SELENIUM BIOCONCENTRATION IN FRESHWATER PERIPHYTON

A Thesis Submitted to the College of

Graduate and Postdoctoral Studies

in Partial Fulfillment of the Requirements

for the Degree Masters of Science

in the Toxicology Graduate Program

University of Saskatchewan

Saskatoon, Saskatchewan, Canada

Blue Markwart

PERMISSION TO USE

In presenting this thesis in partial fulfilment of the requirements for a Postgraduate degree from the University of Saskatchewan, I agree that the Libraries of this University may make it freely available for inspection. I further agree that permission for copying of this thesis in any manner, in whole or in part, for scholarly purposes may be granted by the professor or professors who supervised my thesis work or, in their absence, by the Head of the Program or the Dean of the College in which my thesis work was done. It is understood that any copying or publication or use of this thesis or parts thereof for financial gain shall not be allowed without my written permission. It is also understood that due recognition shall be given to me and to the University of Saskatchewan in any scholarly use which may be made of any material in my thesis. Requests for permission to copy or to make other use of material in this thesis in whole or part should be addressed to:

Chair of the Toxicology Graduate Program

Toxicology Centre

University of Saskatchewan

44 Campus Drive

Saskatoon, Saskatchewan, Canada, S7N 5B3;

Dean

College of Graduate and Postdoctoral Studies

University of Saskatchewan

116 Thorvaldson Building, 110 Science Place

Saskatoon, Saskatchewan, Canada, S7N 5C9

ABSTRACT

Selenium is an essential micronutrient for most forms of life, but it can elicit developmental toxicity in aquatic and semi-aquatic vertebrates, such as fish and waterfowl, through dietary exposure to excess organic Se compounds. When inorganic Se (as selenate or selenite) is introduced into an aquatic ecosystem as a contaminant, it is bioconcentrated by microorganisms and primary producers (algae, periphyton), biotransformed into organic Se compounds and passed on to higher trophic levels through the food chain. The enrichment of Se in algae is difficult to predict due to interspecific differences in Se bioconcentration, which have been demonstrated to vary by several orders of magnitude in planktonic algae. In addition, Se bioconcentration data are largely lacking for freshwater, periphytic species of algae, and for multi-species periphyton biofilms, adding to the challenge of modeling Se transfer in periphyton-based food webs. Therefore, this research project was designed to address specific knowledge gaps related to the enrichment of selenium in different periphyton communities, as defined by differences in photoautrophic assemblage composition. To satisfy this objective, laboratory-grown and naturally-grown periphyton biofilms were exposed to environmentally relevant concentrations of selenite [Se(IV)] or selenate [Se(VI)] (nominal concentrations of 5 and 25 μg Se L^{-1}) under similar, controlled laboratory conditions. Laboratory-grown periphyton biofilm experiments assessed Se oxyanion bioconcentration in single-species, freshwater periphytic biofilms representative of three major algal phyla: Chlorophyta (*Stichococcus bacillaris*), Cyanophyta (*Anabaena flos-aquae*) and Bacillariophyta (*Asterionella formosa*). Results of these experiments revealed that there was different enrichment of selenate versus selenite for the three species of algae tested (e.g., selenite enrichment was significantly higher than selenate enrichment for *A. formosa*). There were also significant differences in Se enrichment when

comparing similar treatments among the three species of algae tested (e.g., enrichment of selenate was 3.6-fold higher in *S. bacillaris* compared to *A. flos-aquae* for the 25 μg Se L^{-1} treatment). Nevertheless, interspecific Se enrichment did not vary by orders of magnitude for freshwater periphyton, but rather by less than one order of magnitude. Naturally-grown periphyton experiments assessed Se oxyanion accumulation in freshwater periphyton communities sampled from five different water bodies. Results revealed that unique periphyton assemblages were derived from the five different field sites, as confirmed by light microscopy and targeted DNA sequencing of the plastid 23S rRNA gene in algae. Selenium accumulation demonstrated a maximum of 23.6-fold difference for selenite enrichment and 2.1-fold difference for selenate enrichment across the periphyton/biofilm assemblages tested. The assemblage from one field site demonstrated both high accumulation of selenite and iron, and was subjected to additional experimentation to elucidate the mechanism(s) of accumulation. Selenite accumulation was assessed in both unaltered and heat-killed periphyton, and in periphyton from the same site grown without light to exclude phototrophic organisms. All periphyton treatments showed similar levels of Se accumulation, indicating that much of the apparent uptake of selenite was due to non-biological processes (i.e., surface adsorption). The results of this study highlight the need for further exploration of the ecological consequences of extracellular adsorption of selenite to periphyton and will also help to reduce uncertainty in the prediction of Se dynamics and food-chain transfer in freshwater environments.

ACKNOWLEDGEMENTS

I will start by expressing my sincere gratitude for the invaluable support and guidance provided by my supervisors, Dr. Lorne Doig and Dr. Karsten Liber, throughout this project. This project has been a truly humbling and wonderful learning opportunity for me, and it would not have been possible without their mentorship. I would also like to thank my advisory committee, Dr. David Janz and Dr. Markus Hecker, as well as my external examiner, Dr. Curtis Eickhoff, for their comments and advice throughout the review process.

Thank you to Ally, Katherine, Yuwei and Wen, whose hard work in the lab and out in the field helped to make this project a success. Thank you to everyone involved in the collaborative selenium project for your input throughout my thesis research. Thank you to all of my friends and colleagues at the Toxicology Centre for inspiring me and making my time as a graduate student so enjoyable. Thank you to the Natural Sciences and Engineering Research Council for providing funding for this research through a Strategic Projects Grant.

Lastly, I would like to thank my friends and family. Your love and support has been, and will always be, a source of strength for me. Thank you, from the bottom of my heart.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

°C	degrees Celsius
μg g^{-1}	micrograms per gram
μg L^{-1}	micrograms per litre
μm	micrometer
Ω	ohm
π	pi
ANOVA	analysis of variance
BC MoE	British Columbia Ministry of Environment
cm	centimeter
CRM	certified reference material
DNA	deoxyribonucleic acid
DO	dissolved oxygen
d.w.	dry weight
EF	enrichment function
g	gram
H_2O_2	hydrogen peroxide
HCl	hydrochloric acid
HDPE	high-density polyethylene
HNO_3	nitric acid
HPLC	high-performance liquid chromatography
ICP-MS	inductively-coupled plasma mass spectrometry
L	litre
LC	liquid chromatography
m	meter
mg	milligram
mL	millilitre
OM	organic matter
PAR	photosynthetically active radiation

PTFE	polytetrafluoroethylene
RNA	ribonucleic acid
rpm	revolutions per minute
rRNA	ribosomal ribonucleic acid
RO	reverse osmosis
S (conductance)	siemens
SD	standard deviation
Se	selenium
SE	standard error
SeO_3^{2-}	selenite
SeO_4^{2-}	selenate
Se(IV)	selenium in +4 oxidation state
Se(VI)	selenium in +6 oxidation state
TTF	trophic transfer function
US-EPA	United States Environmental Protection Agency

PREFACE

This thesis is organized as manuscripts for publication; as such, there is some repetition of the Introduction and Materials and Methods sections in this thesis. Chapter 2 will be submitted to the journal Science of the Total Environment in April 2019. Chapter 3 was accepted for publication (pending minor revision) by Ecotoxicology and Environmental Safety on February 13, 2018. Supplementary information that will be published from Chapters 2 and 3 has been included in Appendix A. Additional methods and rationale that will not be published from Chapter 3 has been included in Appendix B. The full citations are as follows:

Markwart B, Liber K, Raes K, Hecker M, Janz D, Doig L. 2019. Selenium bioconcentration in lab-grown, single-species periphyton biofilms representative of three major algal phyla: Chlorophyta, Cyanophyta and Bacillariophyta. Science of the Total Environment (in preparation).

Markwart B, Liber K, Xie Y, Raes K, Hecker M, Janz D, Doig LE. 2019. Selenium oxyanion bioconcentration in natural freshwater periphyton. Ecotoxicology and Environmental Safety (accepted pending minor revision, February 2019).

CHAPTER 1

INTRODUCTION

1.1 An introduction to selenium chemistry

Selenium was accidentally discovered in 1817 by two Swedish chemists, Jons Jacob Berzelius and Johan Gotliebb Gahn, who were working in a chemical plant that produced sulfuric acid. They found that the newly discovered element had similar chemical properties to that of tellurium, named after the Latin word tellus meaning "Earth", and so Berzelius named the new element after the Greek word selene, meaning "Moon" (Weeks 1932).

1.1.1 Physical properties

Selenium is a polyatomic non-metal, sometimes considered a metalloid, that occurs mainly in four different oxidation states: -2, 0, 4+ and 6+ (Young et al. 2010). It is classified as a chalcogen, located in group 16 of the periodic table, where it is placed below sulphur ($_{16}$S) and above tellurium ($_{52}$Te). Selenium nuclei contain 34 protons ($_{34}$Se) with an overall atomic mass of 78.971 amu (Lide 1994). It's solid at room temperature, with a melting point of 494K and a boiling point of 958K (Lide 1994). Selenium occurs in chemical forms that are analogous to sulfur compounds (Fan et al. 2002; Ohlendorf 2003; Wallschlager and Feldmann 2010; Yang et al. 2011).

1.1.2 Important selenium compounds

This review will be limited to only those selenium compounds that are pertinent to this study: selenate, selenite and select organoselenium compounds.

1.1.2.1 Selenate

Selenium in the form of selenate exists in the +6 oxidation state as a selenium oxyanion with the chemical formula SeO_4^{2-}. The selenate anion is analogous to the sulphate anion (SO_4^{2-}) and has similar chemistry, such as high solubility in water at room temperature. This inorganic oxyanion has selenium in its most oxidized state and is typically the form taken up by organisms requiring selenium as a micronutrient (Ohlendorf 2003). As is typical of other oxyanions, the solubility of selenate increases with increasing pH; a trend opposite to that of base metals.

1.1.2.2 Selenite

In selenite, selenium is present in the +4 oxidation state as the SeO_3^{2-} oxyanion. As with selenate, selenite is highly water soluble at ambient temperature and increases in solubility with rising pH. Selenite has an especially high affinity for sulfhydryl groups (-SH), which allows it to be readily incorporated into stable organic compounds through interaction with thiols (Ganther 1968). Selenite also participates in more sorption reactions than selenate due to its relatively higher particle reactivity (Foster et al. 2003; Wiramanaden et al. 2010).

1.1.2.3. Organoselenium compounds

In organoselenium compounds, selenium is complexed or covalently bound to an organic moiety and typically exists in the -2 oxidation state (Wallschlager and Feldmann 2010). These

compounds can be classified as either proteinaceous/amino acid selenium or non-protein amino acid/biochemical intermediate selenium. Protein/amino acid selenium refers to selenium incorporated into cysteine or methionine amino acids while the latter group refers to a diverse range of biological compounds. Proteinaceous selenium may be formed intentionally (selenoproteins) through genes that are specifically encoded to incorporate selenopeptides, or unintentionally (Se-containing proteins) through non-specific incorporation into amino acids (due to selenium being a sulfur analogue) and subsequent non-specific incorporation into proteins (Moroder 2005; Wessjohann et al. 2007; Young et al., 2010).

1.2 Sources and speciation of selenium in freshwater ecosystems

Selenium occurs in a range of geological formations and is naturally enriched in a wide rang of different sedimentary marine deposits, including: carboniferous shale, coal deposits, phosphate deposits and crustal rock. While natural processes such as volcanic activity, lithospheric weathering and wildfires can release substantial amounts of selenium into the environment (Nriagu 1989), anthropogenic sources are typically the most toxicologically significant, especially on a regional scale (Maher et al. 2010; Presser et al. 1990). Human activities that cause significant land disturbance, such as agriculture and mining, in areas with seleniferous soils are major contributors to selenium loading in aquatic ecosystems. When seleniferous soils/rocks are exposed to weathering, in waste rock piles or tailings ponds for example, dissolved selenium compounds are readily released into the aquatic environment, especially in uncontrolled settings where regulations are weak (Muscatello and Janz 2009; Presser et al. 1990). Fossil fuel combustion, oil refining and metal ore smelting are also important sources of selenium loading to aquatic ecosystems (Ohlendorf 2003; Young et al.

2010). Selenium loading can occur through either non-point sources, such as run-off and atmospheric deposition, or through point sources such as refinery and wastewater effluents.

Total dissolved selenium concentrations in ambient waters are usually between 0.1 and 0.4 μg Se L^{-1}, which is quite low relative to other trace metals (Wallschlager and Feldmann 2010). The speciation of selenium in aquatic ecosystems depends heavily on the nature of the industrial processes leading up to the release, as well as biogeochemical processes that occur within the water body. In general, the majority of particulate and dissolved selenium in industrial discharges is in the form of selenate or selenite (Maher et al. 2010). For example, selenium loading from agricultural run-off is typically in the form of the selenate oxyanion, while leachate from coal fly ash typically produces selenite oxyanions in the receiving environment (Gao et al. 2007; Zhang et al. 1999; Wang et al. 2007). Selenate and selenite are typically stable in water due to the slow oxidation kinetics of selenite and the thermodynamic stability of selenate; both Se(IV) and Se(VI) require a biological or chemical catalyst to participate in redox reactions in any appreciable way (Cutter and Bruland 1984; Lindemann et al. 2000). In lentic systems, where water is slow moving and retention times are high, low oxygen concentrations and high total organic carbon levels favor the reduction of selenate to selenite (Orr et al. 2006). In lotic systems, which are characterized by low retiontion times (high flow rates) and lower productivity, selenate is typically the dominant species of selenium found in the water column (Orr et al. 2006). The reduction of selenate to selenite is typically a unidirectional process with the ratio of selenite to selenate increasing as you sample farther downstream of a source (Luoma and Presser 2009). Once taken up into primary producers, the majority of Se is converted to highly bioavailable organoselenium compounds (Besser et al. 1993, 1994; Stewart et al. 2010; Janz et al. 2014).

1.3 Essentiality of selenium in freshwater ecosystems

Selenium was recognized as an essential micronutrient in 1957 due to its vital role as a component of Se-containing proteins (Mayland 1994). Selenium-containing proteins can be selenoproteins, Se-binding proteins, or proteins in which selenium has been non-specifically incorporated, usually as selenomethionine (Hesketh 2008; Young et al. 2010; Moroder 2005). Selenoproteins are essential for the continuity of life in all living organisms, except for some higher plants and yeasts (Hesketh 2008). These integral proteins carry out a diverse array of functions, of which only some have been characterized. Known selenoproteins and associated functions include: glutathione reductases (catalyze redox reactions), thioredoxin reductases (catalyze redox reactions), iodothyronine deidonases (activates/inactivates thyroid hormone), selenophosphate synthetase (synthesis of selenocysteine), and selenoprotein P (Se transport protein) (Reilly 2006). To date, there are over 20 other selenoproteins that have been identified in vertebrates whose function have yet to be clarified (Hesketh 2008). Overall, selenium's essentially is inexorably linked to the role that selenoproteins play in defense against oxidative stress.

1.4 Toxicity of selenium in freshwater ecosystems

In freshwater ecosystems, oviparous vertebrates, such as fish and waterfowl, are the most sensitive taxa with respect to selenium toxicity. In egg-laying vertebrates, selenium is considered to be a unique micronutrient due to the narrow window of essentiality between deficiency and toxicity (Janz et al. 2010). Essentially all exposure to selenium occurs through the diet of an aquatic animal rather than waterborne uptake, with approximately 90% of Se body burdens resulting from dietary uptake of organoselenium compounds (Zhang and Wang 2007; Stewart et

al., 2010). At sufficiently high levels, populations of sensitive bird and fish species can be negatively impacted, and in some cases completely extirpated, from a contaminated ecosystem (for a review of site-specific case studies and ecosystem level effects, see section *1.4.2 Relevant case studies* below) leaving only the most tolerant animals and resulting in a complete shift in community dynamics leading to eventual ecosystem collapse (Lemly 1997; Janz et al. 2010). Relative to fish and waterfowl, most other freshwater organisms are much less sensitive to selenium toxicity. Algae, plants and bacteria are generally very tolerant to selenium exposure at relevant environmental concentrations and can acquire high tissue Se burdens with little to no measurable effect (Baines and Fisher 2001). Differential selenium bioconcentration in planktonic algal species has been shown to result in up to 5 orders of magnitude difference in tissue selenium concentrations (Baines and Fisher 2001). Some bacterial species have been shown to accumulate approximately twice as much selenium as phytoplankton at relevant environmental concentrations, also with no measurable impairment (Baines et al. 2004). Freshwater macroinvertebrates are more sensitive than algae, plants and bacteria, but are still considered to be generally tolerant to selenium toxicity. Case studies of contaminated sites have not been able to link selenium exposure with effects on macroinvertebrate communities, but an assessment by deBruyn and Chapman (2007) has suggested that sensitive species within invertebrate communities may be affected at concentrations considered safe for their predators.

1.4.1 Toxicity of selenium to oviparous vertebrates

Selenium has several proposed mechanisms of toxicity but the relative importance of each is still a subject of debate, especially in oviparous vertebrates. Proposed mechanisms include: non-specific substitution of selenium for sulfur in amino acids causing protein

dysfunction, increasing oxidative stress, and/or suppression of immune function (Stewart et al. 2010). Regardless of the specific mechanism(s) of action, the most toxicologically important ecosystem-level effects occur as a result of maternal transfer of Se to eggs during vitellogenesis. When embryos are maternally exposed to selenium, it can result in reduced/impaired hatchability, edema and permanent developmental abnormalities, such as spinal curvature and missing or deformed fins (Hamilton 2003; Lemly 1993; Maier et al. 1988). Widespread embryo and early-life-stage mortality in fish and waterfowl can result in population and community level impacts to aquatic ecosystems.

1.4.2 Relevant case studies

The Kesterson Reservoir, located in the San Joaquin Valley, California, U.S.A, is a striking example of the potential population level effects arising from Se contamination in the aquatic environment. A large-scale, subsurface drainage system for collecting agricultural irrigation runoff (to mitigate potential salinization of irrigated croplands) was installed in the arid San Joaquin Valley, with work beginning in the 1970's (Young et al. 2010). Due to the intensity of agricultural practices in the region, wetlands within the valley were diverted for irrigation, resulting in the disappearance of more than 90% of the wetlands in the valley (Ohlendorf et al. 1990). The Kesterson Reservoir, a series of shallow, interconnected ponds, was constructed adjacent to the agricultural drainage system with the intention of creating/replacing wetland habitat and controlling flow within the drainage system, although the end result was that the reservoir acted as a series of evaporative ponds (Young et al. 2010). Unbeknown to the architects of the project, irrigation drainage entering the Kesterson Reservoir was high in selenium due to the leaching of selenate from seleniferous soils in the region (Ohlendorf et al. 1988). Water-

column Se concentrations upon entering the Kesterson Reservoir averaged 340 μg Se L^{-1} in 1983, occurring mostly as dissolved selenate (98%) in the first ponds receiving agricultural drainage and increasing in dissolved selenite content as the pond series progressed (20-30% by the terminal pond) (Ohlendorf et al. 1990). In 1983, a local fish extirpation event occurred, resulting in the elimination of up to 8 warm-water species and leaving only one tolerant species, the mosquitofish, in the region (Saiki and Lowe 1987). Deformity and death in embryos and hatchlings of aquatic bird populations were widespread, coined by researchers as the "Kesterson Syndrome" (Skorupa 1998; Ohlendorf et al. 1988). Local waterfowl populations showed severe reproductive impairment, with over 40% of nests having one or more dead embryos and nearly 20% of embryos or chicks showing deformities (Ohlendorf et al. 1990). By 1986, irrigation drainage inputs were ceased and the severe, population level effects measured in the region triggered a review of other wildlife refuges receiving agricultural runoff in 13 western U.S. states (Presser et al. 1994; Young et al. 2010).

Belews Lake and Hyco Lake, both located in North Carolina, U.S.A., were impounded in the late 1960's/early 1970's to serve as cooling reservoirs for coal burning power plants (Young et al. 2010). In both cases, clarified ash sluice water was returned to the impoundments after use. Selenium released from coal combustion waste occurs primarily as selenite and, due to this design, the wastewater being returned to the water bodies had up to 100-200 μg Se L^{-1} as selenite (Cutter 1991). Adverse effects observed in these lakes included: failure of downstream fisheries, fish kills, fish recruitment failure and the elimination of sensitive species from the area, resulting in community shift and the dominance of tolerant species (Crutchfield 2000; Cumbie and Van Horn 1978). After the elimination of selenium inputs to these lakes, fish communities improved but were still showing signs of impact up to a decade later (Lemly 1997)

The Elk River Valley, located in southeast British Columbia, Canada, is an area rich in high-grade coal deposits that have been mined since the late 1800's (Young et al. 2010). Land disturbance and large volumes of waste rock have led to the weathering and leaching of pyrite-associated Se, released as selenate and draining into the Elk River (Martin et al. 2008). Effluent concentrations have been measured as high as 300 μg Se L^{-1}, with selenium levels in the Elk River ranging from 9.6 μg Se L^{-1} near the area of mining to 5.8 μg Se L^{-1} 60 km downstream. The levels of selenium in the Elk River Valley have not been shown to produce population level effects in fish and waterfowl of the region, although there is potential for localized adverse effects (Canton et al. 2008).

