Selenium in the Prairie Waters of Southern Manitoba: Concentration, Speciation, and Temporal Variation

By
Xiaoxi Hu

A thesis submitted to the Faculty of Graduate Studies of
The University of Manitoba
in partial fulfillment of the requirements of the degree of

Master of Environment

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Abstract

The prairie waters of southern Manitoba share many similarities in geology, climate, water chemistry and hydrology with those in the western United States, where elevated concentrations of selenium from irrigation practices have resulted in adverse impacts on fish and waterfowl. To assess the current range of selenium exposure in southern Manitoba, the concentration, speciation and temporal variation of selenium were studied at Delta Marsh, Stephenfield Reservoir and the South Tobacco Creek Watershed between July 2005 and July 2007. The sites were selected based on their perceived level of impact from agricultural practices. Delta Marsh was chosen as a reference site and was to provide a background level of selenium in southern Manitoba. Stephenfield Reservoir and the South Tobacco Creek Watershed were chosen as potentially selenium contaminated areas by irrigation and other agricultural activities, respectively. A new analytical technique was developed to determine the selenium speciation in natural waters, which was applied to examine the fate of selenium under elevated conditions by a mesocosm study.

At Delta Marsh and Stephenfield Reservoir, the total selenium concentrations were low (<1 μg/L) in the surface water, less than 1.3 μg/g (dry weight) in the sediment cores, and less than 0.12 μg/g (dry weight) in the macrophytes tested. At the South Tobacco Creek Watershed, elevated selenium concentrations (>11 μg/L) in the surface water were found during the spring freshet in 2007 with lower levels observed for the rest of the year, indicating a potential selenium source from airborne particles accumulated during the winter and/or increased soil erosion during snowmelt.

A high performance liquid chromatography-inductively coupled plasma-dynamic reaction cell-mass spectrometry (HPLC-ICP-DRC-MS) method was developed for determination of selenium speciation in natural waters. It allows for the isocratic separation of selenite, selenate, and several organoselenium species (e.g., selenomethionine,

Se-methylselenocysteine, selenocystine) in a rapid fashion. No pre-concentration or pre-reduction step is required, which minimizes the risk of cross-contamination and alterations in speciation, and increases the sample throughput. In the surface water samples from the South Tobacco Creek Watershed, selenium speciation is dominated by selenate, with a small fraction being in the form of selenite. No detectable organoselenium species were found.

A mesocosm study on the transport and transformation of selenium was carried out from May to August 2007, in Crescent Pond of Delta Marsh. Three mesocosms were deployed in the pond, two of which were spiked with 6.3 and 15.7 μg/L ^{82}Se(IV), respectively, in the overlying water column. The concentration of spiked selenium decreased to 2 μg/L in 20 days after addition, and was almost completely removed from the surface water and below the detectable concentration in about 50 days.

The overall results of this thesis indicate that selenium concentrations in the prairie waters of southern Manitoba are low at present, and if selenium is discharged as selenite, it will be quickly removed from the surface water. However, selenium can be elevated during the snowmelt season and in areas of intensive agricultural activities such as at the South Tobacco Creek Watershed. A direct link between surface water selenium concentration and irrigation cannot be established at this time, but continuing of the monitoring of selenium in the prairie waters in southern Manitoba is warranted given the projected increase in irrigation and other agricultural activities in this area.

Acknowledgments

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Chapter 1. Introduction

1.1 Selenium and its Speciation

Selenium (Se) is a metalloid with an atomic number of 34 and an atomic mass of 78.96 amu. Se belongs to Group 6 (Group VIA) of the Periodic Table, located between nonmetallic sulfur (S) and metallic tellurium (Te), and resembles sulfur both in its oxidation states and in its compounds. The six stable isotopes of Se are ^{74}Se, ^{76}Se, ^{77}Se, ^{78}Se, ^{80}Se, and ^{82}Se, which naturally occur with approximate abundances of 0.89, 9.36, 7.63, 23.78, 49.61, and 8.73%, respectively. Se exists in four oxidation states (-II, 0, +IV, +VI) and in a variety of compounds (oxides/hydroxides, organoselenium compounds, selenides and sulfides).

The speciation of Se in the aquatic environment is complex and highly dependent on pH, redox reactions and surface complexion reactions, as well as the solubility of its salts, microbiological activity, and reaction kinetics (Belzile et al., 2000; Frankenberger et al., 1994). Selenate, selenite and organic Se species are water-soluble that can be taken up by aquatic plants and animals, be transformed into volatile Se species (e.g., $(CH_3)_2Se$), be reduced into elemental Se or adsorbed onto algae, particulate/colloidal matter and surficial sediments (Lemly, 1987).

1.1.1 Redox Reactions

pe-pH diagrams are a type of phase diagrams. These diagrams depict the relationships among minerals and aqueous species as a function of pH and redox conditions. Based on the equilibrium constants (Séby et al., 2001; summarized in Table 1 and 2 of Appendix 1), a pe-pH diagram (Figure 1.1) was created showing the stability of aqueous Se species with a total dissolved Se concentration of 10^{-8} M. In the pe-pH diagram, each species of Se shows their stability under a certain pe-pH

range.

Se(+VI) is thermodynamically favored and forms the selenate (SeO_4^{2-}) oxyanion under oxidizing conditions (pe > 6). Selenic acid, H_2SeO_4, is a strong acid and is rapidly disassociated to $HSeO_4^-$ and SeO_4^{2-} in water, with SeO_4^{2-} being the dominant form when pH > 2. Selenate salts are very soluble (Elrashidi et al., 1987), and selenate is the form of Se most readily taken up by plants (Eisler, 1985). Se(+IV) forms selenite (SeO_3^{2-}) and is favored under mildly oxidizing conditions (pe = 4.5 - 6). Selenous acid, H_2SeO_3, is a weak acid with $pKa_1 = 2.70$ and $pKa_2 = 8.54$. Most selenite salts are less soluble than the corresponding selenate salts (Elrashidi et al., 1987). Se(+IV) may be further reduced to Se(0) under reducing conditions when pe = 2 - 4. Se(0) is an insoluble non-metallic solid that is resistant to reduction. The pe-pH diagram also indicates that Se(-II) exists in reducing environments as hydrogen selenide (H_2Se) and as metal selenides. Microbial processes can produce volatile methylated derivatives of Se such as dimethyl diselenide ($(CH_3)_2Se$) or dissolved organoselenium compounds (Simmons and Wallschläger, 2005).

1.1.2 Surface Complexion Reactions and the HFO model

Dissolved Se species can be bonded to Fe- or Mn-oxyhydroxides and organic matter at or near the sediment-water interface, while in reduced sulfidic systems, Se-pyrite or ferroselite can be formed (Belzile et al., 2000).

In the case of Fe oxides, bonding of Se is predominantly by inner-sphere rather than outer-sphere complexation. Spectroscopic studies (Collinsa et al., 1999) have indicated that two oxygen atoms from a given Se oxyanion bond to adjacent surface structural Fe atoms. With inner-sphere complexation, Se competes with surface structural H_2O and OH^- groups for coordination of surface structural Fe. Thus Se is

chemically bonded to the surface and is considered to be a chemical component of the mineral surface. Se is strongly bonded to both poorly crystalline Fe oxides (e.g., ferrihydrite) and well crystalline Fe oxides (e.g., goethite, hematite) (Lemly and Smith, 1987).

The adsorption of Se species onto aquatic particles, hydrous ferric oxide (HFO), was simulated by a program named MINEQL+ Chemical Equilibrium Modeling System (Hartog, 2000), which is a chemical equilibrium model capable of calculating aqueous speciation, solid phase saturation states, precipitation-dissolution, and adsorption. Since HFO is only stable in the oxic environment, the results below apply to oxic conditions, e.g., in surface waters.

In this HFO double-layer surface complexion model (SCM) (Dzombak and Morel, 1990), HFO is considered an amorphous solid, with a molecular weight of 89 g/mol (i.e., $Fe_2O_3 \cdot H_2O$) and a specific surface area of 600 m^2/g. The model distinguishes two adsorption sites on HFO. Site density for "strong" sites is 0.005 mol/mol Fe, which accounts for a smaller set of high-affinity cation binding sites. Site density for "weak" sites is 0.2 mol/mol Fe.

The adsorption of selenite and selenate on HFO surfaces (Figure 1.2 A and B) as a function of pH were individually estimated using MINEQL+. A total Se concentration of 10^{-8} M and a total Fe^{3+} concentration of 10^{-3} M were used in the calculation. As can be seen from the Figure 1.2, lower pH favors the adsorption of both selenite and selenate. The majority of selenite is adsorbed onto HFO when pH is lower than 8.7, while selenate is most adsorbed when pH is lower than 5.8. In other words, selenate is less adsorbed onto HFO than selenite in neutral or slightly alkaline waters, making it more mobile in the surface water of most natural water bodies. In the surface prairie waters, where the pH is normally in the range of 6 - 9, selenate is

expected to be the dominant dissolved Se species.

1.1.3 Microbial processes

Some bacteria can use selenate and selenite as electron acceptors, or can oxidize elemental Se; it is likely that most of the important redox transformations of Se are microbially mediated. The transformations are affected by a variety of factors influencing the growth of microorganisms, including the presence of nutrients, temperature, moisture, oxygen availability and pH (Frankenberger and Karlson, 1994).

1.2 Se Essentiality and Toxicity

Se is an essential micronutrient for microbes, animals and humans where it is a component of about 25 selenoproteins and plays important roles in regulating various physiological functions (Levander, 1999). As part of the enzyme glutathione peroxidase (GSH-Px), which converts peroxide into water and acts as the catalyst in the scavenging of free radicals by glutathione, Se is considered essential to protect biological tissues against oxidative damage (Lemly, 1998). To meet the minimum nutritional level, diets containing Se ranging from 0.1 to 0.3 mg/kg (dry weight) is required for fish, birds and mammals (Ullrey, 1992) and around 40 μg/day for humans (Whanger, 1998). Se deficiency causes infertility, liver necrosis, muscular dystrophy, and white muscle in livestock, and can result in Keshan disease and Kaschin-Beck disease in humans (Ullrey, 1992; Tan and Huang, 1991).

However, excess dietary Se is toxic, because it behaves as an analog to sulfur, erroneously replacing sulfur atoms in proteins, which results in dysfunction of enzymes and proteins (Lemly, 1998). For animal diets containing Se higher than 5

mg/kg (dry weight), chronic toxicity such as anemia, nephropathy, liver damage and reproductive toxicity in fish (Saiki and Ogle, 1995), cattle (Tinggi, 2003) and birds (Hoffman, 2002) can result. Chronic toxicity in humans, caused by diets with Se levels above 0.85 mg Se/day (Yang and Zhou, 1994), results in a condition termed selenosis, characterized by hair loss, nail loss and brittleness, gastrointestinal problems, skin rash, garlic breath odor, and nervous system abnormalities (Yang et al., 1983).

Se toxicity is complex due to the antagonistic and additive effects resulted from its interactions with other metals. Cadmium (Cd), mercury (Hg), silver (Ag) and thallium (Tl) toxicity appear to be reduced by Se (Furst, 2002; Marier and Jaworski, 1983), while the toxicity of other metals, such as copper (Cu), antimony (Sb), germanium (Ge) and tungsten (W) may be enhanced (Chen et al., 1985; Marier and Jaworski, 1983). In the case of Se and arsenic (As)(III), the toxicity of both interacting trace elements is reduced (Marier and Jaworski, 1983).

1.3 Se in the Environment

1.3.1 Sources of Se

Se occurs naturally in the environment. The average concentration in the earth's crust is around 0.12 μg/g (Wedepohl, 1995). Shales typically contain higher levels of Se than other rocks, with an average value of 1.4 μg/g (Presser and Ohlendorf, 1987). Coal is also relatively rich in Se with an average concentration of 3 μg/g (Cooper et al., 1974). Se concentrations in the ocean waters are very low, averaging 4 μg/L. Se is generally found at even lower concentrations in freshwater, commonly less than 1 μg/L (Milne, 1998). Natural atmospheric releases of Se result from volatilization of Se by plants and bacteria, and from volcanic activity.

Human activities including mining, irrigation, petroleum refining, and coal combustion may greatly increase the levels of Se in the environment, especially in surface water and soil (Lemly, 1999).

1.3.2 Se Contamination in the Western United States

The decline and even disappearance of certain species of fish from the San Luis Drain and Kesterson Reservoir in southern California was first noticed in 1981 (Engberg et al., 1998). Elevated levels of Se were then detected in fish in 1982, and in 1983 there were reports of deformed embryos, adult mortality, and poor reproductive success of water birds (Engberg et al., 1998). Tests indicated that Se poisoning was the most probable cause (Ohlendorf et al., 1986). The Se concentrations in flowing irrigation drainage water were found to average about 300 μg/L and the source of Se was traced to the shallow groundwater in the drainage collector system area (Ohlendorf et al., 1986). Since then, extensive research has been carried out on the behavior of Se in wetlands in the western United States (Table 1.1).

Aside from the disposal of seleniferous agricultural wastewater in wetlands, the disposal of fly ash from coal-fired power plants is another source of Se. A study was conducted to document patterns of accumulation and toxicity of Se to organisms in a power plant cooling reservoir in North Carolina (Lemly, 1985). Se entered the reservoir by way of effluent from the coal ash disposal basin, which contained 100 - 200 μg Se/L. Concentrations of Se in the lake water averaged 10 μg Se/L, but it was found accumulated in biota.

Another study conducted in nine stream sites in the Blackfoot River, Salt River, and Bear River watersheds in southeast Idaho (Hamilton and Buhl, 2005) indicated that Se concentrations in the sediment, aquatic plants, aquatic invertebrates and fish (>

4 μg/g in whole body) were sufficiently elevated by mining of the phosphatic shales.

1.4 The Nature of Canadian Prairie Wetlands and Their Similarities to Wetlands in the Western United States

Wetlands are defined as areas where wet soils are prevalent, having a water table near or above the mineral soil for part of the year while supporting hydrophilic vegetation (Mitsch and Gosselink, 1986). Most prairie wetlands in Canada are concentrated in the Continental Prairie Wetland Region, which lies in the southern portions of Alberta, Saskatchewan, and Manitoba. Marshes, both fresh and saline, are the main wetland type and are usually associated with semi-permanent ponds (Glooschenko et al., 1993). These wetlands have properties in common with the western United States in geology, climate, water chemistry and hydrology (Glooschenko et al., 1993; Outridge et al., 1999).

1.4.1 Geology and Climate

Cretaceous to Eocene shales extending from North Dakota to central California in the western United States contain elevated Se concentrations (Presser and Ohlendorf, 1987), and the arid environment of this region allows Se generated by weathering to accumulate in soils (Presser and Ohlendorf, 1987). The Canadian prairie wetlands have a similar Cretaceous geology with relatively high Se concentrations (Garret et al., 2008), and the climate of prairie wetlands is also characterized as semi-arid (Glooschenko et al., 1993). However, extreme weathers in prairie wetlands with cold winters and hot summers may result in higher seasonal variations of the concentrations of major ions (LaBaugh, 1989).

1.4.2 Water Chemistry

Mainly due to the weathering of carbonates, most of the prairie wetlands are alkaline and pH values as high as 10.8 have been reported (LaBaugh, 1989). For instance, Delta Marsh, situated along the south shore of Lake Manitoba, has a pH ranging from 8.2 to 9.0 (Batt, 1998). Similarly, high pH values were found in the western United States. In Benton Lake National Wildlife Refuge, a wetland system in Montana, the pH was as high as 9.72 (Zhang and Moore, 1996). In agricultural drainage waters in the San Joaquin Valley, California, the pH was found in the range of 7.50 - 8.75 (Presser and Ohlendorf, 1987). As shown in Figure 1.2, at high pH values, selenate and selenite are less adsorbed onto aquatic particles such as HFO and thus tend to be more mobile.

According to the classification of wetlands and lakes by Cowardin et al. (1979), most prairie wetlands are brackish or oligosaline, with conductivity ranging from 800 to 10,000 μS/cm (Bierhuizen and Prepas, 1985; Rozkowski, 1969; Barica, 1975). Based on the Practical Salinity Scale for salinity (Appendix 2) recommended by the Joint Panel on Oceanographic Tables and Standards, the salinity of the prairie wetlands ranges from < 2 to 5.6. Conductivity of as high as 472,000 μS/cm was reported in some prairie wetlands (LaBaugh, 1989), making them more saline than seawater. In agricultural drainage waters in the San Joaquin Valley, California, the conductivity was measured as high as 31,000 μS/cm (Presser and Ohlendorf, 1987), which was equivalent to a salinity of 19.3.

Major ions in Delta Marsh were dominated by Na^+, HCO_3^- and Cl^- (Batt, 1998), while those in the agricultural drainage water in San Joaquin Valley, California, were dominated by Na^+ and SO_4^{2-} (Presser and Ohlendorf, 1987). Seasonal variation in major ions is affected by: concentration under ice cover, dilution due to snowmelt and

runoff, concentration by evaporation, dilution from rainfall, and interaction with groundwater (LaBaugh, 1989).

1.4.3 Hydrology

Prairie wetlands are characterized by high seasonal and annual variations in water levels (Adams, 1988), and low velocity of water movement (Simmons and Wallschläger, 2005). Sudden increases in water levels may be caused by snow melting in the spring and heavy infrequent rains in the summer; while evaporation counts for 35 - 55% lost from semi-permanent and permanent prairie wetlands, and the rates increase with the air temperature (Adams, 1988).

1.5 Se in Prairie Wetlands

1.5.1 Se Speciation

The redox potential and pH are the most important parameters that determine the chemical speciation and stability of Se compounds in the aquatic environment (Séby et al., 2001). Since the creek or drainage usually has a more oxic environment with a constant mixing, selenate is generally the dominant species of dissolved Se. Reduction of selenate occurs in anoxic sediments or in ponds where reducing conditions prevail. Microbial processes will also facilitate the reduction of selenate. For example, Benton Lake National Wildlife Refuge (Montana), a wetland system, was found to contain moderate Se levels with selenate accounting for 90% of total dissolved Se entering the ponds through Lake Creek (Zhang and Moore, 1996). In Kesterson pond waters, 20% to 30% of the total Se was present as selenite, while in drainage water selenite only accounted for 2% and the rest was in selenate.

Selenite has been shown to have a stronger affinity for sorption sites on sediment

than selenate in laboratory experiments (Balistrieri and Chao, 1990) and by the HFO model (Figure 1.2) at pH ranging from 8 to 9, which is the range the majority of prairie wetland waters will exhibit. However, selenate and organic Se were the major Se species found within the adsorbed fraction of Se in the sediment (Zhang and Moore, 1996). Fujii and Deverel (1989) observed that selenate (51%) and selenite (49%) were in almost equal proportion of the adsorbed Se in soil in the western San Joaquin Valley, California. Tokunaga et al. (1991) found that selenate and selenite consisted of 35% and 65% of total Se, respectively, in soil at Kesterson, CA. The inconsistency between the results from the laboratory and the field indicated that the adsorption process was related to the dissolved Se species present. When there was less selenite to compete with selenate for adsorption sites, it would result in smaller portion of selenite in the adsorbed Se fraction.

Microbial reduction of selenate to elemental Se, Se uptake by organisms and incorporation of these organisms into sediments are the major processes removing Se from water and transporting it to sediment (Zhang and Moore, 1996).

Several bacteria strains have been isolated from Se-contaminated environments that use selenate as an electron acceptor for growth, including *Thauerea selenatis*, *Sulurospirillum barnesii*, and *Enterobacter cloacae* (Losi and Frankenberger, 1997). Various groups of bacteria and fungi can transform Se species, including selenate, selenite, elemental Se and various organoselenium compounds, into volatile forms by assimilatory reduction of extracellular Se to organoselenide compounds (Frankenberger and Karlson, 1994). The production of volatile Se compounds is thought to be a protective mechanism used by microorganisms to avoid Se toxicity in contaminated ecosystems.

1.5.2 Ecological Effects of Se

In the aquatic environment, in addition to the total Se concentration, Se toxicity is also dependent on speciation. It is generally accepted that selenate has lower toxicity than selenite, and that inorganic Se species have lower toxicity than seleno-amino acids (Simmons and Wallschlager, 2005) (also see Table 1.2), which are thought to be most bioavailable to primary consumers (Riedel et al., 1991). These organic species have also been found to bioconcentrate (Besser et al., 1996) and bioaccumulate (Fan et al., 2002) more substantially than inorganic forms. Extensive studies have proven Se as a primary element of concern because of its bioaccumulation potential in food webs: from water and sediment to aquatic plants and aquatic invertebrates, and to fish and waterfowl (Hamilton, 2004; Lemly, 1996; Presser et al., 1994). The bioaccumulation may result from the chemical similarity with sulfur and its essentiality to organisms.

Although there is no conclusive evidence that plants require Se, Se uptake, accumulation, and metabolism vary among different plant species. Plants can be classified by their ability to tolerate and accumulate Se. Primary hyperaccumulators, including golden weed (*Oonopsis condensate*), yellow prince's plume (*Stanleya pinnata*), and some species of the genus *Astragalus*, are found only on highly seleniferous soil where they accumulate 1000's of mg/kg of Se, mostly in organic forms (Fox et al., 2003). These plants have evolved intriguing biochemical mechanisms not only to tolerate Se, but to accumulate it to concentrations higher than in soil. Se-accumulating plants *A. bisculcatus* and *A. pectinatus* were found averaging from 3 to 1125 mg/kg in Canadian prairie wetlands (Outridge et al., 1999). Secondary accumulators have Se concentrations of 100's of mg/kg of Se, and can be found on soils with a wide range of Se concentrations. Members of this group include the large

family *Brassicaceae* and *Allium* species, such as garlic and onions (Fox et al., 2003). These species are able to volatilize Se and often accumulate the same organic forms found in the hyperaccumulators, only to a lesser extent. The growth of non-accumulators, however, is inhibited by the presence of Se in the soil. Most common crop and forage plants fall under this category. Zhang and Moore (1996) found the water milfoil (Myriophyllum spp.) in a wetland in Montana had Se concentrations from about 360 to 1200 times higher than those found in the water (0 - 14.5 μg/L) in which it was growing and 1-3 times as high as those in surrounding sediment (0.41 - 5.76 μg/g).

Se accumulation by organisms eaten by fish and waterfowl is usually the major pathway leading to its toxicity (Hamilton, 2004). Se in the selenite form is less readily incorporated into proteins than Se that occurs naturally in foods, particularly in plants. Dietary plant Se is easily absorbed by animals; 85% to 100% of Se from plants is biologically available, while only 20% to 50% of Se present in fish is absorbed by birds eating them (Ohlendorf, 1989). Se concentration in fish is influenced by the concentration in the water and diet. In some areas, Se concentrations as low as 3 μg/L may lead to potentially harmful bioaccumulation in higher tropic levels such as migratory waterfowl or shorebirds (Engberg et al., 1998). Zhang and Moore (1996) found the chironomid larvae in a wetland in Montana had Se bioconcentration factors from 3.5 to 20 over sediment concentrations (0.41 - 5.76 μg/g) and from 1700 to 8600 (0.41 - 5.76 μg/g) over water concentrations. In the laboratory, chinook salmon exposed to selenite in the simulated water for 60 days had a bioconcentration factor of 55 and mortality was found (Hamilton et al., 1986).

In the Canadian prairies, Outridge et al. (1999) summarized the Se in eggs for a number of Canadian bird species, including black guillemot, black-legged kittiwake,

Canada goose, common eider, common loon, glaucous gull, king eider, northern fulmar, and thick-billed murre. The highest Se concentrations were in the eggs of northern fulmars (*Fulmarus glacialis*) (4.01μg/g dry weight) and black-legged kittiwakes (*Rissa tridactyla*) (4.39 μg/g dry weight). They were almost twice as high as in other near-shore and terrestrial species, but were still well below those thresholds to cause Se-induced embryonic toxicity (>10 μg/g dry weight). Se concentrations in higher trophic level feeders, such as the predominantly carnivorous diving ducks, tended to be greater than in omnivorous dabbling ducks. For most species, median Se concentrations tended to be higher in populations in western Canada. These migrating western Canadian waterfowl typically follow the Pacific and Central flyways, and are thus more likely to come into contact with high-Se environments in the western United States than are eastern Canadian waterfowl, which typically follow the Mississippi and Atlantic flyways (Outridge et al., 1999).

Se concentrations of wetland pond waters (n = 238) from southwest Manitoba, southern Saskatchewan and southeast Alberta were reported generally lower than 1.0 μg/L, but elevated Se in rivers and lakes, up to 120 μg/L, were found in some locations in these prairie provinces (Outridge et al., 1999) (Table 1.1).

In southern Manitoba, the agriculture industry has recently shifted from grain production to more intensive livestock and value added crops. In 1988 there were 32,000 acres irrigated and the irrigated acreages have doubled by 2001 (AAFC-PFRA, 2003). In light of the increasing irrigation activities and the properties of prairie wetlands, which share great similarities to the wetlands in the western United States, we hypothesize that Se levels in wetlands in southern Manitoba may become elevated and could pose adverse effects on the ecosystem in the future.

1.6 Objectives

The objectives of this research are to study the biogeochemistry of Se in prairie waters in southern Manitoba, and its potential ecosystem implications. Three specific objectives are addressed in this thesis:

1) To assess temporal and spatial variations in Se levels in surface water, porewater, sediment and aquatic plants in Southern Manitoba.

2) To develop an analytical method for the analysis of Se speciation in natural waters, and to apply the method for prairie water samples. And,

3) To study the transport and transformation of selenite in prairie waters by conducting a mesocosm experiment at Delta Marsh.

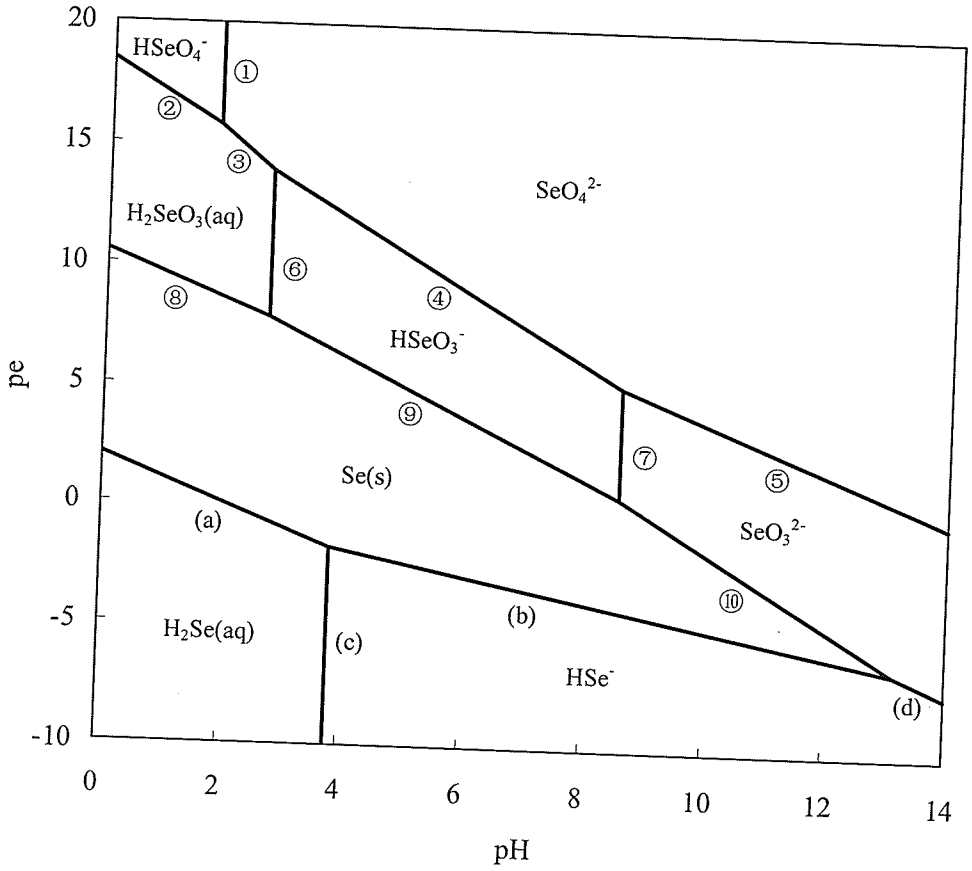

Figure 1.1 Se pe-pH diagram at 25℃, 1 atm and I = 0 for a dissolved Se concentration of 10^{-8} M.

