.

Leaf Concentration Ratio	Petiole Concentration Ratio
424.1	761.2
536.3	472.7
549.6	670
557.9	1147.1
646.1	1104.3
706.2	756.9
710.9	664.1
720.8	995
748.8	834
880	1410
911.2	551.5
1035.3	1084.7
1452.5	1840.8
1461.5	1098.5
1463	2942
1564.2	1666.7
1862.2	2415.6
2054.2	2545.8
2578.2	1744.5
4870	7743.3
6295	6400
9646.7	9806.7
mean= 1894.3 ± 2256.04	**2211.61 ± 2495.97**

APPENDIX 7.13

Results of regressions: Total Hg concentrations in sediments versus the organic content of the sediment (LOI), dissolved organic carbon (DOC) versus Alkalinity (Alk.), and dissolved organic carbon (DOC) versus pH for the St. Lawrence River wetlands.

Regression	F Value	p value	r^2	slope
Sed Hg * LOI	27.414	<0.001	0.566	5.920
DOC * Alk.**	7.85	0.011	0.272	0.00019
DOC * pH	1.218	0.282	0.055	-2.011 n.s.

n.s. = not significant at the p=0.05 level.
** = DOC concentration used in the regression is the common log.

NOTE: A significant relationship was found between logDOC and Alkalinity, however, this is due to 1 point (high DOC) and this result should be viewed with caution.

APPENDIX 7.14

Results of regressions: Total Hg concentrations in water versus pH; alkalinity; dissolved organic carbon; and the total Hg concentration in sediments for the St. Lawrence River wetlands.

Variable	F Value	p value	r^2	slope
pH	0.381	0.544	0.018	2.191 n.s.
alkalinity	0.034	0.856	0.0016	0.00039 n.s.
DOC	0.217	0.646	0.010	0.193 n.s.
[Hg] sediment	8.776	0.007	0.295	0.026

n.s. = not significant at p=0.05 level.

APPENDIX 7.15

Results of regressions: Total Hg concentrations in *Nuphar variegatum* leaves versus pH; alkalinity; dissolved organic carbon; total Hg in water; % organic content of sediment; total Hg in sediment; and the total Hg concentration in *Nuphar* petioles for the St. Lawrence River wetlands.

Variable	F Value	p value	r^2	slope
pH	0.036	0.851	0.00171	0.608 n.s.
alkalinity	0.216	0.647	0.010	0.00087 n.s.
DOC	0.055	0.817	0.003	0.087 n.s.
[Hg] water	0.002	0.968	0.000077	0.008 n.s.
LOI	0.188	0.669	0.009	0.031 n.s.
[Hg] sediment	0.182	0.674	0.009	0.004 n.s.
[Hg] petiole	5.846	0.025	0.218	0.191

n.s. = not significant at p=0.05 level.

APPENDIX 7.16

Results of regressions: Total Hg concentrations in *Nuphar variegatum* petioles versus pH; alkalinity; dissolved organic carbon; total Hg in water; % organic content of sediment; total Hg in sediment; and the total Hg concentration in *Nuphar* leaves for the St. Lawrence River wetlands.

Variable	F Value	p value	r^2	slope
pH	1.786	0.196	0.078	10.060 n.s.
alkalinity	0.011	0.916	0.00054	0.00049 n.s.
DOC	1.605	0.219	0.071	1.115 n.s.
[Hg] water	0.001	0.973	0.000055	0.016 n.s.
LOI	0.575	0.457	0.027	0.133 n.s.
[Hg] sediment	0.004	0.952	0.000177	0.00138 n.s.
[Hg] leaves	5.846	0.025	0.218	1.141

n.s. = not significant at p=0.05 level.

APPENDIX 7.17

The sediment based concentration ratios (*Nuphar* Hg/sediment Hg) for the leaves and petioles of *Nuphar variegatum* for the 23 St. Lawrence River wetlands. These values are arranged in order of increasing leaf concentration ratios with their corresponding petiole concentration ratios located in a separate column. Values represent the mean of 5 plant, and 3 sediment measurements.

Leaf Concentration Ratio	Petiole Concentration Ratio
0.04	0.02
0.05	0.04
0.05	0.10
0.06	0.05
0.07	0.10
0.07	0.07
0.07	0.14
0.08	0.28
0.09	0.18
0.09	0.08
0.10	0.09
0.11	0.07
0.12	0.10
0.14	0.22
0.15	0.19
0.15	0.37
0.15	0.18
0.16	0.19
0.20	0.26
0.20	0.20
0.27	0.39
0.34	0.42
1.08	0.98
mean= 0.167 ± .21	**0.204 ± .21**

APPENDIX 7.18

The water based concentration ratios (*Nuphar* Hg/water Hg) for the leaves and petioles of *Nuphar variegatum* for the 23 St. Lawrence River wetlands. These values are arranged in order of increasing leaf concentration ratios with their corresponding petiole concentration ratios located in a separate column. Values represent the mean of 5 plant, and 3 water measurements.