The Key Lake uranium milling operation, located in northern Saskatchewan, Canada produces effluent high in dissolved selenium, in the form of selenate (Dube et al. 2011). Between 2008 and 2012, selenium concentrations in treated effluent from the Key Lake Operation were reduced from an average of 40 μg Se L^{-1} to 16 μg Se L^{-1}, although a recent study conducted by Janz et al. (2014) showed that fish tissues sampled from local populations had not yet shown a similar decrease. Although no effects to local fish and waterfowl were able to be directly linked to elevated selenium exposure during study of this site, the results of site-specific work in the region has highlighted the importance of periphyton/biofilm Se biotransformation in the trophic transfer of Se in cold, freshwater environments (Janz et al. 2014).

1.5 The selenium cycle

Selenium cycling occurs as a result of a complex system of biogeochemical processes that affect the fate and transport of selenium compounds through different environmental compartments (Maher et al. 2010; Masscheleyn 1993; Ohlendorf 2003; Wallschlager and

Feldmann 2010). The degree to which selenium is affected by each aspect of the process is site-specific because the rates for each process vary widely with differing conditions. To date, various studies have identified temperature, organic carbon content, selenium concentration and speciation/redox status, microbial activity, growth rates, food web dynamics, iron/magnesium hydroxide formation and competing anions (ie. sulphate, phosphate, carbonate) as some of the important factors that affect selenium cycling (Dhillon and Dhillon 2003; Howard 1977; Luoma and Presser 2009; Ohlendorf 2003, Wallschalger and Feldmann 2010). In general, selenium cycling is a competition between processes that immobilize selenium in non-bioavailable compartments versus those that remobilize selenium and make it available for biological uptake. In freshwater environments, selenium redox reactions are the most important controls for speciation, methylation, solubility and sorption dynamics (Maher et al. 2010; Ohlendorf 2003).

1.5.1 Physical processes that influence selenium fate and transport

The partitioning of selenium between solid and aqueous phases is fundamentally linked to iron geochemistry and, to a lesser extent, the geochemistry of manganese and other minerals (Howard 1977). Dissolved selenite has a very high affinity for Fe and Mn oxy-hydroxide minerals which adsorb selenium (largely due to electrostatic attraction); Se strongly partitions to the solid phase when oxy-hydroxides are present (Maher et al. 2010; Balistrieri and Chao 1987, 1990). Conversely, selenate does not adsorb to manganese oxides and has an intermediate affinity for ferrous oxy-hydroxides so that only a portion of aqueous selenate partitions to the solid phase (Foster et al. 2003; Balistrieri and Chao 1987, 1990).

Adsorption of selenium on oxy-hydroxide minerals is primarily influenced by changes in pH, temperature and competing anions. The effect of pH on selenium partitioning to sediments is

especially complex because the sorption of selenate and selenite on oxy-hydroxide minerals increases under acidic conditions but increased acidity can also lead to the dissolution of oxy-hydroxide minerals, which decreases the number of binding sites (Foster et al. 2003; Balistrieri and Chao 1987, 1990). Maximal selenate and selenite adsorption occurs around a pH of 7, which means that in typical natural fresh waters (pH 7-8) the equilibrium partitioning lies slightly toward desorption (Davis et al. 1978). Increasing temperature can also slow adsorption rates due to the exothermic nature of adsorption reactions; the surface energy of an adsorbent is decreased therefore heat energy must be released to satisfy the First Law of Thermodynamics (Balistrieri and Chao 1990). Competition for sorption sites among naturally occurring or anthropogenically enriched anions can influence the degree to which selenium species adsorb to Fe and Mn oxy-hydroxides. The main competing ions are sulphate, phosphate and carbonate, all of which compete (to varying degrees) with selenate and selenite for binding sites on mineral surfaces and organic ligands (Dhillon and Dhillon 2003; Lo et al. 2015). Ambient sulphate concentrations are high enough in many fresh waterbodies to outcompete selenate and make selenate-to-mineral adsorption almost non-existent (Dhillon and Dhillon 2003).

1.5.2 Biological processes that influence selenium fate and transport

Although there are several important abiotic processes and parameters that influence selenium cycling in freshwater environments, biochemical processes are typically of higher toxicological significance when considering selenium uptake into food-webs. The most important step in the accumulation and food-chain transfer of selenium is the initial bioconcentration of inorganic selenium species in algae and bacteria (Stewart et al. 2010, Presser and Luoma 2010). In many cold, freshwater ecosystems, the majority of algal biomass occurs as

periphyton in the form of a biofilm (Ennis and Albright 1980). Biofilms are defined by the IUPAC (2014) as an "Aggregate of microorganisms in which cells adhere to each other and/or to a surface. These adherent cells are frequently embedded within a self-produced matrix of extracellular polymeric substances." Extracellular matrices are generally composed of DNA, proteins and polysaccharides in various configurations (IUPAC 2014). In freshwater environments, the majority of selenium biotransformation processes and accumulation at the base of the food-web occurs within these biofilms (Luoma and Presser 2009; Janz et al. 2014).

1.5.2.1 Selenium biotransformation and accumulation in bacteria

Selenium is readily metabolized by many species of bacteria through a variety of metabolic functions including: assimilation, methylation/detoxification and anaerobic respiration (Stotz et al. 2006). Depending on the nature of the interaction, selenium bioavailability will increase or decrease accordingly. Freshwater bacteria have also been shown to bioconcentrate selenium oxyanions, with 34-74% of selenite uptake in water (in the absence of light) occurring in the microbial fraction (0.2 to 1.0 μm) (Baines et al. 2004).

1.5.2.1.1 Assimilation, methylation and anaerobic respiration

Prokaryotes take up selenium through a currently unknown pathway and incorporate it into essential selenoproteins, largely in the form of selenocysteine [Se (-II)] (Stoltz et al. 2006).

Dimethyl selenide and dimethyl diselenide are the most common methylated forms of selenium, with selenium present in the -2 oxidation state (Stolz et al. 2006; Wallschlager and Feldman 2010). Methylation is a common method of detoxification for prokaryotes (Heider and Bock 1993). This method of detoxification is also a significant driver of selenium loss from

aquatic environments as methylated selenides are typically volatile under ambient conditions; they tend to partition to air rather than water (Cooke and Bruland 1987; Tessier et al. 2002).

There are a number of ubiquitous bacteria that have been identified as selenate reducers (typically sulphate reducers as well) that use selenium oxyanions as terminal electron acceptors in anaerobic respiration (Herbel et al. 2003; Stoltz et al. 2006). These bacteria usually reduce selenium to its elemental (0) or -2 oxidation state (Stoltz et al. 2006). In slow moving, low oxygen waters, selenium undergoes rapid microbial biotransformation from selenate (+6) to selenite (+4) to elemental Se (0) [and sometimes to organoselenium (-2)] metabolites (Stoltz et al. 2006; Zhang et al. 2004). Elemental selenium can also be oxidized by some species of bacteria, typically into its +6 oxidation state as selenate (Oremland et al. 1991, 2004).

1.5.2.2 Selenium biotransformation and accumulation in eukaryotic algae

1.5.2.2.1 Uptake & biotransformation

As an essential nutrient for algal growth, selenium is taken up by algal cells through a carrier-mediated active transport process (Baines and Fisher 2001; Riedel et al. 1991, 1996). Uptake of selenate, selenite and organic selenides display typical Michaelis-Menten saturation kinetics with uptake occurring quickly at first and slowing as carriers become saturated, leading to a non-linear relationship between [dissolved Se] vs. [tissue Se] in algae (Baines and Fisher 2001; Fournier et al. 2006; Riedel et al. 1996). Competitive interaction for uptake between sulphate and selenate strongly suggests that they are both taken up through the same carrier protein (sulphate membrane transporters), while organic selenides appear to have a different, unknown high affinity membrane transporter (Fournier et al. 2006; Riedel et al. 1991). Although selenite uptake appears to follow similar saturation kinetics and is taken up at a significantly

higher rate than selenate, there is not enough evidence to suggest that this is entirely an active process. Riedel et al. (1991) found heat-killed algal cells took up almost as much selenite as living cells when exposed under similar conditions. This suggests that a portion of apparent selenite uptake may be occurring as a result of sorption processes whereby selenite is adsorbed onto the surface of algal cells. Experiments using terrestrial plants and environmentally relevant Se concentrations indicate that selenite and phosphate show competitive inhibition for sorption sites, much in the same way that sulphate vs. selenate does but to a lesser degree (Hopper and Parker, 1999). In contrast, Baines and Fisher (2001) determined that the relationship between Se content and cell volume was inconsistent between algal taxa; metals that are primarily taken up by adsorption processes should display a strong relationship between uptake and surface area for a broad range of biological and inorganic particulates (Fisher and Reinfelder 1995). In reality, it is likely that selenite uptake is controlled by both adsorption processes and interspecific differences due to the presence of an unknown carrier protein.

Regardless of the route of uptake, it is generally agreed that inorganic selenium taken up into algal cells, as selenate and selenite, is rapidly enzymatically reduced to organic selenides through the same metabolic pathway as sulphur (Besser et al. 1993, 1994; Stewart et al. 2010; Terry et al. 2000). The biotransformation of intracellular selenate (to organoselenium compounds) in plants is hypothesized to occur in a stepwise fashion, starting with selenate and proceeding through adenosine phosphoselenate (APSe), selenite, selenide(s), and then to selenocysteine (SeCys) via cysteine synthase (Terry et al. 2000). Selenocysteine can be directly incorporated into proteins, methylated to form volatile Se species or transformed to selenomethionine, which occurs after transformation to selenocystathionine and Se-methylselenocysteine (Terry et al. 2000). There is strong evidence to support that Se taken up

into microorganisms (primary producers and bacteria) at the base of the food web is preserved or even concentrated as it is passed up through the trophic levels (Luoma and Presser 2009).

1.5.2.2.2 Enrichment functions and interspecific differences in accumulation

Unlike many metal contaminants in aquatic systems, selenium bioaccumulation and toxicity cannot be predicted based solely on the concentration of dissolved selenium found in the environment; thermodynamic/equilibrium-based constants alone cannot describe this complex relationship (Stewart et al. 2010). To address this problem, selenium researchers have come up with experimentally derived [dissolved Se] to [algal tissue Se] ratios, known as "enrichment functions", that are specific to each plant or microbe (Stewart et al. 2010). Simply put, an enrichment function is the concentration of particulate selenium (in algae and bacteria) divided by the ambient concentration of selenium in the aquatic environment (water). Enrichment functions in different species of algae vary greatly, displaying up to 5 orders of magnitude difference in [tissue Se] under the same exposure conditions (Baines and Fisher 2001). This substantial, interspecific difference in Se accumulation complicates the prediction of selenium body-burdens at the base of food-webs. It is likely that interspecific accumulation patterns arise as a result of differences in cellular Se requirements, as well as differing strategies to regulate Se uptake (Stewart et al. 2010; Baines and Fisher 2001).

1.5.2.2.3 Community composition

Due to the high degree of interspecific variability in selenium uptake among primary producers, algal community structure is hypothesized to influence the initial bioconcentration step and the subsequent food-chain transfer of Se compounds (Baines and Fisher 2001; Presser

and Luoma 2010). The composition of algal communities is greatly influenced by a number of important parameters, such as: salinity (Cloern and Dufford 2005), nutrient concentrations/ratios (Chisholm 1992), differences in light intensity and differences in temperature/vertical stratification (Margalef 1978), and selective grazing pressure (Smetacek et al. 2004). The dynamic nature of algal communities engenders a high degree of spatial and temporal variability regarding selenium enrichment in a particular ecosystem.

1.5.2.2.4 Growth phase, growth dilution and bloom dilution

The degree of Se enrichment has been shown to vary according to physiological state in some (not all) species of algae (Baines and Fisher 2001). For species that display this pattern, rapidly growing cells are much less enriched than cells entering senescence. This may occur as a result of growth dilution, where algal biomass is increasing within each cell at a rate outstripping its capacity for Se uptake; intracellular Se is diluted with photosynthetically fixed carbon (Hills and Larsen 2005). Growth phase may be considered the driving factor behind this phenomenon, with cells that are entering senescence slowing in growth while continuing to take up selenium (Baines and Fisher 2001). It also appears that many algal species are capable of taking up excess Se and storing it intracellularly until biological needs arise (Baines and Fisher 2001), much the same as what occurs for many other essential nutrients. Bloom dilution is a result of increased competition for Se uptake when an algal bloom occurs (Hills and Larsen 2005). Increased competition for Se decreases the available pool of selenium for uptake, which in turn decreases the overall algal tissue-Se.

1.6 Food chain transfer of selenium in freshwater ecosystems

Similar to the enrichment of selenium at the base of the food chain, trophic transfer patterns for selenium are unique to each producer-consumer/predator-prey relationship in a given ecosystem. The Trophic Transfer Function (TTF) describes the species-specific relationship between [tissue Se] and [Se in food] in the organism of interest (Stewart et al. 2010). As with the enrichment function, trophic transfer is represented as a function due to the non-linear relationship between Se exposure and accumulation. Typical TTFs for algae to primary consumers range from 0.6 to 23, while TTFs for invertebrates to fish usually range from 1 to 3 (Stewart et al. 2010; Zhang and Wang 2007). Se has the potential to biomagnify wherever TTF values are above 1, indicating that Se is being efficiently assimilated and loss rates are low (Zhang and Wang 2007).

1.6.1 Factors affecting trophic transfer functions

Although there is limited information regarding the relationship between assimilation efficiency (AE) and [prey Se], an inverse relationship has been experimentally demonstrated between the two (Guan and Wang 2004). At low [dietary Se] high affinity uptake pathways result in high assimilation efficiency; however, as the carrier proteins become saturated (when exposed to high dietary selenium) the overall AE decreases. Feeding behaviour can also influence TTFs through changes in ingestion rate (which influences total Se exposure and gut residence time), or through processes like selective grazing and prey selectivity (Stewart et al. 2010). TTFs can be especially difficult to interpret correctly due to differences in migration, habitat utilization and tissue allocations among aquatic consumers and high-level predators (Stewart et al. 2010).

1.7 Ecosystem-scale selenium modelling

Ecosystem-scale selenium models are largely site-specific due to the absence of a direct relationship between dissolved selenium concentration and toxicity in higher order predators (Luoma and Presser 2009). Models must accurately link the biogeochemical processes that affect uptake at the base of the food-web with trophic transfer and subsequent toxicity in higher-order predators. The movement of dissolved selenium, as well as the transformation of selenium between dissolved and particulate (i.e., algae and bacteria) phases, is largely controlled by speciation. The concentration at the base of the food web determines how much selenium will be available for primary consumers. Similarly, the concentration of selenium in primary consumers determines the exposure and toxicity of Se to higher-order predators, such as fish and waterfowl. Understanding the ecology/food web dynamics of an ecosystems is essential to being able to predict the fate and transport of selenium in different aquatic environments.

1.8 Research goals and objectives

The primary goal of this project was to assess the relative influence of differing periphyton community composition on the uptake and bioconcentration of waterborne selenium oxyanions at environmentally relevant concentrations. To attain this goal, research was divided into two specific objectives:

1. Compare selenium bioconcentration in lab-grown, single-species periphyton biofilms representative of major algal phyla (Chlorophyta, Cyanophyta and Bacillariophyta*)* exposed to environmentally relevant concentrations of inorganic selenium (as selenate or selenite) under controlled, laboratory conditions.

H_0: There is no difference in mean periphyton-Se concentration, for a given Se treatment, across different lab-grown periphyton biofilms ($\mu_1 = \mu_2 = \mu_3$).

2. Compare selenium bioconcentration in genetically different (as defined by differences in plastid 23S rRNA gene sequences), field-collected freshwater periphyton assemblages exposed to environmentally relevant concentrations of inorganic selenium (as selenate or selenite) under controlled, laboratory conditions.

H_0: There is no difference in mean periphyton-Se concentration, for a given Se treatment, across genetically different periphyton assemblages ($\mu_1 = \mu_2 = \mu_3 = \mu_4 = \mu_5$).

The secondary goal of this project was to determine the relative influence of adsorption processes and uptake by non-phototrophic bacteria on the apparent uptake of Se in natural periphyton known accumulate high levels of selenite. To address this goal, research was again divided into two objectives:

1. Compare selenite bioconcentration in untreated, field-collected periphyton with selenite accumulated by the same field-collected biofilm that has been heat-killed to cease all biological processes that result in active Se uptake.

H_0: There is no difference in mean periphyton-Se concentration, for a given selenite treatment, for untreated, field-collected periphyton and heat-killed, field-collected periphyton ($\mu_1 = \mu_2$).

2. Compare selenite bioconcentration in untreated, field-collected periphyton with selenite bioconcentration in bacterial biofilms grown under similar conditions but without light to exclude phototrophs.

   H_0: There is no difference in mean periphyton-Se concentration, for a given selenite treatment, for untreated, field-collected periphyton and bacterial biofilms grown under similar conditions but excluding phototrophs ($\mu_1 = \mu_2$).

CHAPTER 2

SELENIUM OXYANION BIOCONCENTRATION IN LAB-GROWN, SINGLE-SPECIES PERIPHYTON BIOFILMS REPRESENTATIVE OF MAJOR ALGAL PHYLA: CHLOROPHYTA, CYANOPHYTA AND BACILLARIOPHYTA.

Preface

The research in this chapter was designed to assess the bioconcentration of inorganic selenium, as selenate or selenite, in lab-grown, single-species periphyton biofilms representative of three major algal phyla: Chlorophyta, Cyanophyta and Bacillariophyta. This chapter will be submitted (with minor modification) to Science of the Total Environment. The full citation is: Markwart B, Liber K, Raes K, Hecker M, Janz D, Doig L. 2019. Selenium bioconcentration in lab-grown, single-species periphyton biofilms representative of three major algal phyla: Chlorophyta, Cyanophyta and Bacillariophyta. Science of the Total Environment (in preparation).

The author contributions to chapter 2 of this thesis were as follows:

Blue Markwart (University of Saskatchewan) collected, processed and analyzed all samples, performed all statistical analyses and drafted the manuscript.

Karsten Liber (University of Saskatchewan) helped design the study, provided scientific input and guidance; reviewed and revised the manuscript, providing comments and corrections; procured (with co-PIs) and provided funding required to conduct the research.

Katherine Raes (University of Saskatchewan) helped design the study and provided scientific input.

Markus Hecker (University of Saskatchewan) helped design the study, provided scientific input and guidance; reviewed and revised the manuscript, providing comments and corrections; procured (with co-PIs) and provided funding required to conduct the research.

David Janz (University of Saskatchewan) helped design the study, provided scientific input and guidance; reviewed and revised the manuscript, providing comments and corrections; procured (with co-PIs) and provided funding required to conduct the research.

Lorne Doig (University of Saskatchewan) helped design the study, provided scientific input and guidance; reviewed and revised the manuscript, providing comments and corrections.

2.1 Abstract

When inorganic Se (as selenate or selenite) is introduced into an aquatic ecosystem as a contaminant, it is bioconcentrated by microorganisms and primary producers (algae, periphyton), biotransformed into organic Se compounds and passed on to higher trophic levels through the food chain. The enrichment of Se in algae is difficult to predict due to interspecific differences in Se bioconcentration, which have been demonstrated to vary by several orders of magnitude in planktonic algae when exposed to similar ambient Se concentrations. Previous studies have largely focussed on Se bioconcentration in planktonic algal species, many of which are marine. To better predict Se dynamics in periphyton dominated, freshwater ecosystems, this study assessed Se oxyanion bioconcentration in singe-species, freshwater periphytic biofilms representative of three major algal phyla: Chlorophyta (*Stichococcus bacillaris*), Cyanophyta (*Anabaena flos-aquae*) and Bacillariophyta (*Asterionella formosa*). Monoculture periphytic biofilms were grown in batches before being exposed to dissolved inorganic Se, as selenite or selenate, at two nominal treatment concentrations of 5 and 25 μg Se L^{-1}. Results revealed that there was different enrichment of selenate versus selenite for the three species of algae tested (e.g., selenite enrichment was significantly higher than selenate enrichment for *A. formosa*), as well as significant differences in Se enrichment when comparing similar treatments among the three species of algae tested (e.g., enrichment of selenate was 3.6-fold higher in *S. bacillaris* compared to *A. flos-aquae* for the 25 μg Se L^{-1} treatment). Despite small differences, interspecific Se enrichment did not vary by orders of magnitude for freshwater periphyton, but rather by less than one order of magnitude. These observations will help to reduce uncertainty when modelling Se bioaccumulation and toxicity in freshwater ecosystems.