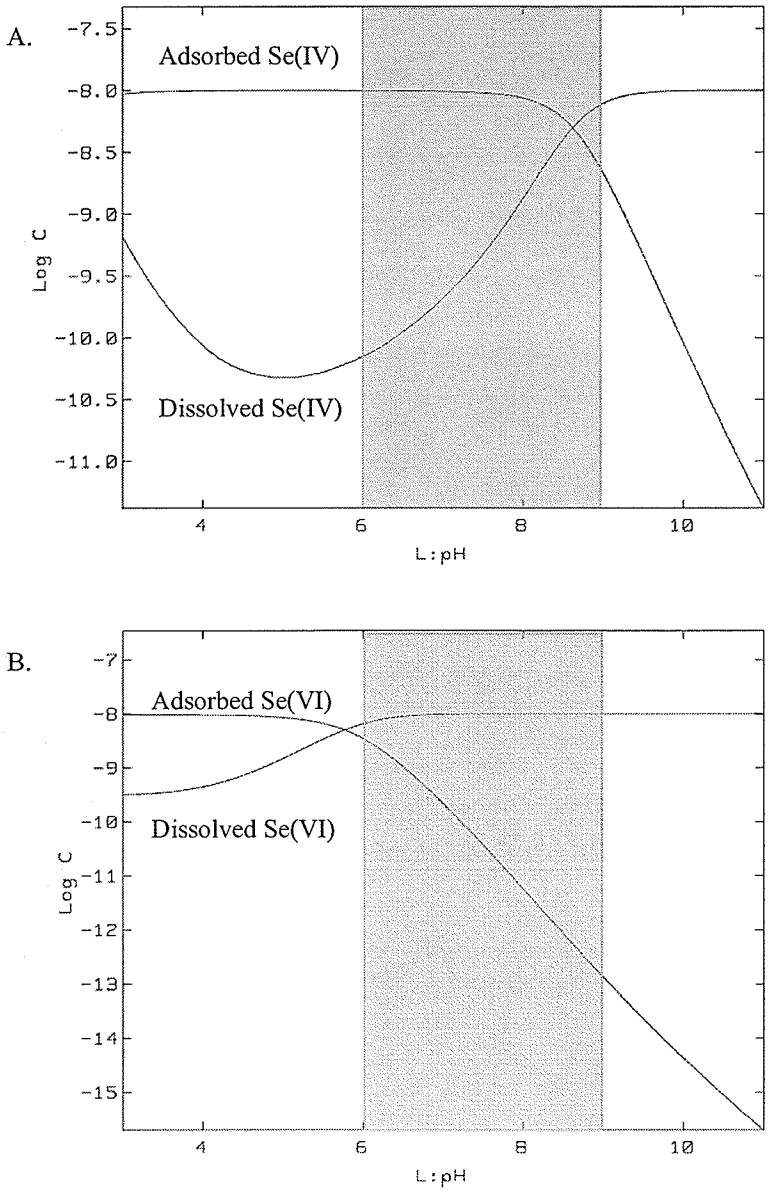

Figure 1.2 The adsorption of 10^{-8} M Se on 10^{-3} M hydrous ferric oxide as a function of pH, at 25 °C, 1 atm, and I = 0.1 M, as modeled by the MINEQL program, assuming no other solids containing Se is formed. A: Selenite (SeO_3^{2-}); B: Selenate (SeO_4^{2-}). The shadow area presents the normal pH range of prairie waters, which is from 6 to 9.

Table 1.1 Se concentrations and speciation in the surface water in the western U.S and Canada.

	Location	pH	Conductivity (μS/cm)	[Se] (μg/L)	Se Species	References
Montana	Benton Lake					
	Inflow	4.33 - 9.72	n/a	5.81 - 562	Se(VI)	Zhang and Moore, 1996
	Pond system	8.27 - 9.43	n/a	0.74 - 26.3	Se(VI), org Se	
California						
	Kesterson National Wildlife Refuge	7.87 - 8.75	12,000 ~ 23,000	15 - 350		
	San Luis Drain	7.5 - 8.7	350 ~ 11,000	<2 - 340	Se(VI)	Presser and Ohlendorf, 1987
	Inflow to SLD	7.8 - 8.5	5200 ~ 31,000	140 - 1400		
	Irrigation supply water	7.75 - 7.95	330 ~ 350	<2		
Southeast Idaho						
	Blackfoot, Salt, and Bear River Watershed	7.6 - 8.7	390 ~ 1050	<2 -11	n/a	Hamilton and Buhl, 2005
Utah						
	Stewart Lake Waterfowl Management Area	n/a	n/a	<1 - 140	n/a	Stephens and Waddell, 1998
Alberta						
	Oldman River, Taber, 1988-93	n/a	n/a	3.0[a,b]	n/a	Outridge et al., 1999
	Milk River, at US border 1988-93	n/a	n/a	2.0[a,b]	n/a	Outridge et al., 1999
Saskatchewan						
	Five lakes surrounding Key Lake uranium mine	5.1 - 7.0	23.0 - 2111.7	0.13 - 7.67	n/a	Pyle et al., 2001
	Yak Creek, downstream of Key Lake mine, 1985-96	n/a	n/a	22[b]	n/a	Outridge et al., 1999
	Beaver Creek, downstream of Flin Flon, 1985-96	n/a	n/a	120[b]	n/a	Outridge et al., 1999
	S. Saskatchewan River, Clarkboro Ferry, 1985-96	n/a	n/a	3[b]	n/a	Outridge et al., 1999
	Coronach Reservoir	n/a	n/a	14[b]	n/a	Outridge et al., 1999
Manitoba						
	Pembina River	n/a	n/a	2.8[a,b]	n/a	Outridge et al., 1999

Note: n/a: no information available;[Se] stands for the total Se concentration in the surface water, except [a] for dissolved Se. [b] concentrations were maximum [Se].

Table 1.2 Ecotoxicity data of selenium compounds on algae and fish. (Source: USEPA Ecotox Database.)

Species Scientific Name	Species Common Name	End-point	Effect	Se Compound	Exposure Type	Exposure Duration (days)	Concentration (μg/L)	References
Ankistrodesmus falcatus	Green algae	EC50	growth	sodium selenite	static	14	33	Vocke et al., 1980
Chlorella pyrenoidosa	Green algae	EC50	population	sodium selenite	renewal	67	800	Bennett, 1988
Microcoleus vaginatus	Blue-green algae	EC50	growth	sodium selenite	static	14	8511	Vocke et al., 1980
Pseudokirchner-iella subcapitata	Green algae	EC50	growth	sodium selenite	static	14	277	Vocke et al., 1980
Chlorella vulgaris	Green algae	LOEC	population	sodium selenite	flow through	7	82.6	Dobbs, 1996
Chlorella vulgaris	Green algae	NOEC	population	sodium selenite	flow through	7	9.1	Dobbs, 1996
Oncorhynchus mykiss	Rainbow trout	LOEC	accumulation	selenomethionine	food	120	10500	Holm, 2002
		NOEC	accumulation	selenomethionine	food	120	3250	Holm, 2002
		LC50	mortality	sodium selenite	static	5	2700	Adams, 1976
		LC50	mortality	sodium selenate	static	4	32300	Buhl and Hamilton, 1991
Danio rerio	Zebra danio	LC5	mortality	selenocystine	renewal	10	12000	Niimi and Laham, 1976
		LC50	mortality	sodium selenite	renewal	4	15000	
		LC50	mortality	sodium selenate	renewal	4	81000	
Pimephales promelas	Fathead minnow	LC50	mortality	sodium selenite	static	4	11300	Adams, 1976
		LC50	mortality	sodium selenate	static	4	11800	Adams, 1976

Note: EC50 refers to the concentration of the chemical in water which induces a response halfway between the baseline and maximum. LOEC stands for the lowest observed effect concentration. NOEC stands for the no observed effect concentration. LC5 refers to the concentration of the chemical in water that kills 5% of the test species in a given time. and LC50 refers to the concentration of the chemical in water that kills 50% of the test species in a given time.

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Chapter 2. Selenium Concentrations in Prairie Waters of Southern Manitoba

Abstract

The prairie waters of southern Manitoba share many similarities in geology, hydrology and ecology with those in the western United States, where elevated concentrations of selenium from irrigation practices have resulted in adverse impacts on wildlife populations. To assess the current state of selenium in prairie waters in southern Manitoba, selenium concentrations were quantified at three water bodies between July 2005 and July 2007. The sites included Delta Marsh, Stephenfield Reservoir and the South Tobacco Creek Watershed; they were selected based on their perceived level of impact from agriculture practices. Selenium concentrations in surface water, sediment cores, sediment porewater and macrophytes were determined. At Delta Marsh and Stephenfield Reservoir, the selenium concentrations were <1 μg/L in the surface water, < 1.3 μg/g (dry weight) in the sediment, and < 0.12 μg/g (dry weight) in the macrophytes, relatively low comparing to similar wetlands in the Prairies. At the South Tobacco Creek Watershed, elevated selenium concentrations (>11 μg/L) were found in the surface water during the spring freshet in 2007 with lower levels observed during the rest of the year. Profiles of dissolved selenium and other water chemistry properties, such as pH, dissolved organic carbon (DOC), sulfide and sulphate, in the sediment porewater were also characterized at Delta Marsh. Dissolved selenium showed a strong dependence on the redox conditions. Higher concentrations of selenium up to 1.66 μg/L were found above the redox transition layer (~8-12 cm below the sediment-water interface), while lower concentrations of selenium (0.47 - 0.90 μg/L) were found below the layer, where the environment was less oxic. The results indicate that at present selenium concentrations are generally low in the prairie waters of southern Manitoba; however, Se can be elevated during the snowmelt season and by intensive agricultural

activities such as in the South Tobacco Creek Watershed. A direct link between surface water Se concentration and irrigation activities cannot be established at this time, probably due to the limited irrigation activities in the study areas. Based on literature studies on Se bioaccumulation in other regions with higher irrigation activities, continuing monitoring of Se concentrations in the prairie waters in southern Manitoba is warranted given the projected increase in irrigation and other agricultural activities in this area.

2.1 Introduction

Selenium (Se) is a metalloid existing in four oxidation states (-II, 0, +IV, +VI) and in a variety of chemical forms (elemental selenium, oxides, oxyanions, selenides, and organoselenium compounds). In the aquatic environment its speciation is complex and highly dependent on pH, redox reactions and surface complexion reactions, as well as the solubility of its salts, microbiological activity, and reaction kinetics (Belzile et al., 2000; Frankenberger et al., 1994). Dissolved Se species can be taken up by aquatic plants and animals, be transformed into volatile Se species (e.g., $(CH_3)_2Se$), or be reduced into elemental Se or adsorbed onto algae or aquatic particles (Lemly, 1987). They can be bonded to Fe- or Mn-oxyhydroxides and organic matter at or near the sediment-water interface, while in reduced sulfidic systems, Se-pyrite or ferroselite can be formed (Belzile et al., 2000).

Se is an essential micronutrient for microbes, animals and humans. As part of the enzyme glutathione peroxidase (GSH-Px), which converts peroxide into water and catalyzes the scavenging of free radicals by glutathione, Se can protect biological tissues against oxidative damage (Lemly, 1998). Diets containing Se ranging from 0.1 to 0.3 mg/kg (dry weight) is considered minimum nutritional requirement for fish, birds and mammals (Ullrey, 1992). However, diets containing Se higher than 5 mg/kg (dry weight) is deemed toxic, because it behaves as an analogue to sulfur and can erroneously replace sulfur atoms in

proteins, resulting in dysfunction of enzymes and proteins (Lemly, 1998).

Se occurs naturally in the earth's crust with an average concentration around 0.12 μg/g (Wedepohl, 1995), and is found higher in shale with an average value of 1.4 μg/g (Presser and Ohlendorf, 1987). Se is released to the aquatic environment by the erosion of seleniferous soils and weathering of rocks (Seiler, 1998) and is commonly less than 1 μg/L in freshwaters (Milne, 1998). However, human activities including mining, petroleum refining, coal combustion, and agricultural irrigation may greatly increase the concentrations of Se in the environment, especially in surface water and soil (Lemly, 1999).

One of the most documented Se contaminations is in the western United States where high geological Se background and irrigated agriculture have resulted in elevated Se levels in many wetlands and upland ecosystems with impacts observed on fish populations (Presser et al., 1994). Cretaceous marine sedimentary rocks rich in reduced sulfur compounds, such as pyrite and other sulfide minerals, are known as important sources of Se. They form the near-surface bedrock beneath about 805,000 km^2 of land in the 17 conterminous western United States (Berrow and Ure, 1989; Seiler, 1998). Se in the seleniferous soils being irrigated is mobilized by infiltrating irrigation water. It is discharged either to surface water by subsurface drainage or to ground water by percolation. Moreover, the semi-arid climate and high evaporation rate favor the accumulation of Se in those ecosystems (Presser et al., 1994). An example of agricultural practices resulting in elevated Se can be found in the 26 irrigated areas in the western United States and their downstream receiving environments where Se concentrations in the surface water reached as high as 265 μg/L (Seiler, 1998).

Similar to the western United States, the prairie waters in southern Manitoba are also characterized by Cretaceous geology with relatively high Se concentrations in soils (Garret et al., 2008), semi-arid climate, saline surface water (LaBaugh, 1989; Outridge et al., 1999) and high seasonal and annual variations in water levels (Adams, 1988). Farmlands and

irrigation-dependent agriculture are also widespread. Negative water balance, a characteristic of Canadian prairies, stimulated the industry of high value irrigated crops, such as potatoes, in southern Manitoba (Hoppe, 1999). Moreover, southern Manitoba has been experiencing drought since the 1980s. As a result, the region has seen rapid increase in agricultural irrigation activities; the irrigated acreages have doubled from 32,000 acres by 2001 (AAFC-PFRA, 2003).

In light of the geological background and the increasing irrigation and other agricultural activities, we hypothesize that the Se concentrations in the prairie waters of southern Manitoba are likely to rise and potentially pose adverse effects on the aquatic ecosystem. This study aims to assess the temporal and spatial variations in Se concentrations in areas representing a range of agricultural activities in southern Manitoba.

2.2 Methods and Materials

2.2.1 Field Sites

Three prairie waters in southern Manitoba, Delta Marsh, Stephenfield Reservoir, and the South Tobacco Creek Watershed (Figure 2.1) were chosen as the study sites. They all share similar Cretaceous geology but have experienced different agricultural activities. A comparison among three sites was listed in Table 2.1.

With limited irrigation activities (Hoppe, 1999), Delta Marsh was chosen as a reference site and was to provide a background level of Se in southern Manitoba. It is a eutrophic brackish prairie wetland on the south shore of Lake Manitoba, comprising a surface area of 150 km^2 with large open bays and channels up to 3 m in depth and smaller shallow bays less than 1 m in depth (Batt, 1998). Surface water samples were collected across the entire wetland in June 2004 (by a summer student H. Poklitar of our group) and June 2006 (Figure 2.2). Additional samples of surface water, sediment porewater, sediment cores, and aquatic

plants were collected from Forster's Bay (50°10'38"N, 98°24'06"W), a shallow bay with a depth less than 1 m, and connected with other parts of the marsh by the Blind Channel.

Stephenfield Reservoir (49°31'34"N, 98°18'22"W) was chosen as a site with some irrigation activities (Hoppe, 1999). It was dammed on the Boyne River in 1963 due to concerns about the area's water supply. The reservoir is 5 km long, 0.5 km wide and over 9 m deep, and lies in the southeast end of the Stephenfield Lake watershed, which has a drainage area of approximately 960 km^2. It collects the runoff from two major waterways: the Boyne River and the Roseisle/Lyles Creek. At the Stephenfield Lake watershed, agricultural cropland is a major land use of this area, which consists 81.6% of the watershed (Figure 2.3) (Wood, 2005).

The South Tobacco Creek is a small catchment (76 km^2) located in the upper reaches of the Red River Basin (Figure 2.4). The watershed is situated in the Aspen Parkland Ecoregion with headwaters draining from the uplands of the Manitoba Escarpment (Glozier et al., 2006). Steppler Reservoir (site designation STC-3) (49°20'13"N, 98°21'39"W), located at the base of the watershed with a depth up to 3 m, was chosen as a site characterized by farmlands with tillage practices. The gradient of the South Tobacco Creek, descending about 200 m over a distance of 50 km, and the extensive agricultural settlement from the 1870s, which has largely cleared the vegetation within the upland portion of the creek, resulted in extreme runoff events and prompted further water management in the watershed. Of 50 small-scale headwater retention dams, 26 were built within the watershed, and the Steppler Dam is one of them. It was built in 1989 and has effectively decreased peak flows during spring runoff and summer rainstorm events by retaining water for a short period of time to reduce flow rates. Surface water samples were collected from STC-3 and two other sites in the watershed, STC-1 and STC-2 (Figure 2.4), which have been monitored for the water quality by Glozier et al. (2006) from 1992 to 2001. STC-1, designated as Highway 240 Station (49°21'55"N,

98°20'44"W), is located on a 2nd order stream, which drains the southern portion of the watershed and is upstream of the confluence with the northern arm. STC-2, designated as Upland Wood Station (49°22'48"N, 98°25'11"W), is located in a private property, surrounded by woods and bushes in the upland.

2.2.2 Sample Collection

Samples of surface waters, sediment porewaters, sediment cores, and aquatic plants were collected at the above sites from July 2005 to July 2007. Detailed sampling dates and locations are summarized in Table 2.2.

All the sampling device preparation were done at the metal-free, Class 10-1000 cleanrooms of the Ultra-Clean Trace Elements Laboratory (UCTEL) of the University of Manitoba. All the chemicals were purchased from Fisher Scientific, Canada. Milli-Q Element ultrapure water (>18 MΩ•cm; Millipore Corporation, USA) located inside UCTEL was used as the laboratory water ("ultrapure water" hereafter). Prior to sampling in the field, 15-mL polypropylene Corning centrifuge tubes (for Se and trace elements) (Corning, Canada) were pre-cleaned in a 4 M HNO_3 (Reagent A.C.S) acid bath. They were then rinsed with ultrapure water, dried in a Class 10 laminar flow workstation, spiked with 0.5% Optima HNO_3 and single bagged in Ziploc bags at UCTEL. The parts of the portable filtration system (filter holder, connectors, clamps, and Teflon tubing) and the pipette tips (Fisher, Canada) were pre-cleaned in a 4 M HCl (Reagent A.C.S) acid bath. They were then rinsed with ultrapure water, dried, and double bagged in Ziploc bags at UCTEL. Two 1-L amber HDPE bottles (Fisher Scientific) were also pre-cleaned in a 4 M HCl acid bath and rinsed by ultrapure water. They were filled with ultrapure water on the same day of sampling, double bagged in Ziploc bags at UCTEL, transported in coolers with ice packs and used as field blanks.

Surface water samples were collected following the "clean hand, dirty hand" ultraclean

sampling techniques (Fitzgerald, 1999). Raw surface water samples were collected from approximately 10 cm below the surface at all the sampling sites by grab sampling with the Corning tubes pre-spiked with HNO_3 for the analyses of total waterborne Se (Se_T) and other trace elements. Samples for dissolved Se (Se_D) were collected by *in situ* filtration with 0.45-μm GHP hydrophilic polypropylene membrane filters (Pall Corporation, USA). The filtration system was conditioned for five min before the filtrates were collected in the pre-spiked Corning tubes. The field blanks were prepared by filling the pre-spiked Corning tubes with water from the ultrapure water brought to the field (in 1-L amber HDPE bottles). Samples and blanks were stored in coolers with ice packs and transported to the laboratory, where they were kept refrigerated at 4°C till analysis.

Dialysis samplers ("peepers") (Figure 2.5 A and B) described by Carignan et al. (1985) were used to collect sediment porewaters *in situ* at a vertical resolution of 1cm. A peeper is a plastic plate with cells containing deoxygenated ultrapure water, fitted with a dialysis membrane, and placed vertically into the sediment. The peeper method is based on the principle that solutes in the sediment porewater diffuse through the membrane until they reach equilibrium with the water within the peeper (Hesslein, 1976). Each peeper consisted of a 30 × 15 × 1 cm Plexiglas plate in which two columns of 0.6 × 7.0 × 0.6 cm compartments spaced 1 cm center to center were machined. A 0.2-μm hydrophilic polysulfone membrane (HT-200, Gelman, USA) to cover the bottom plate was laid between the bottom plate and a 0.2-cm thick Plexiglas cover sheet, which has windows matching the compartments on the bottom plate. They were assembled by screws. The Plexiglas bottom plates and the cover sheets were pre-cleaned in a 4 M HNO_3 acid bath at 70 - 80°C for 2 days, rinsed with ultrapure water and kept under a pure N_2 atmosphere for two weeks to remove traces of oxygen from the material. At UCTEL, the compartments in the bottom plate of each peeper were filled with ultrapure water, covered with the membrane and the cover sheet, and

assembled in ultrapure water to avoid oxygen bubbles in the cells. The assembled peepers were then placed under a pure N_2 atmosphere for another seven days for further deoxygenation before placement in the sediment.

Five peepers were deployed, about 20 cm apart, in Forster's Bay at Delta Marsh on October 4, 2005. After three weeks of equilibration in the field, the peepers were retrieved individually from the sediment (Figure 2.5 C and D) and samples processed within 1 hour at a field laboratory at the Delta Marsh Field Station. Samples (1 mL) for pH were collected with 1-mL polypropylene syringes (Fisher) and injected into a 4-mL amber glass vial; the pH was measured with an Orion pH meter (Model 250) and Accumet pH electrode (Model 290) (Thermo Fisher Scientific) within 30 min at the field laboratory. Samples (3 mL) for inorganic sulfide $\Sigma[H_2S]$ were collected with 3-mL polypropylene syringes (Fisher) and injected immediately through Teflon septa into N_2-purged amber glass vials containing the Cline reagents (Cline, 1969). The concentrations of the Cline reagents were 5.2 mM N,N-dimethyl-*p*-phenylenediamine (DMPD) and 5.5 mM $FeCl_3 \cdot 6H_2O$ (120 μL of each). Samples (2 mL) for major cations and anions were collected with 3-mL polypropylene syringes and injected into 2-mL polypropylene centrifuge vials. Samples (2 mL) for dissolved organic carbon (DOC) were collected with 3-mL polypropylene syringes and injected through Teflon septa into 2-mL N_2 purged amber glass vials. Samples (5 mL) for dissolved Se (Se_D) were obtained with an Eppendorf pipette fitted with acid cleaned plastic pipette tips (Fisher) and transferred into the 15-mL pre-spiked Corning tubes. Three field blanks, obtained from 1-L HDPE bottle filled with ultrapure water, for $\Sigma[H_2S]$, major ions, DOC or Se_D were treated in the same way as the samples. All the samples and blanks were stored in coolers with ice packs and transported to the laboratory, where they were kept refrigerated at 4°C till analysis.

Sediment cores were collected from both Delta Marsh and Stephenfield Reservoir. The sediment cores from Forster's Bay in Delta Marsh were collected 1 m away from the

sediment porewater sampling location. The sediment cores from Stephenfield Reservoir were collected from the flooded upland soil close to the shore. A metal-free polycarbonate tube corer (id. 46 mm x 1.5 m) was manually inserted vertically into the sediment as deep as possible (20 - 40 cm, depending on the nature of the sediment). The top end was filled up with the surface water from the pond and then sealed by a rubber stopper. The corer was slowly pulled out from the water and its bottom end was immediately sealed by another rubber stopper (Figure 2.5 E). The sediment core was held vertically until it was transported to the shore where it was sliced. To slice the sediment core, the bottom stopper was removed, and the corer was allowed to sit on a plastic cylinder on top of a metal rod. The top stopper was then removed and the corer was pushed down vertically. With the water flowing out, the sediment was extruded out slowly from the top of the corer and sliced by a metal-free polycarbonate ring with the proper length. The top 20 cm of the core was sliced into 1-cm sections, and the rest into 2-cm sections. Each section was collected in a Ziploc bag, stored in coolers with ice packs and transported to the laboratory, where they were kept frozen at -24°C till analysis.

Aquatic plants were collected by hand from Delta Marsh and Stephenfield Reservoir and bagged in Ziploc bags. Coontail (*Ceratophyllum demersum*) and cattail (Typha sp.) with leaves and roots were collected from both sites. Bladderwort (*Utricularia macrorhiza*) and bulrush (*Scirpus tabemaemontani*) with leaves and roots were collected from Delta Marsh only, while watermilfoil (Myriophyllum spp.) was collected from Stephenfield Reservoir only. Plant samples were stored in coolers with ice packs and transported to the laboratory. After rinsed three times with distilled water, each plant sample was bagged in a clean Ziploc bag and kept frozen at -24°C till analyses.

2.2.3 Acid Digestion of the Sediment and Aquatic Plant Samples

Sediment cores and aquatic plants were freeze dried and then digested in a block digester in the laboratories of Fisheries and Oceans Canada in Winnipeg. Two mL of concentrated HNO_3 (Fisher) and 6 mL concentrated HCl (Fisher) were added to 0.4 g freeze dried samples of sediment cores in 50-mL glass test tubes. The tubes were covered with a thin plastic film and samples were predigested over night. The next day the samples were boiled in the block digester at 80°C for 30 minutes and then at 125°C for another 30 minutes. The samples were then allowed to cool, diluted to 25 mL with distilled water. 5 mL of each digest was collected in an acid-cleaned 15-mL Corning tubes (Corning) and was stored at 4°C in the fridge till analysis for Se_{SED}. Two blanks and a certified reference materials (MESS-3, marine sediment, from National Research Council of Canada) were treated in the same way as the samples. Due to the matrix effect and the high acidity, the digests were diluted 100 fold prior to analysis by ICP-MS.

Five mL of concentrated HNO_3 (Fisher) and 0.5 mL concentrated H_2SO_4 (Fisher) were added to 1 g freeze dried samples of aquatic plants in 50-mL glass test tubes with two 2-mm bumping beads. After 20 min of predigestion, the acid-mixed samples were heated in the block digester at 80°C until the organic matter had oxidized and no more bubbles appeared. At this point, the samples were allowed to digest at 125°C for approximately 20 hours. A small volume of 30% H_2O_2 was added to the digests to complete the oxidation process. The samples were then allowed to cool, diluted to 25 mL with distilled water. Five mL of each digest was collected in an acid-cleaned 15-mL Corning tubes and was stored at 4°C in the fridge till analysis of Se_{PLA}. Two blanks and a certified reference materials (SRM1572, citrus leaves, from National Institute of Standards and Technology of Canada) were treated in the same way as the samples. Due to the matrix effect and the high acidity, the digests were diluted 100 fold prior to analysis by ICP-MS.