Leaf Concentration Ratio	Petiole Concentration Ratio
610	2234.6
780.7	426
790	1095
1065.8	1920
1254.5	1105.5
1396.2	1159.1
1439.1	1307.3
1592.9	3095.7
1658	1649
1665	1359.3
1895	1821.7
2010	2612.5
2263.8	1455
2372.5	2083.8
2478.6	2934.3
2662.5	2467.5
2895	3328.3
3060	4454.3
3427.5	4170
3667.5	8900
3900	5100
3976.7	7363.3
4086	6662
mean= 2215.1 ± 1083.35	2987.1 ± 2202.13

APPENDIX 7.19

Results of t-Test analyses.

Variables	t value	degrees of freedom	p value
Leaf Hg*Petiole Hg (South-Central wetlands)	-2.194	42	0.034
Leaf Hg(South-Central wetlands)* Leaf Hg(St. Lawrence River wetlands)	2.871	43	0.006
Sediment Hg(South-Central wetlands)* Sediment Hg(St. Lawrence River wetlands)	3.145	43	0.003
CR(leaf Hg/water Hg) *CR(petiole Hg/water Hg)(St. Lawrence River wetlands)	-1.509	44	0.139 n.s.

n.s. No statistically significant differences between the mean values of the 2 variables at the p=0.05 level.

APPENDIX 7.20

Results of Mann-Whitney Rank Sum Test analyses.

Variables	T value	n(small)	n(big)	p value
Alk.(South-Central wetlands)* Alk.(St. Lawrence River wetlands)	253	22	23	<0.001
DOC (South-Central wetlands)* DOC (St. Lawrence River wetlands)	288	23	22	<0.001
LOI (South-Central wetlands)* LOI (St. Lawrence River wetlands)	318	23	22	<0.001
Water Hg(South-Central wetlands)* Water Hg(St. Lawrence River wetlands)	378	23	22	<0.001
Petiole Hg(South-Central wetlands)* Petiole Hg(St. Lawrence River wetlands)	460	23	22	0.120 n.s.
Leaf Hg* Petiole Hg (St. Lawrence River wetlands)	468	23	23	0.114 n.s.
CR(leaf Hg/sed Hg)* CR(petiole Hg/sed Hg) (South-Central wetlands)	435	22	22	0.162 n.s.
CR(leaf Hg/water Hg)* CR(petiole Hg/water Hg)(South-Central wetlands)	458	22	22	0.392 n.s.

n.s. No statistically significant differences between the median values of the 2 variables at p=0.05 level.

APPENDIX 7.20 continued

Variables	T value	n(small)	n(big)	p value
CR(leaf Hg/sed Hg)* CR(petiole Hg/sed Hg) (St. Lawrence River wetlands	490	23	23	0.272 n.s.
CR(leaf Hg/sed Hg) (South-Central wetlands)* CR(leaf Hg/ sed Hg)(St. Lawrence River wetlands)	434	22	23	0.104 n.s.
CR(petiole Hg/sed Hg) (South-Central wetlands)* CR(petiole Hg/sed Hg)(St. Lawrence River wetlands)	431	22	23	0.091 n.s.
CR(leaf Hg/water Hg) (South-Central wetlands)* CR(leaf Hg/ water Hg)(St. Lawrence River wetlands)	401	22	23	0.018
CR(petiole Hg/water Hg)(South-Central wetlands)* CR(petiole Hg/water Hg)(St. Lawrence River wetlands)	405	22	23	0.022

n.s. No statistically significant differences between the median values of the 2 variables at p=0.05 level.

APPENDIX 7.21

Results of multiple regression for total concentration of Hg in *Nuphar variegatum* leaves for the 45 Ontario wetlands. Independent variables include location (South-Central Ontario or St. Lawrence River); pH; alkalinity; DOC; LOI; water Hg; and sediment Hg)

Variable	p value	slope
location	0.082	-6.353 n.s.
pH	0.932	-0.116 n.s.
alkalinity	0.634	0.000129 n.s.
DOC	0.821	-0.053 n.s.
LOI	0.534	-0.037 n.s.
[Hg] water	0.054	-0.200 n.s.
[Hg] sediment	0.403	0.011 n.s.

n.s = not significant at the p= 0.05 level.

APPENDIX 7.22

Results of multiple regression for total concentration of Hg in *Nuphar variegatum* petioles for the 45 Ontario wetlands. Independent variables include location (South-Central Ontario or St. Lawrence River); pH; alkalinity; DOC; LOI; water Hg; and sediment Hg)

Variable	p value	slope
location	0.460	4.560 n.s.
pH	0.901	-0.293 n.s.
alkalinity	0.817	0.000126 n.s.
DOC	0.183	0.540 n.s.
LOI	0.193	0.135 n.s.
[Hg] water	0.386	-0.152 n.s.
[Hg] sediment	0.482	-0.015 n.s.

n.s= not significant at the p= 0.05 level.