2.2 Introduction

Selenium (Se) contamination of aquatic ecosystems occurs as a by-product of various important economic activities, including energy production, mining and irrigated agriculture (Young et al. 2010). Se is typically released to the environment as an inorganic oxyanion in the form selenate or selenite, depending on the source and processing of parent material (Maher et al. 2010). Se impacted waters typically contain no more than 10 – 100 μg Se L^{-1} (Maher et al. 2010).

Selenium is an essential micronutrient with an unusually narrow window of essentiality in vertebrate animals (Renwick 2006). Selenium toxicity in aquatic and semi-aquatic vertebrates, such as fish or waterfowl, can result in result in reproductive impairment and is primarily driven by dietary exposure (trophic transfer) to organic Se compounds (Stewart et al. 2010). The most important, and highly variable, step in the food-web transfer of Se occurs when dissolved Se is bioconcentrated by microorganisms and primary producers (algae, periphyton), biotransformed to organic Se compounds, and then passed on to subsequent trophic levels (Fan et al. 2002; Presser and Luoma 2010). The enrichment of Se in algae is difficult to predict due to interspecific differences in Se bioconcentration, which reportedly can vary by several orders of magnitude in planktonic algae when exposed to similar ambient Se concentrations (Vandermeulen and Foda 1988; Baines and Fisher 2001). Interspecific differences in Se bioconcentration, when subject to similar environmental conditions, are likely related to differences in cellular Se requirements, but may also be related to the ability of algal cells to regulate Se uptake (Stewart et al. 2010).

Ambient concentration and oxidation state of inorganic Se compounds [Se(IV) vs Se(VI)] are two other important variables that influence the concentration of Se in algae. Se(IV), as

selenite, is generally the most available form of inorganic Se to phytoplankton (Baines et al. 2001; Conley et al. 2013; Hu et al. 1997; Riedel et al. 1991; Stewart et al. 2010; Vandermeulen and Foda 1988), but to our knowledge this has not been demonstrated in periphyton consisting of a single species of algae. Previous studies have demonstrated up to 10^6-fold enrichment of Se from water to phytoplankton at environmentally relevant concentrations (Baines and Fisher 2001). Accumulation of either inorganic Se compound in algae may or may not be proportional to ambient Se concentration, depending on saturation of Se accumulation mechanisms and Se concentration ranges (Baines and Fisher 2001; Fournier et al. 2006).

Previous studies have largely focussed on Se bioconcentration in planktonic algal species, many of which were marine (Baines and Fisher 2001; Fournier et al. 2006; Hu et al. 1997; Kiffney and Allen 1990; Riedel et al. 1991; Riedel and Sanders 1996). Moreover, many of the previous studies measured Se uptake rates rather than Se concentrations in cells, making it difficult to assess the potential ecological impact of differential Se uptake in algae. To better predict Se dynamics in periphyton dominated, freshwater ecosystems, this study assessed Se oxyanion bioconcentration in freshwater periphytic biofilms representative of three major algal phyla; Chlorophyta, Cyanophyta and Bacillariophyta. The goal of this study was to determine if there were significant differences in Se bioconcentration in periphytic biofilms across different algal phyla. This could have significant implications for risk assessment in periphyton dominated, freshwater aquatic environments. This goal was addressed by exposing single-species periphyton biofilms to environmentally relevant concentrations of Se oxyanions, selenite or selenate, under controlled laboratory conditions.

2.3 Materials and methods

2.3.1 Test organisms and culturing

Each experiment involved the growth and testing of lab-grown periphyton monocultures representing three main algal phyla: Cyanophyta, Chlorophyta and Bacillariophyta. The three species selected for experimentation were: *Anabaena flos-aquae* (CPCC #631), a cyanophyte (cyanobacteria), *Stichococcus bacillaris* (CPCC #177), a chlorophyte, and *Asterionella formosa* (CPCC #69), a diatom. Algal strains were obtained from the Canadian Phycological Culture Centre (CPCC) at the University of Waterloo. Algal species were selected based on ability to form biofilms and ease of culturing under non-sterile laboratory conditions (due to experimental design).

All glassware and plasticware for culturing and experimentation were acid-washed with 1M HCl before use. Any glassware or plasticware that directly contacted culturing or testing solutions were also disinfected with 5% sodium hypochlorite for 30 minutes before use. Single-species periphyton biofilms were cultured in large (25-L), aerated polypropylene (PP) containers to limit differences in algal growth among replicates before the start of each experiment. After several failed attempts at growing *A. formosa* in large, PP containers, cultures were successfully grown in batches inside 6-L Erlenmeyer flasks before being seeded onto sampling plates, 3 days prior to the exposure phase. Different algal species were cultured in a growth medium specific to each type of algae. *A. flos-aquae* was cultured in ASM No. 8a medium (O'Flaherty and Phinney 1970), *S. bacillaris* was grown in Bold's Basal medium (Stein 1973) and *A. formosa* was cultured in CHU-10 medium (Stein 1973) modified to have no added Se. Growth media for all species were made up by mixing concentrated stock nutrient solutions with reverse osmosis water in 50-L batches and balancing pH to 6.8 with sodium hydroxide (NaOH) or hydrochloric

acid (HCl) as required. The pre-exposure growing period ranged from 3 to 5 weeks depending on the rate of growth for each species. Culturing and testing took place in an environmental chamber at the Toxicology Centre, University of Saskatchewan, with 16 h light: 8 h dark cycle and the temperature set at 24 ± 2°C. Portions of periphyton biofilm were sampled for identification, using traditional light microscopy, throughout the test to ensure biofilms were dominated by the algal species of interest. Although biofilm composition was confirmed for each algal species, it is likely that there was some degree of microbial contamination in the periphyton biofilms as a result of the non-sterile nature of the culturing and testing apparatus. All algal species were grown and exposed under similar conditions so differential microbial contamination is unlikely to be the cause of differential Se bioconcentration in the algae tested.

2.3.2 Experimental setup

Each Se-algae treatment had three replicates, with each replicate consisting of a 5.5-L polypropylene vessel with eight 10 cm x 10 cm x 4 mm frosted borosilicate glass plates placed vertically in a high-density polyethylene holder. The glass sampling plates and holders were fully submerged in test solution. Each exposure vessel had a lid to limit evaporation from the test system. More sampling plates than necessary were included to provide additional samples if needed. Each replicate also received aeration to ensure that the exposure media was oxic and well mixed. The Se exposure period was initiated after algal growth was sufficient to produce ≥10 mg (d.w.) of algae per sampling plate. The bench-top position of each exposure replicate was randomized to account for minor spatial differences in light and temperature.

2.3.3 Exposure period

Se exposure length was originally set for 14 days based on the assumption that pseudo-steady state (no statistical difference in biofilm Se concentrations over two consecutive sampling days) would be reached within that time period. Test duration was extended to 21 days for *A. formosa* due to a slow growth rate after being transferred into the test system. For reasons unclear, diatom growth was suppressed for the first 10 days of exposure, but began to increase by day 14. The increase in biological activity on day 14 is the reason for continuing the diatom exposure phase for an additional week, to day 21.

Test water was made according to specifications provided in Environment Canada's "Biological Test Method: Growth Inhibition Test Using Freshwater Alga" (2007) (Table A.1). Test water was made up in a similar manner to growth media, but with lower nutrient concentrations. This water was formulated to regulate algal growth and supply the nutrients required to maintain biological function. The test water was also low in dissolved sulphate (SO_4^{2-}) and phosphate (PO_4^{3-}), anions known to compete with selenium oxyanions for uptake in primary producers (Fisher and Went 1993; Fournier et al. 2010; Lo et al. 2015; Riedel and Sanders 1996; Williams et al. 1994; Yu and Wang 2004). There are limited data available from studies that have examined selenate-sulphate antagonism at a range of sulphate concentrations comparable to the test water used in this study (nominal concentration of 3.6 mg SO_4^{2-} L^{-1}). Riedel and Sanders (1996) found that selenate uptake in the planktonic green algae, *Chlamydomonas reinhardtii,* was significantly reduced when SO_4^{2-} concentrations where increased from 4.8 and 9.6 mg L^{-1}; the concentration of sulphate in test water used for this study (list your concentration) should therefore have had minimal effect on selenate uptake. Riedel and Sanders (1996) also found that selenite uptake in *C. reinhardtii* was significantly depressed when

the concentration of K_2HPO_4 in test medium was increased from 0.871 and 1.742mg L^{-1}, which is substantially higher than the nominal concentration of 0.65mg L^{-1} used in this study.

2.3.4 Treatments

Selenium speciation and concentrations were selected to produce four different selenium treatments, plus a control, for a total of five different treatments. Nominal treatment conditions for the exposure phase included three replicates (*n*=3) each for: a control (no added selenium compounds), 5 and 25 µg Se L^{-1} as sodium selenite (Na_2SeO_3, Sigma-Aldrich, St. Louis, MO, USA) and 5 and 25 µg Se L^{-1} as sodium selenate (Na_2SeO_4, Sigma-Aldrich, St. Louis, MO, USA). Over the course of the exposure phase, water changes occurred every 2 days and consisted of replacing 50% of the exposure medium (2.75-L) with new test solution to prevent depletion of Se by algal accumulation. Regular water changes also helped to avoid "bloom dilution", where a high rate of algal growth increases competition for available Se in a static system, thus decreasing the available pool of Se for uptake and, in turn, decreasing the overall algal tissue-Se (Hills and Larsen 2005).

2.3.5 Sampling design

Samples for measurements of water quality (dissolved oxygen (DO), temperature, conductivity, pH, total hardness and total alkalinity) were taken from all replicates on days 0, 7 and 14 for all tests, and also on day 21 for the diatom test, to ensure that test conditions were consistent throughout the duration of each experiment. DO and temperature were measured with a portable meter (Orion Star A Series, Thermo Fisher Scientific, Mississauga, ON, Canada),

conductivity and pH with bench top probes (ATI Orion Model 170 and Orion 370, respectively, Thermo Fisher Scientific, Mississauga, ON, Canada), and hardness and alkalinity by titration (HACH digital titrator, HACH Company, Loveland, CO, USA). During water quality sampling, photosynthetically active radiation (PAR) was also measured at the center of the top surface of each exposure vessel using a 2π quantum sensor (Model MQ-500, Apogee Instruments, Logan, UT, USA). Water samples for dissolved selenium analysis were taken on the same days, but were collected in acid washed 8-mL HDPE sample bottles using syringe filters (0.45 μm pore size, polyethersulfone membrane, VWR International, Radnor, PA, USA) and acidified using high-purity nitric acid (Omnitrace Merck KGaA Darmstadt, Germany). Ten percent of the samples taken for dissolved Se measurement were method blanks, consisting of ultrapure water (17.4 MΩ-cm; Barnstead, Thermo Scientific, Waltham, MA, USA) passed through syringe filters, acidified and stored in a similar manner to test water samples. Table 2.1 summarizes water quality measurements of all test solutions.

Periphyton biofilms were sampled on days 0, 3, 7, 10, 14 and 21 (when applicable) to capture Se accumulation, ideally to a pseudo-steady state phase, for each species of algae tested. Periphyton biofilms were sampled by randomly selecting one of the sampling plates in each replicate and removing it from the exposure vessel. Biofilm from each plate was then scraped with a ceramic blade into 50-mL HDPE centrifuge tubes (BD Biosciences, Bedford, MA, USA). Each algae sample was then resuspended in ultrapure water, centrifuged at 2750 rpm for 15 min and the supernatant decanted. This was repeated twice, for a total of three rinses. After rinsing,

Table 2.1: Mean (± SD) water quality and dissolved Se measurements for each species of algae tested and all experimental treatments. Mean ± SD was calculated using all treatment replicates from water collected on all sampling days.

Algal Species	Algal Phylum	Treatment	DO ($mg\ L^{-1}$)	Temp. (°C)	Conductivity ($\mu S\ cm^{-1}$)	pH	Alkalinity ($mg\ CaCO_3\ L^{-1}$)	Hardness ($mg\ CaCO_3\ L^{-1}$)	PAR ($\mu mol\ m^{-2}\ s$)	Water Se ($\mu g\ Se\ L^{-1}$)
Anabaena flos-aquae	Cyanophyta	Control	7.9 ± 0.2	24.1 ± 0.2	75 ± 2	7.5 ± 0.2	26 ± 3	6 ± 1	19 ± 1	<LoD*
		5 µg Se(IV) L^{-1}	8.0 ± 0.1	23.9 ± 0.2	75 ± 1	7.4 ± 0.1	28 ± 3	8 ± 1	21 ± 2	4.01 ± 0.75
		25 µg Se(IV) L^{-1}	8.0 ± 0.1	23.9 ± 0.2	75 ± 1	7.5 ± 0.2	27 ± 3	7 ± 1	21 ± 3	23.07 ± 1.09
		5 µg Se(VI) L^{-1}	8.0 ± 0.1	23.7 ± 0.2	74 ± 1	7.4 ± 0.1	26 ± 3	6 ± 1	22 ± 2	4.84 ± 0.09
		25 µg Se(VI) L^{-1}	8.0 ± 0.2	23.8 ± 0.5	75 ± 2	7.5 ± 0.1	27 ± 3	7 ± 1	21 ± 3	24.07 ± 0.36
Stichococcus bacilliarus	Chlorophyta	Control	8.0 ± 0.2	23.9 ± 0.3	69 ± 3	7.7 ± 0.7	24 ± 5	5 ± 2	37 ± 2	<LoD*
		5 µg Se(IV) L^{-1}	8.0 ± 0.1	23.8 ± 0.4	69 ± 3	7.4 ± 0.2	24 ± 5	5 ± 1	36 ± 3	4.09 ± 0.66
		25 µg Se(IV) L^{-1}	8.0 ± 0.1	23.6 ± 0.3	69 ± 1	7.5 ± 0.2	26 ± 3	6 ± 1	35 ± 3	21.19 ± 1.63
		5 µg Se(VI) L^{-1}	8.0 ± 0.1	23.6 ± 0.4	68 ± 3	7.7 ± 0.7	25 ± 5	5 ± 1	34 ± 3	4.39 ± 0.28
		25 µg Se(VI) L^{-1}	8.1 ± 0.1	23.8 ± 0.4	67 ± 3	7.5 ± 0.3	27 ± 8	5 ± 1	38 ± 1	21.43 ± 1.42
Asterionella formosa	Bacillariophyta	Control	8.2 ± 0.2	23.8 ± 0.3	82 ± 11	7.6 ± 0.2	22 ± 2	7 ± 1	47 ± 8	<LoD*
		5 µg Se(IV) L^{-1}	8.2 ± 0.3	23.9 ± 0.2	78 ± 11	7.6 ± 0.2	25 ± 4	7 ± 1	46 ± 11	4.74 ± 0.52
		25 µg Se(IV) L^{-1}	8.2 ± 0.5	24.0 ± 0.2	78 ± 4	7.6 ± 0.2	25 ± 1	6 ± 0	50 ± 7	22.84 ± 2.53
		5 µg Se(VI) L^{-1}	8.1 ± 0.3	24.0 ± 0.2	79 ± 11	7.6 ± 0.2	24 ± 1	8 ± 2	48 ± 6	4.89 ± 0.19
		25 µg Se(VI) L^{-1}	8.1 ± 0.3	24.1 ± 0.2	78 ± 11	7.7 ± 0.3	24 ± 2	7 ± 1	50 ± 10	24.37 ± 0.67

*Instrumental limit of detection ranged from 0.032 to 0.21 µg Se L^{-1} for all Se analyses.

periphyton samples were frozen at -20°C and later freeze dried. Freeze dried materials were weighed in entirety to determine mass/area (mg d.w./cm^2) on sampling plates and later digested for Se analysis.

2.3.6 Se analysis

All Se concentrations were measured using ICP-MS operated in collision cell mode (8800 ICP-MS Triple Quad, Agilent Technologies, Santa Clara, CA, USA). Dissolved Se was measured directly from acidified test water and biofilm Se was measured in solution after digesting lyophilized algal tissue. Periphyton biofilms were microwave digested by weighing a known amount (10 − 20 mg d.w., depending on available tissue) of homogenized, freeze-dried algae into PTFE digestion vials. After weighing, 2 mL of high purity, 69% HNO_3 and 1.4 mL of high purity, 30% H_2O_2 (Sigma Aldrich, St. Louis, MO, USA) were added to each digestion vial. Sample vials were then capped and placed in a MARS-5 microwave digestion system (CEM Corporation, Matthews, NC, USA), ramping to 160°C for 20 min. When the digestion process was complete and samples had cooled, digests were transferred to 30-mL acid washed HDPE containers. PTFE digestion vials were then rinsed with 10 mL of ultrapure water, which was combined with the rest of the digested sample. Digested samples were filtered (0.45 μm pore size, polyethersulfone membrane) and diluted to 2% HNO_3 using ultrapure water before analysis.

The instrumental certified reference material (CRM) was "1640a – Trace Elements in Natural Water" (National Institute of Standards and Technology, Gaithersburg, MD, USA). The mean of the 1640a analyses for Se was 100.1 ± 1.6 % (mean ± SE) of the certified value, and measured concentrations of Se in all method blanks (10% of total number of samples) were

below the limit of detection for water (0.032 - 0.21 μg Se L^{-1}) and low relative to the measured concentrations of tissue-Se (all tissue-Se concentrations were blank-subtracted). TORT-3 (lobster hepatopancreas) from NRC Canada (Institute for Environmental Chemistry, Ottawa, CA) served as the tissue CRM. Three separate tissue digestions were required for each species of algae tested (9 digestions total), with measured CRM values of 102.6 ± 2.4 %, 103.9 ± 11.5 % and 94.3 ± 2.6 % (mean ± SE) of the certified value for *S. bacillaris*, *A. flos-aquae* and *A. formosa*, respectively. The instrumental limit of detection ranged from 0.032 to 0.21 μg Se L^{-1} for all Se analyses.

2.3.7 Data analyses

Mean Se concentrations in periphyton biofilms were compared among sampling days, for each algal species tested, using one-way repeated measures analysis of variance (ANOVA) followed by Tukey's honest significant difference *post-hoc* test to determine time to pseudo-steady state for each treatment. Biofilm data were $\log_{10}$-transformed when necessary to achieve normality and homoscedasticity. Periphyton biofilms were considered to be at pseudo-steady state when biofilm Se was statistically similar between subsequent sampling days for a given species of algae.

The enrichment function of Se, from water to biofilm, was calculated as described in Eq. 2.1 using mean biofilm Se concentration for all treatment replicates on a given sampling day and overall mean water Se values for each treatment.

$$\text{Enrichment Function (EF)} = \frac{\text{Biofilm Se (mg Se/kg d.w.)}}{\text{Water Se (mg Se/L)}} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (2.1)$$

Biofilm Se and EF data from the last day of sampling were used for statistical comparison between treatment levels and among algal species tested. These data were selected as

a best estimate for pseudo-steady state. Mean biofilm Se and EFs, at pseudo-steady state, were compared using one-way ANOVA followed by Student–Newman–Keuls method for multiple comparisons. One-way ANOVA on ranks was employed when data were non-parametric and transformation was unsuccessful in achieving normality and homoscedasticity.

Percent change in biomass per unit area (growth) over the duration of the test, was calculated for each treatment replicate. Mean percent change in biomass was compared across algal species using a one-way ANOVA on ranks (data were non-parametric and transformation was unsuccessful).

2.4 Results

2.4.1 Algal growth

Algal growth (as % change in biomass/cm^2) over the course of the exposure phase was statistically similar across all three species tested (p=0.916). Mean (±SD) percent changes in biomass/cm^2 over each test duration were: 206 ± 105 %, 198 ± 80 % and 167 ±20 % for *S. bacillaris*, *A. flos-aquae* and *A. formosa*, respectively. Algal biomass/area (mg d.w./cm^2) for each sampling day is shown in Figure 2.1.