2.2.4 Sample Analyses

The pH of the surface water was measured *in situ* with an Orion pH meter (Model 250) and pH electrode (Model 9107) (Thermo Fisher Scientific). $\Sigma[H_2S]$ was determined on a Varian Cary 50 Visible-Ultraviolet (UV-Vis) spectrophotometer (Varian Inc., USA) at 670 nm (Cline, 1969) with a 1-cm light path quartz cuvette. The detection limit was 0.13 μM. DOC was analyzed using the high-temperature combustion method (APHA, 1998) on a Shimadzu TOC-5000A carbon analyzer by Enviro-Test Laboratories (now ALS Environmental), Winnipeg. The detection limit was 1 mg/L. Cations ($[Na^+]$, $[K^+]$, $[Mg^{2+}]$, $[Ca^{2+}]$, and $[NH_4^+]$,) and anions ($[Cl^-]$, $[SO_4^{2-}]$, $[Br^-]$, $[NO_3^-]$ and $[PO_4^{3-}]$) were analyzed on an ICS-1000 Ion Chromatograph System (Dionex Corporation, USA) in Dr. Mario Tenuta's laboratory in the Department of Soil Science, following recommended procedures (Dionex Corporation, 2003). The detection limit for each ion was 0.1 mg/L.

Sediment cores were dated by the laboratories of Fisheries and Oceans Canada. Five to 15 grams of the sample were sealed in 60 x 15 mm plastic Petri dishes, aged for 21 days and counted on a gamma spectrometer (Canberra Industries, CT, USA) with a Hyperpure Ge crystal coaxial gamma detector (Ortec, TN, USA) for the determination of Cs-137 and Ra-226. One gram of the sample was analyzed for Pb-210 by leaching in 6N HCl in the presence of a Po-209 tracer. Polonium was autoplated onto a silver disc (Flynn 1968) which was counted on an alpha spectrometer (Model 576, Ortec, TN, USA) to determine Pb-210 via its Po-210 daughter. Ra-226 was determined on selected slices by the radon de-emanation technique (Mathieu 1977; Wilkinson 1985). Excess Pb-210 was determined in each slice by subtracting the Ra-226 activity from the Pb-210 activity.

Se_T, Se_D, Se_{SED}, Se_{PLA} and other trace elements were analyzed at UCTEL on a Perkin-Elmer Elan DRC II Inductively Coupled Plasma-Mass Spectrometer (ICP-MS).

Operating conditions of ICP-MS are shown in Table 2.3. Nebulizer gas flow, lens voltage, and auto lens calibration were optimized daily. A 5-point linear calibration curve was plotted daily for each element from a series of calibration standards with concentrations of 0.1, 1, 10, 50 and 100 μg/L, which were prepared from a 10 mg/L multi-element solution (CLMS-2, SPEX CertiPrep, Inc., U.S.A.). The coefficient of determination (r^2) for each calibration curve was above 0.999. The method detection limit (MDL) was defined as 3σ, where σ is the standard deviation obtained from 7 runs of the lowest calibration standard. The MDL determined for Se was 5 ng/L. Certified reference materials used in the analysis included NIST 1640 (National Institute of Standards and Technology, USA), and TM-Rain 95 (National Water Research Institute, Canada), and were found to be within 90%-110% of the certified value of 21.96 μg L^{-1} and 0.74 μg L^{-1}, respectively.

2.2.5 Statistical Test

An one-sample t test was carried out to determine the significance level of the difference between Se_D and Se_T. The null hypothesis was "no difference", and the alternative hypothesis was "$Se_T > Se_D$".

2.3 Results

2.3.1 Selenium Concentrations at Delta Marsh

Se concentrations in the surface water of Delta Marsh are shown in Table 2.4 and Figure 2.2. Two wetland-wide surveys were done in June 2004 and June 2006, respectively. In addition, the surface water of Forster's Bay was monitored in July 2005, October 2005 and June 2006. Overall, the Se concentrations in the surface waters were relatively low, ranging from 0.53 to 1.54 μg/L. There was no significant difference between dissolved Se (Se_D) and total Se (Se_T) concentrations in the surface water samples (significance level α=0.025; p was

between 0.02 and 0.025) (Table 2.4), suggesting the majority of Se was present in the dissolved form.

Figure 2.6 shows the profile of dissolved Se and other water chemistry parameters across the sediment-water interface at Forster's Bay of Delta Marsh in October 2005. Although efforts were taken when deploying the peepers so that the sediment-water interface was between the 5th and 6th cells, fluctuations occurred during the 3-week equilibration period due to the dynamics of the sediment-water interface in this shallow bay. The "averaging" water-sediment interface location on the peeper (the dashed line in Figure 2.6) was thus determined by the appearance of the peeper when it was taken out from the sediment. The upper surface which stayed mostly in the water column was yellowish and dirty; while the lower part, which stayed in the sediment, was colorless and clean. As shown in, Se_D in the overlying water and upper layer porewaters was fluctuating around 1.19 μg/L between the depth of -10 cm and 5 cm (a negative depth indicates above the sediment-water interface), but decreased sharply to 0.53 μg/L at 10 cm depth. The Se_D gradient occurred at the same depth where profiles of DOC, dissolved sulfide, sulphate, Fe, and Mn (Figure 2.6), suggesting the control of the redox potential (pe) in the distribution of Se_D.

Se concentrations in the sediment core (Se_{SED}) from Delta Marsh ranged from 0.6 to 1.3 μg/g dry weight (Figure 2.7 A). They were at the lower end of the range of 0.1 to 6 μg/g dry weight reported in seleniferous soils in Canadian prairie provinces (Outridge et al., 1999), and were lower than the US Geological Survey upper limit of the expected 95% baseline for soils of the western United States reported at 1.4 μg/g dry weight (Presser et al., 1994). No obvious changes were found with depth, which was in accordance with the dating results (Figure 2.7 B), indicating the vertical mixing of the sediments. Although the core showed a slight exponential decay in the excess Pb-210 activities, the excess Pb-210 flux was only 34 $Bq/m^2/yr$ (Appendix 3), much lower than the normal range of 130 $Bq/m^2/yr$ for a depositional

site (Lockhart et al., 1998).

Se concentrations in aquatic plants from Delta Marsh (Se_{PLA}) ranged from <MDL (0.01 μg/g) to 0.06 μg/g dry weight (Table 2.5), which was much lower than the reported range of 3 to 1125 μg/g in Se-accumulating plants in Canadian prairie provinces (Outridge et al., 1999), and the range of 1.2 to 22.6 μg/g in non-Se-accumulating plants in wetlands (Zhang and Moore, 1996). Se_{PLA} in the roots of bulrush and cattail were found to be higher than those in the leaves.

2.3.2 Se Concentrations at Stephenfield Reservoir

Se concentrations in the surface water from Stephenfield Reservoir were slightly below 1.0 μg/L in July 2005 (Table 2.4). In the sediment core, Se_{SED} ranged from 0.3 to 0.8 μg/g dry weight (Figure 2.7 A), which was lower than the Se_{SED} range in Delta Marsh, and at the lower end of the range of 0.1 to 6 μg/g dry weight reported in seleniferous soils in Canadian prairie provinces (Outridge et al., 1999). No obvious changes were found with depth, which may result from the mix of the sediment when the dam was built in 1960s. In aquatic plants from Stephenfield Reservoir, Se_{PLA} ranged from 0.02 μg/g to 0.12 μg/g dry weight (Table 2.5). Se_{PLA} in the roots of cattail was found higher than that in the leaves.

2.3.3 Se Concentrations in the South Tobacco Creek Watershed

Total Se concentrations (Se_T) varied among the three sampling sites in the South Tobacco Creek Watershed. At each site, Se concentration reached the highest value in April (Table 2.4). Throughout the four sampling events, Site STC-2 had the lowest Se concentrations, ranging from 0.87 to 1.95 μg/L. The highest Se concentration of 11.58 μg/L was found at Site STC-3 in April 2007.

2.4 Discussion

2.4.1 Spatial Distribution of Se in the Surface Water of Southern Manitoba

Among the three study sites in southern Manitoba, the South Tobacco Creek Watershed had higher Se level in the surface water. At Stephenfield Reservoir, although the Se level in the surface water was close to the Canadian water quality criteria (CCME, 1987), which is 1 μg/L, it seemed not elevated when compared with Delta Marsh. Except for STC-2 which is an upland wood station, STC-1 and STC-3 of the South Tobacco Creek Watershed showed much higher surface water Se concentrations, ranging from 1.73 to 6.26 ug/L and 1.81 to 11.58 μg/L, respectively, for the period of September 2006 to July 2007, indicating potential impact from the agricultural activities on the farmlands, such as tillage.

Agricultural activities have greatly disturbed the original vegetation distribution in the South Tobacco Creek Watershed. Prior to extensive agriculture settlement in the 1870s, vegetation within the upland portion of the creek consisted of Aspen forest and scrub brush, and the large Boyne Marsh was historically located at the base of the escarpment with native prairie wetlands. The large clearance of the vegetation and tillage practices in these areas for farmlands accelerated soil erosion from upland and degraded the water quality in the runoff (Glozier et al., 2006). Located in the upper headwaters of the watershed and completely surrounded by native forest and brush, STC-2 represents the least disturbed area in the watershed and thus shows the lowest Se concentrations. The higher Se concentrations at STC-1 and STC-3 are likely due to the agricultural development in the area, which may facilitate soil erosion and bring Se from the soil into the surface water.

Tillage practices could lower soil organic matter levels, cause poor soil structure, and result of compacted contribute to increases in soil erodibility. During spring months, runoff from the agricultural land may be greatest when the soils are usually saturated, snow is melting and vegetative cover is minimal (Ward and Robinson, 2000).

2.4.2 Seasonal Variations of Se in the Surface Water

Seasonal variations of Se were investigated at South Tobacco Creek Watershed. As shown in, Se levels in the surface water of STC-1, STC-2 and STC-3 varied throughout the year, with the highest values observed in the spring freshet in April. Elevated Se in the watershed was also reported by the Manitoba Water Stewardship (2007) with a concentration of 9.3 μg/L in April 2004 in the South Tobacco Creek at Miami. Glozier et al. (2006) noted three hydrologically distinct periods in the watershed: i). the spring snowmelt, from March to April, when the discharge is driven by snowmelt; ii) the summer, from May to August, when the discharge is driven by rain events; and iii) the fall and winter, from September to February, when the system has the lowest flow or was ice-covered. The highest Se concentration in April coincides with the high flow period associated with the spring snowmelt (Figure 2.8), suggesting a potential Se source from airborne particles accumulated during the winter and/or increased soil erosion during snowmelt. Elevated trace element concentrations have been reported during the spring freshet season in many large river systems (Rember and Trefry, 2004).

2.4.3 Diagenetic Processes of Selenium in Sediments

Diagenetic processes of selenium in sediments were studied at Forster's Bay of Delta Marsh. The sediment profile of Se did not show major changes, due to the extensive vertical mixing of particles in the shallow bay which was confirmed from the dating result. Such mixing could be due to wind-induced wave mixing in summer and freezing and thawing in winter and spring. It could also be due to bioturbation of the extensive plant root systems and invertebrates (DeVries and Wang, 2003), and the increasing population of carp.

Obvious gradients are, however, observed from the porewater profiles, with a redox

transition zone located at around 8 m to 12 m below the sediment-water interface (Figure 2.6). Above this depth was an oxic layer, where the sulphate concentration was much higher than the sulphide concentration, and dissolved iron and manganese remained very low due to their precipitation as ferric hydroxides ($Fe(OH)_3(s)$) and manganese oxides ($MnO_2(s)$), respectively. Within the redox transition layer, however, sulphate is rapidly reduced to sulphide, and dissolved Mn and Fe increased due to reductive dissolution of their respective hydroxides.

Selenium is a redox-sensitive element, and its profile in the sediment porewater is also related to the major diagenetic constituents. Based on the relationship between pe and pH in aquatic systems for Se (Figure 1.1), indicating Se speciation highly depends on redox conditions, it is predicted that the dominant Se species will change from selenate (VI), to selenite (IV), and eventually to elemental selenium (0) and selenide (-II) when the water transits from the oxic to anoxic redox conditions. As shown in Chapter 1, although selenate and selenite are mainly present as dissolved species, both of them can be adsorbed onto the surfaces of oxides with the latter having a high affinity to the surfaces. Therefore, as selenate is reduced to selenite, the fraction of the dissolved Se would decrease due to the stronger scavenging of selenite by Fe and Mn oxides. As the redox potential pe continues to decrease into the deeper sediment layer, more dissolved Se will be removed from the porewater due to the formation of elemental Se, the precipitation of metal selenides (e.g., ferroselite ($FeSe_2$) or achavalite (FeSe)), and the incorporation of selenide into organic matter (Myneni et al., 1997; Takayanagi and Belzile, 1988; Tokunaga et al., 1997). The low concentrations of dissolved Fe and Se below the redox transition layer were in accordance with the above processes. Similar porewater profiles have been reported in a wetland system in Montana, USA (Zhang and Moore, 1996), and in two lakes in the Sudbury area, Ontario, Canada (Belzile et al., 2000).

2.4.4 Potential Impacts of Se on the Aquatic Ecosystem in Southern Manitoba

Se concentrations in the surface waters from the three wetlands in southern Manitoba are generally lower than the U.S. Environmental Protection Agency chronic criterion of 5 μg/L for the protection of aquatic life (USEPA, 1987). While most of values are also lower than the CCME guideline (CCME, 1987) of 1 μg/L for the protection of aquatic life in freshwater, exceedance of the CCME guideline occurs sporadically, particularly at the South Tobacco Creek, suggesting a low to marginal ecological risk. However, much higher Se concentrations can be associated with the snowmelt season, which raises potential concern in some of the wetlands. The relative low Se concentrations in the sediments and plants from Delta Marsh and Stephenfield Reservoir also suggested the systems are at low risk at present.

Although Stephenfield Reservoir was studied as a site with potential Se contamination resulting from irrigation, no elevated Se levels in the surface water, sediment and plants were observed relative to those in Delta Marsh. Due to the limited quantities of data, the results may not fully reflect the effects of irrigation on Se levels in this area. Other agricultural activities, such as tillage practices on farmlands, could potentially explain the elevated Se levels in Steppler Reservoir at South Tobacco Creek Watershed.

2.5 Conclusion

This study on Se distribution in the prairie waters in southern Manitoba indicated that Se levels in the surface water, sediment and plants are relatively low at present. Se concentrations, however, can be elevated during the snowmelt season and by intensive agricultural activities such as in the South Tobacco Creek Watershed. A direct link between surface water Se concentration and irrigation cannot be established at this time, potentially due to the limited irrigation activities in the study areas.

Studies in the wetlands in the western United States and Canadian Prairie provinces have

proved bioaccumulation of Se can occur even when Se levels were low in the water and sediment (Lemly, 1999b; Presser, 1994). Therefore, it is important to continue the monitoring of Se concentrations in the prairie waters in southern Manitoba given the projected increase in irrigation and other agricultural activities in this area.

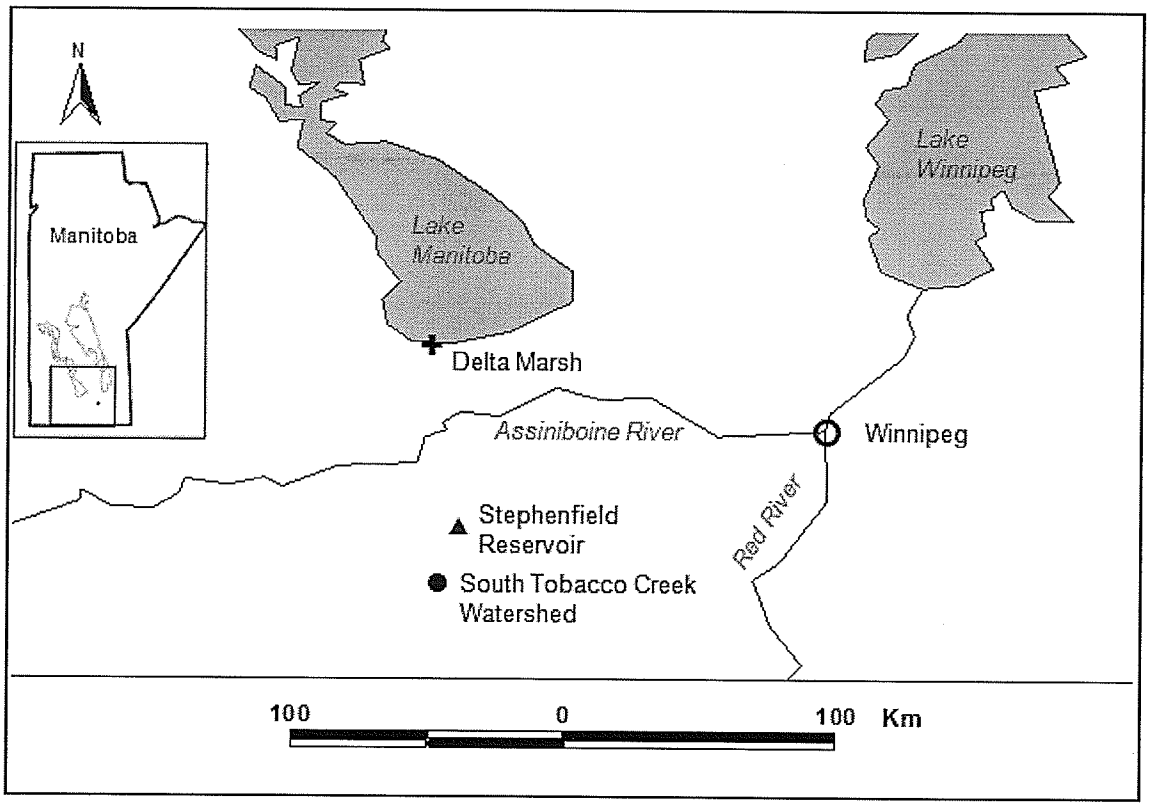


Figure 2.1 Locations of the study sites in southern Manitoba.

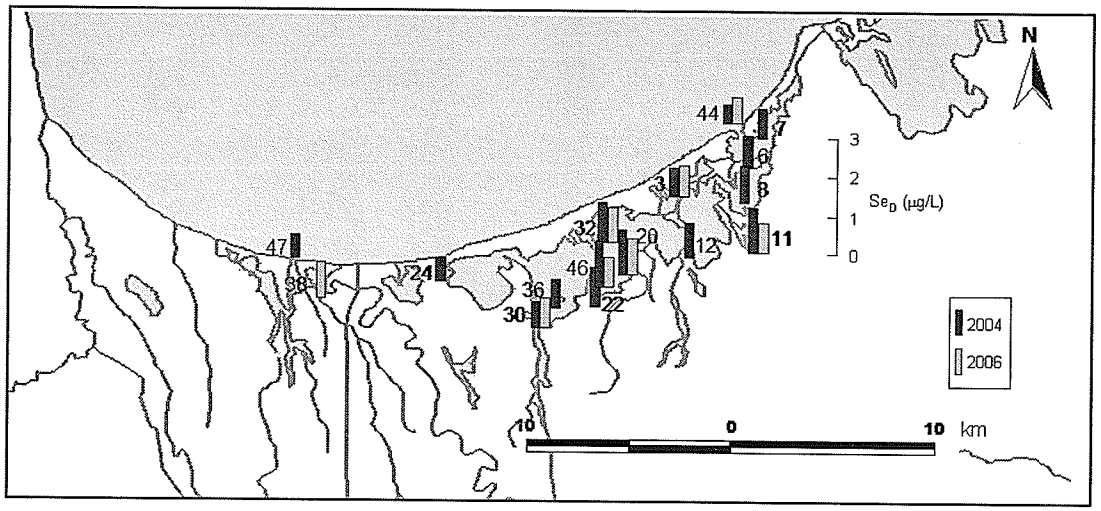


Figure 2.2 Sampling sites (numbers refer to the IDs in Table 2.4) and the dissolved Se (Se_D) concentrations in the surface water of Delta Marsh.

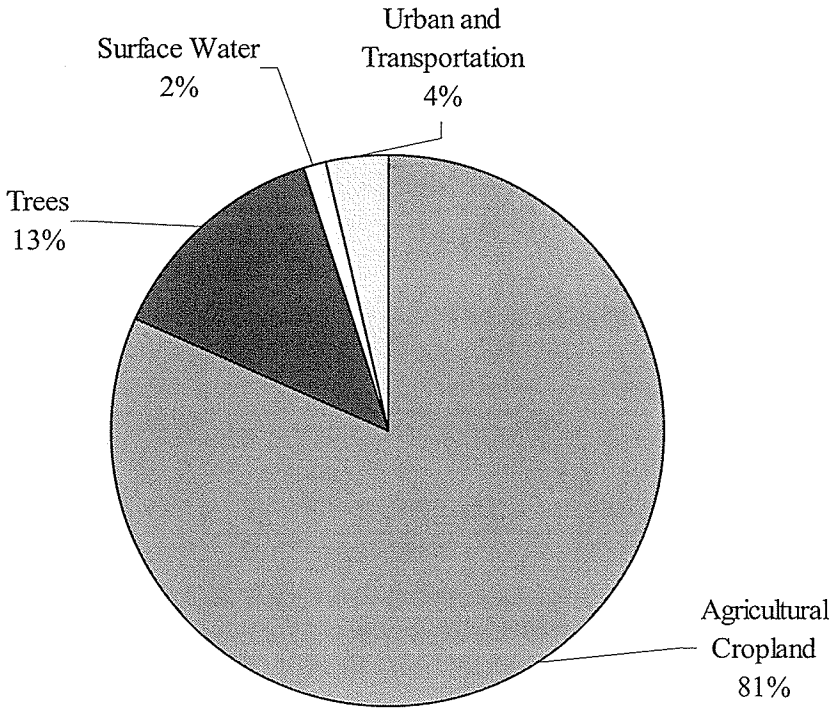


Figure 2.3 Land use at the Stephenfield Lake watershed. (Data were derived from Wood, 2005).

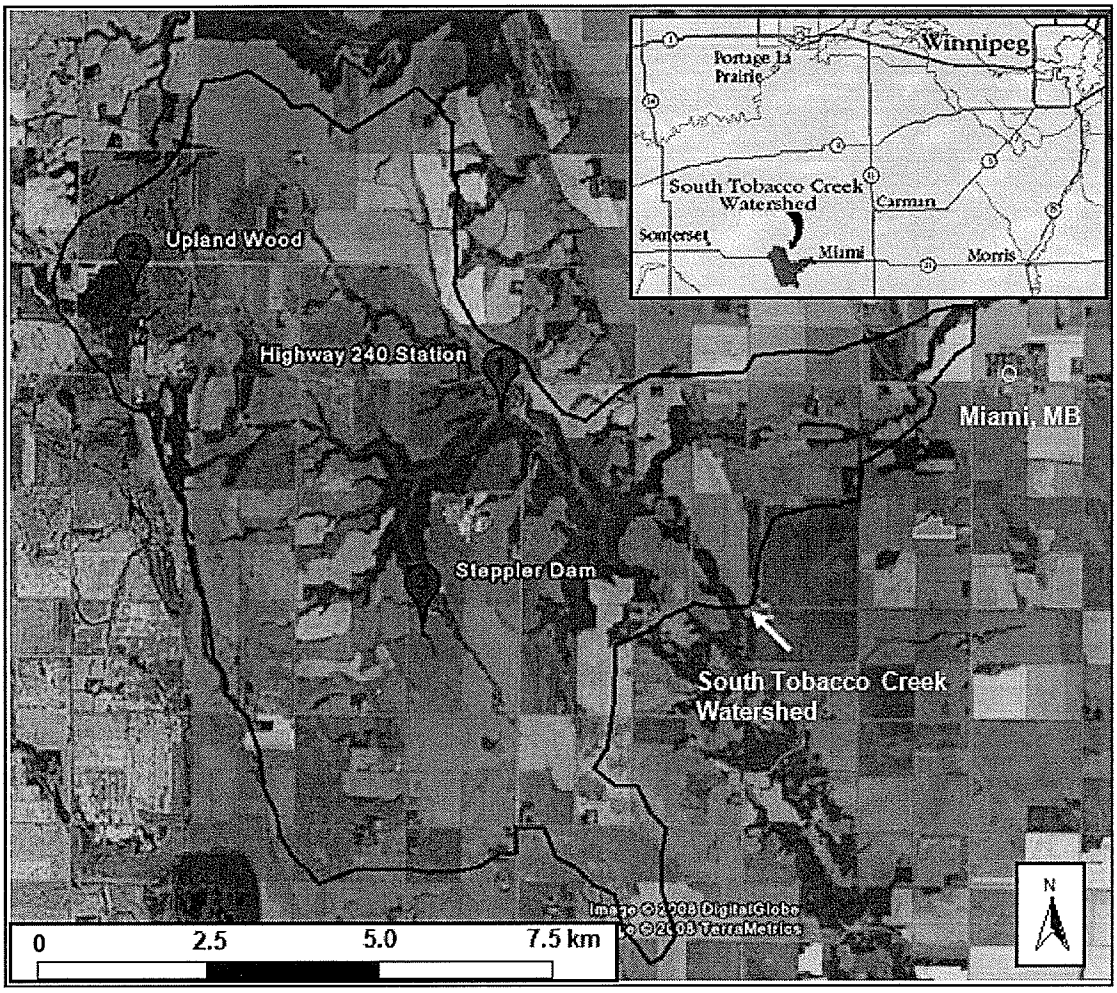
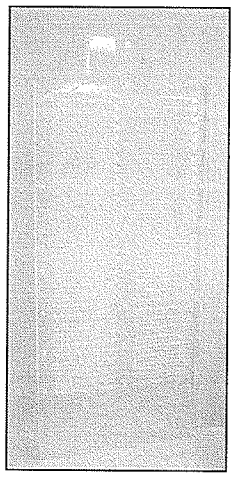
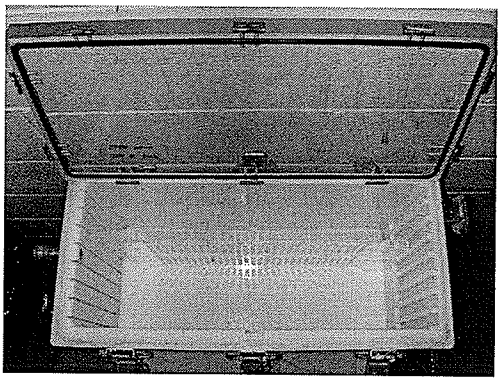


Figure 2.4 Locations of the sampling sites (STC 1-3) at the South Tobacco Creek Watershed.

A.

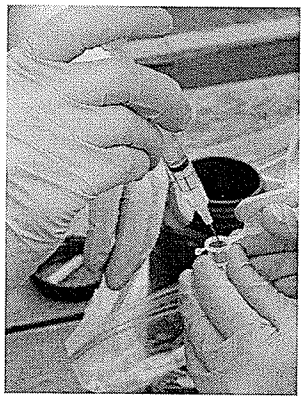


B.



C.

D.



E.

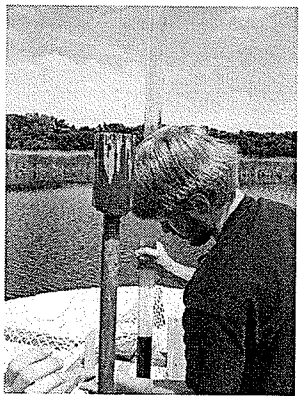


Figure 2.5 Pictures showing porewater and sediment sampling: A) the *in situ* sediment porewater sampler ("peeper"); B) A peeper in the degassing box to be degassed; C) Peeper retrieval; D) Peeper sampling; E) Retrieval of the sediment core.

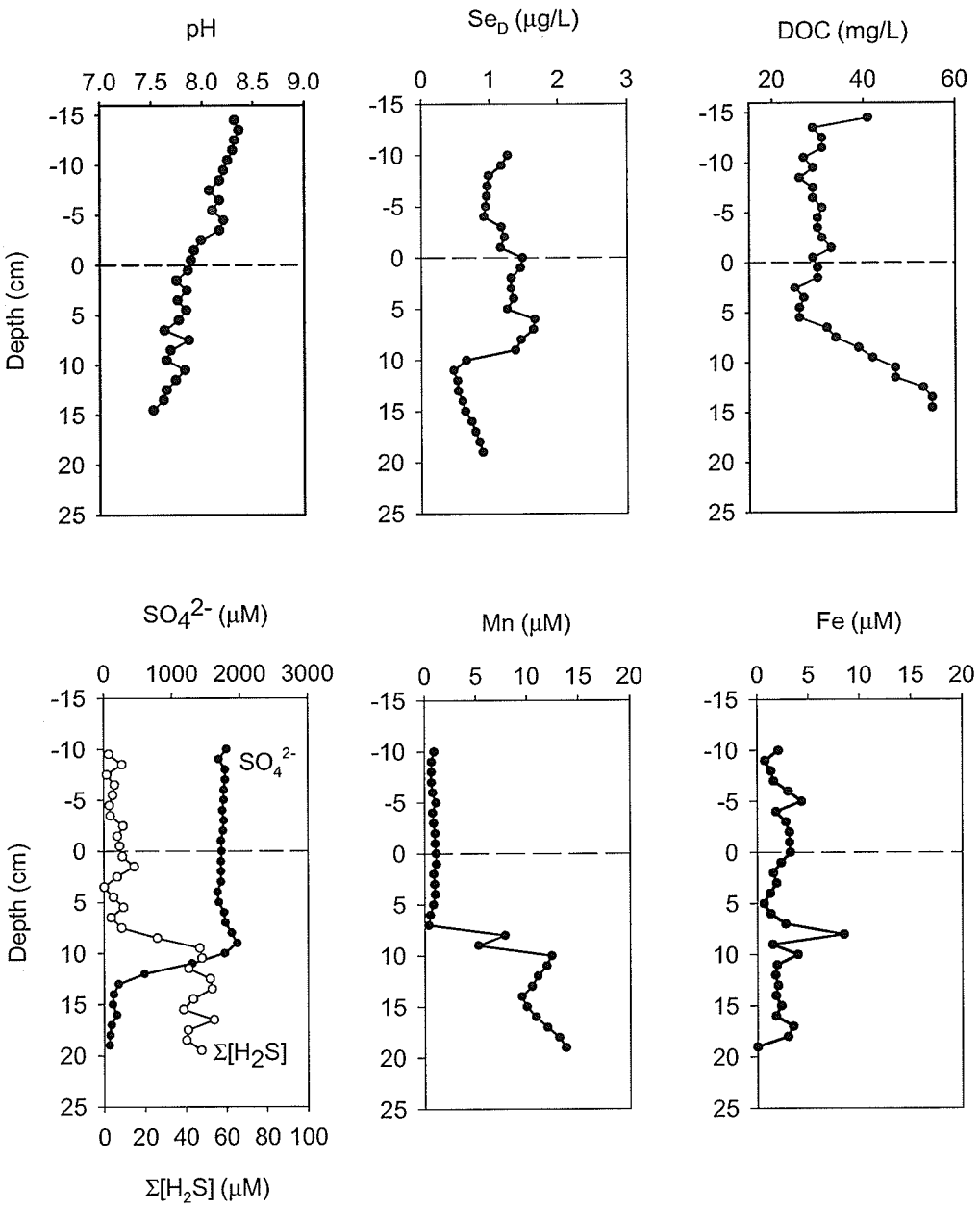
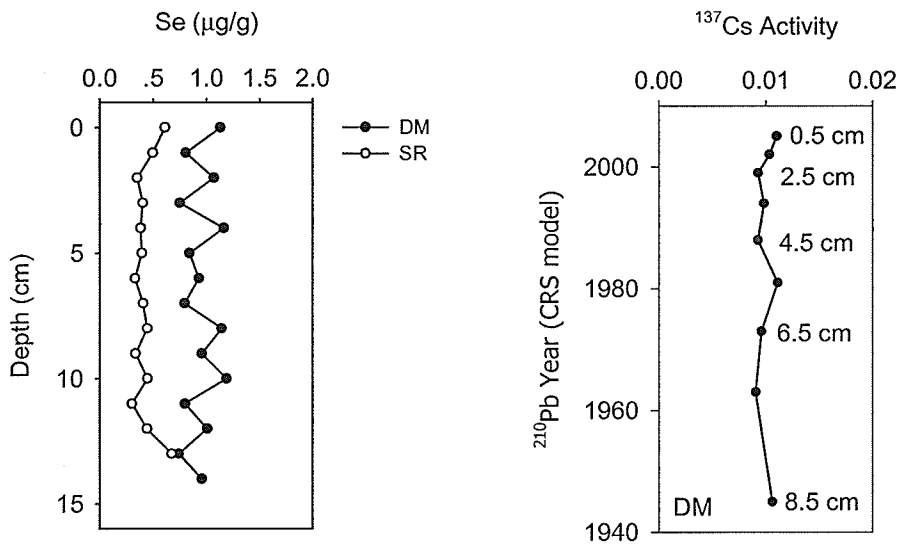


Figure 2.6 Profiles of dissolved Se (Se_D), pH, dissolved organic carbon (DOC), total sulfide, sulphate, dissolved Fe and Mn in the sediment porewaters at Forster's Bay, Delta Marsh, in October 2005. The dashed line represents the water-sediment interface.



A) B)

Figure 2.7 A) Profiles of Se_{SED} (µg/g, dry weight) in the sediment cores from Forster's Bay, Delta Marsh (DM) and Stephenfield Reservoir (SR) in July 2005. B) ^{210}Pb dated profile of the sediment core from Forster's Bay.

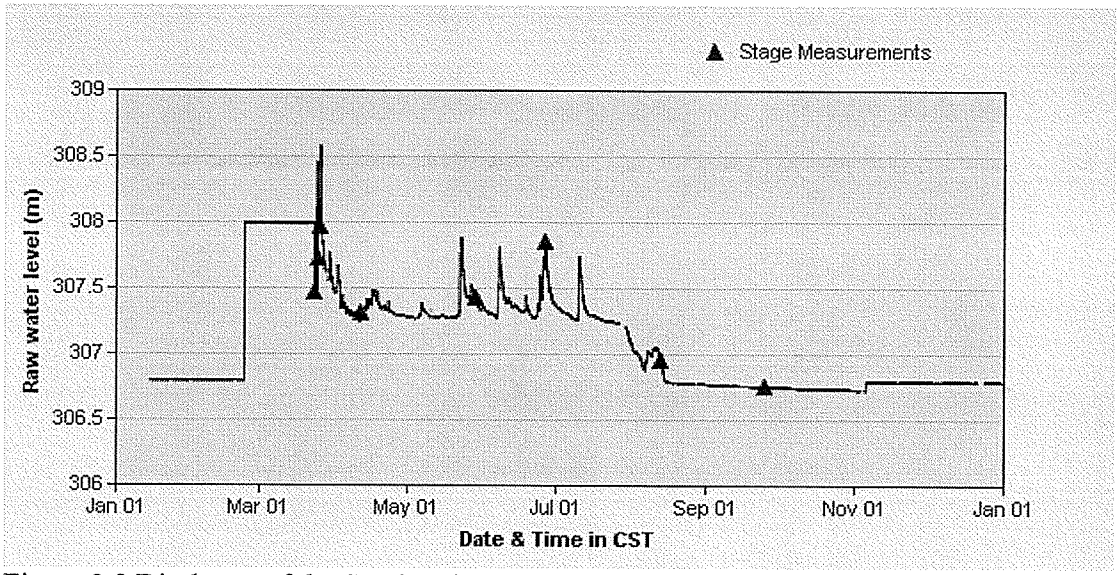


Figure 2.8 Discharge of the South Tobacco Creek Watershed at Miami (2007) (published data were from Environment Canada, Water Survey of Canada). Data of 2006 were not available.

Table 2.1 Comparison of three sampling sites in southern Manitoba.

Sampling Site	Location	Surface Area (km^2)	Maximum Depth (m)	Dominant Plants	[Se] in Surface Soil (μg/g)
Delta Marsh	*(Reference)* 50°10' N, 98°24' W	150	<1 - 3	Typha sp. Phragmites sp.	0.5 - 0.7 (Garrett, 2008)
Stephenfield Reservoir	*(Potential Se contamination caused by irrigation)* 49°31' N, 98°18' W	~ 2.5	~ 9	Typha sp. Myriophyllum sp.	0.5 - 0.7 (Garrett, 2008)
South Tobacco Creek Watershed (STC-3, Steppler Reservoir)	*(Potential Se contamination caused by agricultural activities)* 49°20' N, 98°21' W	~ 0.1	< 3	Typha sp.	0.5 - 0.7 (Garrett, 2008)

Table 2.2 Sampling locations and dates in southern Manitoba.

Sample Type	Location	Date
Surface water	Delta Marsh	July 6-7, 2005
	Stephenfield Reservoir	July 7, 2005
	Delta Marsh	October 4, 2005
	Delta Marsh	October 28, 2005
	Delta Marsh	June 19-21, 2006
	South Tobacco Creek Watershed	September 6, 2006
	South Tobacco Creek Watershed	October 12, 2006
	South Tobacco Creek Watershed	April 17, 2007
	South Tobacco Creek Watershed	July 18, 2007
Sediment porewater	Delta Marsh	Peepers in: October 4, 2005 Peepers out: October 28, 2005
Sediment cores	Delta Marsh	July 7, 2005
	Stephenfield Reservoir	July 7, 2005
Aquatic plants	Delta Marsh	July 7, 2005
	Stephenfield Reservoir	July 7, 2005

Table 2.3 ICP-MS instrument and operating conditions for the analysis of Se and other trace elements.

ICP-MS instrument	Perkin-Elmer Elan (DRC II)
Plasma conditions:	
RF power	1100 W
Plasma gas flow	15 L min^{-1}
Nebulizer gas flow	~ 0.85 L min^{-1} (optimized daily)
Dynamic Reaction Cell (DRC) settings:	
DRC gas	CH_4
DRC gas flow	0.5 mL min^{-1}
RPa	0
RPq	0.55
Cones:	
Cones used	Platinum cones
Mass spectrometer settings:	
Dwell time per (AMU)	100 ms
Sweeps/readings	10
Readings/replicates	1
Replicates	3
isotopes monitored	^{80}Se, ^{82}Se

Table 2.4 Se concentrations (Mean ± standard deviation, μg/L) in the surface water of southern Manitoba.

Sampling Sites	Jun. 2004	Jul. 2005		Oct. 2005		Jun. 2006		Sept. 2006	Oct. 2006	Apr. 2007	Jul. 2007
	Se_D [a]	Se_D	Se_T	Se_D	Se_T	Se_D	Se_T	Se_T	Se_T	Se_T	Se_T
Delta Marsh											
3. Riley Bay	0.81	-	-	-	-	0.88±0.05	0.81	-	-	-	-
6. Mid Clandeboye Bay	0.91	-	-	-	-	-	-	-	-	-	-
7. Souix Pass	0.85	-	-	-	-	-	-	-	-	-	-
8. Mid Waterhen Bay	1.04	-	-	-	-	-	-	-	-	-	-
11. St. Mark's Lake	1.24±0.07	-	-	-	-	0.83±0.01	0.71	-	-	-	-
12. Mid-east Bluebill Bay	0.93	-	-	-	-	-	-	-	-	-	-
20. South Blackfox Lake	1.24	-	-	-	-	1.00±0.02	0.81	-	-	-	-
22. Home Bay	1.05	-	-	-	-	-	-	-	-	-	-
24. Delta Channel	0.71±0.04	-	-	-	-	-	-	-	-	-	-
30. Portage Creek	0.73	-	-	-	-	0.83±0.11	0.86	-	-	-	-
32. 22 Bay	1.12	-	-	-	-	0.97±0.03	0.91	-	-	-	-
36. Mid Big Lake	0.82±0.10	-	-	-	-	-	-	-	-	-	-
38. Forster's Bay	-	0.43±0.02	0.38±0.02	0.56±0.09	0.72±0.14	0.99±0.05	0.69	-	-	-	-
41. Portage Diversion	-	-	-	-	-	-	1.54±0.33	-	-	-	-
42. East Blind Channel	-	-	-	-	-	-	0.85±0.09	-	-	-	-
44. Lake Manitoba (East)	0.53	-	-	-	-	0.76±0.01	0.74	-	-	-	-
46. Mid Simpson Bay	1.28	-	-	-	-	0.84±0.02	0.83	-	-	-	-
47. Lake Manitoba	0.70	-	-	-	-	-	-	-	-	-	-
Crescent Pond	0.73±0.18	-	-	-	-	-	0.75±0.06	-	-	-	-
Stephenfield Reservior	-	0.99±0.06	0.89±0.11	-	-	-	-	-	-	-	-
South Tobacco Creek Watershed											
STC-1. Highway 240 Station	-	-	-	-	-	-	-	2.41±0.12	1.73±0.08	6.26±0.75	3.36±0.08
STC-2. Upland Wood	-	-	-	-	-	-	-	1.08±0.05	0.87±0.04	1.95±0.31	1.27±0.09
STC-3. Steppler Reservoir	-	-	-	-	-	-	-	2.23±0.22	1.81±0.02	11.58±1.65	1.60±0.12

a. Data were from Holly Poklitar, a summer research student working in the lab in 2004.
"-" Not calculated.

Table 2.5 Se concentrations (μg/g, dry weight) in the aquatic plants (Se_{PLA}) from southern Manitoba.

Forster's Bay (Delta Marsh) Sample	Se_{PLA}	Stephenfield Reservoir Sample	Se_{PLA}
Bladderwort, *Utricularia macrorhiza*	0.03	Watermilfoil, *Myriophyllum spp.*	0.07
Coontail, *Ceratophyllum demersum*	0.06	Coontail, *Ceratophyllum demersum*	0.12
Cattail, *Typha latifolia*		Cattail, *Typha latifolia*	
Leaves	< MDL	Leaves	0.02
Roots	0.02	Roots	0.07
Bullrush, *Scirpus tabernaemontani*			
Leaves	< MDL		
Roots	0.02		

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Chapter 3. Characterizing Selenium Speciation in Natural Waters Using HPLC-ICP-DRC-MS

Abstract

A high performance liquid chromatography-inductively coupled plasma-dynamic reaction cell-mass spectrometry (HPLC-ICP-DRC-MS) method was developed for the determination of selenium speciation in natural waters. A hydroxide selective anion-exchange column, IonPac® AS18, was used as the stationary phase in combination with 23 mM KOH as the mobile phase, which allowed the isocratic separation of selenite, selenate, and several organoselenium species (e.g., selenomethionine, Se-methylselenocysteine, selenocystine) in pure water. ^{80}Se and ^{82}Se in the separated selenium species were detected on-line by ICP-MS equipped with a quadrupole dynamic reaction cell. No pre-concentration or pre-reduction step is required, which minimizes the risk of cross-contamination and alterations in speciation, and increases the sample throughput. This method was applied to a number of surface water samples from western Canada, and revealed that selenium speciation is, in general, dominated by selenate, with a small fraction being in the form of selenite. No detectable organoselenium species were found in the surface water samples analyzed.

3.1 Introduction

The chemical species of selenium (Se) can be divided into two main groups: inorganic and organic. Inorganic Se species include selenite (Se(IV)), selenate (Se(VI)), elemental selenium, and inorganic selenide (Se(-II)); hydrogen selenide (H_2Se) is the only volatile inorganic form. Organic Se species include: methylated Se compounds, selenoamino acids, selenoproteins and their derivatives. The known volatile organic forms are dimethylselenide (DMSe) and dimethyldiselenide (DMDSe). The predominant species of Se in natural fresh

waters are selenate and selenite, and organic Se species are found in the waters with high microbial activity (Xu et al., 1997; Zhang and Moore, 1996). Because those different forms of Se have different chemical reactivities, toxicities and bioavailabilities, there is a need to separate and analyze them individually rather than in total.

A variety of analytical techniques have been applied for the determination of total Se in different sample matrices. Hydride generation atomic absorption spectrometry (HGAAS) (Xu et al., 1997), graphite furnace atomic absorption spectrometry (GFAAS) (Kashiwagi *et al*, 1997) and inductively coupled plasma-mass spectrometry (ICP-MS) (B'Hymer and Caruso, 2006; Miekeley et al., 2005; Pedersen and Larsen, 1997) are most commonly used for environmental samples because of their relatively low detection limits.

Analytical methods are also available for determining the speciation of Se in water, sediment, soil, air, plants, animals and other environmental samples. Those methods typically employ hyphenated techniques, combining separation techniques and high resolution element detection techniques. High performance liquid chromatography (HPLC), capillary elctrophoresis (CE) and gas chromatography (GC) are most widely used separation techniques, which are often combined with ICP-MS and electrospray mass spectrometry (ESI-MS) for both elemental determination and molecular identification (McSheehy et al., 2002; Michalke et al., 1999; Uden, 2002).

Since total Se in natural waters is often below 1 μg/L (Nriagu, 1989; see also Chapter 2), one challenge for the analytical method is its detection limit, particularly when dealing with organoselenium species that tend to be present at much lower concentrations. A common practice for estimating organic Se species is to calculate their concentrations by subtracting the concentrations of Se(IV) and Se(VI) from the total Se concentration (Xu et al., 1997; Zhang and Moore, 1996). This is an indirect approach, may not be reliable, and could not identify and quantify individual organoselenium species. Although ESI-MS is a powerful

technique for molecular identification, its detection limit is usually 100 times higher than that of ICP-MS (Kotrebai et al., 2000), which renders it less useful in identifying organic Se species in natural waters.

The objective of this study was to develop a simple and robust analytical method for the determination of both inorganic and organic Se species in natural waters.

3.2 Methods and Materials

3.2.1 Instrumentation

The chromatographic separations were carried out using a Waters 626 non-metallic liquid chromatograph system (Waters Associates, MA, USA) at 20°C. Samples were injected manually through the Rheodyne model 9125 injector equipped with a 100 μL poly-ether-ether-ketone (PEEK) sample loop with a non-metallic pump. The separation method was adapted from the quality assurance report of IonPac® AS18 (Dionex Corporation, 2006). The analytical column was an IonPac® AS18 anion-exchange column (250mm x 4mm i.d.; Dionex, USA), with an IonPac® AG18 as a guard column. The mobile phase was a 23 mM potassium hydroxide (KOH). Injection volume was fixed at 100 μL and the mobile phase was delivered at 1 mL/min isocratically.

A Perkin-Elmer Elan ICP-MS (DRC II) (PerkinElmer, MA, USA) equipped with a quadrupole dynamic reaction cell was used to detect the Se isotopes (^{80}Se and ^{82}Se) in the effluent on-line, which was directly pumped into the quartz concentric nebulizer via PEEK tubing. Platinum sampler and skimmer cones were used. The chromatographic peaks were quantified by their peak areas calculated by Chromera 1.2 (PerkinElmer). Important instrumental parameters are summarized in Table 3.1. Nebulizer Ar gas flow, lens voltage, and auto lens calibration were optimized daily, before the speciation analysis.

3.2.2 Chemicals

All the preparation and analyses of standards and samples were done at the metal-free, Class 10-1000 Ultra-Clean Trace Elements Laboratory (UCTEL) of the University of Manitoba. Milli-Q Element ultrapure water (>18 MΩ•cm; Millipore Corporation, USA) located inside UCTEL was used as the laboratory water ("ultrapure water" hereafter) throughout the study.

Stock solutions of sodium selenite (Na_2SeO_3) (anhydrous, 99.75% min.; Alfa Aesar), sodium selenate (Na_2SeO_4) (anhydrous, SigmaUltra grade; SigmaAldrich), $_L$-Selenomethionine ($CH_3SeCH_2CH_2NH_2COOH$) (≥98%, Calbiochem, USA), $_L$-Selenocystine ($HCOONH_2CH_2SeSeCH_2NH_2COOH$) (≥98%, Aldrich), and Se-methyl-seleno-$_L$-cysteine ($CH_3SeCH_2NH_2COOH$) (purum grade, Fluka) were prepared at a concentration of 2 mg Se/L in ultrapure water and stored refrigerated. All standards were based on atomic weight of Se. Individual and mixed working standards (0.5 - 100 μg Se /L) were freshly prepared from the stock solutions. The 23 mM KOH (Certified A.C.S. grade, Fisher Scientific) mobile phase was prepared by dissolving KOH in 1 L ultrapure water was degassed before use.

3.2.3 Sampling and Sample Preparation

Surface water samples were collected in June 2007 from Delta Marsh, a typical prairie wetland, and in April 2007 from South Tobacco Creek Watershed in Manitoba, with intensive agriculture activities. They were filtered *in situ* by 0.45-μm GHP hydrophilic polypropylene membrane filters (Pall Corporation, USA) (see section 2.2.2), stored refrigerated and analyzed within one month from the sampling date. Other water samples, collected in April 2007 from coal-mining affected lakes in southwest Alberta by Dr. Alice Hontela's group at the University of Lethbridge, were stored frozen and shipped to UCTEL in dry ice. They were then stored refrigerated at UCTEL. All samples were allowed to stand in the lab to reach

room temperature before analysis. Samples were then directly injected into the LC system as described above. No preconcentration, pre-reduction, or heating, were performed.

3.3 Results

3.3.1 Separation of Se Species

Figure 3.1 shows a typical chromatogram of a mixed standard of five compounds: selenomethionine, Se-methylselenocysteine, Se(IV), Se(VI) and selenocystine at 10-15 μg Se/L. All five species were resolved, with a retention time of 4.02 min, 4.56 min, 8.45 min, 13.94 min and 16.65 min, respectively.

3.3.2 Calibration Curves and Detection Limits

Figure 3.2 shows typical calibration curves based on the peak areas for Se(IV), Se(VI), and Se-methylselenocysteine (as an example of organoselenium compounds) in ultrapure water. In all the cases the R^2 was greater than 0.999. Detection limits for Se in all species were calculated to be of 0.36 μg/L or lower, as shown in Table 3.2. They were obtained by three times the standard deviation of seven results of the lowest calibration standard.

Since organoselenium species were not at detectable concentrations in most of the water samples analyzed, subsequent efforts were focusing on Se(IV) and Se(VI) only. Figure 3.3 shows a chromatogram of a mixed standard containing Se(IV) and Se(VI) only. The entire analysis can be done within 16 minutes.

3.3.3 Se Speciation in Surface Waters from the Prairies

This HPLC-ICP-DRC-MS method was applied for speciation of Se(IV) and Se(VI) in a few natural water samples from the Canadian prairies. Figures 3.4 and 3.5 are two example chromatograms representing the Se species in surface waters from Alberta and southern

Manitoba respectively. Shown in Table 3.3, the sum of Se(IV) and Se(VI) was in good agreement with the total Se independently measured by ICP-MS (See Chapter 2). Se speciation in those surface waters were found to be dominated by Se(VI), followed by Se(IV); organic forms of Se were not at detectable concentrations.

3.4 Discussion

The IonPac® AS18 column was designed for the determination of inorganic anions and low-molecular weight organic acids (Dionex, 2003). This hydroxide-selective anion-exchange column has been shown to be capable of separating Se(IV) and Se(VI) at mg/L (ppm) levels with a gradient KOH up to 52 mM on a conductivity detector (Dionex 2003 and 2006).

By using an ICP-MS with a dynamic reaction cell, we were able to extend this method for samples with Se concentrations at μg/L (ppb,) or sub-ppb levels. Mobile phase flow rate used for the AS18 column was 1.0 ml/min and was compatible with the quartz concentric nebulizer and a standard spray chamber. However, potassium salts introduced from KOH were found to build-up at the nebulizer orifice, resulting in clogging of the nebulizer, as well as the residue left upon the sampler and skimmer cones of ICP-MS and this has been as observed before (B'Hymer and Caruso, 2006). We found that this could be improved by using a less concentrated KOH as the mobile phase, though it would prolong the retention time. Therefore, a concentration of KOH 23 mM (Dionex, 2006) was used in this study to shorten the separation within 16 minutes, although the sampler and skimmer cones need to be frequently checked and cleaned.

The anion exchange is based on the mechanism of exchange equilibria between a stationary phase containing positively charged surface ions and negatively charged ions in the mobile phase. The pH of the mobile phase containing 23 mM KOH was over 12. Under this

very basic condition, inorganic and organic species were all negatively charged, thus can be theoretically separated with anion-exchange chromatography.

By using methane gas as the reaction gas, we were able to remove the polyatomic Ar_2^+ interference and thus both ^{82}Se and ^{80}Se could be detected and used for quantification (see Figure 3.2). Since the natural abundance of ^{80}Se is much higher than that of ^{82}Se, it can greatly improve the detection limit.

This HPLC-ICP-DRC-MS method was applied to six surface water samples from end pit lakes in a mining area in Alberta, and a surface water sample from Steppler Reservoir at South Tobacco Creek Watershed in southern Manitoba. Se(VI) was found to be the major species in most of the samples except Pit 24, in which Se(IV) was dominant, indicating a relative reducing environment. No organic Se was observed from the chromatograms (eg. Figure 3.4 and Figure 3.5). This is in general agreement with literature studies on Se speciation in most spring water and tap water samples (Bueno et al., 2007; Jakubowski et al., 1996; Wang et al. 2001) (see Table 3.4).

Several studies have reported the existence of organoselenium species (by subtracting Se(VI) and Se(VI) from total Se) in waters with high organic matter and microbial productivity. For example, organoselenium was found to account for 1% - 59% of total dissolved Se species in the surface water in a wetland system in Montana (Zhang and Moore, 1996). Organoselenium up to 0.36 μg/L was reported in sediment porewaters from two lakes in the Sudbury area, ON (Belzile et al., 2000). In seawater, organic Se was found to be the dominant species at 0.12 μg/L in the upper 250 m in the Orca Basin (Takayanagi and Wong, 1985). The lack of measurable organoselenium species in the prairie waters, particularly in Steppler Reservoir, which is a prairie wetland, is thus surprising. This might be due to several analytical reasons. First, the detection limit of the present method may not be low enough to detect organic Se in these waters. Second, the organic Se species may have been oxidized to

Se(VI) during sample storage. Third, although the organic Se can be well separated in standard solutions, it may not be detectable in real samples owing to matrix effects, for example, with high content of salts. Further work is needed to improve the method for organoselenium analysis.