2.4.2 Pseudo-steady state

By the final two sampling days, pseudo-steady state for each treatment level, was achieved for: all Se treatments in *S. bacillaris* (chlorophyte) and *A. flos-aquae* (cyanophyte) and all Se treatments except for 25 μg Se L^{-1} selenate in *A. formosa* (diatom) (Figure 2.2). For *A.*

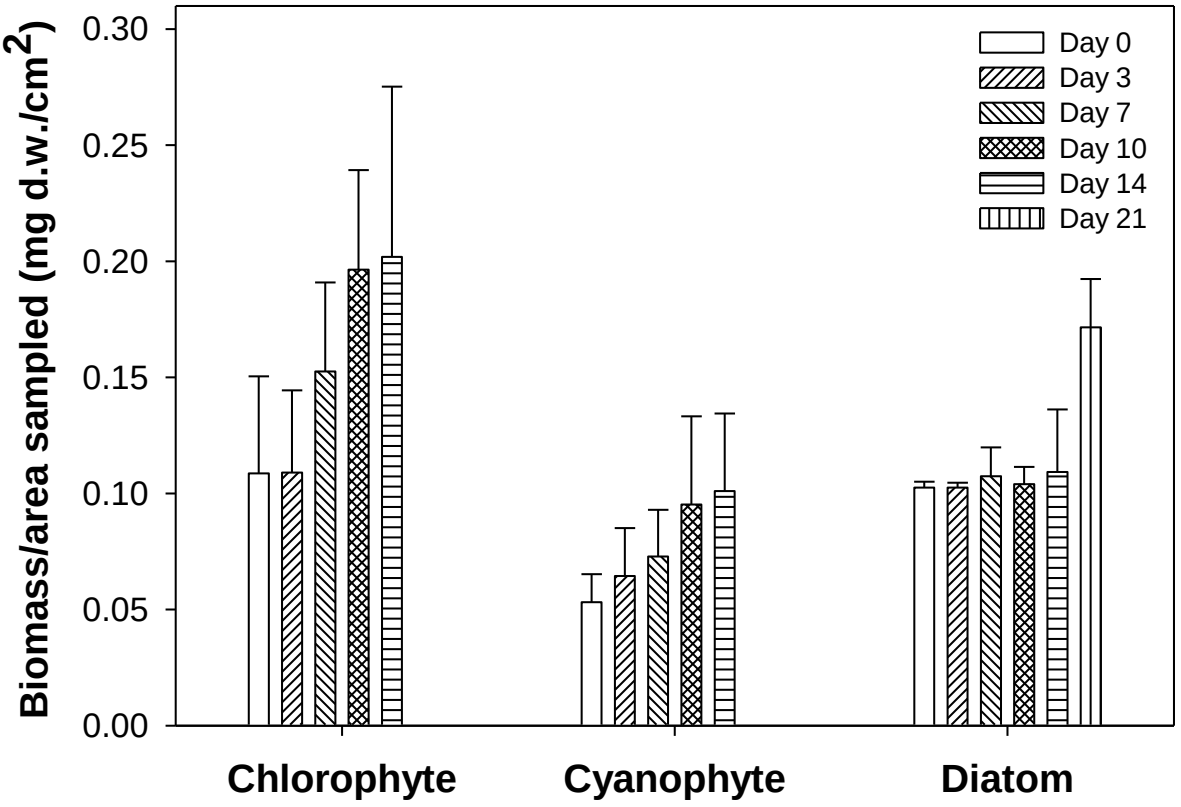

Figure 2.1: Mean dry weight biomass per unit area for biofilm sampled on days 0, 3, 7, 14 and 21 (when applicable), for three different algal species. Error bars represent one standard deviation.

formosa, biofilm Se in the 25 μg Se L^{-1} selenate treatment increased significantly between days 14 and 21 (p<0.001). In general, biofilm Se concentrations from the final sampling day were assumed to serve as best estimates for pseudo-steady state across all exposures and treatment levels.

2.4.3 Biofilm Se and EFs

Mean biofilm Se concentrations at the end of the exposure period were significantly different among treatment groups for all three species of algae tested (p<0.001, p<0.001 and p=0.011 for *S. bacillaris* [Figure 2.2A], *A. flos-aquae* [Figure 2.2B], and *A. formosa* [Figure 2.2C], respectively). *Post-hoc* statistical differences among treatment groups (p<0.05) are indicated graphically.

Mean EFs for all Se treatments in the *S. bacillaris* experiment (Figure 2.3A) were not statistically different on the last sampling day (p=0.090), ranging from 758 to 2566 with a high degree of variability within treatments. Conversely, mean EFs for Se treatments in the *A. flos-aquae* test (Figure 2.3B) were statistically different on the final sampling day (p<0.001), ranging from 343 to 6050. Mean EFs for the *A. formosa* test (Figure 2.3C) were also significantly different on the last sampling day, ranging from 1036 to 4375, with greater EFs observed for selenite.

Comparison of biofilm Se concentrations and EFs between similar treatments in different algal species at pseudo-steady state (Table 2.2) showed that statistical differences among species were typically similar when comparing biofilm Se or EFs. This indicates that measured dissolved Se concentrations (summarized in Table 2.1) were similar among tests and consistent for the length of the exposure phase in all tests. If dissolved Se concentrations had been different among

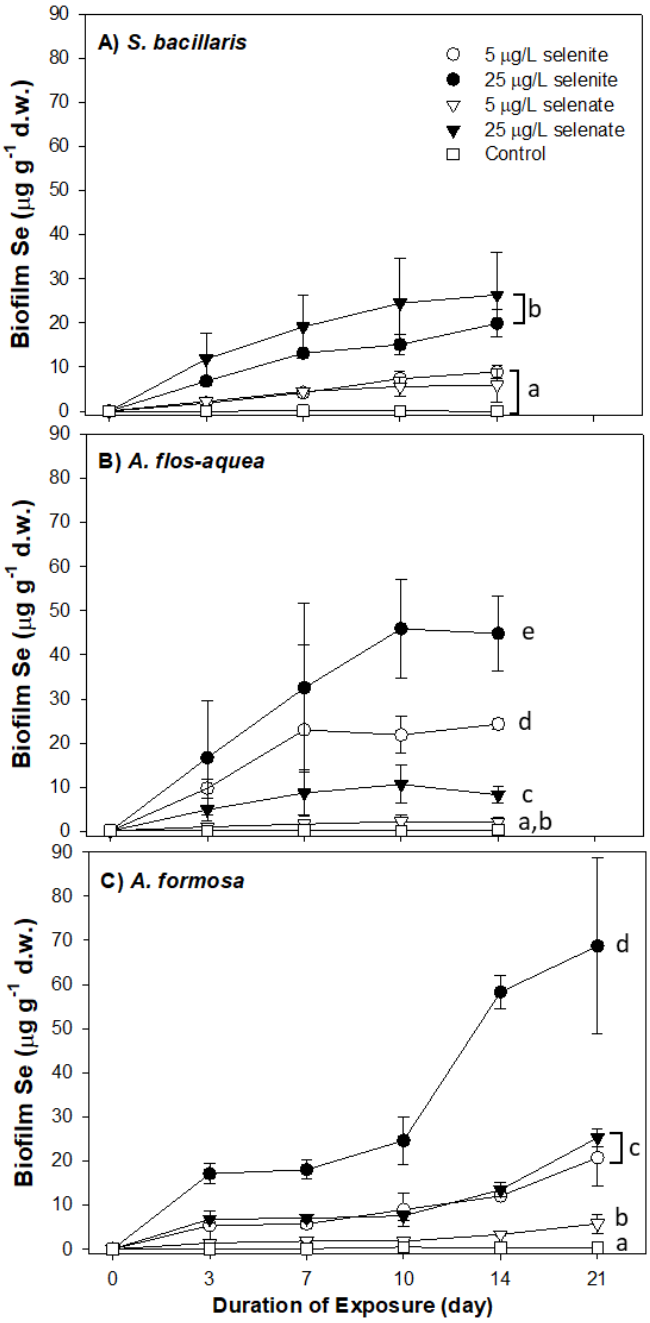

Figure 2.2: Mean biofilm Se concentration on sampling days 0, 3, 7, 14 and 21 (when applicable) in (A) *Stichococcus bacillaris* (Chlorophyta), (B) *Anabeana flos-aquea* (Cyanophyta) and (C) *Asterionella formosa* (Bacillariophyta). Error bars represent one standard deviation and statistical differences are indicated by different letters.

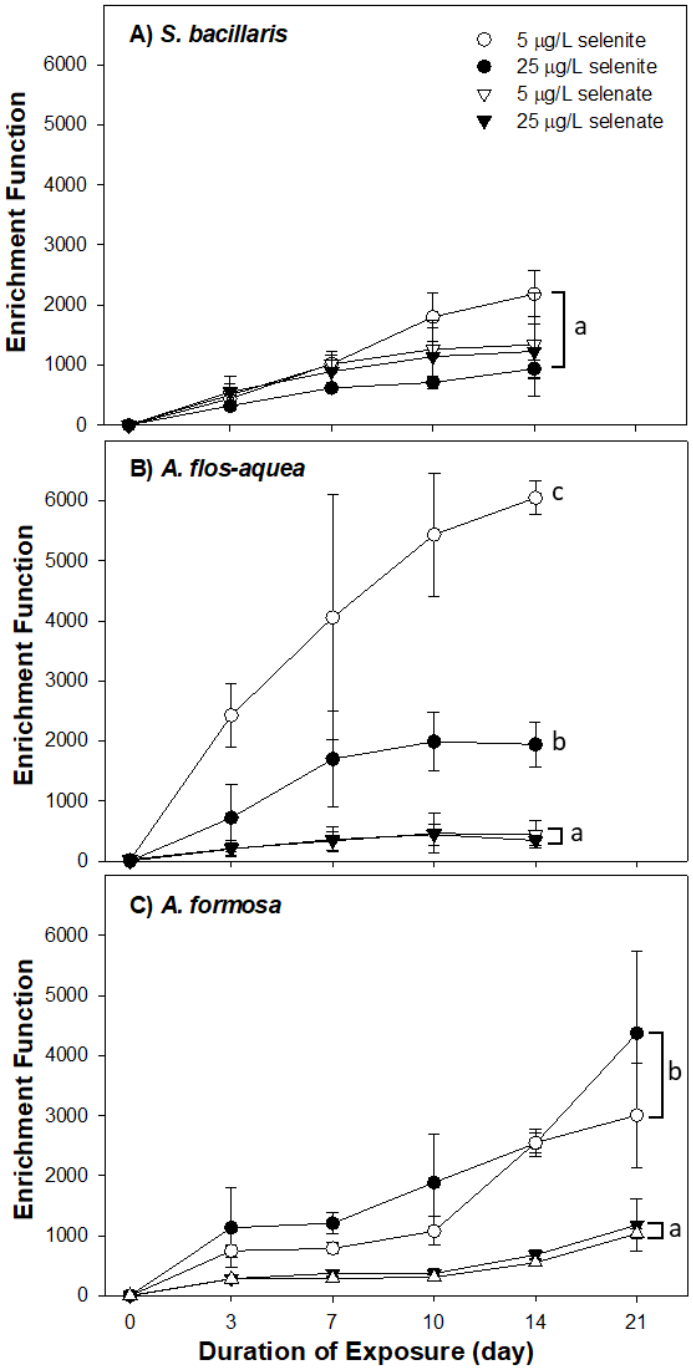

Figure 2.3: Mean enrichment functions on sampling days 0, 3, 7, 14 and 21 (when applicable) in (A) *Stichococcus bacillaris* (Chlorophyta), (B) *Anabeana flos-aquea* (Cyanophyta) and (C) *Asterionella formosa* (Bacillariophyta). Error bars represent one standard deviation and statistical differences are indicated by different letters.

Table 2.2: Mean biofilm Se concentrations and enrichment functions at the end of the exposure phase (best estimate of pseudo-steady state). Statistical differences among algal phyla tested, for each Se treatment, are indicated with different letter.

	Biofilm Se at Pseudo-steady State (μg g^{-1} d.w.)			Enrichment Function at Pseudo-steady State		
Treatment	Chlorophyte	Cyanophyte	Diatom	Chlorophyte	Cyanophyte	Diatom
5 μg L^{-1} selenite	8.9 ± 1.6^{a}	24.3 ± 1.1^{b}	20.8 ± 6.5^{b}	2184 ± 383^{a}	6050 ± 280^{c}	4375 ± 1370^{b}
25 μg L^{-1} selenite	19.9 ± 3.1^{a}	44.8 ± 8.5^{ab}	68.7 ± 19.9^{b}	938 ± 146^{a}	1943 ± 369^{ab}	3008 ± 870^{b}
5 μg L^{-1} selenate	5.9 ± 3.8	2.2 ± 1.1	5.8 ± 2.1	1338 ± 865	444 ± 235	1179 ± 435
25 μg L^{-1} selenate	26.3 ± 9.6^{b}	8.2 ± 1.8^{a}	25.3 ± 2^{b}	1229 ± 447^{b}	343 ± 76^{a}	1036 ± 80^{b}

tests, the EFs would show different statistical trends than biofilm Se due to the way it is calculated (Eq. 2.1); small differences in water Se concentration result in large changes to the EF due to the large difference in magnitude between the numerator and denominator. For the 5 μg Se L^{-1} selenite treatment, final day, mean biofilm Se (p=0.004) and mean EFs (p=0.007) were statistically different among algal test species. *Post-hoc* analysis revealed that biofilm Se was lower for *S. bacillaris* compared to *A. flos-aquae* and *A. formosa*, and that EFs for all three algal species were statistically different from one another. For the 25 μg Se L^{-1} selenite treatment, final day biofilm Se (p=0.009) and EFs (p=0.011) were statistically different among algal test species as well: *S. bacillaris* and *A. formosa* were statistically different while *A. flos-aquae* was not different from either of the other test species. There were no statistical differences in biofilm Se (p=0.217) or EFs (p=0.217) among algal test species for the 5 μg Se L^{-1} selenate treatment. For the 25 μg Se L^{-1} selenate treatment, biofilm Se (p=0.003) and EFs (p=0.002) were different among algal test species: *A. flos-aquae* had significantly lower biofilm Se and EFs than *S. bacillaris* and *A. formosa*, which were statistically similar. See Table 2.2 for a summary of mean biofilm Se and EFs on the final sampling day, including statistical differences.

2.5 Discussion

2.5.1 Growth

Mean biomass/area for each species of algae increased as the exposure phase progressed, indicating that periphyton biofilms were growing throughout the test. The initial stagnation in growth for *A. formosa*, and subsequent growth spike, may be a result of unfavourable conditions in the test system relative to culturing vessels (where nutrient concentrations, such as silica, were much higher) which required an acclimation period. An alternative explanation is that the diatom

biofilms were very loosely associated with sampling plates at the beginning of the test, but by the end were more consolidated due to production of extracellular polymeric substances (Stevenson 1996).

Growth dilution, where algal biomass is increasing at a rate outstripping the cellular capacity for Se uptake, has the potential to create variations in Se enrichment due to the dilution of intracellular Se with photosynthetically fixed carbon (Hills and Larsen 2005). Apparent differences in Se enrichment due to growth dilution could confound those that occur as a result of taxon-specific differences in Se enrichment, which were the focus of this study. Because growth was statistically similar among algal test species, and biofilm Se concentrations were generally stable between final sampling days (pseudo-steady state), it is reasonable to conclude that growth dilution was not the cause of observed differences in Se enrichment between algal test species.

2.5.2 Intraspecific differences in biofilm Se and EFs at pseudo-steady state

For *S. bacillaris* (Chlorophyta), selenate and selenite were similarly bioconcentrated in a concentration-dependent manner, where increasing ambient Se concentration resulted in higher Se bioconcentration. The concentration-dependent nature of Se bioconcentration indicates that Se accumulation mechanisms, for both inorganic Se compounds, were not saturated at the range of dissolved Se tested (Baines and Fisher 2001; Fournier et al. 2006). Statistical similarities among all EFs for this species of algae provide further proof that Se accumulation was not saturated at the concentrations tested. However, it is difficult to determine with certainty whether the relationships between ambient Se and biofilm Se were proportional or non-proportional (partial saturation) due to the variability within treatment groups; a proportional/linear

relationship between ambient Se and biofilm Se would result in statistically similar EFs with increasing ambient Se concentration. Regardless, the results are consistent with the findings of Fournier et al. (2006), who demonstrated that the accumulation of selenate and selenite in the green freshwater algae *Chlamydomonas reinhardtii* (Chlorophyta) was not saturated when ambient Se concentration was below 1000 μg Se L^{-1}.

Selenite was bioconcentrated to a greater degree than selenate at both test concentrations for *A. flos-aquae* (Cyanophyta). This suggests that *A. flos-aquae* has a higher capacity for the accumulation of selenite compared to selenate. Selenite bioconcentration was concentration-dependent (an increase in ambient selenite concentration resulted in an increase in biofilm-Se), but the relationship was not proportional to ambient Se concentrations, as indicated by declining EFs between low and high concentration treatment groups (6050 vs. 1943). This suggests that selenite accumulation mechanisms were becoming saturated in the range of Se concentrations used for this exposure (Baines and Fisher 2001). Unlike selenite, selenate bioconcentration occurred in a linear, concentration-dependent fashion indicative of unsaturated accumulation, as demonstrated by statistical similarities between EFs in low and high concentration selenate groups.

Selenite was also preferentially bioconcentrated over selenate at both test concentrations for *A. formosa* (Bacillariophyta), and bioconcentration of both inorganic Se compounds occurred in a concentration-dependent manner. Similarities between EFs for low and high concentration groups, for each Se compound, indicate that Se accumulation mechanisms were not saturated and that Se enrichment was proportional to ambient Se concentration at the range of Se concentrations tested.

2.5.3 Interspecific differences in biofilm Se and EFs at pseudo-steady state

The results of these experiments show that selenite enrichment was higher in the cyanophyte, *A. flos-aquae*, than in the other test species for the low concentration treatment (5 µg Se L^{-1} as selenite). This relationship was not consistent with increasing ambient selenite concentration, as the diatom, *A. formosa*, showed statistically similar Se enrichment to *A. flos-aquae* for the high concentration treatment (25 µg Se L^{-1} as selenite). The chlororphyte, *S. bacillaris*, had the lowest enrichment of selenite for both treatments, but EFs were statistically similar between *S. bacillaris* and *A. flos-aquae* in the high concentration treatment. Overall, *S. bacillaris* exhibited lower selenite enrichment than *A. formosa*, which is consistent with findings of previous selenite bioconcentration tests using phytoplankton species from algal phyla similar to those used in this study; chlorophytes typically exhibit lower selenite enrichment compared to diatoms (Riedel et al. 1991; Baines and Fisher 2001). Conversely, Riedel et al. (1991) observed that *A. flos-aquae* took up less selenite than other algal species tested (*C. reinhardtii* and *Cyclotella meneghiania*) over the course of a 24-hour selenite exposure. It is not possible to determine whether this trend would have continued if the test length was extended to a duration comparable to the one used here.

In the low concentration selenate treatment (5 µg Se L^{-1}), Se enrichment was not statistically different among the three algal species, but the trend was similar to the statistical differences observed at the high selenate concentration (25 µg Se L^{-1}). In the high concentration selenate treatment, *S. bacillaris* and *A. formosa* showed higher Se enrichment than *A. flos-aquae*. This is consistent with the findings of previous studies, where diatoms and chlorophytes accumulated more selenate than cyanophytes did under similar conditions (Riedel et al. 1991). In general, selenate showed a lower degree of enrichment relative to selenite, which supports the

consensus that selenite is the more available form of inorganic Se to algae (Baines et al. 2001; Conley et al. 2013; Hu et al. 1997; Riedel et al. 1991; Stewart et al. 2010; Vandermeulen and Foda 1988).

Although some trends in Se bioconcentration observed in this study matched well with those reported by others, overall, enrichment was lower than observed in some previous studies. The highest degree of Se enrichment in this study, observed in *A. flos-aquae* 5 μg Se L^{-1} as selenite treatment, was a mean of 6050-times the mean ambient Se concentration. Compared to the potential 10^6-fold enrichment observed by Baines and Fisher (2001) in marine phytoplankton species, this is quite low. This difference is likely related to the lower Se concentration range used for the Baines and Fisher (2001) study (0.01 - 0.36 μg Se L^{-1}), given that EFs tend to decrease with increasing ambient Se concentration due to saturation of Se accumulation mechanisms (Baines and Fisher 2001; Fournier et al. 2006). At exposure concentrations similar to our study (i.e., 10 μg Se L^{-1} as selenate and selenite), comparable EFs of 1580 (selenate) and 2030 (selenite) were observed in multi-species periphyton exposed for 8 days (100% static-renewal of test solutions every 24 hours) (Conley et al. 2013). It should be noted that ambient sulphate concentrations were comparatively high, at a nominal concentration of 40.6 mg L^{-1}, and this likely inhibited selenate accumulation (Lo et al. 2015). The degree of selenate enrichment (ranging from 343 to 1338) observed in our study is also comparable to that observed by Lo et al. (2015), where the concentration of tissue-Se in the green alga, *Pseudokirchneriella subcapitata*, was 249-times the water-Se concentration, when exposed to 10 μg Se L^{-1} for a period of 7 days with an ambient sulphate concentration of 5 mg L^{-1} (a similar concentration as used in our study). However, the Se enrichment observed by Lo et al. (2015) was likely biased downward due to the lack of test solution renewal during the exposure (i.e., bloom dilution).

Differences in Se enrichment among the algal species and Se compounds tested suggests that algae (here periphyton) from different phyla differentially bioconcentrated inorganic Se compounds and that mechanisms controlling the accumulation of Se in different types of algae can have different capacities for each Se oxyanion. In addition, interspecific differences in Se enrichment under similar environmental conditions (similar water quality variables and ambient Se concentration) were much smaller than previously observed in planktonic algae, which have been reported to vary by several orders of magnitude (Baines and Fisher 2001). A maximum of a 3.6-fold difference in enrichment was observed among 25 μg Se L^{-1} as selenate treatment groups, across three diverse algal taxa characteristic of freshwater ecosystems. These findings are similar to those observed by Friesen et al. (2017), where a broad range of genetically different periphyton communities showed only a 6.7-fold difference in Se enrichment when ambient Se concentrations were similar. This may be a result of the test organisms existing as part of a biofilm, where the protective nature of biofilms decrease exposure to dissolved nutrients/metals (Stevenson 1996). Regardless, these observations will help to reduce uncertainty when modelling Se fate and transport in periphyton dominated, freshwater ecosystems. EFs, often the largest uncertainty factor, do not appear to vary by orders of magnitude for freshwater periphyton when exposure conditions are similar, but rather by less than one order of magnitude when ambient Se concentrations are high enough to exceed water quality guidelines (1.5 – 3.1 μg Se L^{-1}, depending on the receiving environment [US EPA, 2016]). Although the specific (algal) species present in a Se-contaminated freshwater ecosystem may be important for predicting Se accumulation in algae, other factors, such as productivity (growth dilution, bloom dilution) and water quality (competing ions), may be of similar or greater importance under certain circumstances.

2.5.4 Acknowledgements

This research was supported by an NSERC Strategic Projects Grant to M. Hecker, K. Liber, and D. Janz (grant # STPGP 479047-15).

CHAPTER 3

SELENIUM OXYANION BIOCONCENTRATION IN NATURAL FRESHWATER PERIPHYTON

Preface

The research in this chapter was designed to assess the bioconcentration of inorganic selenium, as selenate or selenite, in natural freshwater periphyton sampled from different waterbodies. The secondary objective of the research presented herein was to assess the relative contribution of adsorption processes and uptake in non-phototrophic organisms on the apparent bioconcentration of selenite in periphyton known to be a high accumulator. This chapter has been accepted by the journal Ecotoxicology and Environmental Safety. The full citation is: Markwart B, Liber K, Xie Y, Raes K, Hecker M, Janz D, Doig LE. 2019. Selenium oxyanion bioconcentration in natural freshwater periphyton. Ecotoxicology and Environmental Safety (accepted pending minor revision).

The author contributions to chapter 3 of this thesis were as follows:

Blue Markwart (University of Saskatchewan) collected, processed and analyzed all samples, performed all statistical analyses and drafted the manuscript.

Karsten Liber (University of Saskatchewan) helped design the study, provided scientific input and guidance; reviewed and revised the manuscript, providing comments and corrections; procured and provided funding required to conduct the research.

Yuwei Xie (University of Saskatchewan) provided scientific input and helped conduct complex statistical analyses.

Katherine Raes (University of Saskatchewan) helped design the study and provided scientific input.

Markus Hecker (University of Saskatchewan) helped design the study, provided scientific input and guidance; reviewed and revised the manuscript, providing comments and corrections; procured and provided funding required to conduct the research.

David Janz (University of Saskatchewan) helped design the study, provided scientific input and guidance; reviewed and revised the manuscript, providing comments and corrections; procured and provided funding required to conduct the research.

Lorne Doig (University of Saskatchewan) helped design the study, provided scientific input and guidance; reviewed and revised the manuscript, providing comments and corrections.

3.1 Abstract

Selenium (Se) enrichment has been demonstrated to vary by several orders of magnitude among species of planktonic algae. This is a substantial source of uncertainty when modeling Se biodynamics in aquatic systems. In addition, Se bioconcentration data are largely lacking for periphytic species of algae, and for multi-species periphyton biofilms, adding to the challenge of modeling Se transfer in periphyton-based food webs. To better predict Se dynamics in periphyton dominated, freshwater ecosystems, the goal of this study was to assess the relative influence of periphyton community composition on the uptake of waterborne Se oxyanions. Naturally grown freshwater periphyton communities, sampled from five different water bodies, were exposed to environmentally relevant concentrations of selenite [Se(IV)] or selenate [Se(VI)] (nominal concentrations of 5 and 25 μg Se L^{-1}) under similar, controlled laboratory conditions. Unique periphyton assemblages were derived from the five different field sites, as confirmed by light microscopy and targeted DNA sequencing of the plastid 23S rRNA gene in algae. Selenium accumulation demonstrated a maximum of 23.6-fold difference for Se(IV) enrichment and 2.1-fold difference for Se(VI) enrichment across the periphyton/biofilm assemblages tested. The assemblage from one field site demonstrated both high accumulation of Se(IV) and iron, and was subjected to additional experimentation to elucidate the mechanism(s) of uptake/accumulation. Selenite accumulation was assessed in both unaltered and heat-killed periphyton, and in periphyton from the same site grown without light to exclude phototrophic organisms. All periphyton treatments showed similar levels of Se accumulation, indicating that much of the apparent uptake of Se(IV) was due to non-biological processes (i.e., surface adsorption). The results of this study will help reduce uncertainty in the prediction of Se dynamics and food-chain transfer in freshwater environments. Further exploration of the

ecological consequences of extracellular adsorption of Se(IV) to periphyton, rather than intracellular absorption, is recommended to further refine predictions related to Se biodynamics in freshwater food webs.

3.2 Introduction

Although naturally occurring at low background concentrations, selenium (Se) is released to the aquatic environment as a by-product of various economically important activities, including coal-fired energy production, crude oil refinement, and the mining of coal, phosphate, copper and uranium (Janz et al. 2014; Lemly 2004; Young et al. 2010). Selenium is mobilized and transported to aquatic ecosystems via effluent, or when a Se-containing matrix, like mine tailings or coal fly-ash, comes into contact with water (Young et al. 2010). Anthropogenic Se is typically released as an inorganic oxyanion, either as selenate (+6 oxidation state) or selenite (+4 oxidation state), depending on the source or processing of Se-bearing materials (Maher et al. 2010). For example, in the Elk Valley, BC, Canada, selenium is released primarily as selenate from weathering of coal mine waste rock (Martin et al. 2011). Regardless of the source, Se impacted waters typically contain no more than 10 – 100 μg Se L^{-1} (Maher et al. 2010).

Selenium is an essential trace element with a narrow margin between nutritionally optimal and potentially toxic dietary exposures in vertebrate animals (Mayland 1994; Renwick 2006). In aquatic ecosystems, oviparous vertebrates, such as fish and waterfowl, have the lowest thresholds for Se toxicity (Stewart et al. 2010), with reproductive failure and teratogenicity occurring at dietary exposures of only 7–30 times optimal levels (Hodson and Hilton 1983). At sufficiently high levels, Se contamination can result in the extirpation of local fish or bird populations. In aquatic ecosystems, dissolved Se is bioconcentrated by microorganisms and

primary producers (e.g., algae, periphyton), biotransformed into organic Se compounds, and passed on to higher trophic levels primarily through dietary exposure (trophic transfer) (Fan et al. 2002; Presser and Luoma 2010). The enrichment of Se in algae appears to be highly variable, with several orders of magnitude difference in Se bioconcentration reported for different species of phytoplankton at a given concentration of ambient Se (Baines and Fisher 2001). Interspecific differences for Se bioconcentration in algae may be the result of different cellular requirements for Se or different cellular capacity to regulate uptake (Stewart et al. 2010). There is a large body of evidence to indicate that the uptake of inorganic Se in algae is a carrier mediated, active transport process that can be saturated, as described by Michaelis-Menton kinetics (Baines and Fisher 2001; Fisher and Wente 1993; Fournier et al. 2006; Riedel et al. 1991). Consequently, the relationship between ambient Se concentration and Se concentration in algae may be non-linear if ambient Se concentration approaches or exceeds transport saturation limits for a particular species of algae (Baines and Fisher 2001).

The speciation of inorganic Se in water (Se[IV] vs Se[VI]) is also an important factor that can affect Se bioconcentration in algae because there are different transport pathways for different Se species (Wallschlager and Feldmann 2010). The pathway for Se(IV) uptake (as selenite) is not well characterized at the current time, but there is evidence to suggest that Se(IV) is taken up competitively via the phosphate transporter in plants (Hopper and Parker 1999). Conversely, the Se(VI) (as selenate) uptake pathway in algae is well described; Se(VI) is taken up competitively through the sulphate pathway (Fisher and Went 1993; Lo et al. 2015). As such, the accumulation of different dissolved, inorganic Se species in algae can be influenced by competing ions when ambient concentrations of such are sufficiently high (Fisher and Wente 1993; Lo et al. 2015; Ponton et al. 2018; Riedel and Sanders 1996). Se speciation can also be

important for adsorbent-adsorbate interactions. Selenite is known to adsorb strongly to iron oxyhydroxides, whereas selenate shows no meaningful interaction (Balistrieri and Chao 1990). Reduction of Se(VI) to Se(IV) is typically a unidirectional process, with the ratio of Se(IV) to Se(VI) increasing as you sample farther downstream from a selenate source due to the thermodynamic unfavorability of Se(IV) oxidation to Se(VI) under natural conditions (Cutter and Bruland 1984; Luoma and Presser 2009).

Periphyton, defined as a complex mixture of algae (green algae, cyanobacteria, diatoms, etc.), heterotrophic bacteria and detritus on or associated with submerged substrata (Stevenson 1996), can vary greatly in composition depending on environmental factors (Chisolm 1992; Cloen and Dufford 2005; Lowe 1996). In shallow bodies of water where a large portion of the benthic zone receives enough light to support photosynthesis, periphyton can dominate carbon fixation (Lowe 1996). In general, cells in biofilms are better protected from chemical, physical and biological stress than are planktonic forms (Singh et al. 2006). Previous studies regarding Se uptake and bioconcentration in algae have focused primarily on free-floating species (phytoplankton) cultured in the lab (Baines and Fisher 2001; Fournier et al. 2006; Hu et al. 1997; Kiffney and Allen 1990; Riedel et al. 1991; Riedel and Sanders 1996). Additionally, many of the species of algae used in previous studies have been marine species (Baines and Fisher 2001). There have been a number of recent studies that have utilized complex periphyton assemblages for Se accumulation experiments (Conely et al. 2009; Conely et al. 2013; Friesen et al. 2017), but to our knowledge no other published research has involved the testing of differential Se bioconcentration in field-grown periphyton exposed under similar conditions.

To better predict Se dynamics in periphyton dominated, freshwater ecosystems, the main goal of this study was to assess the relative influence of differing periphyton community

composition, as defined by differences in the phototrophic (algal) assemblage, on the uptake and bioconcentration of waterborne Se oxyanions. This goal was addressed by exposing genetically different field-collected periphyton communities to environmentally relevant concentrations of Se oxyanions (selenite or selenate) under controlled laboratory conditions. Using a complex periphyton community demonstrating high Se accumulation, the secondary goal of this study was to investigate the relative influence of other important components and processes (adsorption and uptake in non-phototrophic microorganisms) on the bioconcentration of Se(IV) (as selenite). This was done by comparing Se bioconcentration in natural periphyton with Se accumulation in similar periphyton that had been heat-treated to cease all biological processes, and in biofilms that were grown under similar conditions, but without light, to exclude phototrophs.

3.3 Materials and methods

3.3.1 Field sites

A total of fifteen different lakes and ponds within the Boreal Plains ecozone in Saskatchewan, Canada, were sampled in May 2016 and analyzed for basic water chemistry parameters (dissolved oxygen (DO) concentration, temperature, total hardness, alkalinity, conductivity, pH, sulphate, total nitrogen and orthophosphate. Of these water bodies, five lakes (located approximately 300 to 330 km northeast of Saskatoon, SK) with different characteristics were selected as sampling sites; sites that were predicted to produce different communities of periphyton as a result of the different environmental requirements across algal taxa (Chisolm 1992; Cloen and Dufford 2005; Lowe 1996). Field-sites were designated with a site number rather than the name of the water body as some of the water bodies were unnamed. The

following are the site numbers with the accompanying GPS coordinates: Site 1 (Cub Lake) – 54°17'39.84"N, 104°33'23.46"W; Site 2 (Summit Lake) – 54° 9'49.80"N, 104°45'43.14"W; Site 3 (Chris' Lake) – 54°17'7.50"N, 104°40'21.36"W; Site 4 (Unnamed) – 54°17'2.40"N, 104°38'30.66"W; Site 5 (Unnamed) – 53°44'26.82"N, 104°35'38.64"W. (See Figure A.1 for a map of the field-site locations). Site 5 was the focus of the second field season in the summer of 2017. Table 3.1 summarizes the water quality parameters measured at the periphyton sampling sites during both field seasons.

3.3.2 Collection of natural periphyton

Periphyton samplers (Figure 3.1) were designed and constructed in-house at the Toxicology Centre, University of Saskatchewan. Each sampler was constructed from PVC pipe and held five pieces of frosted soda-lime-silicate glass (20 cm x 20 cm x 5 mm) as substrates for periphyton colonization. The frame was slotted at regular intervals and was designed to allow each of the five glass plates to be oriented in a vertical position to reduce the deposition of settling materials. Different algal species have different substrate requirements and some are not able to adhere to polished glass surfaces (Tarkowska-Kukuryk and Mieczan 2012). Therefore, the glass plates were frosted to enhance colonization. Periphyton samplers were deployed at a depth of approximately 1 m, at the sediment-water interface in each of the five selected lakes. In total, each lake received five samplers (five plates per sampler), for a total of 25 glass sampling plates per lake. After a six-week colonization period, samplers were collected and immediately transported in coolers filled with site water to the Toxicology Centre, University of Saskatchewan. Due to logistical limitations, periphyton from each lake was tested individually, with a new test being initiated every week for five weeks.

Table 3.1: Routine water quality, nutrient levels and dissolved Se measured in site water from each of the five sampling sites. For each site, the top number in each column represents measurements taken from water sampled at the beginning of the field season (June 2016; July 2017 for Site 5, year 2) and the bottom number represents water sampled at the end of the field season, when samplers were removed from the field-sites (Aug-Sept 2016; Sept 2017 for Site 5, year 2).

Periphyton Treatment	Se Treatment	DO ($mg\ L^{-1}$)	Temp. (°C)	Conductivity ($\mu S\ cm^{-1}$)	pH	Alkalinity ($mg\ CaCO_3\ L^{-1}$)	Hardness ($mg\ CaCO_3\ L^{-1}$)	PAR ($\mu mol\ m^{-2}\ s$)	Water Se ($\mu g\ Se\ L^{-1}$)	CV for Water Se (%)
Natural	Control	8.6 ± 0.2	19.3 ± 0.6	70 ± 1	7.5 ± 0.0	23 ± 2	6 ± 1	17 ± 2	<LoD	-
	5 µg Se(IV)/L	8.6 ± 0.1	19.3 ± 0.5	69 ± 1	7.5 ± 0.1	24 ± 1	7 ± 1	19 ± 2	4.26 ± 0.42	9.9
	25 µg Se(IV)/L	8.6 ± 0.1	19.4 ± 0.6	70 ± 0	7.5 ± 0.0	24 ± 2	7 ± 1	20 ± 1	21.25 ± 2.0	9.6
Heat Killed	Control	8.7 ± 0.1	18.9 ± 0.3	70 ± 2	7.5 ± 0.0	20 ± 3	6 ± 1	<LoD	<LoD	-
	5 µg Se(IV)/L	8.8 ± 0.1	18.7 ± 0.3	70 ± 1	7.5 ± 0.0	21 ± 2	6 ± 1	<LoD	4.27 ± 0.54	12.6
	25 µg Se(IV)/L	8.8 ± 0.1	18.7 ± 0.4	70 ± 1	7.5 ± 0.0	21 ± 2	6 ± 1	<LoD	21.21 ± 2.5	12.1
Shade Cloth	Control	8.7 ± 0.1	19.1 ± 0.5	69 ± 1	7.5 ± 0.0	23 ± 2	6 ± 1	<LoD	<LoD	-
	5 µg Se(IV)/L	8.7 ± 0.1	19.2 ± 0.5	68 ± 1	7.5 ± 0.1	24 ± 2	6 ± 1	<LoD	4.38 ± 0.20	4.6
	25 µg Se(IV)/L	8.6 ± 0.1	19.2 ± 0.5	69 ± 1	7.5 ± 0.0	24 ± 2	7 ± 1	<LoD	22.56 ± 0.6	2.8
FeO(OH)	Control	8.8 ± 0.1	18.8 ± 0.4	69 ± 1	7.5 ± 0.1	22 ± 2	6±1	<LoD	<LoD	-
	5 µg Se(IV)/L	8.8 ± 0.1	18.7 ± 0.4	69 ± 1	7.5 ± 0.0	22 ± 1	6±1	<LoD	2.28 ± 1.75	76.8
	25 µg Se(IV)/L	8.8 ± 0.1	18.7 ± 0.5	70 ± 1	7.5 ± 0.0	21 ± 2	7±1	<LoD	13.37 ± 7.5	56.2

Limits of Detection for: water selenium – 0.026 µg Se L^{-1}; total nitrogen – 0.011 - 0.013 mg TN L^{-1}; sulphate – 0.006 mg $SO_4\ L^{-1}$; phosphate – 0.017 mg $PO_4\ L^{-1}$.

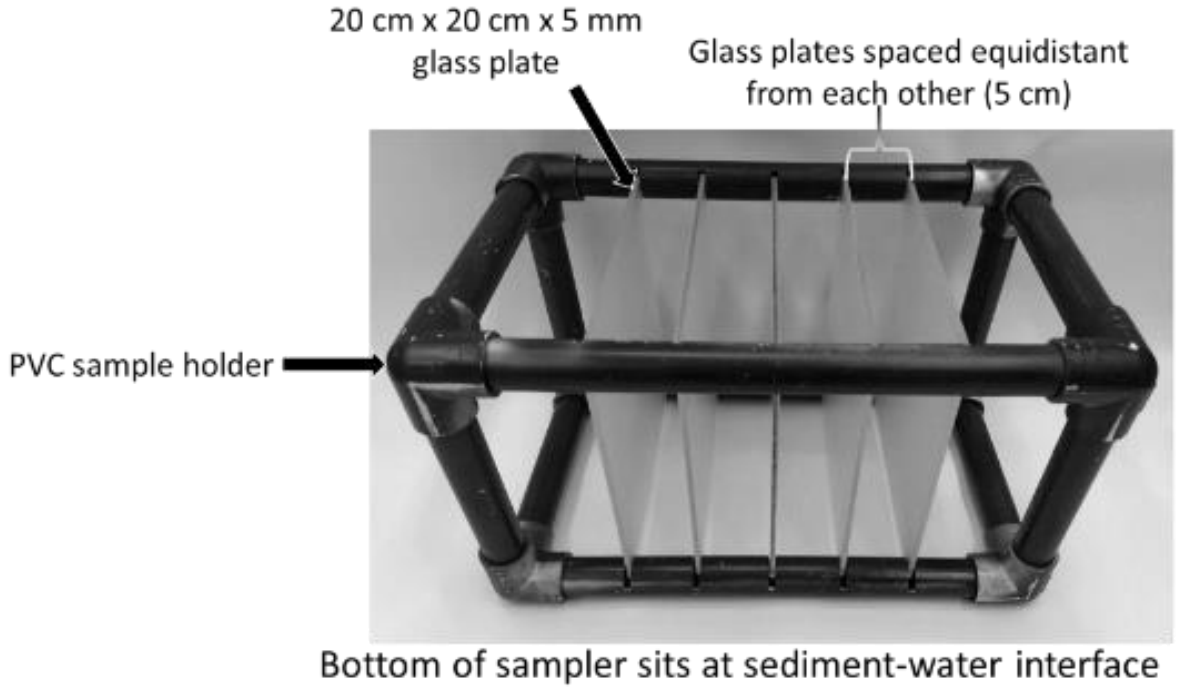

Figure 3.1: Picture of periphyton sampling device. Samplers were placed at the sediment-water interface for a colonization period of six weeks before collection, transport and testing for selenium accumulation.

Site 5 was selected for further investigation in a second field season (summer 2017) because of the enhanced ability of periphyton from this site to accumulate Se(IV). Periphyton from this site had comparatively high iron content and visible orange-red precipitates on the surface of the sampling substrates that appeared to be iron oxyhydroxides.

3.3.3 Experimental setup

All periphyton Se exposures were conducted in the Aquatic Toxicology Research Facility (ATRF) at the Toxicology Centre, University of Saskatchewan. Temperature was regulated to 18 ± 1°C with a 16 h: 8 h light:dark cycle. Each experimental treatment had five replicates, with each replicate consisting of a 5.5-L polypropylene exposure vessel and a colonized, glass periphyton sampling plate fully submerged in test solution. Each replicate was aerated to ensure that each unit was oxic and well mixed. All exposure vessels were covered with a translucent lid (lids reduced PAR inside containers by <5%) to limit evaporation. Exposure vessels were set-up, filled with test water and spiked with the appropriate selenium solution the day before retrieving the field samplers. All glassware and plasticware used for experimentation were acid-washed with 1M HCl and rinsed with ultrapure (17.4 MΩ-cm; Barnstead, Thermo Scientific, Waltham, MA, USA) water prior to use. Any glassware or plasticware that directly contacted the test solutions were also disinfected with 5% sodium hypochlorite before use. Before initiation of the exposure period, each periphyton sampler was closely examined for macroinvertebrates and macroscopic bits of detritus, which were removed, and then carefully rinsed with clean test water before being placed in the test system.