The method was also used for selenium speciation in the surface water and sediment porewater from Delta Marsh. Instead of Se(IV) and Se(VI), an unknown species was the only Se species found in the surface water of the pond and overlying water in mesocosms before the addition of Se(IV). By an addition test using a standard solution of Se(IV) and Se(VI), this unknown species was considered as an organic Se species. This will be further discussed in Chapter 4.

3.5 Conclusion

The HPLC-ICP-DRC-MS method is rapid, sensitive and robust for the analysis of Se(IV), Se(VI) and organic Se species (e.g., selenomethionine, Se-methylselenocysteine and selenocystine). No pre-concentration or pre-reduction step is required in this method, which minimizes the risk of cross-contamination and alterations in speciation, and increases the sample throughput. When applied to a number of surface water samples from western Canada, it showed that selenium speciation is, in general, dominated by selenate, with a small fraction being in the form of selenite, while no detectable organoselenium species were found in those surface water samples. Further development is warranted to improve the detection limits of organoselenium species.

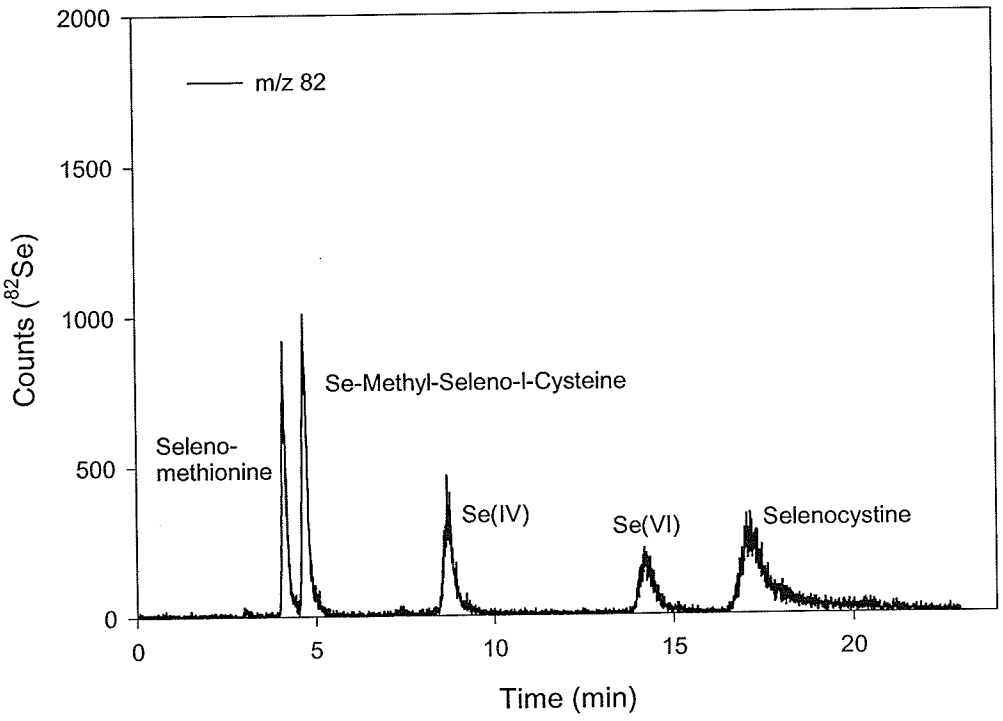

Figure 3.1 Chromatogram of a mixed standard solution containing 10-15 µg Se /L of each selenium species: selenomethionine, Se-methylselenocysteine, Se(IV), Se(VI) and selenocystine; 100 µL injected, Dionex IonPac® AS18 column, 23 mM KOH as mobile phase at 1.0 mL/min.

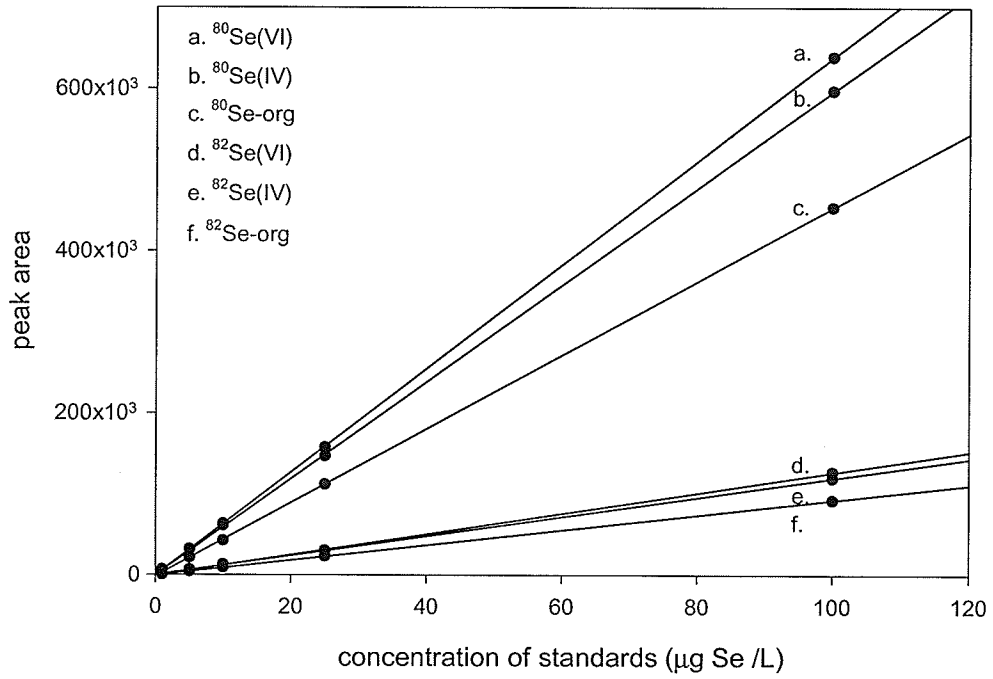

Figure 3.2 Calibration of Se(IV) and Se(VI) working standards from 0.5 µg/L to 100 µg/L; for all three species, R^2>0.9999; Se-methylselenocysteine was used as an example of organic selenium species (Se-org) that can be determined using this method.

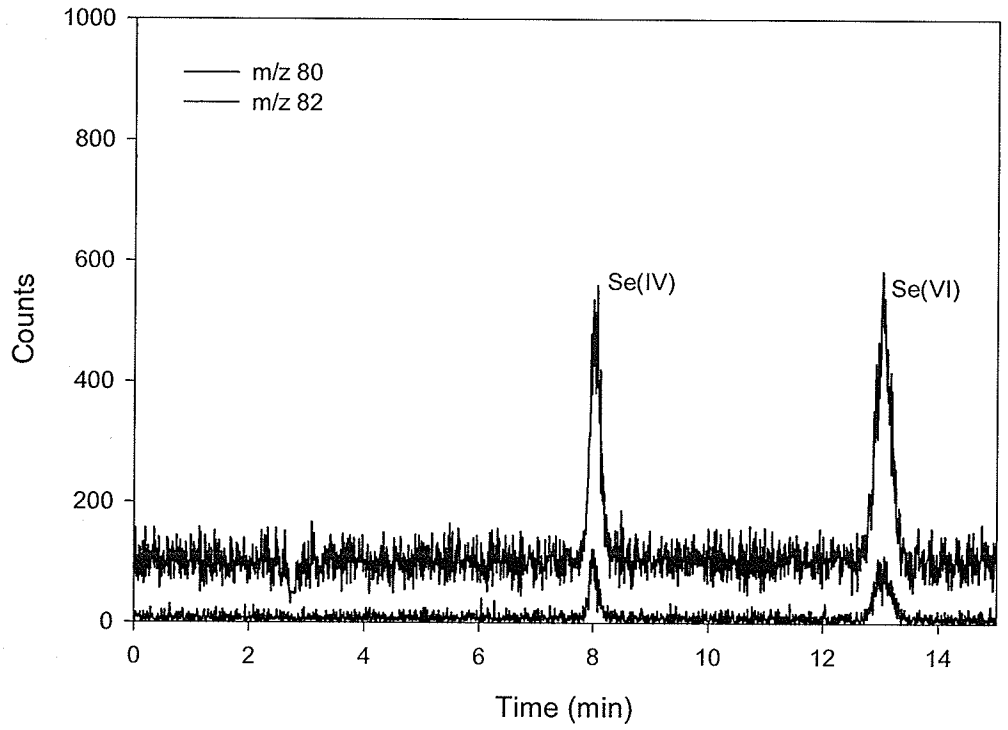

Figure 3.3 Chromatograms of 1 μg Se /L standard containing Se(IV) and Se(VI); 100 μL injected, Dionex IonPac® AS18 column, 23 mM KOH as mobile phase at 1.0 mL/min.

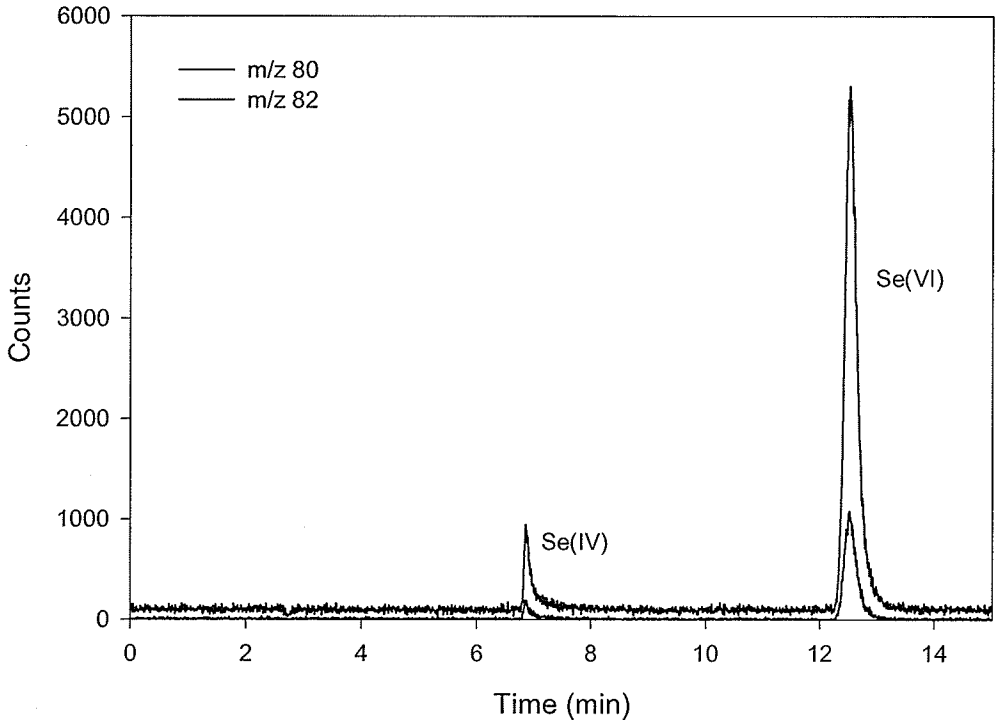

Figure 3.4 Chromatogram of surface water from End Pit Lake C4 in a mining area in Alberta, Canada.

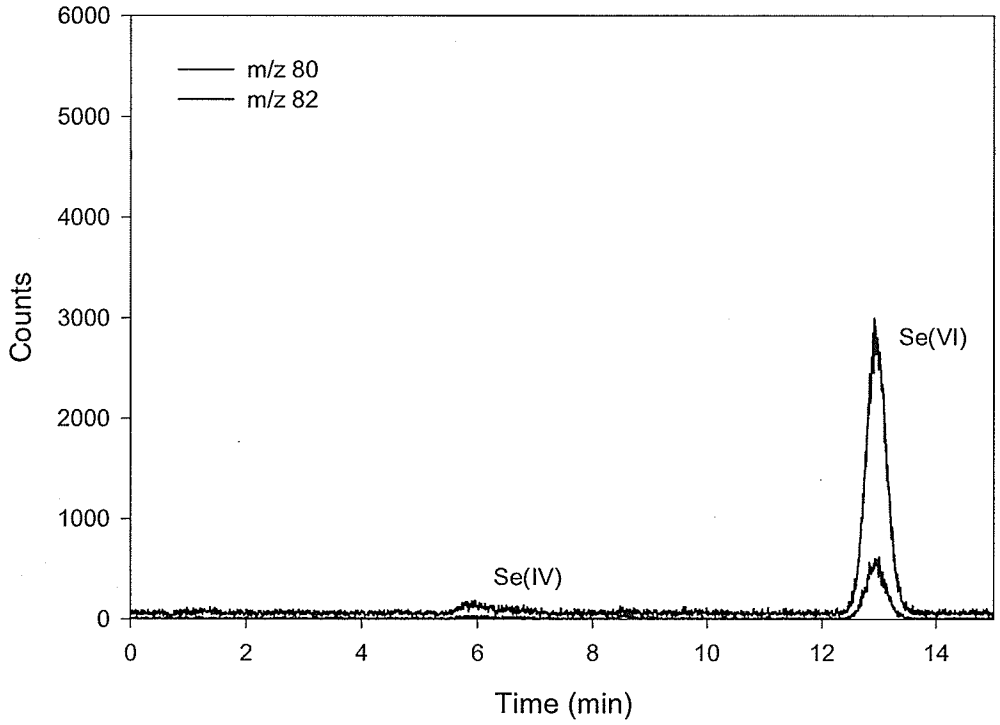

Figure 3.5 Chromatogram of surface water from Steppler Reservoir at South Tobacco Creek Watershed, Manitoba, Canada.

Table 3.1 Instrumental parameters for Perkin-Elmer Elan DRC II ICP-MS for the analysis of Se speciation in natural water samples.

Parameter	Value
Plasma conditions:	
RF power	1100 W
Ar plasma gas flow	15.0 L/min
Ar nebulizer gas flow	~ 0.85 L/min (optimized daily)
Dynamic Reaction Cell (DRC) settings:	
DRC gas	CH_4
DRC gas flow	0.5 mL/min
RPa	0
RPq	0.55
Mass spectrometer settings for Se speciation:	
Dwell time per AMU	250 ms
Sweeps/readings	10
Readings/replicates	170
Replicates	1
isotopes monitored	^{80}Se, ^{82}Se

Table 3.2 Detection limits of selenium species by HPLC-ICP-DRC-MS (corrected by the natural isotopic abundance).

Se species	^{80}Se ($\mu g\ L^{-1}$)	^{82}Se ($\mu g\ L^{-1}$)
Se(IV)	0.22	0.18
Se(VI)	0.36	0.17
Se-org*	0.32	0.29

* Se-methylselenocysteine was used as an example of organic selenium species that can be determined using this method.

Table 3.3 Selenium concentrations determined in different natural waters. (Se isotope monitored: ^{80}Se)

Sample ID	Sampling Date and Location	Total Se (μg/L)[1]	Se(IV) (μg/L)[2]	Se(VI) (μg/L)[2]	Other Se Species
Pit 24	June 7,2007, AB	0.42	0.44[3]	< MDL	not detected
Pit 44	June 7, 2007, AB	2.30	< MDL	1.87	not detected
Livingston Creek	June 1, 2007, AB	0.73	< MDL	0.51	not detected
Sphinx Lake	n/a[4]	1.41	< MDL	1.37	not detected
Pit C4	n/a	15.50	2.18[3]	10.81	not detected
Luscar Lake	n/a	21.49	1.71[3]	16.87	not detected
Steppler Reservoir	April 17, 2007, MB	11.96	< MDL	10.22	not detected

1. The method detection limit (MDL) for total Se is 5 ng/L.
2. The method detection limit (MDL) is 0.22 μg/L for Se(IV) and 0.36 μg/L for Se(VI).
3. Se(IV) in these samples was eluted at a shorter retention time than in the standards due to the matrix effect. This was confirmed by spiking with a standard with known Se species.
4. Sampling date was not specified, which probably was in May or June 2007.

Table 3.4 A comparison of analytical methods applied in Se speciation in waters.

Pre-treatment	Separation	Detection	Species[2]	Duration	Detection Limits	Reference
n/a	HPLC: IonPac® AS18, 23 mM KOH	Perkin-Elmer Elan ICP-MS (DRC II),quadrupole dynamic reaction cell	Se(IV), Se(VI), SeMet, SeCyst, Methyl-SeCys	23 min	≤0.36 µg/L (100 µL injection)	This Study
n/a	HPLC[1]: Hamilton PRPX-100, 5 mM ammonium citrate buffer (pH adjusted to 5.2) and 2% v/v methanol	Agilent 7500ce ICP-MS, with an Octopole Reaction System cell	SeCyst, Se(IV), SeMet, Se(VI)	14 min	^{80}Se 15 ng/L (100 µL injection)	Bueno et al., 2007
n/a	HPLC: Metrosep A Suppl, 3 mM cyanuric acid (pH adjusted to 10.9) and 2.5 mM perchlorate and 2% v/v acetonitrile	ELAN 5000A ICP-MS	Se(IV), Se(VI), $SeCN^-$	8.5 min	≤0.09 ng (500 µL injection)	Miekeley et al., 2005
n/a	HPLC: Hypercarb (porous graphitized carbon, 500 mM formic acid	ThermoElemental PQ-Excell ICP-MS with a hexapole collision cell	Se(IV), Se(VI)	5.8 min	≤24 ng/L (500 µL injection)	Mazan et al., 2002
n/a	HPLC: C18 Eurospher RP 100-5, 0.1M tetrabutylammonium acetate	ICP-MS (VG PlasmaQuad 2 TruboPlus)	Se(IV), Se(VI)	5 min	0.14 µg/L (200 µL injection)	Jakubowski et al., 1996
pre-reduction for Se(VI) and pre-oxidation for Se-org	n/a	HGAAS	Se(IV) Se(VI) and Se-org - calculated	n/a	0.1 ng Se	Xu et al., 1997
	HPLC: Nova-Pac® C18, acetonitrile and cyclohexane (v/v 100:2)	fluorescence spectrometer	Se-2.3-diamino-naphthalene (DAN) complex	5.1 min	0.2 ng Se	
pre-concentration (reduction) on activated carbon	n/a	square-wave voltammetry	Se(IV) and Se(VI)	n/a	10 ng/L	Bertolino et al., 2006

1. HPLC method is summarized as the anion exchange column with the mobile phase.
2. SeCyst: selenocystine; SeMet: selenomethionine; SeIV: selenite; SeVI: selenate; $SeCN^-$: selenocyanate; Methyl-SeCys: Se-methylselenocysteine.

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Chapter 4. Movement of Newly Added Se(IV) in a Typical Prairie Wetland: A Mesocosm Study

Abstract

An aquatic mesocosm study on the transport and transformation of selenium in a prairie wetland was carried out from May to August, 2007, in Crescent Pond of Delta Marsh, Manitoba. Three mesocosms were deployed in the pond, two of which were spiked with 6.3 and 15.7 μg/L ^{82}Se(IV), respectively, into the overlying water column and the third was a control. The concentration of spiked selenium in the water columns of both spiked systems decreased to 2 μg/L in 20 days after addition. It adsorbed to the sediment from the water with a rate coefficient approximately 0.1 day^{-1}. It became undetectable in the surface water after 50 days after addition. This is the first mesocosm study on the transport and transformation of selenium in prairie waters, and revealed the challenges of low detection limits, matrix effects and biological data in examining selenium under natural conditions that need to be considered in future.

4.1 Introduction

In the aquatic environment, selenium (Se) toxicity is dependent on its speciation to which biota is exposed. It is generally accepted that selenate has lower toxicity than selenite, and that inorganic Se species have lower toxicity than seleno-amino acids (e.g., selenomethionine) (Simmons and Wallschlager, 2005), which are thought to be most bioavailable to primary consumers (Riedel et al., 1991).

As shown in Chapters 2 and 3, Se concentrations in the wetlands of southern Manitoba are considered relatively low at present. However, given the projected increase in agricultural irrigation in the region (AAFC-PFRA, 2003), it is possible that Se in the surface waters could

rise in the future. Thus, it is important to study the cycling, transformation and fate of Se in those prairie systems prior to increased agricultural activities. A few models have been developed to elucidate the relative importance of various processes that may influence Se behavior in the environment. Tokunaga et al. (1997) described a mass transfer model for the transport of selenate (Se(VI)) and selenite (Se(IV)) between well-mixed surface waters and shallow sediment. Guo et al. (2000, 2001) simulated the transforming process of Se in soil using a five-compartment model that included Se(VI), Se(IV), elemental Se (Se^0), organic Se and dimethyl selenide (DMSe). Fujita et al. (2005) developed a seven-compartment model describing the fate of Se in an aquatic environment containing a water-sediment boundary.

To evaluate how prairie wetlands would respond to elevated Se input, a mesocosm experiment was carried out at Delta Marsh where stable isotope-labeled Se was added as Se(IV). This paper describes the transport and transformation of the newly added Se(IV) among various compartments, and its relevance in assessing potential ecological risk of elevated selenium in prairie ecosystems.

4.2 Methods and Materials

4.2.1 Field Site

The mesocosm study was carried out at Crescent Pond (50°10'05"N, 98°24'25"W) of Delta Marsh. Delta Marsh is a eutrophic brackish prairie wetland on the south shore of Lake Manitoba, comprising a surface area of 150 km^2 with large open bays and channels up to 3 m in depth and smaller shallow bays less than 1 m in depth (Batt, 1998). As shown in Chapter 2, the selenium concentration in the surface water of Delta Marsh was the lowest among three field sampling sites in southern Manitoba. Crescent Pond was chosen as the field site because it is a small littoral sheltered pond that is isolated from the rest of the wetland except for unusually high water-level years. The pond is surrounded predominantly by the vegetation

Typha sp. and *Phragmites* sp. (Hann, 1995). Moreover, a similar mercury addition in the form of ^{202}Hg experiment was conducted in this pond in 2003 and 2004, and as such more recent water quality parameters, such as pH, total sulfide and dissolved organic carbon, were available (Page, 2005).

4.2.2 Mesocosm Deployment

Three large polyethylene mesocosms (1.5 m diameter, 2.4 m long, with a wall thickness of 1/4 inches) were deployed in the west side of Crescent Pond on May 1, 2007. One mesocosm was used as the control with no addition of $^{82}Se(IV)$. One mesocosm received a one-time dosage of $^{82}Se(IV)$ as $Na_2{}^{82}SeO_3$ with a total Se concentration of approximately 10 times the average background level of Se (~1 μg/L; Chapter 2) at Delta Marsh. The third mesocosm received a one-time dosage of $^{82}Se(IV)$ as $Na_2{}^{82}SeO_3$ with a total Se concentration of approximately 25 times the average background level of Se at Delta Marsh.

The three mesocosms were set up in the shape of an equilateral triangle, with a distance of no greater than 3 m in between. They were hammered into the sediments as deep as possible and had about 1.3 m of the mesocosms emerging from the water column. Water depth within the mesocosms was around 80 cm from the sediment surface at the time of their deployment.

4.2.3 Preparation of the $^{82}Se(IV)$ Stock Solution

One hundred milligrams of ^{82}Se-enriched elementary Se (99.72% ^{82}Se) were purchased from Trace Sciences International (Richmond Hill, Ontario). Five Se isotopes made up the remaining 0.28% in the following abundance: ^{74}Se 0.00%, ^{76}Se 0.08%, ^{77}Se 0.02%, ^{78}Se 0.03% and ^{80}Se 0.15%. All the glassware used during the preparation were pre-cleaned in a 4 M HNO_3 acid bath, rinsed with Milli-Q Element ultrapure water (>18 MΩ•cm; Millipore

Corporation, USA; "ultrapure water" hereafter), dried under the Class10-100 laminar flow workstation at UCTEL.

Four millilitres of concentrated HNO_3 (Optima, Fisher Scientific) and 8.00 mL concentrated HCl (CMOS, J.T. Baker) were slowly added into the round-bottom flask containing 100 mg of the enriched ^{82}Se. Connected with a Vigreux distilling column, the flask was then heated to 100 °C. Once the elementary Se was completely dissolved, it was heated and refluxed for another 30 min. After cooling to room temperature, the Se(IV) solution was brought to 500 mL with ultrapure water (personal communication, Y. Chen, Laurentian University).

Five hundred millilitres of 4.88 mM sodium hydroxide (NaOH), dissolved from NaOH pellet (Certified A.C.S., Fisher Scientific), were mixed with the Se(IV) solution to make 1 L of 100 mg Se/L $Na_2{}^{82}SeO_3$ stock solution. The $Na_2{}^{82}SeO_3$ stock solution was then transferred to a pre-cleaned 1-L HDPE bottle.

The 100 mg Se/L $Na_2{}^{82}SeO_3$ stock solution was immediately checked for the concentration and speciation on HPLC-ICP-DRC-MS at the University of Manitoba Ultra Clean Trace Elements Laboratory (UCTEL). After being diluted 100 times twice, it was analyzed using the method described in Chapter 3. The results demonstrated $^{82}Se(IV)$ was the only measurable species at a concentration of 98.8 mg Se/L.

The 98.8 mg Se/L $Na_2{}^{82}SeO_3$ stock solution was transferred to two acid-cleaned 500-mL labeled polypropylene bottles in 100 mL and 250 mL respectively, which contained 9.88 mg and 24.7 mg of $^{82}Se(IV)$ correspondingly. Then the two bottles were stored refrigerated at 4°C till field addition on the next day.

4.2.4 Field Addition

Three weeks after the mesocosms were deployed in Crescent Pond, sediments were

assumed having settled from the disturbance and regained equilibrium. Treatment with $^{82}Se(IV)$ was then conducted into the mesocosms resulting in the targeted levels reported in Table 4.1. For each treatment, $Na_2{}^{82}SeO_3$ stock solution prepared on the previous day was slowly and evenly poured into the mesocosm. The bottle containing the stock solution was rinsed three times using the pond water, and the water after rinse was poured into the mesocosm as well.

4.2.5 Se and Water Quality Sampling

The pH, water level, total Se concentration and Se speciation in the surface water of Crescent Pond itself and the overlying water ("surface water" thereafter) of the three mesocosms were monitored throughout the study from May to August 2007, once per week in the first month and once per 10 - 20 days for the remainder of the study. Sediment cores were collected on the same day as sediment porewaters (peeper retrieval), which were collected after 40 days and 96 days of $^{82}Se(IV)$ addition (on June 25 and August 20, 2007, respectively). Before retrieval, peepers were allowed to stand in the sediment for 3 weeks for the equilibrium of ions inside and outside the membrane. In general, samples in different environmental compartments were collected in the sequence of surface water, sediment and sediment porewater, to minimize disturbance caused during the retrieving of peepers for sediment porewater samples. Samples were also collected in a sequence of sites with Se concentrations from low to high: the pond, Mesocosm Control, Mesocosm 10x and Mesocosm 25x ("Control", "10x" and "25x" thereafter). The pond was assumed to have similar Se levels to Control.

The pH was measured *in situ* with an Orion pH meter (Model 250) and pH electrode (Model 9107) (Thermo Fisher Scientific). The water level was estimated by vertically inserting a dry wood stick into the water until it touched the sediment, and measuring the

length of the water mark on the stick from the wet end. Surface water samples were collected following the "clean hand, dirty hand" ultraclean sampling techniques (Fitzgerald, 1999). Samples for dissolved Se (Se_D) were collected by *in situ* filtration with 0.45-μm GHP hydrophilic polypropylene membrane filters (Pall Corporation, USA). The filtration system was conditioned for five minutes before the filtrates were collected in the 15-mL acid-cleaned and pre-spiked polypropylene VWR centrifuge tubes (VWR, Canada) with 0.5% Optima HNO_3 (Fisher Scientific, Canada). For speciation analysis, the filtrates were collected in the acid-cleaned VWR tubes. The field blanks were prepared by filling the pre-spiked VWR PP tubes with water from the ultrapure water brought to the field (in 1-L amber HDPE bottles). Samples and blanks were stored in coolers with ice packs and transported to the laboratory, where they were kept refrigerated at 4°C till analysis.