Additionally, setup for the Site 5, year 2 experiments included four different periphyton pre-treatments to help separate processes that may have been responsible for the high

accumulation of Se(IV) in periphyton from this site. Periphyton/substrate pre-treatments were as follows: natural (negative control), heat-killed, shade cloth, and iron oxy-hydroxide precipitate (FeO(OH), positive control). The heat-killed periphyton pre-treatment involved submerging each glass sampling plate in 80-85°C water for 8 minutes (similar to the method used by Riedel et al., (1991) but with a higher temperature and longer immersion time because algae were incorporated into periphyton rather than being free-floating) to cease biological activity while still leaving the physical structure of the periphyton intact. Comparison of heat-killed periphyton to natural periphyton served as a proxy for comparing Se(IV) incorporation in periphyton to surface adsorption. Two weeks before the exposure phase, a sub-sample of colonized periphyton sampling plates were retrieved, heat-killed and then placed in a nutrient rich growing solution for algae (Bold's Basal Medium: Stein 1973) for a week to ensure the heat treatment was effective; no new growth was observed. Examination of algal cells using light microscopy also showed that internal membranes had been disrupted while the outer cell walls remained mostly intact. The purpose of the shade cloth periphyton pre-treatment was to grow a biofilm that largely excluded phototrophs. The intention of this treatment was to separate the accumulation of Se in algae (phototrophs) with Se accumulation in the remaining organisms that make up periphyton (non-photosynthetic bacteria, fungi, etc.). A positive iron oxy-hydroxide treatment was included in this experiment to characterize Se(IV) adsorption to FeO(OH)s on the sampling surface used for these tests (glass plates), under standardized, experimental conditions. FeO(OH)s were produced by placing sampling plates in aerated reverse osmosis (RO) water containing an excess of dissolved Fe(II) in the form of ferric chloride tetrahydrate (4g $FeCl_2 \bullet 4H_2O$ L^{-1}). As the aqueous iron was oxidized to the +3 state, poorly soluble FeO(OH)s were deposited onto the surface of the sampling plates (Domingo et al. 1994). The intent was to produce Fe oxyhydroxides in a

manner similar to naturally occurring Fe oxyhydroxides in well-oxygenated waterbodies, which are typically in the form of ferrihydrite and amorphous Fe oxyhydroxides (Carlson and Schwertmann 1981). Each periphyton/substrate pre-treatment included 15 replicates (*n*=15) for the exposure phase (described below) and were only tested for Se(IV) accumulation.

3.3.4 Exposure period

Exposure duration was set at 8 days to balance the need for algae to reach a pseudo-steady state regarding Se concentration, and the need to limit changes to periphyton community structure as a result of different environmental conditions in the test system relative to field conditions. Previous research has shown that Se bioconcentration in freshwater primary producers tends to peak within 6-14 days of exposure (Dobbs et al. 1996).

Test water was made according to specifications provided in Environment Canada's "Biological Test Method: Growth Inhibition Test Using Freshwater Alga" (2007) (Table A.1) with slight modification. Nutrient concentrations in test media were modified to have 5 μg L^{-1} phosphorus (P) (instead of 0.12 mg P L^{-1}) to better reflect the low nutrient status of the field-sites where the periphyton was collected, and to further minimize periphyton community shift when placed in the test system. This test water was also selected because it had low dissolved sulphate (SO_4^{2-}) and phosphate (PO_4^{3-}); anions known to compete with selenium oxyanions for uptake in primary producers (Lo et al. 2015; Williams et al. 1994; Riedel and Sanders 1996).

3.3.5 Selenium treatments

Selenium speciation and exposure concentration were varied to produce four different selenium treatments, plus controls, for a total of five different treatments. Nominal treatments for

the exposure phase included five replicates (*n*=5) each for: a control (no added selenium), 5 and 25 µg Se L^{-1} as sodium selenite (Na_2SeO_3, Sigma-Aldrich, St. Louis, MO, USA), and 5 and 25 µg Se L^{-1} as sodium selenate (Na_2SeO_4, Sigma-Aldrich, St. Louis, MO, USA). Treatments herein will be referred to as either 'low' or 'high' in regard to Se concentration (nominal 5 and 25 µg Se L^{-1}, respectively) followed by the oxidation state of the Se compound being tested; Se(IV) for selenite and Se (VI) for selenate [e.g., Low Se(IV) refers to the nominal 5 µg Se L^{-1} as selenite treatment]. Conely et al. (2013) demonstrated that natural periphyton biofilms (grown in an artificial stream) exposed to Se(VI) generated detectable quantities of aqueous Se(IV) after 96 hours of static exposure. Therefore, water changes occurred every 2 days and consisted of replacing 4-L of the exposure media with new test water to minimize the likelihood that significant quantities of aqueous, biogenically reduced Se(IV) was formed in the Se(VI) treatment groups. No Se(VI) treatments were included for experiments with periphyton sampled from Site 5 during the second field season.

3.3.6 Sampling regime

Measurements and samples for water quality analysis (DO concentration, temperature, conductivity, pH, total hardness and alkalinity) were taken from all replicates on days 0, 4 and 8 for all tests to ensure that exposure conditions were consistent throughout the duration of each test. Temperature and DO concentration were measured with a portable meter (Orion Star A Series, Thermo Fisher Scientific, Mississauga, ON, Canada), conductivity and pH with bench top probes (ATI Orion Model 170 and Orion 370, respectively, Thermo Fisher Scientific, Mississauga, ON, Canada), and hardness and alkalinity by titration (HACH digital titrator, HACH Company, Loveland, CO, USA). During water quality sampling, photosynthetically

active radiation (PAR) was also measured at the center of the top surface of each exposure vessel using a 2π quantum sensor (Model MQ-500, Apogee Instruments, Logan, UT, USA). Tables A.3 and A.4 summarize mean (±SD) light intensity and water quality measurements for the Se exposure phase.

Samples for dissolved Se analysis were collected from all replicates at the beginning and end of each test (days 0 and 8). Dissolved Se samples were also collected from a sub-set of replicates (n=3) on day 4 of the exposure phase, before and one hour after water changes (for a total of 6 dissolved Se samples collected for each Se treatment, on day 4). Day 4 sampling was included to capture the immediate change in dissolved Se concentration that may have occurred when old test water was replaced with new test water, which was minimal. The coefficient of variation [CV(%) = (SD/mean)(100%)] for Se exposures ranged from 2.0 to 11.6% for the year 1 (2016) periphyton community experiments, with all Se treatments for periphyton from four sites having ≤8.1% CV; the exception was the low Se(IV) treatment from Site 5 (11.6% CV). The coefficient of variation for the Site 5, year 2 (2017) experiments ranged from 2.8 to 76.8%; all Se treatments were at or below 12.6% variance, with the exception of those in the FeO(OH) pre-treatment group (52.6 – 72.8% CV) (see Tables A.3 and A.4 for summarized CV values). The CV was high in the FeO(OH) pre-treatment groups because selenite was adsorbed at such a rate that it was depleted significantly between water changes; this resulted in a high degree of variance between sampling days. Samples for dissolved Se analysis were collected in acid washed 8-mL HDPE sample bottles using syringe filters (0.45 μm pore size, polyethersulfone membrane, VWR International, Radnor, PA, USA) and acidified using high-purity nitric acid (Omnitrace Merck KGaA Darmstadt, Germany). Ten percent of the samples taken for dissolved Se measurements were method blanks, consisting of ultrapure water passed through syringe

filters, acidified and stored in a similar manner to test water samples. Tables A.3 and A.4 summarize mean dissolved Se concentrations (±SD and coefficients of variation) measured in test waters.

Periphyton was sampled from all replicates on days 0 and 8. Periphyton was sampled by scraping a known (measured) area of periphyton with a ceramic blade, into 50-mL HDPE centrifuge tubes (BD Biosciences, Bedford, MA, USA). When sampling, plates were scraped from top to bottom, and samples collected in entirety, to account for spatial variability due to the heterogenous nature of periphyton. Each periphyton sample was then resuspended in ultrapure water, centrifuged at 3000 rpm for 15 min and the supernatant decanted. This was repeated until periphyton had been rinsed three times. After rinsing, periphyton samples were resuspended in 50 mL of ultrapure water, homogenized at low speed using a tissue homogenizer and a sub-sample of 1 mL removed for algae identification using light microscopy. The remaining periphyton sample was spun down again, decanted, flash frozen in liquid nitrogen and then stored at -20°C until being freeze-dried. Freeze dried materials were weighed to determine mass/area (mg d.w. cm^{-2}) on sampling plates and then a sub-sample of 10 – 20 mg (d.w.) was digested for trace metals analysis. The remaining periphyton from day 8 was used for the determination of ash-free dry-weight (AFDW) and calculation of organic matter (OM) content.

Samples for periphyton community characterization using light microscopy and targeted metagenomic analysis were collected from all replicates on day 8 of the exposure phase. Samples for algal identification using light microscopy were collected as described above. Light microscopy samples were preserved in 0.5 – 1 % glutaraldehyde and stored in the dark at 4°C until analysis. Samples for targeted metagenomic analysis were taken directly from periphyton plates before scraping for other analyses. All materials for genetic sampling were autoclaved

before use. Samples for metagenomic analysis were flash frozen in liquid nitrogen and stored at -80°C until analysis.

3.3.7 Analyses

Selenium and iron concentrations (water and tissue) were measured using ICP-MS (8800 ICP-MS Triple Quad, Agilent Technologies, Santa Clara, CA, USA) operated in collision cell mode. Dissolved Se concentrations were measured directly from filtered (0.45 μm) and acidified (2% HNO_3) test water. Periphyton Se and Fe content were measured in solution after digestion procedures were complete. Periphyton was digested by homogenizing lyophilized sample and weighing 10 to 20 mg (d.w.) into PTFE digestion vials. High purity, 69% nitric acid (2 mL) and high purity, 30% hydrogen peroxide (1.4 mL) (Sigma Aldrich, St. Louis, MO, USA) were added to each vial before being capped and placed in a MARS-5 microwave digestion system (CEM Corporation, Matthews, NC, USA). Digests were brought to 160°C for 20 min. Digested samples were filtered (0.45 μm pore size, polyethersulfone membrane) and diluted to 2% HNO_3 before analysis.

The instrumental certified reference material (CRM) for Se and Fe analysis was "1640a – Trace Elements in Natural Water" (National Institute of Standards and Technology, Gaithersburg, MD, USA). The mean (± SE) of the 1640a analyses for Se and Fe were 99.6 ± 1.9 % and 103.2 ± 4.7 % of the certified value, respectively. Measured concentrations of Se and Fe in method blanks (10% of total number of samples) were mostly (99.5% of all blanks) below the instrumental limit of detection for water (0.0053 - 0.21 μg Se L^{-1}; 0.019 - 0.027 μg Fe L^{-1}) and low relative to measured tissue concentrations (all tissue-Se and tissue-Fe concentrations were blank-subtracted). TORT-3 (lobster hepatopancreas) from NRC Canada (Institute for

Environmental Chemistry, Ottawa, Canada) served as the tissue CRM. Eight separate tissue digestions were required for tissue-Se and tissue-Fe analysis. Measured CRM values were 92.6 ± 3.2 %, (mean ± SE) of the certified value for tissue-Se analysis, and 99.5 ± 7.0 % for tissue-Fe analysis.

Microscopic identification and counting of algal cells/colonies in preserved periphyton samples was performed using a Palmer counting cell, in accordance with US EPA Rapid Bioassessment Protocols for Use in Wadeable Streams and Rivers (1999). Algae from three replicates (n=3) from each site were identified to the lowest taxonomic level possible using a key for frequently occurring freshwater algae (Bellinger and Sigee 2010) and a minimum of 10 counting units were measured with an ocular micrometer to determine average size for each identified group. Size measurements were entered into the equations of Hillebrand et al. (1999) and multiplied by counts to determine the relative biovolume for each taxon. Traditional light microscopy methods were employed as a compliment to the higher statistical power of targeted metagenomic sequencing, providing a quantitative assessment of the organisms present in the algal portion of periphyton, as well as a measure of the relative biovolume of species identified.

Targeted DNA sequencing was performed by Contango Strategies Ltd. to identify cyanobacteria and eukaryotic algae via polymerase chain reaction (PCR) amplification of the plastid 23S rRNA gene (Sherwood and Presting 2007; Steven et al. 2012). DNA extraction, sequencing and data analysis methods followed those outlined by Friesen et al. (2017; see Appendix B.2 for additional DNA extraction, sequencing and data analysis methods). Operational taxonomic units (groupings of organisms) were based on 97% identity threshold for the gene sequenced.

3.3.8 Data analyses

The enrichment function of Se, from water to periphyton, was calculated as described in Eq. 3.1 using mean periphyton Se concentration for all treatment replicates from each site over mean, measured water Se values. The distribution coefficient of Se, from water to substrate, was calculated in a similar manner, described in Eq. 3.2.

$$\text{Enrichment Function (EF)} = \frac{\text{Periphyton Se (mg Se/kg d.w.)}}{\text{Water Se (mg Se/L)}} \quad \text{.....(3.1)}$$

$$\text{Distribution Coefficient } (K_d) = \frac{\text{Substrate Se (mg Se/kg d.w.)}}{\text{Water Se (mg Se/L)}} \quad \text{.....(3.2)}$$

Some replicates were removed from statistical comparison as outliers due to the presence of large, freshwater sponges that dominated the periphyton biomass; freshwater sponges unevenly colonized some sampling plates and the focus of this study was on the algal component of periphyton. Three to five replicates remained in each treatment, for each site, after the removal of outliers (n=3 to 5). Periphyton Se concentrations and enrichment functions were normalized to organic matter content to account for differences in inorganic material content between sampling sites (See Tables A.5 and A.6 for raw data; additional rationale for normalizing periphyton-Se and EF to organic matter content provided in Appendix B.1). Normalized, mean Se concentrations and enrichment functions were compared among periphyton from different sampling sites (1 to 5) for each Se treatment using one-way analysis of variance followed by Tukey's honest significant difference *post hoc*. Site 5, year 2 periphyton and substrate Se and Fe concentrations were compared among periphyton pre-treatment groups using similar statistical techniques. Periphyton Se concentrations from all Se treatments were compared to respective controls in a similar statistical manner, but instead using Dunnett's test as the *post hoc* comparison. EFs for low vs. high exposure concentrations, for each Se species, were compared

using *t*-tests. Data were $\log_{10}$-transformed when necessary to achieve normality and homoscedasticity. Analysis of variance on ranks was employed when transformation was unsuccessful, followed by Dunn's method for multiple comparison *post hoc*.

Statistical analyses of targeted DNA sequencing of plastid 23S rRNA genes were performed using R (http://www.R-project.org/) and PRIMER V7 with PERMANOVA+ add-on software (PRIMER-E Ltd, Plymouth, UK) (Clarke and Gorley 2015; KR et al. 2014). Principal coordinate analysis (PCoA) was performed to visualize the level of dissimilarity of algae assemblages based on the weighted UniFrac distance. The beta-diversities of algae communities were compared using permutation-based analyses of variance (PERMAVOVA) (Lozupone and Knight 2005) with weighted UniFrac distance matrices followed by pair-wise, *post-hoc* comparisons between structures of algae assemblages from different sampling sites (1-5). Statistical significance was set at $p<0.05$ and the number of permutation test replicates was set at 9,999.

Comparison of periphyton mass/area at the beginning and end of the exposure phase (day 0 and 8, respectively) was used to determine whether there was growth after being placed in the test system. Mass/area data from all replicates for each site were combined and tested with paired *t*-tests to determine if there was significant change over the course of the test.

3.4 Results

3.4.1 Periphyton composition

Permutational multivariate analysis of variance of periphyton assemblage composition confirmed that community composition varied significantly among sampling sites, according to targeted DNA sequencing of the plastid 23S rRNA gene (PERMANOVA: pseudo-F = 6.9524,

p<0.001). According to this analysis, periphyton assemblage composition from Site 1 was similar to those from Sites 3 and 4, with the other periphyton assemblages being different from one another. Principal coordinates analysis (PCoA) (Figure 3.2) demonstrated differences in periphyton assemblages across sampling sites.

Percent algal biovolumes for genera of algae identified using light microscopy are presented in Table 3.2. In general, algal biovolume for Sites 1 through 5, respectively, was dominated by: 1) chlorophytes, 2) diatoms, 3) chlorophytes and diatoms, 4) chlorophytes and diatoms, and 5) cyanophytes and chlorophytes. Periphyton sampled from Site 5 in year 2 showed an increase in percent diatom biovolume largely at the expense of chlorophytes. Diatoms were identified and the data pooled into morphologically similar groups of genera. No algal cells were observed in the shade cloth periphyton pre-treatment (Site 5, year 2 experiment).

Periphyton mass/area (mg cm^{-2}) measured at the beginning and end of the Se exposure phase (days 0 and 8) was not statistically different (p>0.05) for Sites 1, 3 and 5. Periphyton mass/area changed significantly during the exposure phase for Site 2 (p=0.045; mean decrease 11%) and Site 4 (p=0.004; mean increase 32%). Mean organic and inorganic composition of periphyton from each sampling site is presented in Figure 3.3. The inorganic matter component of periphyton was separated into Fe content and remaining inorganic material. Average periphyton mass per sampling area for Sites 1 to 5 were as follows: 0.06 ± 0.01, 0.60 ± 0.09, 0.24 ± 0.07, 0.04 ± 0.01 and 0.35 ± 0.22 mg/cm^2, respectively. Mean (±SD) of mass/area, percent organic matter, inorganic matter and Fe content for periphyton/biofilms used in all experiments are summarized in Table A.2.

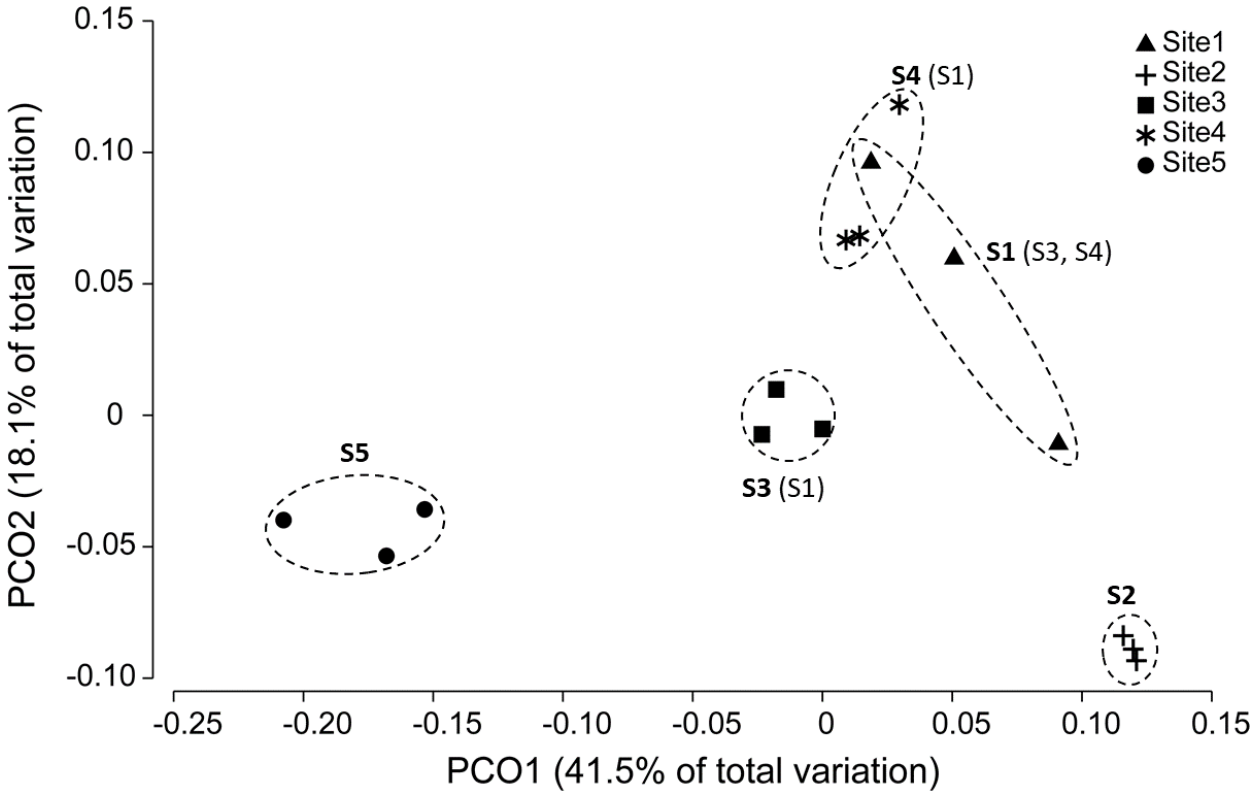

Figure 3.2: Principal coordinate analysis (PCoA) comparing periphyton assemblages across sampling sites based on targeted metagenomic analysis of plastid 23S rRNA gene sequences (n=3). Dotted ellipses show assemblages sampled from different field sites. Community structure is significantly different among groups (PERMANOVA: pseudo-F = 6.9524, p<0.0001 using weighted UniFrac as a distance metric). Brackets show sites that are statistically similar (pairwise *post hoc* comparison, p<0.05).