One sediment core was collected from each mesocosm and also Crescent pond, following the procedure described in Chapter 2. Each section of the core was collected in a Ziploc bag, stored in coolers with ice packs and transported to the laboratory, where they were stored frozen at -24°C till analysis.

The use of dialysis samplers ("peepers") (Carignan et al. 1985; Hesslein 1976) for collecting samples of sediment porewater were described in Chapter 2. Three peepers were deployed, about 20 cm apart, in each mesocosm and also in the pond on June 1 and July 31 respectively. After 3 weeks of equilibration in the field, the peepers were retrieved individually from the sediment and sampled immediately. Samples (1 mL) for pH were collected with 1-mL polypropylene syringes (Fisher) and injected into a 4-mL amber glass vial; the pH was measured *in situ* with an Orion pH meter (Model 250) and Accumet pH electrode (Model 290) (Thermo Fisher Scientific). Samples (3 mL) for inorganic sulfide $\Sigma[H_2S]$ were collected with 3-mL polypropylene syringes (Fisher) and injected immediately through Teflon septa into N_2-purged amber glass vials containing the Cline reagents (Cline

1969). The concentrations of the Cline reagents were 0.295 M N,N-dimethyl-*p*-phenylenediamine (DMPD) and 0.222 M $FeCl_3 \cdot 6H_2O$ (120 μL of each). Samples (2 mL) for major cations and anions were collected with 3-mL polypropylene syringes (Fisher) and injected into 2-mL polypropylene centrifuge vials. Samples (3 mL) for dissolved Se (Se_D) were obtained by piercing the peeper membrane with an Eppendorf pipette fitted with acid cleaned plastic pipette tips (Fisher) and transferred into 15-mL pre-spiked Corning tubes. Samples (2 mL) for selenium species were obtained in the same way as Se_D, and were collected in acid-cleaned 2-mL polypropylene centrifuge vials. Duplicates were sampled for each item except for major ions. Three field blanks, obtained from 1-L HDPE bottle filled with ultrapure water, for $\Sigma[H_2S]$, major ions, and Se_D were treated in the same way as the samples. All the samples and blanks were stored in coolers with ice packs and transported to the laboratory, where they were stored refrigerated at 4°C till analysis.

4.2.6 Microwave Digestion for Sediment Samples

Sediment cores were digested by the CEM Microwave Accelerated Reaction System (MARS) V (CEM Corp., USA). Half gram of freeze dried samples were weighed in 100-mL Teflon XP-1500+ Vessels, and 10 mL concentrated HNO_3 (Reagent A.C.S, Fisher Scientific) were added to each vessel. If the samples contained high organic matter content, a pre-digestion was performed by leaving the HNO_3 reacting with the sample at room temperature for 10 - 15 min with the vessel open, allowing the escape of nitrogen oxide gases, to prevent extreme high pressure or explosion during digestion. The digestion procedure consisted of two stages following the recommends of CEM (CEM, 2006). In stage I, the temperature was ramped from room temperature to 165°C in 2 minutes. In stage II, the temperature was ramped from 165°C to 175°C in 3 minutes and held at that temperature for another 5 minutes. After the system was cooled down, the digestant in each vessel was

transferred to polypropylene Falcon tubes (BD Falcon, Canada), diluted 100 times with ultrapure water, and stored refrigerated untill analysis.

4.2.7 Sample Analyses

pH, $\Sigma[H_2S]$, and major ions of sediment porewaters were analyzed in the same way as described in Section 2.1.4 of Chapter 2. The determination of the concentration of Se in the porewater (Se_D) and sediments (Se_{sed}) followed the same procedure as in Section 2.2.4 of Chapter 2, and the method for Se speciation analysis was described in Chapter 3, except for the results output as the concentration of each Se isotope instead of corrected for the total Se.

4.2.8 Data Processing

The total dissolved Se concentration in a surface water or sediment porewater sample was denoted as $[Se]_D$, which was the sum of each Se isotope, consisting of the added and the background Se, denoted as $[Se]_A$ and $[Se]_B$ respectively. To understand the behavior of added Se, ^{82}Se(IV), calculations were used in the determination of added ^{82}Se from background ^{82}Se.

As the most natural abundant Se isotope and the reliable results on ICP-DRC-MS, ^{80}Se was chosen for the mass balance equations. The concentrations of ^{80}Se and ^{82}Se in the water samples were determined on ICP-MS, denoted as $[^{80}Se]_D$ and $[^{82}Se]_D$ respectively. The natural abundances of ^{80}Se and ^{82}Se were 49.8% and 8.7% respectively; while the added ^{82}Se was 99.72% enriched in ^{82}Se with 0.15% of ^{80}Se and 0.13% of other isotopes.

The mass balance equations were expressed as:

$$[^{82}Se]_D = [^{82}Se]_A + [^{82}Se]_B \quad (1)$$

$$[^{80}Se]_D = [^{80}Se]_A + [^{80}Se]_B \quad (2)$$

where $[^{82}Se]_A$ or $[^{82}Se]_B$ were the product of the abundance ratio and $[^{80}Se]_A$ or $[^{80}Se]_B$ respectively:

$$[^{82}Se]_A = R_A[^{80}Se]_A \quad (3)$$

$$[^{82}Se]_B = R_B[^{80}Se]_B \quad (4)$$

R_A was denoted as the abundance ratio of ^{82}Se to ^{80}Se in added Se and R_B was denoted as the natural abundance ratio. Then $[^{82}Se]_A$ and $[^{82}Se]_B$ in Equation (1) were replaced by Equation (3) and (4):

$$[^{82}Se]_D = R_A[^{80}Se]_A + R_B[^{80}Se]_B \quad (5)$$

$[^{80}Se]_A$ was solved by Equation (2) and (5):

$$[^{80}Se]_A = \frac{[^{82}Se]_D - R_B[^{80}Se]_D}{R_A - R_B} \quad (6)$$

Therefore, $[^{82}Se]_A$ was solved by Equation (5) and (6):

$$[^{82}Se]_A = R_A \frac{[^{82}Se]_D - R_B[^{80}Se]_D}{R_A - R_B} \quad (7)$$

Since $[^{80}Se]_D$ and $[^{82}Se]_D$ could be determined directly by ICP-DRC-MS, and R_A and R_B were calculated by known information:

$$R_A = \frac{99.72\%}{0.15\%} = 664.8$$

$$R_B = \frac{8.7\%}{49.8\%} = 0.176$$

Equation (7) was used for the calculation of the spiked ^{82}Se in the surface water and sediment porewater samples. It was also used for the correction of ^{82}Se in the sediment, where the unit of $[^{80}Se]_D$ and $[^{82}Se]_D$ was mg/kg.

4.3 Results

4.3.1 Surface Water

The pH and water level were monitored throughout this study (Table 4.2 and Figure 4.1). At the beginning of the experiment (May 16, 2007), the pH in the mesocosms was similar or slightly higher than that in the outside pond. However, obvious changes were found in pH within and outside the mesocosms as the study elapsed. By June 8, 2007, the pH of the pond water was 0.5 - 1 lower than that of the surface water in the mesocosms. Thereafter, the pH of the pond exceeded that of the mesocosms and was as high as 9.75 on August 20, 2008. This suggested that the water chemistry inside the mesocosms was much different than the pond water. In the pond water, pH rose due to photosynthetic consumption of aggressive CO_2 which shifts the carbonate buffering system as carbonate, and bicarbonate are converted to CO_2, depleting H^+. In the mesocosms, the change in pH may be due to the decreased photosynthesis and primary productivity in the microcosms by the elevated walls blocking sunlight; photosynthetic nitrate assimilation is an alkalinity generating process (Stumm and Morgan, 1996). It could also be due to the redox differences as the mesocosms seem to be less oxic than the outside pond, due to a thick layer of duckweed covering on the water surface. The water level was similar in all systems and fluctuated over the length of the study and increased 9% in the end.

After the addition to the mesocosms, ^{82}Se(IV) dissipated from the surface water rapidly. Its concentration dropped below 2 μg/L by the 20th day, and was below detectable concentration (0.18 μg/L) after 50 days in both spiked systems (Figure 4.2 A and B).

Problems were encountered when performing the Se speciation analysis by HPLC-ICP-MS. In most cases, the chromatograms of Se in the surface water and porewater in the pond and mesocosms showed only one peak (Figure 4.3 A) with a retention time different from those of Se(IV), Se(VI) and the three organoselenium species studied in

Chapter 3. Standard addition tests further confirmed that it was neither Se(IV) nor Se(VI) (Figure 4.3 B). Efforts were also taken to identify this species by ESI-MS but were not successful due to its low sensitivity. Further studies are needed to identify this unknown Se species.

4.3.2 Sediment

Total Se concentrations in the control sediment core ranged from 0.66 to 1.21 μg/g (Figure 4.4 A), which were calculated from the concentrations of ^{80}Se and its natural abundance. No obvious changes were found with depth, which was in accordance with the results in Chapter 2.

In the 10x mesocosm, spiked ^{82}Se concentrations were only at detectable concentrations (Figure 4.4 B) of less than 1.1 mg/kg in the top 0 - 3 cm of the sediment core, just below the water-sediment interface. No downward movement of spiked ^{82}Se was observed throughout the study.

In the 25x mesocosm, spiked ^{82}Se was found only in the top 0 - 2 cm of the sediment (Figure 4.4 C) after 40 days of $^{82}Se(IV)$ addition, with concentrations lower than 0.65 mg/kg. After 96 days of $^{82}Se(IV)$ addition, spiked ^{82}Se was observed consecutively in the top 0 - 4 cm of the sediment, lower than 0.31 mg/kg, and sporadically in 4 - 19 cm at trace levels, which may be a result of bioturbation.

4.3.3 Sediment Porewater

Although efforts were taken when deploying the peepers in Crescent Pond and three mesocosms in order to keep the sediment-water interface between the 5 th and 6 th cells, fluctuations occurred during two 3-week equilibration periods due to the dynamics of the sediment-water interface. The location of water-sediment interface on each peeper (the

dashed line in all porewater profiles) was thus determined by the appearance of the peeper when it was taken out from the sediment, as described in Chapter 2. The upper surface which stayed mostly in the water column was yellowish and dirty; while the lower part, which stayed in the sediment, was colorless and clean.

Figure 4.5 and Figure 4.6 showed the profiles of dissolved Mn ([Mn]), sulphate ($[SO_4^{2-}]$) and total sulfide ($\Sigma[H_2S]$) across the sediment-water interface in Crescent Pond and Control Mesocosm in June and August, 2007. In Crescent Pond, the surface water and upper layer porewaters were found to be more oxic in August (Figure 4.5 B) than in June (Figure 4.5 A). As shown in Figure 4.5 A, [Mn] in the surface water fluctuated around 20 μM, but decreased to 6.1 μM at 3.5 cm below the water-sediment interface. It fluctuated between the depth of 3.5 cm to 16.5 cm ranging from 5.9 to 9.5 μM, and gradually increased to 25 μM at a depth of 23.5 cm. In the presence of oxygen, Mn will precipitate as manganese oxides ($MnO_2(s)$), so the relatively high [Mn] in the surface water suggests a less oxic environment. In the porewater, [Mn] was low due to the precipitation as MnS. $[SO_4^{2-}]$ gradually decreased from 834 to 70 μM between the depth of -4 cm and 1 cm (a negative depth value indicates a position above the sediment-water interface), however, it increased between the depth of 1 cm and 4 cm with a maximum value around 550 μM at 3 and 4 cm, and then decreased and fluctuated between the depth of 5 cm to 20 cm ranging from 50 to 80 μM. $[SO_4^{2-}]$ increased again between the depth of 21 cm to 24 cm ranging from 280 to 340 μM. $\Sigma[H_2S]$ was higher than 726 μM in the surface water, increased to 947 μM at the water-sediment interface and gradually decreased to 290 μM in the porewater in depth of 24 cm. Different shapes of these profiles were found in August, shown in Figure 4.5 B. [Mn] remained almost constant, at values slightly lower than 10 μM. $[SO_4^{2-}]$ was around 1 mM between the depth of -4.5 cm and 3.5 cm, while $\Sigma[H_2S]$ was almost not detectable; when $\Sigma[H_2S]$ fluctuated between 678 and 835 μM below the depth of 11.5 cm, $[SO_4^{2-}]$ was found to be lower than 126 μM. As

shown in Figure 4.6 B, $\Sigma[H_2S]$ increased with depth in the surface water from 390 μM to 1.1 mM, which was 1.5 cm above the water-sediment interface, and fluctuated between 0.85 and 1.2 mM in the porewater. This suggests a very anoxic condition in the sediment of the control mesocosm. In the 10x and 25x mesocosm, the profiles of [Mn], $[SO_4^{2-}]$ and $\Sigma[H_2S]$ were similar to those in Control, as shown in Figure 4.7 and Figure 4.8.

The profiles of dissolved ^{80}Se and ^{82}Se in Crescent Pond and each mesocosm are summarized in Appendix 4. Due to a series of problems, including the lack of biota data, the unknown Se species, and the abnormal high isotopic ratio of $^{82}Se/^{80}Se$, which will be discussed in next section, it is hard to interpret the transformation of Se(IV). Therefore, for the rest of this chapter we will limit our discussion on the removal of Se(IV) in the surface water, and possible improvements for the mesocosm study in future.

4.4 Discussion

4.4.1 Redox Conditions

In the mesocosms, no obvious trend in pH with time or depth was found between the surface water and sediment porewater. It was typically in the range of 7 - 8, which was similar to the results of a previous mesocosm study at the same site in September 2003 (Page, 2005).

$\Sigma[H_2S]$ in the sediment porewater in Crescent Pond is unusually high compared with most inland waters, due to the geological enrichment of gypsum and anhydrite in the region (DeVries and Wang, 2003). The previous study by Page (2005) demonstrated it was in the sub-millimolar level (approx. 0.5 mM) in the porewater of Crescent Pond in 2003 and 2004, and it was found to be even higher in this study, around 1 mM. Since there was no flow of the surface water in the mesocosms, and the water surface was covered by duckweed during the second half of the experiment, a more reduced environment was found inside ($\Sigma[H_2S] > 0.25$

mM) than outside the mesocosms ($\Sigma[H_2S]$ close to zero) in August.

4.4.2 Removal of $^{82}Se(IV)$ from the surface water

When discharged into aquatic environments, soluble Se(IV) can adsorb onto surfaces of sediment minerals and organic matter (Tokunaga et al., 1997). It can be readily reduced to Se(0) and in turn to Se(-II) by numerous bacteria, especially in anaerobic conditions (Ike et al., 2000; Maiers et al., 1998). The reduction to insoluble elemental Se makes it less biologically unavailable and have less toxicity.

The removal of spiked ^{82}Se(IV) was assumed as a first-order reaction with the mass transfer coefficient k (day^{-1}), and the kinetics was expressed as the following (Fujita et al., 2005).

$$\frac{d[^{82}Se(IV)]_A}{dt} = -k\,[^{82}Se(IV)]_A \qquad (8)$$

$$\ln[^{82}Se(IV)]_{At} = -kt + \ln[^{82}Se(IV)]_{A0} \qquad (9)$$

Equation (9) is the integral form of Equation (8). In the calculation, the initial spiked $[^{82}Se(IV)]_{A0}$ was 6.28 μg/L for 10x mesocosm and 15.7 μg/L for 25x mesocosm. The rate coefficient was fitted to a constant value, which was optimized by the linear regression line (Figure 4.9). In mesocosm10x, the calculated rate coefficient was 0.105 day^{-1}, and it was 0.093 day^{-1} in mesocosm 25x.

Similar results were obtained by Fujita et al. (2005) in Japan in a microcosm study with Se(IV) addition, in which the estimated rate coefficient was 0.19 day^{-1} in the Senri River microcosm, 0.082 day^{-1} in the Yodo River microcosm and 0.54 day^{-1} in the Yamato River microcosm. Higher adsorption rate was related to high organic matter content, which was also reported by Tokunaga et al. (1997).

Se(VI) was below the detectable concentration throughout the experiment, which

indicated the oxidation rate from Se(IV) to Se(VI) was negligible. This was reasonable in the waters of Crescent Pond due to its less oxic environment conditions.

Although the speciation of Se in the sediment was not determined in this experiment, ^{82}Se detected in the surface sediment proved the adsorption of Se(IV) from the surface water to sediment.

4.4.3 Improvements in Future

In the microcosm studies by Tokunaga et al. (1997) and Fujita et al. (2005), the initial Se concentrations were in ppm level, which were about 1000x higher than the background Se level in waters they tested. The modelling of Se chemistry characteristics at such unusually high concentration does not necessarily simulate the processes in natural waters. In this mesocosm study, the concentrations of spiked $^{82}Se(IV)$ were only up to 15 times of the background Se level, which was much closer to the natural conditions. However, a few challenges arose and improvements in sampling and analyses need to be done in future.

First of all, the relatively low spiked Se concentrations required very low detection limits for subsequent Se analyses. Second, the matrix of the water samples from Crescent Pond could be complex with high ionic strength and introduce polyatomic interference in the analysis of ^{82}Se on ICP-MS. Third, an unknown Se species was detected in both surface water and sediment porewater. Without spiked Se(IV), this species was the only form of Se in the waters, so its identification would be very important. Fourth, besides water and sediment samples, biological samples should also been collected, such as aquatic plants and animals.

4.5 Conclusion

In this mesocosm study, isotopic labelled Se(IV), up to 15.7 μg/L, was spiked into the surface water. The spiked Se(IV) was removed from the surface water with a rate coefficient

around 0.1 day^{-1}. Se(IV) was below the detectable concentration in the overlying water by 50 days after addition, and was observed in the upper few centimetres of surface sediment. This is the first mesocosm study on the transport and transformation of Se in prairie waters, and revealed the challenges of low detection limits, matrix effects and data of biota in examining Se under natural conditions need to be considered in the future.

Table 4.1 Dose of $^{82}Se(IV)$ added to each mesocosm.

Mesocosm ID *	Water** Column (m^3)	Bottle to be used	Se added $^{82}Se(IV)$ (mg)	$^{82}Se(IV)$ ($\mu g\ L^{-1}$)	Se_T ($\mu g\ L^{-1}$)
Control	1.57	-	0	0	0
10 x	1.57	Se(IV)-10	9.88	6.28	6.28
25 x	1.57	Se(IV)-25	24.7	15.70	15.70

* The background level of total Se in the surface water at Delta Marsh was around 1 µg/L.
** Based on the diameter of the mesocosm of 1.5 m, and the water depth of 0.89 m measured on the day of Se addition (May 16, 2007).

Table 4.2 pH and water level monitored throughout this study.

Sampling Date	May 16	May 24	June 1	June 8	June 15	June 25	July 12	July 31	Aug 20
Pond									
pH	8.33	8.15	8.09	8.19	8.26	8.11	8.62	9.75	8.48
T (°C)	14.4	12.1	17	14.5	22.7	20.8	20.1	>25	17.1
Depth (cm)	89	91	104.5	98,97	86	104	102	89	96.5
Control									
pH	8.75	8.99	8.73	8.01	7.85	7.82	8.05	7.93	7.8
T (°C)	14	11.3	17	13.8	21.7	20.3	18.6	>25	16.8
Depth (cm)	89	91	101	98	97	102	98	90	99
10x									
pH	8.98	9.21	9.14	8.06	7.87	7.76	7.44	7.23	7.63
T (°C)	14	11.6	17.5	13.7	21.8	20.5	19.2	>25	16.7
Depth (cm)	89	97	103	104	93	104	102	95	97
25x									
pH	8.43	8.85	8.89	7.99	7.93	7.88	7.41	7.38	7.83
T (°C)	13.9	11.4	17.7	13.7	21.7	20.7	18.7	>25	16.8
Depth (cm)	89	97	102.5	112	91	105	97	94	96

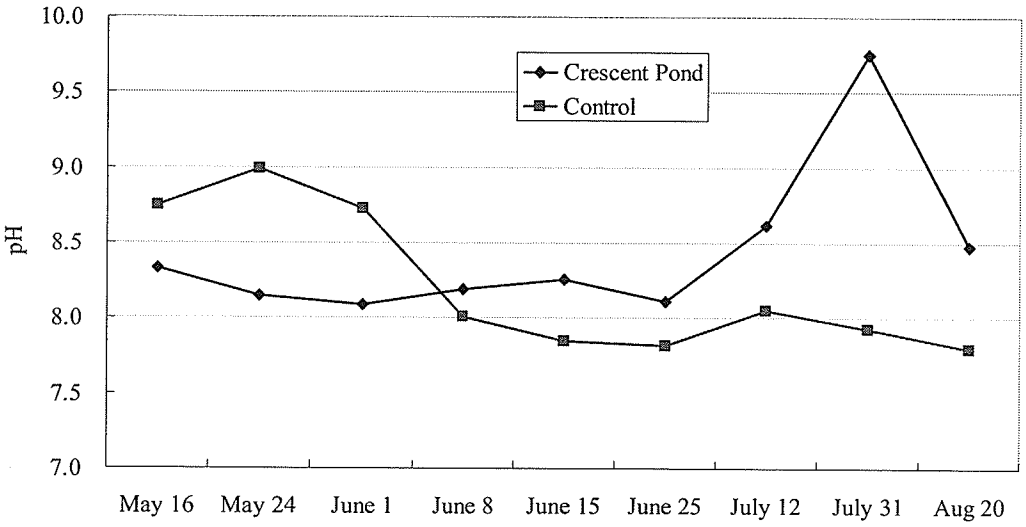

Figure 4.1 pH change in the surface water of Crescent Pond and inside Control Mesocosm from May to August, 2008 at Delta Marsh.

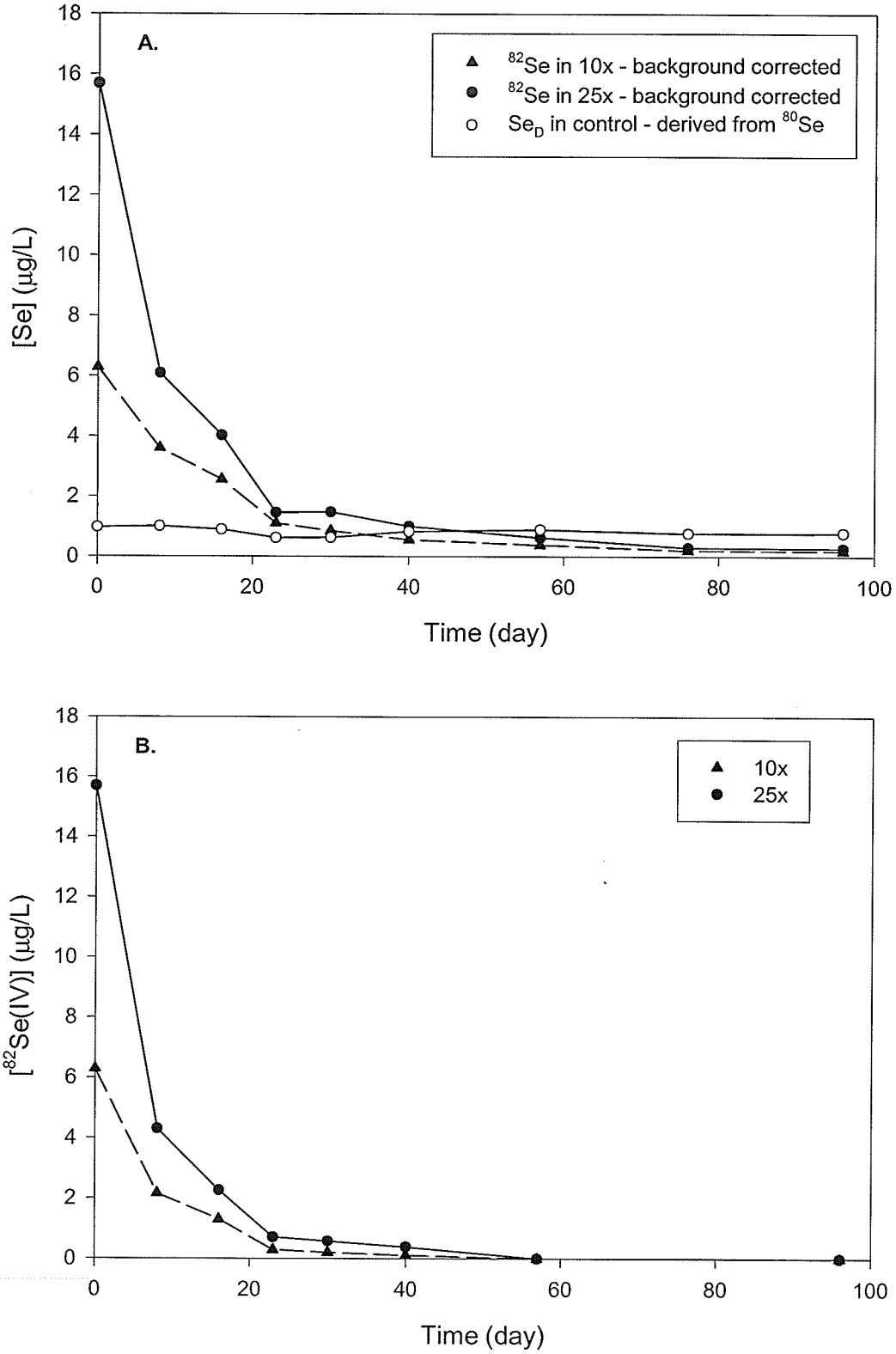

Figure 4.2 Se concentrations (A.) and added ^{82}Se(IV) (B.) in the surface water in mesocosms 10x, 25x or control changing with the time after ^{82}Se(IV) addition.

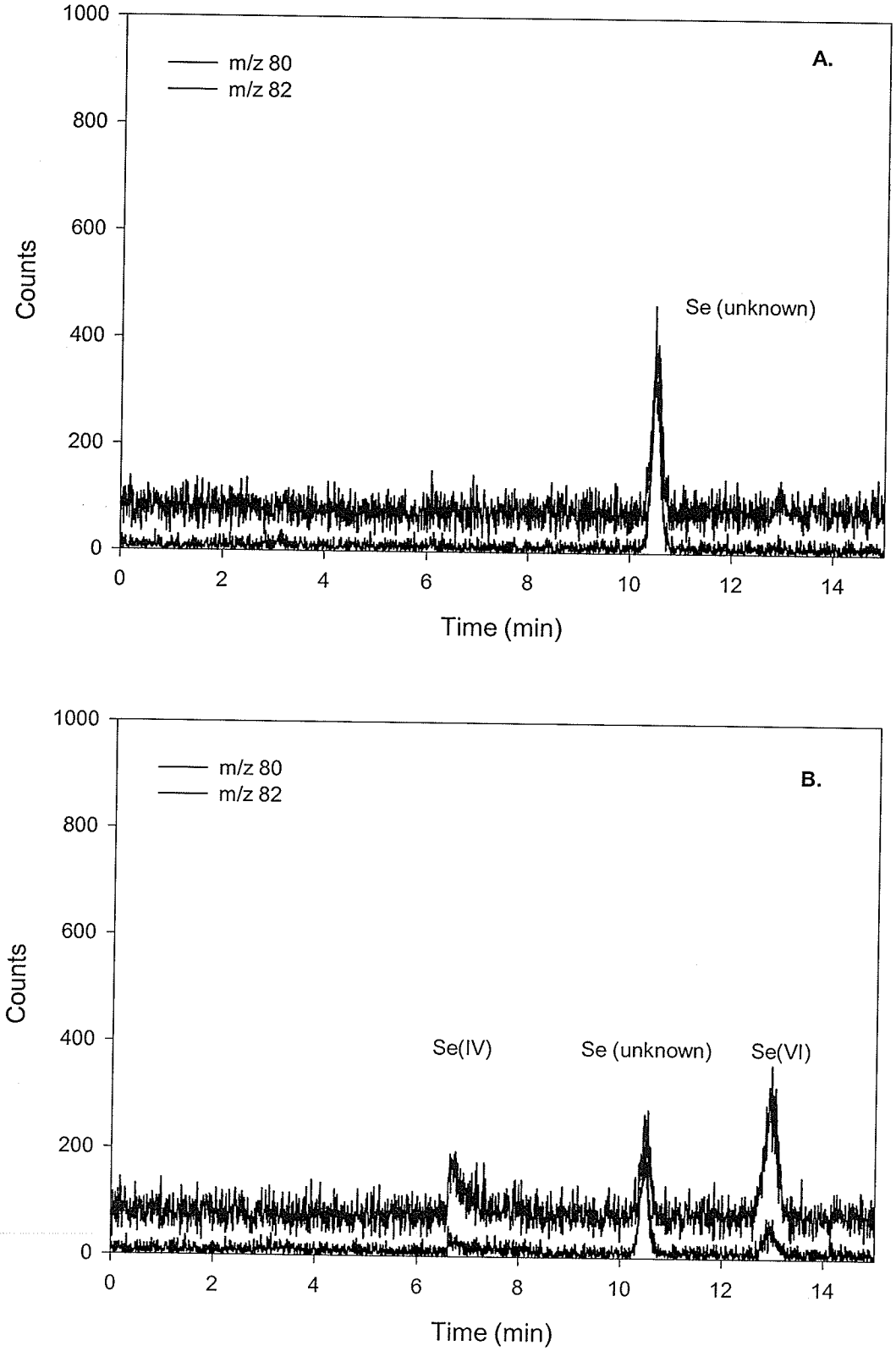

Figure 4.3 Chromatograms of Se in the surface water of Crescent Pond; an unknown Se species was detected (A) and was exclude as an inorganic species by addition test (B).

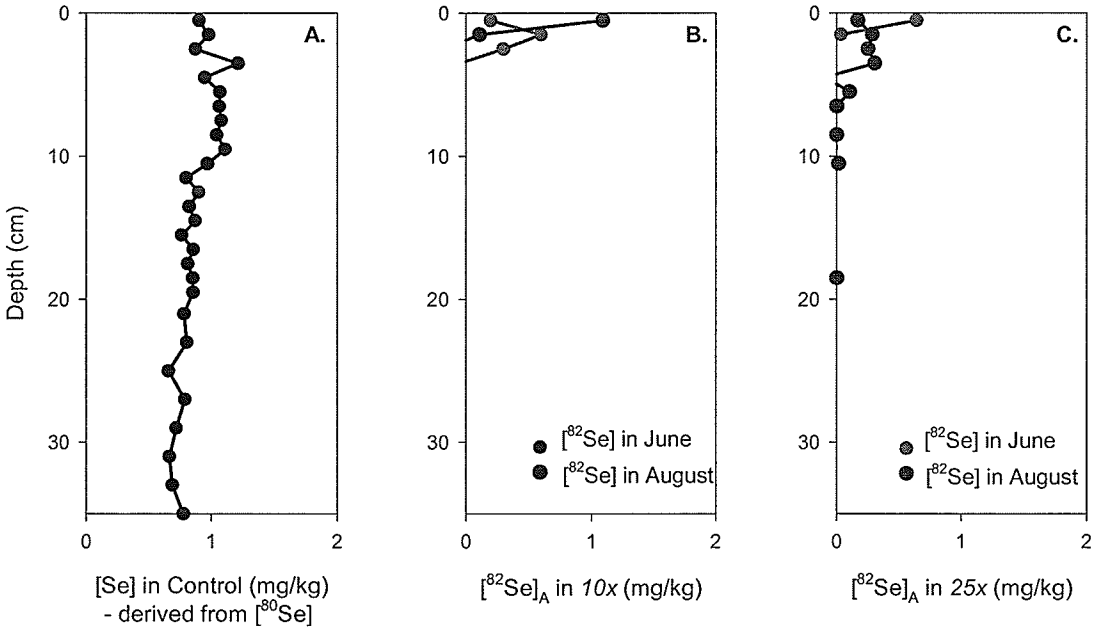

Figure 4.4 Concentrations of ^{80}Se and ^{82}Se in the sediments of (A) Control, (B) 10x, and (C) 25x mesocosms in Crescent Pond of Delta Marsh in 2007.

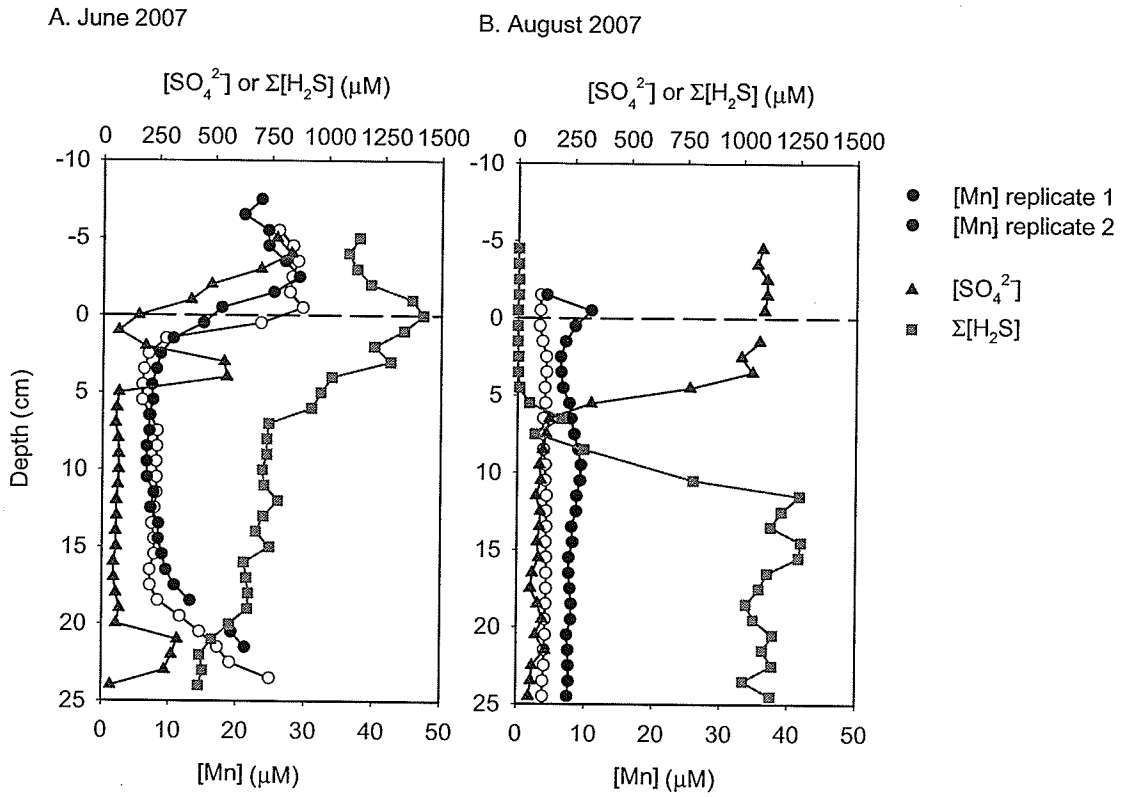

Figure 4.5 Profiles of dissolved manganese ([Mn]), sulphate ($[SO_4^{2-}]$) and total sulfide ($\Sigma[H_2S]$) in the sediment porewater of Crescent Pond in June (A) and in August (B) 2007.

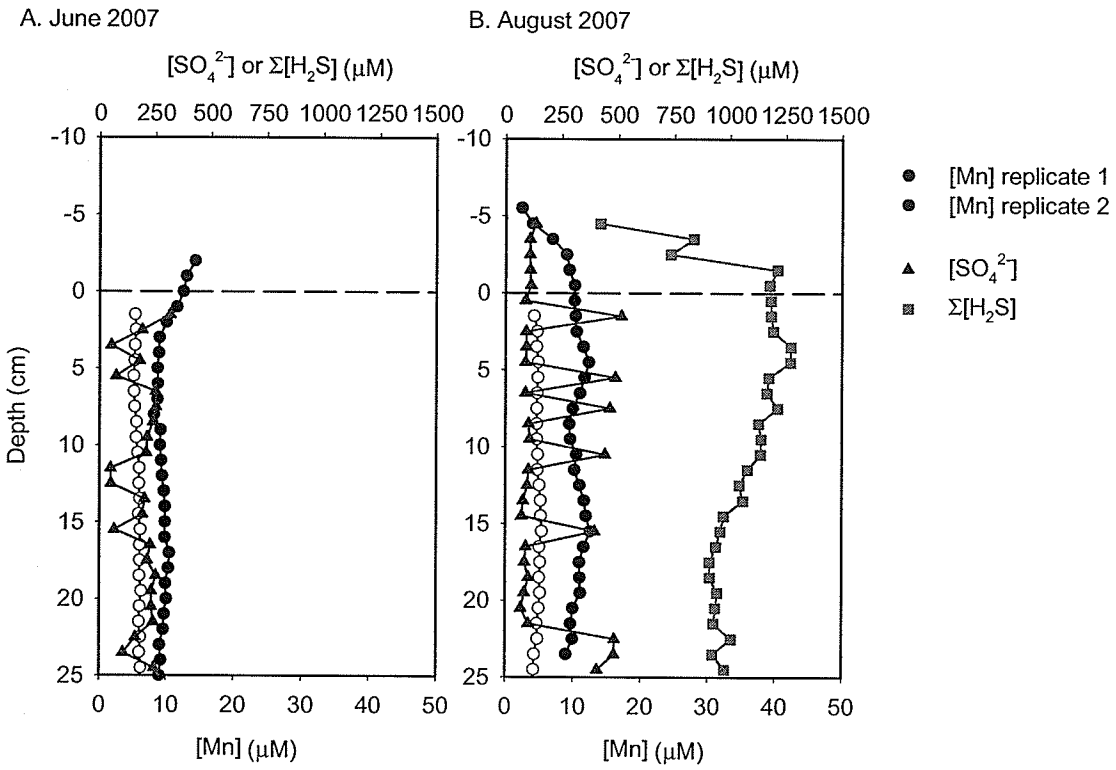

Figure 4.6 Profiles of dissolved manganese ([Mn]), sulphate ($[SO_4^{2-}]$) and total sulfide ($\Sigma[H_2S]$) in the sediment porewater of Control Mesocosm in June (A) and in August (B) 2007.

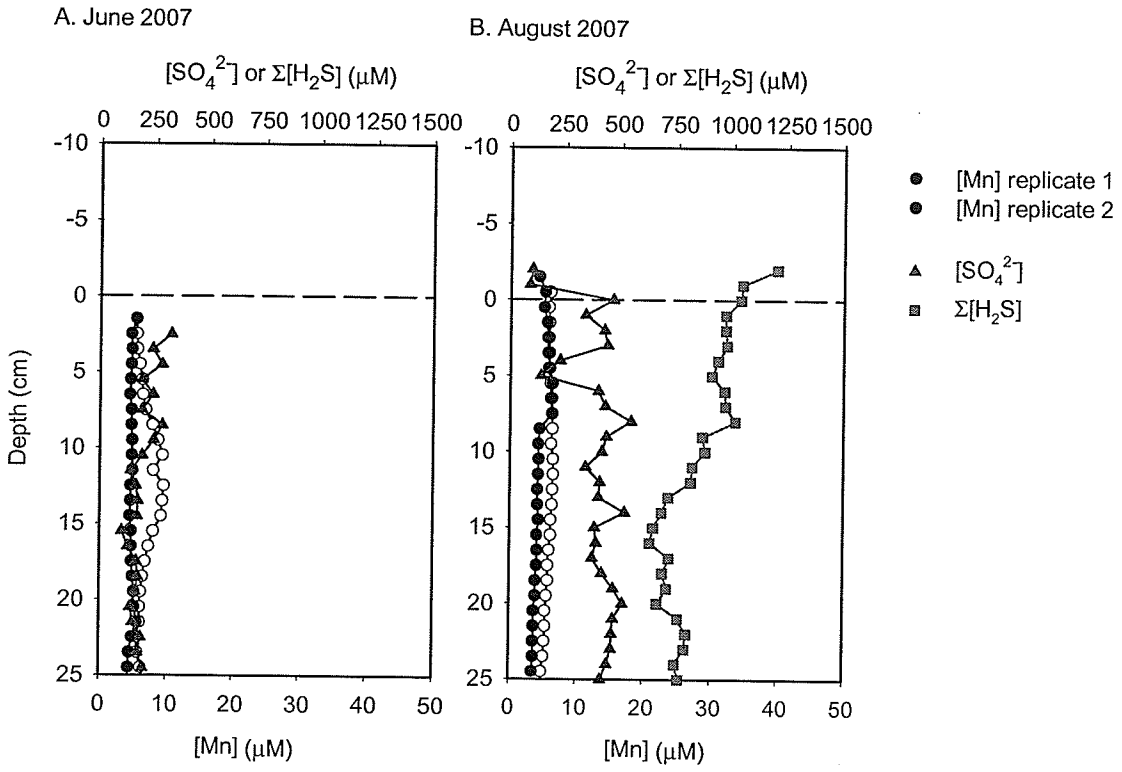

Figure 4.7 Profiles of dissolved manganese ([Mn]), sulphate ($[SO_4^{2-}]$) and total sulfide ($\Sigma[H_2S]$) in the sediment porewater of 10x mesocosm in June (A) and in August (B) 2007.

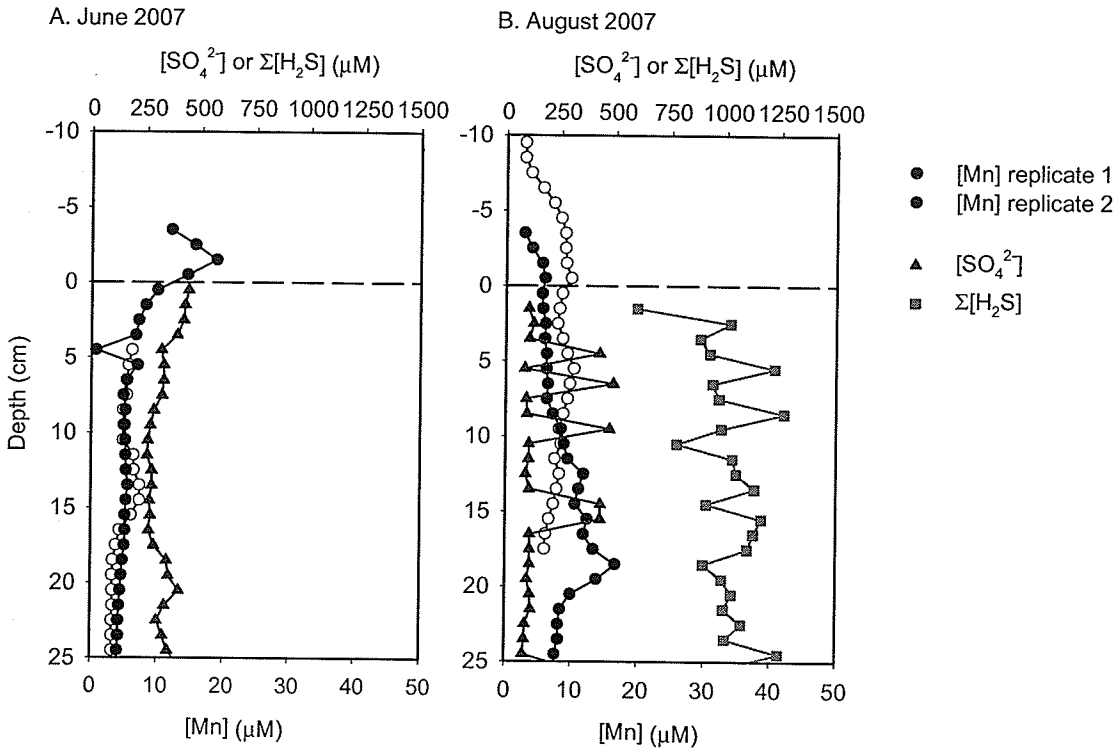

Figure 4.8 Profiles of dissolved manganese ([Mn]), sulphate ($[SO_4^{2-}]$) and total sulfide ($\Sigma[H_2S]$) in the sediment porewater of 25x mesocosm in June (A) and in August (B) 2007.

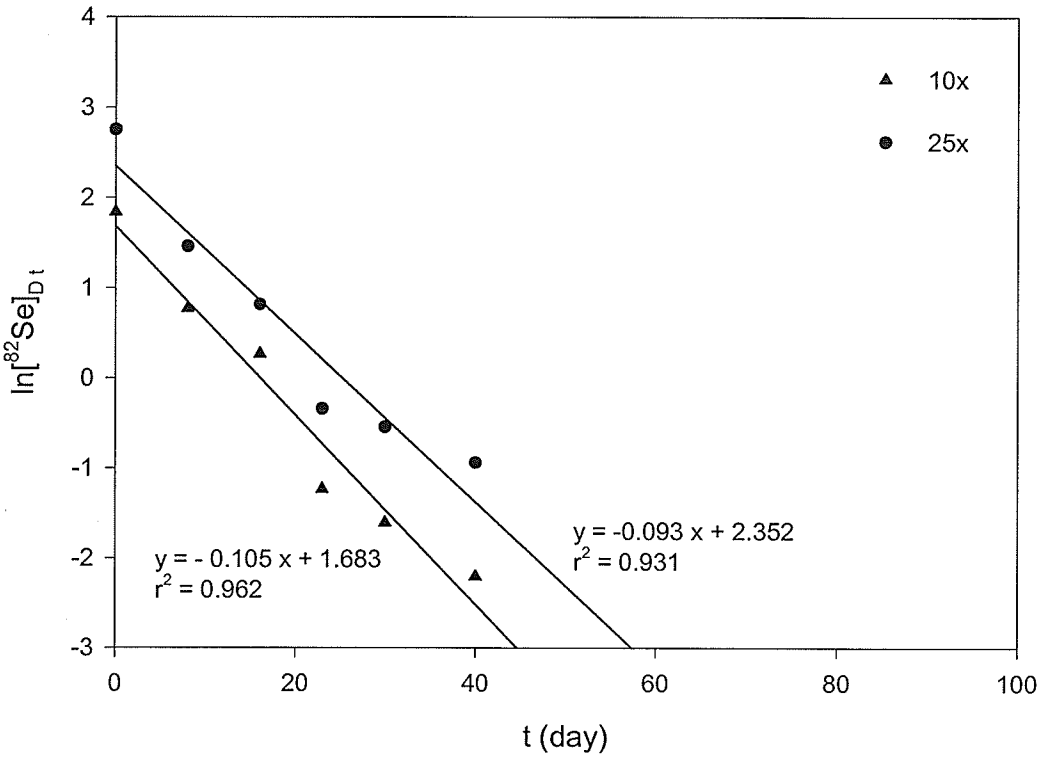

Figure 4.9 Derivation of the mass transfer coefficient (k) for the removal of Se from the overlying water in 10x and 25x mesocosms in Crescent Pond of Delta Marsh in 2007. According to the equation (9), k is the absolute value of the slope of the regression line ($\ln[^{82}Se]_{Dt}$ vs time). Therefore, in 10x mesocosm, k is 0.105 day^{-1}, and in 25x mesocosm, k is 0.093 day^{-1}.

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Chapter 5. Conclusions

The aims of this study were to (1) assess temporal and spatial variations in selenium (Se) levels in the surface water, porewater, sediment and macrophytes in southern Manitoba, (2) develop an analytical method for the analysis of Se speciation in natural waters and apply the method for prairie water samples, and (3) study the transport and transformation of selenite in prairie waters by conducting a mesocosm experiment at Delta Marsh.

Over a period of 3 years, a series of field and laboratory experiments have been carried out to address the objectives of the study as detailed in the preceding chapters. In conclusion, Se levels are relatively low at present in the prairie waters of southern Manitoba. At Delta Marsh and Stephenfield Reservoir, the Se concentrations were <1 μg/L in the surface water, < 1.3 μg/g dry weight in the sediment, and < 0.12 μg/g dry weight in the macrophytes, relatively low comparing to similar wetlands in the Prairies and in western United States. Se concentrations in the surface water, however, can be elevated (>11 μg/L) during the snowmelt season and by intensive agricultural activities such as in the South Tobacco Creek Watershed. Although sharing many similarities in geology, hydrology and ecology with those in the western United States, where elevated concentrations of Se from irrigation practices have resulted in adverse impacts on wildlife populations, in southern Manitoba a direct link between surface water Se concentration and irrigation cannot be established at this time, potentially due to the limited irrigation activities in the study areas.

However, based on literature studies on Se bioaccumulation in other regions with higher irrigation activities, continuous monitoring of Se concentrations in the prairie waters in southern Manitoba is warranted given the projected increase in irrigation and other agricultural activities in this area. A better review of the irrigation and other agricultural activities would be desirable, so that sampling sites could be selected to better represent the current status of Se levels in southern Manitoba. At South Tobacco Creek Watershed, it is

advised that the monitoring of both inter- and intra-annual variations of Se levels in the surface water be continued; sampling and analysis of Se concentrations in sediment and biota, as well as ancillary data on hydrology and other water chemistry are also needed. This can be achieved by collaborating with the Deerwood Soil and Water Management Association or other scientific organizations.

In this study, a new analytical technique was developed to determine the Se speciation in natural waters, which was applied to examine the fate of Se under elevated conditions by a mesocosm study at Delta Marsh. The HPLC-ICP-DRC-MS method developed in this study is rapid, sensitive and robust for the analysis of Se(IV), Se(VI) and organic Se species (e.g., selenomethionine, Se-methylselenocysteine and selenocystine). No pre-concentration or pre-reduction step is required, which minimizes the risk of cross-contamination and alterations in speciation, and increases the sample throughput. In the surface water samples from South Tobacco Creek Watershed, selenium speciation is dominated by selenate, with a small fraction being in the form of selenite. No detectable organoselenium species were found.

However, instead of Se(IV) and Se(VI), an unknown Se species was the only Se species detected in both surface water and sediment porewater of Delta Marsh. By an addition test using a standard solution of Se(IV) and Se(VI), this unknown species was considered as an organic Se species. It may be a metabolite of selenium due to the high productivity in the wetland. In other studies, several organic Se species indentified in natural waters are: non-volatile organic selenides, including selenoamino acids, dimethyl- and trimethyl-selenonium, and the volatile methylated forms: dimethyl selenide and dimethyl diseledide. In the future, it is crucial to indentify this Se species in the waters of Delta Marsh, to better understand Se speciation under an anoxic and sulphidic condition. This may be achieved by electrospray mass spectrometry (ESI-MS), but the challenge is that the detection

limit of ESI-MS is not low enough and a post-column may be required to concentrate Se in the water after the separation by HPLC.

To study the transport and transformation of selenite in prairie waters, three mesocosms were deployed in Crescent Pond of Delta Marsh, two of which were spiked with 6.3 and 15.7 μg/L ^{82}Se(IV), respectively, in the overlying water column. The mesocosm study indicated the spiked ^{82}Se(IV) was removed from the surface water with a rate coefficient around 0.1 day^{-1}. It was below the detection limit (0.18 μg/L) in the surface water after approximately 50 days post-addition, and was observed adsorbed onto the surface sediment.

Unlike the microcosm studies, which could be well controlled, mesocosm studies may be affected by weather conditions and bioturbation of the extensive plant root systems and invertebrates, and can only be conducted at a low Se level to be environmental relevant and responsible. To improve this mesocosm study on Se at Delta Marsh, in the future, there are a few aspects that need to be considered. First, the speciation of Se in the surface water and sediment porewater should be identified. Otherwise, the transformation of Se species cannot be concluded. Since Crescent Pond is relatively isolated, to confirm whether this Se species is representative or not, it is necessary to collect surface waters from other sites at Delta Marsh for Se speciation analysis. Second, selenate may be another option to be spiked, and its results could be compared with those of selenite. Third, other Se isotopes could be used to label the spiked Se to increase the sensitivity of the detection method, because ^{82}Se is less abundant than some other Se isotopes. Last, although the removal of spiked ^{82}Se from the overlying water was studied, it was impossible to quantify whether all the Se lost from the surface water ended up in the sediments. Therefore, to understand the complete cycle of Se between each environmental compartment, data on Se concentration and speciation in biological samples and sediments should be collected at the same frequency as the surface waters.

Appendices

Appendix 1. Thermodynamic data and equations for the Se pe-pH diagram.

Table 1. Acid-base equilibrium constants of H_2Se, H_2SeO_3 and H_2SeO_4 at 25°C and 1 atm (Séby et al., 2001)

Description	Reaction	pK_a
H_2Se	$H_2Se + H_2O \leftrightarrow HSe^- + H_3O^+$	3.8
	$HSe^- + H_2O \leftrightarrow Se^{2-} + H_3O^+$	14
H_2SeO_3	$H_2SeO_3 + H_2O \leftrightarrow HSeO_3^- + H_3O^+$	2.70
	$HSeO_3^- + H_2O \leftrightarrow SeO_3^{2-} + H_3O^+$	8.54
H_2SeO_4	$H_2SeO_4 + H_2O \leftrightarrow HSeO_4^- + H_3O^+$	-2.01
	$HSeO_4^- + H_2O \leftrightarrow SeO_4^{2-} + H_3O^+$	1.8

Table 2. Standard potentials of selenium redox couples at 25°C and 1 atm (relative to normal hydrogen electrode) (Séby et al., 2001)

Description	Reaction	E^o (V)	pe^o [b]
Se(0) / Se(-II)	$Se(s) + 2H^+ + 2e^- \leftrightarrow H_2Se(aq)$	-0.115	-1.949
	$Se(s) + H^+ + 2e^- \leftrightarrow HSe^-$	-0.227[a]	-3.847
	$Se(s) + 2e^- \leftrightarrow Se^{2-}$	-0.641[a]	-10.864
Se(IV) / Se(0)	$H_2SeO_3(aq) + 4H^+ + 4e^- \leftrightarrow Se(s) + 3H_2O$	0.74	12.542
	$HSeO_3^- + 5H^+ + 4e^- \leftrightarrow Se(s) + 3H_2O$	0.78[a]	13.220
	$SeO_3^- + 6H^+ + 4e^- \leftrightarrow Se(s) + 3H_2O$	0.903[a]	15.305
Se(VI) / Se(IV)	$HSeO_4^- + 3H^+ + 2e^- \leftrightarrow H_2SeO_3(aq) + H_2O$	1.090	18.475
	$HSeO_4^- + 2H^+ + 2e^- \leftrightarrow HSeO_3^- + H_2O$	1.008[a]	17.085
	$HSeO_4^- + H^+ + 2e^- \leftrightarrow SeO_3^{2-} + H_2O$	0.760[a]	12.881
	$SeO_4^{2-} + 4H^+ + 2e^- \leftrightarrow H_2SeO_3(aq) + H_2O$	1.139[a]	19.305
	$SeO_4^{2-} + 3H^+ + 2e^- \leftrightarrow HSeO_3^- + H_2O$	1.060[a]	17.966
	$SeO_4^{2-} + 2H^+ + 2e^- \leftrightarrow SeO_3^{2-} + H_2O$	0.811[a]	13.746

[a] Recommended values by Séby et al., 2001.