Table 3.2**:** Percent total algal biovolume for genera identified in periphyton samples using light microscopy (*n*=3) at the end of the Se exposure phase (day 8).

Taxonomic Classification			% Total Algal Biovolume					
Domain	**Phylum**	**Genus/Grouped Genera**	**Site 1**	**Site 2**	**Site 3**	**Site 4**	**Site 5**	**Site 5 Yr 2**
Eukaryota	Chlorophyta	*Asteroccocus*		1.0 ± 1.4				
		Bulbochaete	13.7 ± 2		4.4 ± 3			
		Chaetophora	2.3 ± 1.1		7.1 ± 2.5	46.2 ± 22	2.1 ± 3.7	
		Chlorella				0.2 ± 0.2		
		Coleochaete	53.9 ± 19.5		18.7 ± 7.2	6.2 ± 7.1	12.7 ± 4	
		Mougeotia		5.7 ± 9.9				10.7 ± 9.8
		Oedogonium	16.5 ± 14.3		4.7 ± 0.7		11.3 ± 5.5	5.1 ± 6.7
		Pediastrum				0.8 ± 1.3		7.8 ± 12.7
		Scenedesmus						0.5 ± 0.9
		Spirogyra			6.9 ± 11.9		11.4 ± 6.9	
		Volvox				1.4 ± 2.4		
		Zygnema	1.6 ± 1.8					
		Total	**88.2 ± 39**	**6.8 ± 11.4**	**42.0 ± 25.7**	**55 ± 33.3**	**37.7 ± 20.3**	**24.1 ± 30.1**
Eukaryota	Bacillario-phyta	*Achnanthes*		2.1 ± 0.7				
		Cocconeis		3 ± 1.1				
		Cymbella/Amphora		21.9 ± 5.8	31.8 ± 3.4			
		*D/T/N/D/E**		3.6 ± 0.4	6.9 ± 1.7			
		Fragillaria/Synedra		8.5 ± 7	2.5 ± 2.2			
		Gomphonema		21.7 ± 3.9	0.3 ± 0.6	15.1 ± 9.3		
		Grouped Bacillariophyceae	2.3 ± 2.1			10.5 ± 10.2	5.4 ± 2.4	22.4 ± 6
		Grouped Fragilariophyceae				1.3 ± 0.3	1.8 ± 1.6	1.2 ± 1.5
		Gyrosigma/Pleurosigma			1.0 ± 0.9			
		Navicula/Pinnularia		28.2 ± 4.5	12.1 ± 2.9			
		Total	**2.3 ± 2.2**	**89.4 ± 23.8**	**55 ± 12.1**	**26.9 ± 19.8**	**7.3 ± 4.1**	**23.6 ± 7.5**
Prokaryota	Cyanophyta	*Anabaena*		1.9 ± 2.5		8.1 ± 7	1.8 ± 3.2	0.5 ± 0.8
		Aphanthece		1.9 ± 2.4			0.4 ± 0.7	
		Calothrix/Rivularia				0.3 ± 0.3		
		Chamaesiphon				5.2 ± 4.7		
		Gleotrichia	2.7 ± 0.3					
		Merismopedia					1.2 ± 1.4	1.6 ± 1.4
		Microcystis				3.9 ± 2.5	3.6 ± 6.3	16.6 ± 9.8
		Nostoc					10.1 ± 14.9	
		Oscillatoria	3.8 ± 2.5		3.0 ± 2.2	0.2 ± 0.4	36 ± 20.3	33 ± 11.8
		Spiruline	2.7 ± 2.3				1.5 ± 1.3	
		Total	**9.4 ± 5.3**	**3.8 ± 4.9**	**3.0 ± 2.2**	**17.7 ± 15.1**	**55.4 ± 48.4**	**51.7 ± 23.8**

**Diatoma/Tabellaria/Nitzschia/Denticula/Eunotia*

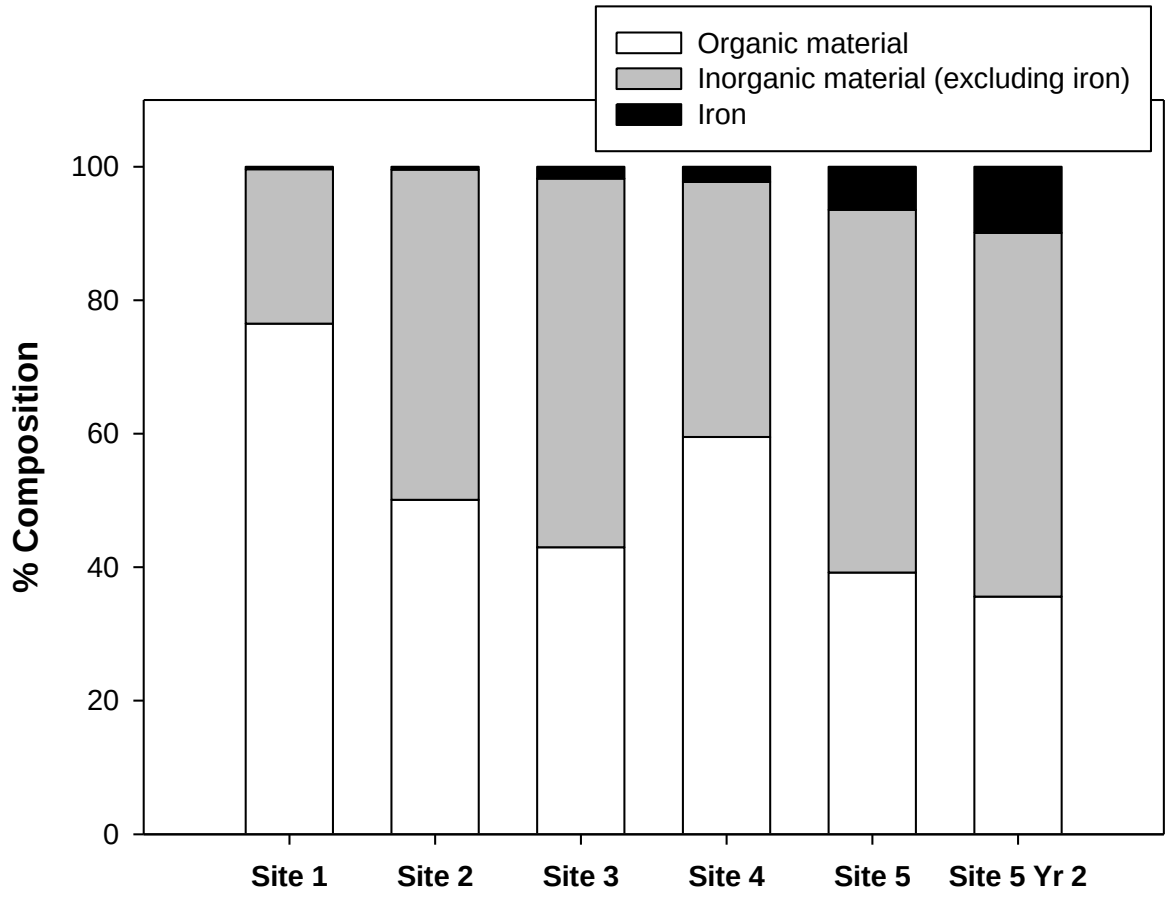

Figure 3.3: Mean percent composition of periphyton from Sites 1 to 5 sampled on day 8, at the end of the Se exposure phase (n=5). Organic and inorganic content was determined by measuring ash-free dry weight. Iron content was determined using ICP-MS.

3.4.2 Periphyton Se and EFs

Mean periphyton Se concentrations (day 8) normalized to percent organic material, for both Se(IV) treatments, showed similar overall trends in Se accumulation (Figure 3.4a; Table A.5). Mean OM-normalized periphyton Se concentrations were statistically different among sites for both the low Se(IV) treatment (ANOVA: f=129.9, p<0.001) and high Se(IV) treatment (ANOVA: f=88.6, p<0.001). Site 2 periphyton was the lowest Se(IV) accumulator (mean of 14.5 and 33.4 μg Se g^{-1} OM d.w. for low and high Se treatment concentrations, respectively). Periphyton sampled from Site 5 was the highest accumulator of Se(IV) at both test concentrations (mean of 222.3 and 567.7 μg Se g^{-1} OM d.w. for low and high Se treatment concentrations, respectively). Normalized periphyton Se concentrations for all Se(IV) treatment groups were statistically different from the respective controls for each site (p<0.05). Compared to Se(IV) treatments, statistical differences in mean normalized periphyton Se concentrations for Se(VI) treatments were much smaller among sites. There were statistically significant differences for mean, OM-normalized periphyton Se among sites at both low Se(VI) (ANOVA: f=11.5, p<0.001) and high Se(VI) (ANOVA on ranks: p=0.007) treatment concentrations. Periphyton from Site 1 was the lowest Se(VI) accumulator at both concentrations (mean of 3.5 and 9.8 μg Se g^{-1} OM d.w. for low and high Se treatment concentrations, respectively), differing from Sites 2 to 5 for the low Se(VI) treatment and differing significantly from only Site 4 (mean of 19.1 μg Se g^{-1} OM d.w.) for the high Se(VI) treatment. Mean, normalized periphyton Se concentrations for all Se(VI) treatment groups were different from their respective controls (p<0.05). Significant differences among periphyton from different sampling sites, for each Se treatment, are shown in Figure 3.4a. Raw periphyton-Se data (before normalization) are presented in Table A.5.

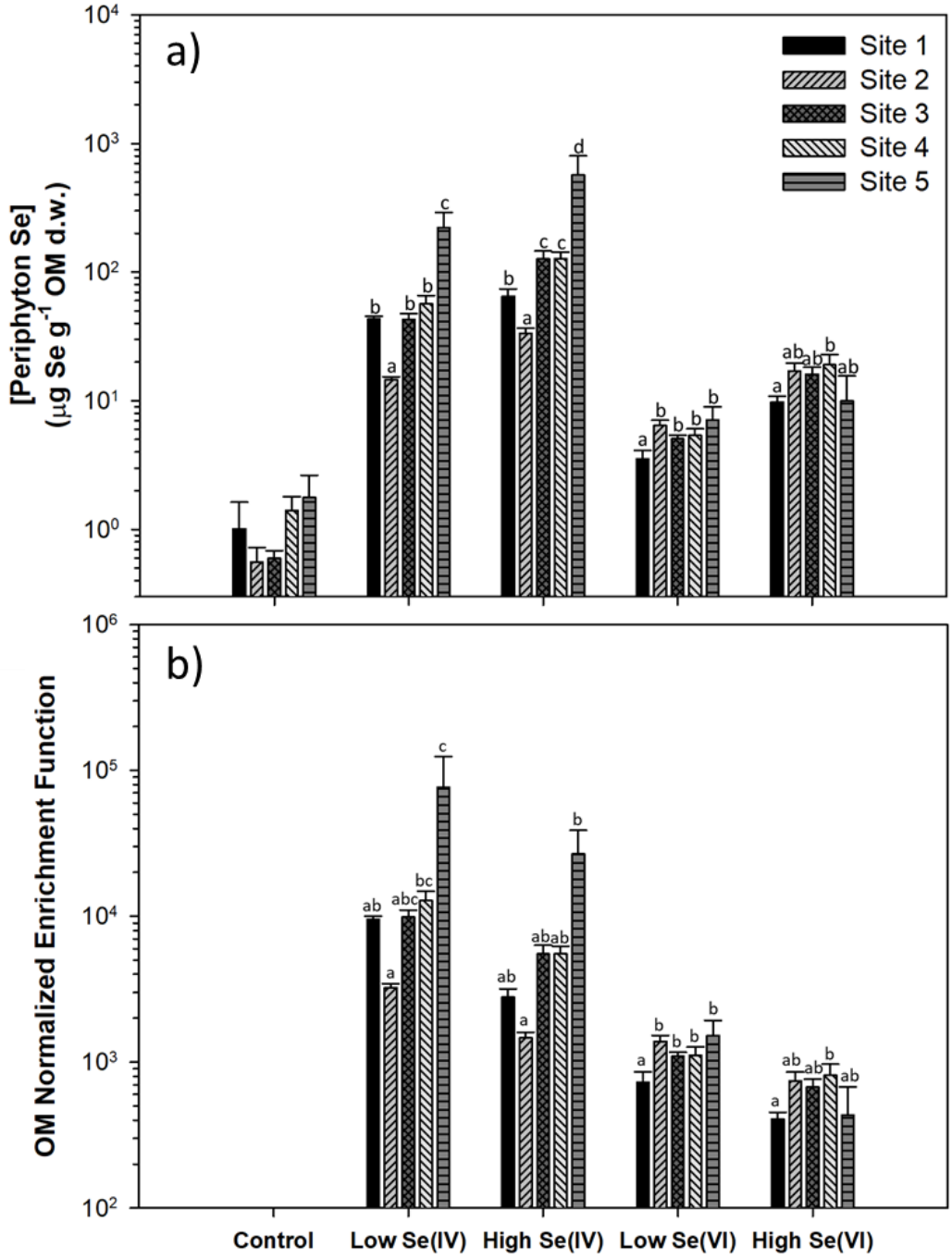

Figure 3.4: Mean, normalized a) Se concentration (µg Se g^{-1} OM d.w.) and b) enrichment function of Se measured in periphyton sampled at the end of the exposure phase (day 8). Periphyton Se and enrichment function were normalized to percent organic matter. 'Low' and 'high' represent nominal treatment concentrations of 5 and 25 µg Se L^{-1} respectively. Letters indicate statistical differences among periphyton sampled from different field sites for each treatment. Error bars represent one standard deviation.

Organic matter-normalized Se(IV) EFs (Figure 3.4b; Table A.6), were statistically different among sites at both low Se(IV) (ANOVA on ranks: $p<0.001$) and high Se(IV) (ANOVA on ranks: $p<0.001$) treatment concentrations. Periphyton sampled from Site 2 had the lowest normalized enrichment at low and high treatment concentrations (mean normalized EFs of 3,243 and 1,465, respectively) and Site 5 had the highest enrichment (mean normalized EFs of 76,599 and 26,796 for low and high, respectively). OM-normalized Se enrichment differed by 23.6-fold among periphyton from different sites for the low Se(IV) treatment and by 18.3-fold among sites for the high Se(IV) treatment. Se(VI) enrichment was also different among sites for both low Se(VI) (ANOVA: f=12.9, $p<0.001$) and high Se(VI) (ANOVA on ranks: $p=0.007$) treatment concentrations. Periphyton from Site 1 showed the lowest Se(VI) enrichment at both exposure concentrations, with mean, OM-normalized EFs of 730 and 408 for low and high Se(VI) treatments, respectively. Periphyton from Site 5 showed the highest Se enrichment for the low Se(VI) treatment concentration (mean normalized EF of 1,523) and periphyton from Site 4 showed the highest enrichment for the high Se(VI) treatment (mean normalized EF of 814). There was a 2.1-fold difference in Se enrichment among sites for the low Se(VI) treatment and a 2.0-fold difference among sites for the high Se(VI) treatment. Comparison of Se(IV) or Se(VI) enrichment for periphyton from the same sampling site, at low vs. high treatment concentrations, showed that all EFs decreased significantly with increasing ambient water Se concentration ($p<0.05$); Se enrichment was inversely related to the concentration of Se in the exposure test water. Raw enrichment function data (before normalization) are presented in Table A.6.

3.4.3 Site 5, year 2 Se accumulation

Periphyton/substrate Se concentrations were significantly different among periphyton pre-treatments for both concentrations of Se(IV) tested (ANOVA: f=144.5, $p<0.001$ for low Se(IV); f=85.8, $p<0.001$ for high Se(IV); Figure 3.5a). At the low Se(IV) concentration, natural, heat-killed and shade cloth pre-treatment groups were not statistically different from each other ($p>0.05$) and similar to periphyton sampled from Site 5 during the previous field season. The FeO(OH) positive control treatment group had the highest concentration of substrate-Se at both concentrations of Se(IV) tested (mean of 632.3 and 3,522.2 μg Se g^{-1} d.w. at low and high Se(IV) exposure concentrations, respectively). At the high Se(IV) test concentration, mean periphyton/substrate Se was similar for natural and heat-killed pre-treatment groups as well as periphyton sampled from the same site during the previous year. Periphyton/substrate Se concentration was significantly higher for the shade cloth pre-treatment group relative to natural periphyton sampled during both field seasons. All Se(IV) treatment groups were significantly different from their respective controls ($p<0.05$) at both Se concentrations tested. There was no statistical difference in periphyton/substrate Fe among any of the periphyton pre-treatment groups (ANOVA on ranks: $p=0.132$), although there was a wide range of Fe concentrations across all of the pre-treatment groups (32.8 – 196.8 g Fe kg^{-1} d.w.). Statistical differences among mean EF or K_ds (Figure 3.5b) were similar to those described above for periphyton/substrate Se (ANOVA: f=55.5, $p<0.001$ for low Se(IV) treatment concentration; f=117.2, $p<0.001$ for high Se(IV) treatment concentration). Statistical differences in EF or K_ds among periphyton pre-treatment groups, for the low Se(IV) test concentration, were the same as those described above for periphyton/substrate Se. Statistical differences in EF or K_ds for the high Se(IV) test concentration were very similar to periphyton/substrate Se as well, but the shade cloth pre-

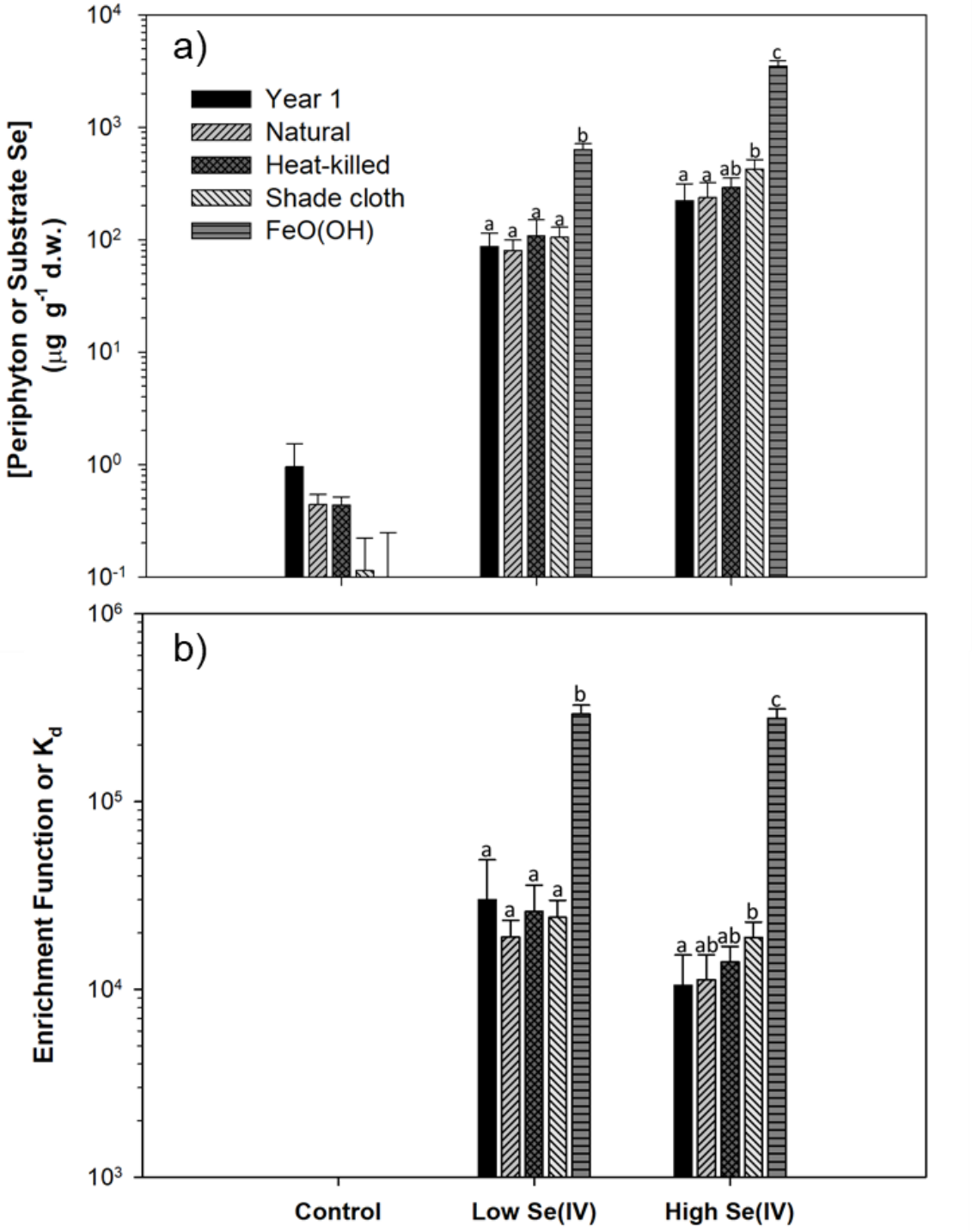

Figure 3.5: Mean a) periphyton/substrate Se concentrations (µg Se g^{-1} d.w.) and b) EF or K_d measured during Site 5, year 2 experiments, at the end of the Se exposure phase. 'Low' and 'high' represent nominal treatment concentrations of 5 and 25 µg Se L^{-1}, respectively. Error bars represent one standard deviation. Statistical differences among treatments are indicated by different letters.

treatment group had a slightly higher mean EF than periphyton sampled at the same site during the previous field season. Mean Se EF and K_ds ranged from 18,993 to 292,652 for different periphyton pre-treatments groups in the low Se(IV) exposure group and from 10,504 to 278,026 in the high Se(IV) exposure group. Natural and heat-killed periphyton pre-treatments showed a significant decrease in EF or K_d values when comparing accumulation in low vs high Se(IV) treatment concentrations for each group (p=0.020 and 0.033 respectively).