[b] pe^o is calculated by $pe^o = \frac{F}{2.3RT}E^o = E^o / 0.059$ (Stumm and Morgan, 1996)

The lines in the pe-pH diagram (Figure 1.1) are characterized by:

① For the equilibrium

$$HSeO_4^- \leftrightarrow SeO_4^{2-} + H^+ \qquad pK_a = 1.8$$

$$\log\frac{[SeO_4^{2-}][H^+]}{[HSeO_4^-]} = -pK_a \quad \rightarrow \quad \log\frac{[SeO_4^{2-}]}{[HSeO_4^-]} - pH = -1.8$$

② For the equilibrium

$$\frac{1}{2}HSeO_4^- + 3H^+ + e^- \leftrightarrow \frac{1}{2}H_2SeO_3(aq) + \frac{1}{2}H_2O \qquad pe^o = 18.475$$

$$pe = pe^o + \log\frac{[HSeO_4^-]^{1/2}[H^+]^{3/2}}{[H_2SeO_3(aq)]^{1/2}} = 18.475 + \frac{1}{2}\log\frac{[HSeO_4^-]}{[H_2SeO_3(aq)]} - \frac{3}{2}pH$$

③ For the equilibrium

$$\frac{1}{2}SeO_4^{2-} + 2H^+ + e^- \leftrightarrow \frac{1}{2}H_2SeO_3(aq) + \frac{1}{2}H_2O \qquad pe^o = 19.305$$

$$pe = pe^o + \log\frac{[SeO_4^{2-}]^{1/2}[H^+]^2}{[H_2SeO_3(aq)]^{1/2}} = 19.305 + \frac{1}{2}\log\frac{[SeO_4^{2-}]}{[H_2SeO_3(aq)]} - 2\,pH$$

④ For the equilibrium

$$\frac{1}{2}SeO_4^{2-} + \frac{3}{2}H^+ + e^- \leftrightarrow \frac{1}{2}HSeO_3^- + \frac{1}{2}H_2O \qquad pe^o = 17.966$$

$$pe = pe^o + \log\frac{[SeO_4^{2-}]^{1/2}[H^+]^{2/3}}{[HSeO_3^-]^{1/2}} = 17.966 + \frac{1}{2}\log\frac{[SeO_4^{2-}]}{[HSeO_3^-]} - \frac{3}{2}pH$$

⑤ For the equilibrium

$$\frac{1}{2}SeO_4^{2-} + H^+ + e^- \leftrightarrow \frac{1}{2}SeO_3^- + \frac{1}{2}H_2O \qquad pe^o = 13.746$$

$$pe = pe^o + \log\frac{[SeO_4^{2-}]^{1/2}[H^+]}{[SeO_3^{2-}]^{1/2}} = 13.746 + \frac{1}{2}\log\frac{[SeO_4^{2-}]}{[SeO_3^{2-}]} - pH$$

⑥ For the equilibrium

$$H_2SeO_3(aq) \leftrightarrow HSeO_3^- + H^+ \qquad pK_a = 2.7$$

$$\log\frac{[HSeO_3^-][H^+]}{[H_2SeO_3(aq)]} = -pK_a \quad \rightarrow \quad \log\frac{[HSeO_3^-]}{[H_2SeO_3(aq)]} - pH = -2.7$$

⑦ For the equilibrium

$$HSeO_3^- \leftrightarrow SeO_3^{2-} + H^+ \qquad pK_a = 8.54$$

$$\log\frac{[SeO_3^{2-}][H^+]}{[HSeO_3^-]} = -pK_a \quad \rightarrow \quad \log\frac{[SeO_3^{2-}]}{[HSeO_3^-]} - pH = -8.54$$

⑧ For the equilibrium

$$\frac{1}{4}H_2SeO_3(aq) + H^+ + e^- \leftrightarrow \frac{1}{4}Se(s) + \frac{3}{4}H_2O \qquad pe^o = 12.542$$

$$pe = pe^o + \log\frac{[H_2SeO_3(aq)]^{1/4}[H^+]}{[Se(s)]^{1/4}} = 12.542 + \frac{1}{4}\log[H_2SeO_3(aq)] - pH$$

⑨ For the equilibrium

$$\frac{1}{4}HSeO_3^- + \frac{5}{4}H^+ + e^- \leftrightarrow \frac{1}{4}Se(s) + \frac{3}{4}H_2O \qquad pe^o = 13.220$$

$$pe = pe^o + \log\frac{[HSeO_3^-]^{1/4}[H^+]^{5/4}}{[Se(s)]^{1/4}} = 13.220 + \frac{1}{4}\log[HSeO_3^-] - \frac{5}{4}pH$$

⑩ For the equilibrium

$$\frac{1}{4}SeO_3^{2-} + \frac{3}{2}H^+ + e^- \leftrightarrow \frac{1}{4}Se(s) + \frac{3}{4}H_2O \qquad pe^o = 15.305$$

$$pe = pe^o + \log\frac{[SeO_3^{2-}]^{1/4}[H^+]^{3/2}}{[Se(s)]^{1/4}} = 15.305 + \frac{1}{4}\log[SeO_3^{2-}] - \frac{3}{2}pH$$

(a) For the equilibrium

$$\frac{1}{2}Se(s) + H^+ + e^- \leftrightarrow \frac{1}{2}H_2Se(aq) \qquad pe^o = -1.949$$

$$pe = pe^o + \log\frac{[Se(s)]^{1/2}[H^+]}{[H_2Se(aq)]^{1/2}} = -1.949 - \frac{1}{2}\log[H_2Se(aq)] - pH$$

(b) For the equilibrium

$$\frac{1}{2}Se(s) + \frac{1}{2}H^+ + e^- \leftrightarrow \frac{1}{2}HSe^- \qquad pe^o = -3.847$$

$$pe = pe^o + \log\frac{[Se(s)]^{1/2}[H^+]^{1/2}}{[HSe^-]^{1/2}} = -3.847 - \frac{1}{2}\log[HSe^-] - \frac{1}{2}pH$$

(c) For the equilibrium

$$H_2Se(aq) \leftrightarrow HSe^- + H^+ \qquad pK_a = 3.8$$

$$\log\frac{[HSe^-][H^+]}{[H_2Se(aq)]} = -pK_a \qquad \rightarrow \qquad \log\frac{[HSe^-]}{[H_2Se(aq)]} - pH = -3.8$$

(d) For the equilibrium

$$\frac{1}{6}SeO_3^{2-} + \frac{7}{6}H^+ + e^- \leftrightarrow \frac{1}{6}HSe^- + \frac{1}{2}H_2O \qquad pe^o = 8.921^1$$

$$pe = pe^o + \log\frac{[SeO_3^{2-}]^{1/6}[H^+]^{7/6}}{[HSe^-]^{1/6}} = 8.921 + \frac{1}{6}\log\frac{[SeO_3^{2-}]^{1/6}}{[HSe^-]^{1/6}} - \frac{7}{6}pH$$

[1] Since the equilibrium: $SeO_3^{2-} + 7H^+ + 6e^- \leftrightarrow HSe^- + 3H_2O$ (log k) could be expressed as the combination of two equilibria: $SeO_3^- + 6H^+ + 4e^- \leftrightarrow Se(s) + 3H_2O$ ($\log k_1$) and $Se(s) + H^+ + 2e^- \leftrightarrow HSe^-$ ($\log k_2$), $\log k = \log k_1 + \log k_2 = n_1pe^o_1 + n_2pe^o_2 = 4 \times 15.305 + 2 \times (-3.847) = 53.526$. Then $pe^o = \frac{1}{n}\log k = \frac{1}{6} \times 53.526 = 8.921$.

Appendix 2. Definition of salinity

Practical Salinity Scale for salinity as conductivity ratio with no units:

$$S = 0.0080 - 0.1692\, R_{15}^{1/2} + 25.3851\, R_T + 14.0941\, R_T^{3/2} - 7.0261\, R_T^2 + 2.7081\, R_T^{5/2} + \Delta S$$

where $R_T = C(S, T, 0) / C(KCl, T, 0)$,

$$\Delta S = [(T-15) / (1 + 0.0162\,(T-15))] + 0.005 - 0.0056\, R_T^{1/2} - 0.0066\, R_T - 0.0375\, R_T^{3/2} + 0.636\, R_T^2 - 0.0144\, R_T^{5/2}$$

For $2 \leq S \leq 42$

where C (S, T, 0) is the conductivity of the water sample at temperature T and standard atmospheric pressure, and C (KCl, T, 0) is the conductivity of the standard potassium chloride (32.4356 g of KCl in a 1 kg of solution) solution at temperature T and standard atmospheric pressure. According to this definition, a seawater sample with a conductivity ratio of 1.0 at 15 °C with the standard KCl solution has a salinity of 35.

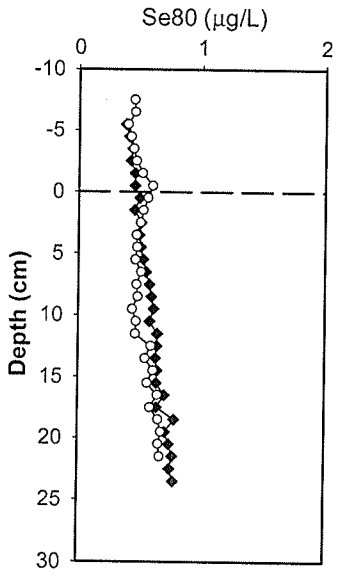
Appendix 3. ^{210}Pb and ^{137}Cs dating of the Delta Marsh sediment core.

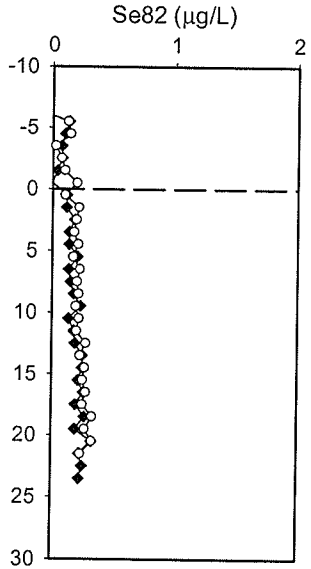
Average Depth (cm)	Excess Pb-210	Pb-210 Activity (Bq/g +/- 2SD)	Cs-137 Activity (Bq/g +/- 2SD)	Linear model		CRS Model		Sedimentation Rate ($g/m^2/yr$)
				# years/slice	Median year of deposition	# years/slice	Median year of deposition	
0.5	$1.63x10^{-2}$	$3.63x10^{-2}$ +/- $1.50x10^{-3}$	$1.10x10^{-2}$ +/- $1.83x10^{-3}$	0.5	2005	1.8	2005	2041
1.5	$1.50x10^{-2}$	$3.50x10^{-2}$ +/- $1.19x10^{-3}$	$1.03x10^{-2}$ +/- $1.41x10^{-3}$	0.8	2005	3.0	2002	2066
2.5	$1.85x10^{-2}$	$3.85x10^{-2}$ +/- $1.39x10^{-3}$	$9.26x10^{-3}$ +/- $7.47x10^{-4}$	0.8	2004	4.3	1999	1493
3.5	$1.75x10^{-2}$	$3.75x10^{-2}$ +/- $1.30x10^{-3}$	$9.79x10^{-3}$ +/- $1.35x10^{-3}$	0.8	2003	4.7	1994	1373
4.5	$2.01x10^{-2}$	$4.01x10^{-2}$ +/- $1.36x10^{-3}$	$9.24x10^{-3}$ +/- $1.07x10^{-3}$	0.9	2002	7.4	1988	990
5.5	$1.73x10^{-2}$	$3.73x10^{-2}$ +/- $1.60x10^{-3}$	$1.11x10^{-2}$ +/- $1.32x10^{-3}$	0.8	2001	6.8	1981	926
6.5	$1.26x10^{-2}$	$3.26x10^{-2}$ +/- $1.74x10^{-3}$	$9.58x10^{-3}$ +/- $1.18x10^{-3}$	1.0	2000	8.2	1973	1005
7.5	$1.28x10^{-2}$	$3.28x10^{-2}$ +/- $1.55x10^{-3}$	$9.03x10^{-3}$ +/- $7.31x10^{-4}$	1.1	1999	12.4	1963	720
8.5	$1.23x10^{-2}$	$3.23x10^{-2}$ +/- $1.66x10^{-3}$	$1.06x10^{-2}$ +/- $1.09x10^{-3}$	1.3	1998	23.7	1945	437
9.5	$1.56x10^{-2}$	$3.56x10^{-2}$ +/- $1.39x10^{-3}$	$8.26x10^{-3}$ +/- $1.04x10^{-3}$	0.9	1997			
10.5		$1.65x10^{-2}$ +/- $9.11x10^{-4}$	$5.98x10^{-3}$ +/- $7.58x10^{-4}$					
		$2.03x10^{-2}$ +/- $1.19x10^{-3}$	0					
		$2.06x10^{-2}$ +/- $1.06x10^{-3}$						
		$2.04x10^{-2}$ +/- $1.17x10^{-3}$						
		$2.19x10^{-2}$ +/- $1.19x10^{-3}$						
		$2.60x10^{-2}$ +/- $1.23x10^{-3}$						
		$2.74x10^{-2}$ +/- $1.23x10^{-3}$						

Appendix 4. Concentrations of ^{80}Se and ^{82}Se in the sediment porewaters of Crescent Pond, Control, 10x and 25x mesocosms. Two replicates were plotted for each graph.

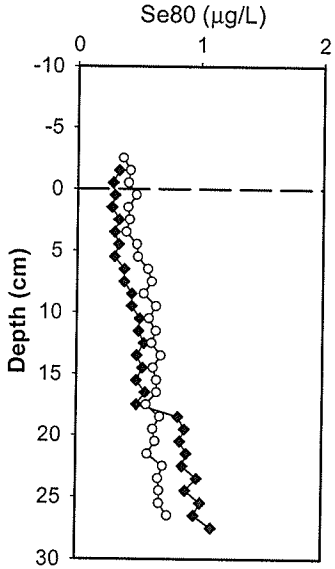
Crescent Pond

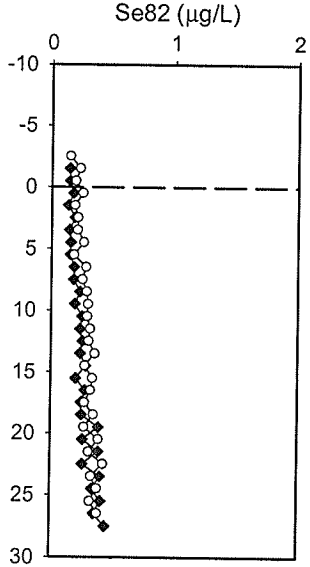
June 2007



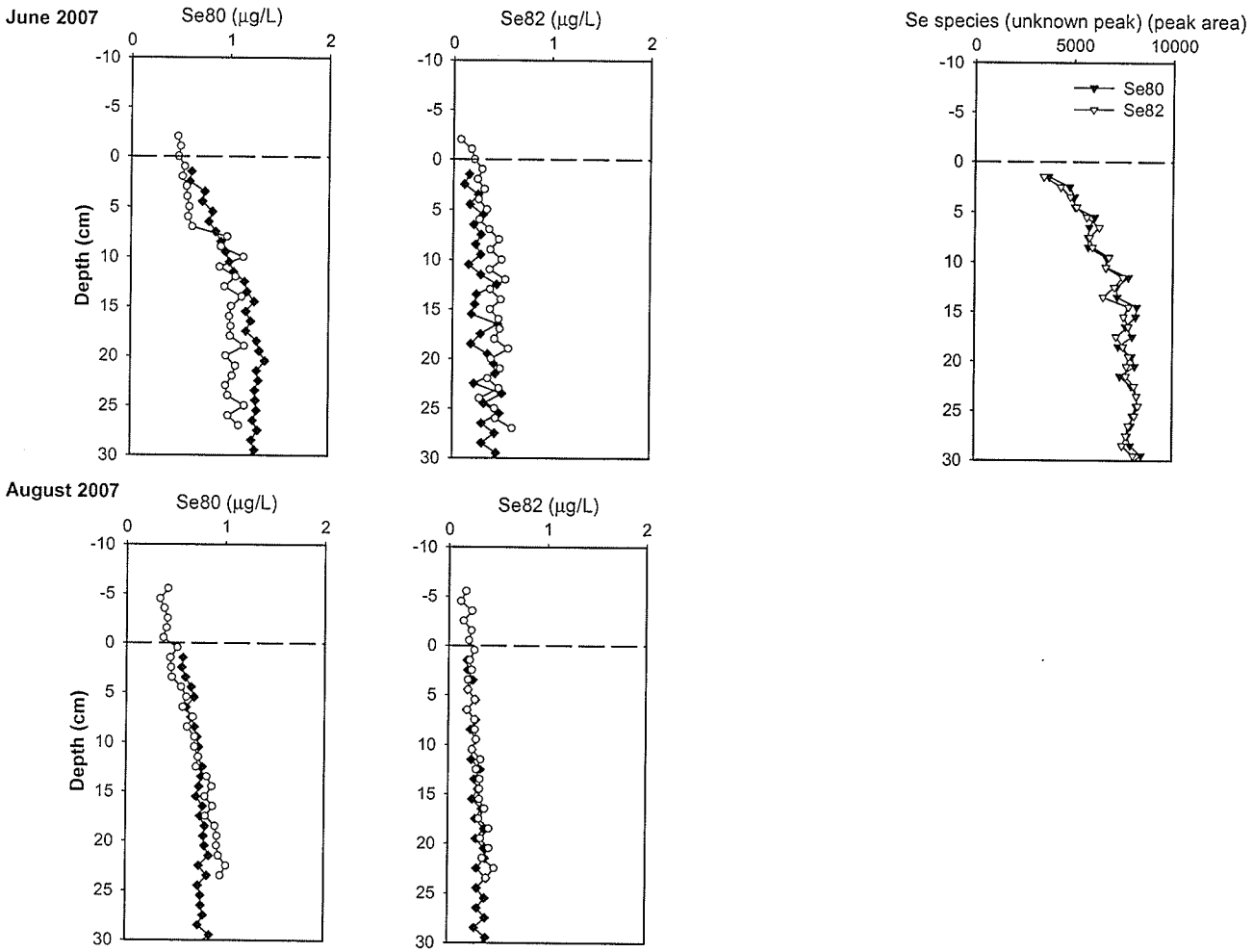


August 2007

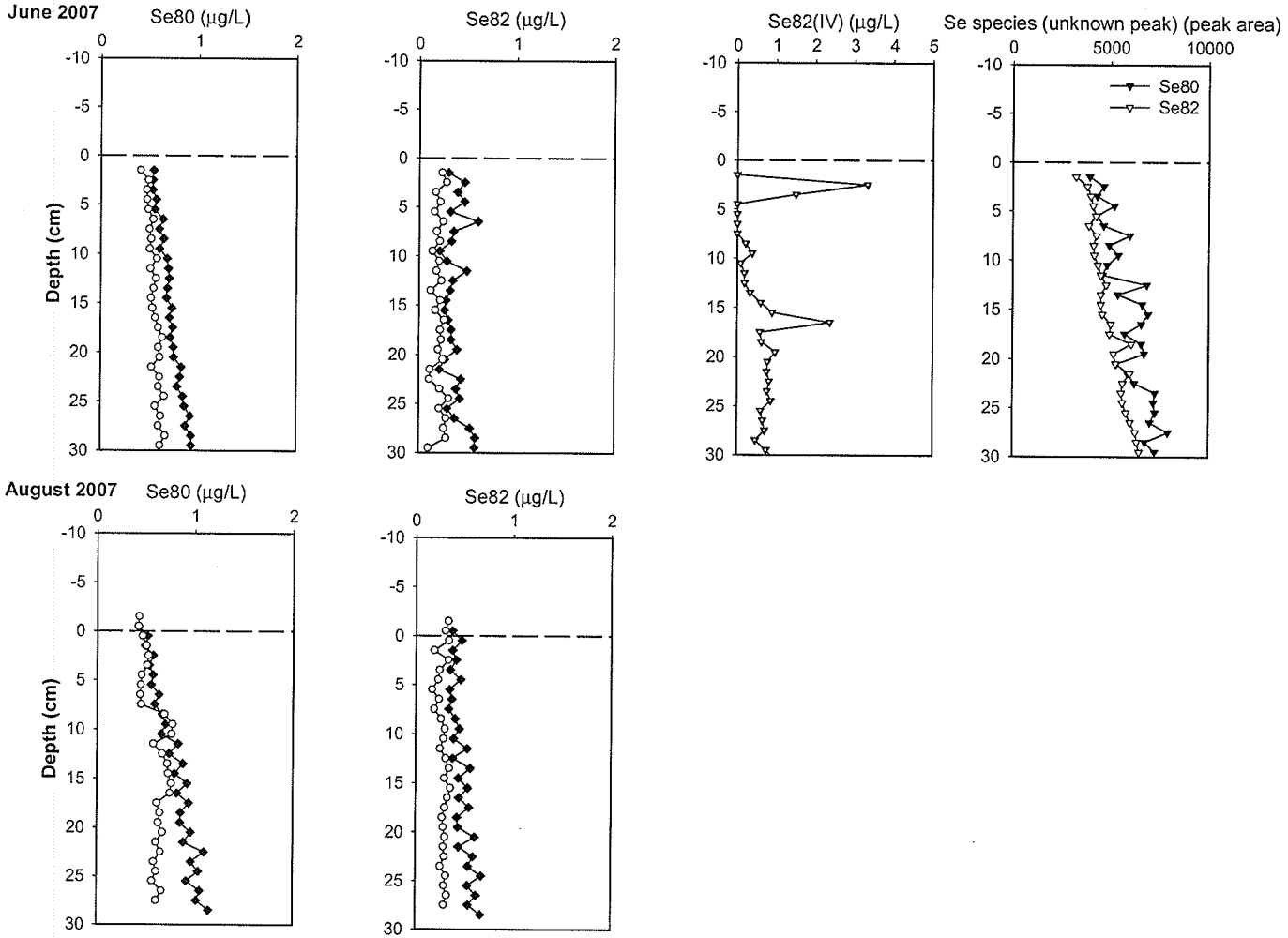




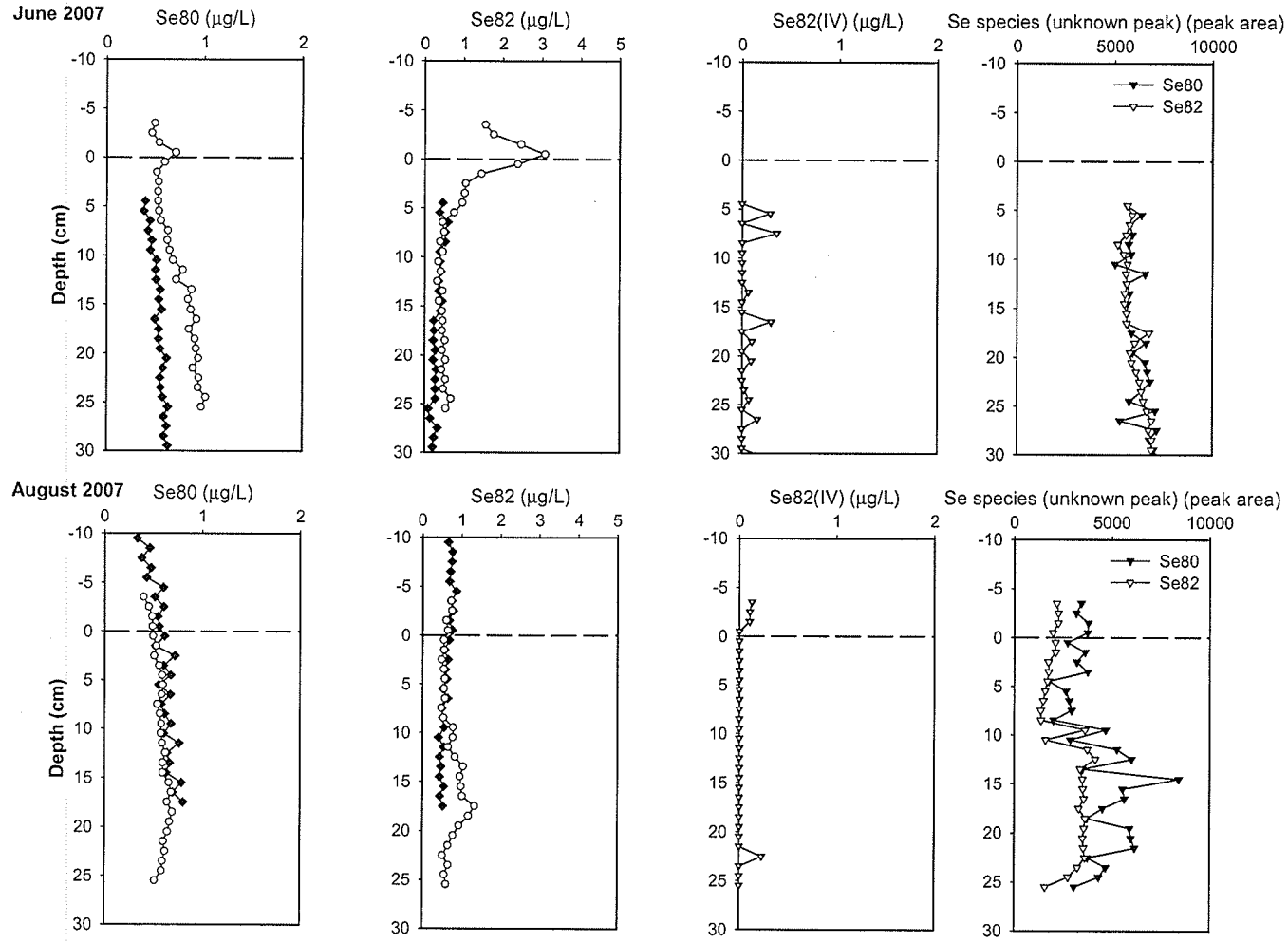
Control


June 2007
Se80 (µg/L)
Se82 (µg/L)
Se species (unknown peak) (peak area)
Se80
Se82
Depth (cm)
August 2007
Se80 (µg/L)
Se82 (µg/L)
Depth (cm)

10x


June 2007
Se80 (µg/L)
Se82 (µg/L)
Se82(IV) (µg/L)
Se species (unknown peak) (peak area)
Se80
Se82
Depth (cm)
August 2007
Se80 (µg/L)
Se82 (µg/L)
Depth (cm)

25x


June 2007
Se80 (µg/L)
Se82 (µg/L)
Se82(IV) (µg/L)
Se species (unknown peak) (peak area)
Depth (cm)
Se80
Se82
August 2007
Se80 (µg/L)
Se82 (µg/L)
Se82(IV) (µg/L)
Se species (unknown peak) (peak area)
Depth (cm)
Se80
Se82