3.5 Discussion

3.5.1 Assessing periphyton composition: metagenomic and microscopy approaches

Periphyton/biofilm typically consists of a heterogenous mixture of microbial taxa. As such, our assessment of Se accumulation among different periphyton communities required a means to quantify taxonomic variability among biofilm samples. Emerging targeted metagenomics techniques were used in combination with traditional light microscopy to help characterise algal assemblage composition. The plastid 23S rRNA gene sequence used in this study allows for the incorporation of both prokaryotic and eukaryotic organisms in the same analysis, facilitating a holistic characterization of the complex algal assemblage for each site (Steven et al. 2012). Gene sequence analysis of periphyton communities demonstrated that distinct algal assemblages were collected from the different field sampling sites (different waterbodies; Figure A.1). This finding was supported by traditional morphological taxonomy (Table 3.2), which also indicated that the different sampling sites produced unique algal assemblages for experimentation. Although plastid 23S rRNA targeted metagenomic analysis provided a strong statistical basis for differentiating complex algal assemblages based on the interspecific genetic diversity of this gene (Steven et al. 2012), there is currently a lack of

consensus on the efficacy of species abundance and biomass estimates using DNA metabarcoding techniques (Elbrecht and Leese 2015). For this reason, light microscopy analysis provided the best estimate of the relative biomass (by way of biovolume) for the algal species identified (see Appendix B.3 for additional rationale for the assessment of periphyton composition using metagenomic and microscopy methods).

3.5.2 Selenium enrichment among periphyton communities

Overall, when ambient Se exposure concentrations were similar, Se(IV) was accumulated to a greater degree than Se(VI) in periphyton collected from all field sites. This is consistent with previous research showing that Se(IV) is the more available species of inorganic Se to algae (Baines and Fisher 2001; Riedel et al. 1991; Simmons and Wallschlager 2011). Periphyton collected from the field site with the highest proportion of diatoms (Site 2; mean of 89% total algal biovolume) demonstrated the lowest accumulation of Se(IV) relative to periphyton sampled from the other four field sites. This is in contrast with previous Se(IV) bioconcentration tests using phytoplankton monocultures, where chlorophytes typically displayed a lower enrichment of Se(IV) than diatoms when exposed under similar conditions (Baines and Fisher 2001; Riedel et al. 1991). Se(IV) enrichment, at both Se concentrations tested here, was most similar among taxonomically similar algal assemblages (Site 1 when compared to Sites 3 and 4), according to plastid 23S rRNA gene sequencing. This supports the hypothesis that periphyton community composition influences Se bioconcentration at the base of the food-chain in freshwater ecosystems.

The inverse, non-linear relationship of EFs with ambient Se concentration supports the observation that Se(IV) accumulation in algae (here, as periphyton) is a carrier-mediated process

that is subject to saturation kinetics (Baines and Fisher 2001; Fisher and Wente 1993; Fournier et al. 2006; Riedel et al. 1991). However, the observation that heat-killed periphyton showed the same trend, raises the possibility that the observed trend may not be a result of biology, but rather based on surface chemistry. In general, our adsorption experiments indicate that a significant portion of apparent Se(IV) bioconcentration may be a result of extracellular adsorption under certain circumstances (e.g., high extracellular Fe content, discussed further below). If selenite accumulation is assumed to be entirely through absorptive mechanisms, differential enrichment among genetically different periphyton assemblages was still low (23.6-fold at ambient selenite concentrations between 4–5 μg Se L^{-1}) compared to the previously reported several orders of magnitude difference among phytoplankton species (Baines and Fisher 2001). Excluding Site 5 Se accumulation data, which are likely elevated due to Fe content, there was only a 4.7-fold difference in Se(IV) enrichment among periphyton communities with fundamentally different periphyton assemblages. Although the range of different periphyton sampled in this study are not exhaustive of all possible periphyton communities, the results indicate that differential periphyton community composition may only have a moderate (several-fold rather than orders of magnitude) influence on Se(IV) accumulation in natural periphyton.

Differences in Se(VI) enrichment among different periphyton assemblages were small, with only an approximately 2-fold difference at both Se exposure concentrations. Se(VI) enrichment appears to be lowest in the periphyton assemblages containing the highest proportion of chlorophytes relative to cyanophytes and diatoms, based on biovolume measurements. This contrasts with previous phytoplankton experimental results demonstrating that Se(VI) was enriched to a higher degree in planktonic freshwater chlorophytes and diatoms compared to cyanophytes (Riedel et al., 1991). However, direct comparison between studies is difficult due to

the much shorter duration of the Se exposure phase (24 hours) in Riedel et al. (1991). Nevertheless, the effect of different periphyton assemblage composition was limited with regard to direct Se(VI) enrichment in the natural, freshwater periphyton use here.

3.5.3 Selenite adsorption and non-phototrophic uptake experiment

Periphyton and non-phototrophic biofilms (sampling plates colonized under shade cloth) grown at Site 5 contained a notably high concentration of Fe, ranging from 3.3 – 19.7% by weight, most likely in the form of Fe-rich precipitates associated with the extracellular environment (Letovsky et al. 2012). Given the high affinity of selenite for Fe oxyhydroxides (Balistrieri and Chao 1990), Fe(III) content was hypothesized to explain the high accumulation of Se by periphyton from this site. The initial bioconcentration experiments were therefore followed up with additional experimentation specific to Site 5 and manipulation of periphyton composition.

The similarity of Se(IV) accumulation in natural, untreated periphyton with accumulation in heat-killed periphyton demonstrated that the bulk of the apparent Se bioconcentration in periphyton sampled from Site 5 was a result of adsorbent-adsorbate interactions rather than active biological uptake. Taken alone, these results are consistent with previous experiments using monocultures of freshwater phytoplankton where accumulation by heat-killed cells was a significant fraction of the accumulation by live cells of the same species; 63-78% of apparent uptake was due to adsorption processes after 12 hours of Se(IV) exposure (Riedel et al. 1991). Additionally, Se accumulation by periphyton in the shade cloth treatment (intended to exclude phototrophic microbes) was similar to the accumulation observed in both the living natural periphyton and the heat-killed periphyton. This provides further support for the hypothesis that

the majority of apparent Se(IV) bioconcentration in periphyton sampled from Site 5 was not accumulated intracellularly by algae, or other organisms in periphyton (bacteria, fungi, etc.), but rather that Se was associated with the extracellular environment of the periphyton/biofilms due to adsorbate-adsorbent interactions.

The form of Fe oxyhydroxide is known to influence the degree of adsorption of various trace elements (Cornell and Schwertmann 1996). The composition of Fe(III) precipitate formed during oxidation is dependent on environmental conditions, and was not determined herein, but selenite sorption data indicates that point zero charge values (which describe the strength of the substrate-adsorbate interactions) of all iron oxyhydroxide polymorphs fall within a narrow range (Benjamin and Leckie 1981; Parida et al. 1996). Other factors known to influence selenite adsorption capacity include pH, availability of competing ions and adsorbate concentration (Benjamin and Leckie 1981). The different periphyton and substrate pre-treatments used here were tested using reconstituted water to avoid artifacts linked to these exposure modifying factors. Therefore, differential accumulation of selenite between the positive FeO(OH) control and other periphyton pre-treatment groups, was likely due to either the presence of adsorbed competing ions (phosphate, silicate and molybdate [Balistrieri and Chao 1990; Carlson and Schwertmann 1981; Riedel and Sanders 1996]), or reduced accessibility to sorption sites resulting from the periphyton matrix in the field-collected materials. In general, the results of the selenite adsorption and non-phototrophic uptake experiment were in agreement with the consensus that Se(IV) strongly adsorbs to Fe oxyhydroxides (Balistrieri and Chao 1990).

Declining EF values, for natural, untreated periphyton (years 1 and 2), with increasing Se exposure concentration could indicate that transport saturation is occurring, suggesting that biological uptake is occurring; however, this trend also appeared in the heat-killed periphyton

pre-treatment group. Additionally, previous work by Balistrieri and Chao (1990) demonstrated that equilibrium constants (K_d) for selenite decrease with increasing adsorption density due to the heterogeneity of Fe oxyhydroxide surfaces. The surface chemistry phenomenon of the saturation of Se(IV) binding sites on Fe oxyhydroxide precipitates can produce similar experimental results to those that are a product of transport saturation kinetics, making interpretation of Se(IV) uptake data in field-collected algae difficult in certain situations. From an ecological perspective, organo-Se compounds produced through biotransformation of absorbed Se(IV) in algae are more bioavailable to primary consumers compared to Se(IV) (Simmons and Wallschlager 2005). Therefore, adsorbed Se(IV) in the diet of primary consumers is likely less bioavailable compared to absorbed and biotransformed Se(IV) at similar total algae-Se concentrations. Whether Se is absorbed or adsorbed to periphyton will have important implications for its uptake and accumulation in primary consumers. Adsorbed Se could also be released by periphytic biofilms periodically or seasonally when Fe oxyhydroxides are reduced to soluble Fe species (Belzile et al., 2000). This process could result in large Se(IV) releases coinciding with environmental conditions at the sediment-water interface, such as low (e.g., <2 mg L^{-1}) dissolved oxygen levels, conducive to reducing Fe oxyhydroxides.

3.5.4 Application to exposure modelling and associated uncertainties

Regardless of periphyton assemblage composition, bulk periphyton-Se in the 5 µg Se L^{-1} treatments, representing both edible and inedible fractions of periphyton/biofilm to invertebrates, were sufficient to potentially exceed recently derived whole-body fish tissue guidelines (8.5 µg g^{-1} d.w. [US EPA 2016]) for all Se(IV) and most Se(VI) (all except Site 3) treatment groups using the mean trophic transfer functions derived by Presser and Luoma (2010; 2.8 for aquatic

insects and 1.2 for fish feeding on invertebrate prey). The US EPA whole-body fish tissue guideline is considerably less conservative than that derived by the British Columbia Ministry of Environment (BC MoE), which is set at 4.0 μg g^{-1} d.w.(whole-body fish tissue), and includes an invertebrate-prey tissue trigger concentration of 4 μg g^{-1} d.w. (BC MoE 2014); bulk periphyton-Se concentrations measured in all 5 μg Se L^{-1} treatment groups were sufficient to potentially exceed BC MoE guidelines for both invertebrate-prey and whole-body fish tissue-Se when applying the mean trophic transfer functions described above.

Ultimately, it is hoped that these findings will be used to model Se biodynamics in periphyton-based food webs similar to those found in the cold, freshwater ecosystems of northern Canada. The relationship between algal assemblage composition and differences in Se accumulation among periphyton communities observed here was substantially lower than what has been previously reported for marine planktonic algal species (Baines and Fisher 2001); these data will help reduce uncertainty when modelling Se biodynamics in cold, freshwater ecosystems. Our results also demonstrate that extracellular adsorption can account for much of the apparent Se(IV) bioconcentration in periphyton under certain circumstances, such as high periphyton/biofilm-Fe content. Consistent with Friesen et al. (2017), these results indicate that bulk-periphyton Se may not be entirely predictive of the subsequent trophic transfer of Se compounds to primary consumers without considering potential exposure modifying factors.

Experiments that utilize complex periphyton communities, such as those used in this study, offer a high degree of ecological relevance relative to those studies using algae monocultures. However, the complexity of natural periphyton communities can also hinder the interpretation of experimental data due to the inherent difficulty in characterizing all biological and physicochemical parameters potentially affecting Se accumulation in a given biofilm. While

the results of this study help to reduce uncertainty regarding biodynamic modelling of Se in freshwater ecosystems, they also highlight the difficulty of interpreting experimental results when using complex, natural periphyton assemblages for Se accumulation experiments. The difficulty of applying experimental results to broader, ecosystem-level applications due to the complex biogeochemical Se cycle is well documented (Presser and Luoma 2010). Further study is required before taxonomically-based periphyton assemblage descriptions can be used to develop predictive relationships between dissolved Se concentrations and Se concentrations in periphyton biofilms.

Prediction of Se accumulation in algae, and subsequent trophic transfer of Se, from algae to primary consumers, is complicated by the potential presence of multiple confounding factors. Exposure modifying factors can influence either the concentration of Se in periphyton, or the trophic transfer of Se from primary producers to primary consumers in aquatic ecosystems. Important parameters that influence the concentration of Se in periphyton include mixed Se speciation in the dissolved phase, competing ions and inorganic material content. In aquatic ecosystems, especially lentic zones, water residence time can be adequately long for a significant proportion of Se(VI) to be biologically reduced by microbes to Se(IV) and released to back into the environment, largely through dissimilatory reduction (Cutter and Bruland 1984; Presser and Luoma 2010; Stoltz et al. 2006). This process has been demonstrated in contaminated lentic systems that have been shown to contain a mixture of dissolved Se species, with different spatial and temporal trends (Cutter and Bruland 1984; Luoma and Presser 2009; Ponton et al. 2018; Wallschlager and Feldmann 2010). In this study, we avoided the potential confounding influence of mixed dissolved Se speciation and site water chemistry by using reconstituted water and regular water changes for all periphyton exposures. However, in the field, variations in water

quality parameters, such as Se speciation and ambient phosphate and sulphate concentrations, will likely modify Se accumulation in periphyton. Biological reduction of Se(VI) and the subsequent effect on Se enrichment in periphyton was not examined in this study, but obviously merits further study in natural systems because of the clear links between Se speciation and EF in periphytic biofilms, as demonstrated in this study. Important parameters that can influence the trophic transfer of Se from periphyton to primary consumers in aquatic ecosystems include selective grazing by primary consumers, nutritional content of foodstuffs (higher rate of consumption or lower body mass to dilute ingested and absorbed Se) and bioavailability of Se compounds in algae (discussed above). Selective grazing has been documented for some species of invertebrates (Bronmark 1989, 1994) and it is likely that this process would influence Se accumulation in primary consumers as a result of differential Se bioconcentration in different species of algae. Selective grazing would in turn influence the trophic transfer of Se to more sensitive receptors in Se-contaminated aquatic ecosystems (fish and aquatic birds).

It is recommended that future work investigating differential Se accumulation in complex, natural periphyton assemblages examine lower exposure concentrations to better inform environmental risk assessment. Future studies involving the exposure of complex periphyton communities to dissolved Se(IV) should, in addition to bulk accumulation, also consider extracellular adsorption versus cellular absorption to better understand site-dependent Se accumulation in primary producer biofilms and potential effects on the trophic transfer of Se to primary consumers. To better characterize the relationship between periphyton-Se and Se accumulated by invertebrate primary consumers, and hence generate reliable trophic transfer functions, we recommend that experiments allowing invertebrates to selectively graze on

selenized periphyton be carried out, in combination with supporting physicochemical characterization of periphyton and Se speciation analysis.

3.5.5 Acknowledgements

This research was supported by an NSERC Strategic Projects Grant to M. Hecker, K. Liber, and D. Janz (grant # STPGP 479047-15).

CHAPTER 4

GENERAL DISCUSSION

4.1 Project rationale and research goals

Although there has been much research attempting to elucidate the phenomenon of differential Se uptake and bioconcentration in algae, data regarding freshwater algal species, particularly those found in complex periphyton biofilms, are lacking. Such data are necessary to develop predictive ecotoxicological models for shallow freshwater systems. Previous studies have largely focused on free-floating species (phytoplankton) cultured under laboratory conditions (Baines and Fisher 2001; Fournier et al. 2006; Hu et al. 1997; Kiffney and Allen 1990; Riedel et al. 1991; Riedel and Sanders 1996), many of which were marine species. In addition, our current understanding of the potential ecological impacts of Se are derived primarily from data gathered from warm-water ecosystems (Janz et al. 2014; Stewart et al. 2010) and may not be applicable to cold, freshwater ecosystems, such as those found in northern Canada. Due to the inherent difficulty in predicting ecological risk associated with different sources of selenium in different aquatic environments, site-specific Se biodynamic models have been recommended as the best method to predict environmental risk (Hodson et al. 2010; Presser and Luoma 2010). Accurate, site-specific Se biodynamic models would aid in assessing the degree of risk associated with a particular source of selenium, and could potentially influence regulatory policy by allowing regulators to better predict how much selenium can safely be released into a given environment without eliciting deleterious effects on higher-order predators.

A thorough understanding of Se bioconcentration in freshwater periphyton would also help to model and create treatment systems with the goal of designing more efficient treatment strategies.

The research presented herein was completed as part of a larger, collaborative, multi-year study designed to assess the accumulation and food-chain transfer of Se in cold, freshwater environments, and the subsequent effects on fish relevant to aquatic ecosystems found in northern Canada. The overall goal of this larger project was to reduce uncertainty when modelling Se biodynamics in cold, freshwater ecosystems. The importance of Se bioconcentration in primary producers, which form the base of most aquatic food-webs, is well documented (Hodson et al. 2010; Presser and Luoma 2010). Therefore, the primary goal of this thesis project was to address the knowledge gap relating to the influence of periphytic algal assemblage composition on Se bioconcentration levels and patterns in complex, freshwater periphyton communities. To assess Se accumulation in simple and complex periphytic biofilms, both single-species periphyton (cultured) and multi-species (field-collected) periphyton were similarly exposed to environmentally relevant concentrations of inorganic Se oxyanions under controlled laboratory conditions; enrichment functions applicable to diverse, freshwater periphyton assemblages were derived using this experimental data. The secondary goal of this project was to determine the relative influence of adsorption processes and uptake by non-phototrophic bacteria on the apparent uptake of Se in natural periphyton known accumulate high levels of selenite. This secondary goal was addressed by assessing selenite accumulation in both unaltered and heat-killed periphyton, and in periphyton from the same site grown without light to exclude phototrophic organisms.

4.2 Summary and integration of project results

4.2.1 Laboratory-grown periphyton experiments

Experiments with laboratory-cultured periphyton, presented in Chapter 2, were designed to assess differential Se bioconcentration in different types of algae that can comprise periphytic biofilms. The species of algae used for these experiments were selected to represent three major taxonomic groups (phyla) of algae: Chlorophyta, Cyanophyta and Bacillariophyta. Bioconcentration of Se in different species of algae has been demonstrated to vary by orders of magnitude in free-floating, planktonic species (Vandermeulen and Foda 1988; Baines and Fisher 2001); however, variability among periphytic biofilms is largely unknown. Lab-grown single-species biofilms were exposed to environmentally relevant concentrations of selenite [Se(IV)] or selenate [Se(VI)] (nominal exposure concentrations of 5 and 25 μg Se L^{-1}) under controlled conditions to assess Se accumulation across taxonomically diverse taxa.

Biofilm-Se concentrations in the three species of algae tested (*Anabaena flos-aquae* [Cyanophyta], *Stichococcus bacillaris* [Chlorophyta], and *Asterionella formosa*, [Bacillariophyta]) demonstrated clear differences at pseudo-steady state. Different trends in Se accumulation were also observed between selenate and selenite. These differences suggest that mechanisms controlling the accumulation of Se-containing compounds in different types of algae can have differing capacity for each Se oxyanion.

The inorganic Se compounds tested showed different trends in accumulation among the species of algae exposed. Selenate and selenite were similarly bioconcentrated in a concentration-dependent manner, where increasing ambient Se concentration resulted in higher Se bioconcentration in *S. bacillaris*. In *A. flos-aquae*, selenite was bioconcentrated to a greater degree than selenate at both test concentrations, suggesting that the mechanisms controlling the

accumulation of selenite in this species have a higher capacity than those responsible for the accumulation of selenate in this species of algae. Selenite bioconcentration in *A. flos-aquae* was not proportional to the ambient Se concentration, suggesting that selenite accumulation was becoming saturated in the range of concentrations tested. Selenate bioconcentration in *A. flos-aquae* occurred in a linear, concentration-dependent pattern indicative of unsaturated accumulation. Selenite was also preferentially bioconcentrated over selenate at both test concentrations for *A. Formosa*, with bioconcentration of both inorganic Se compounds occurring in a concentration-dependent, proportional manner.

Selenium enrichment among the different species of algae tested showed different trends within each treatment. Selenite enrichment was highest in the cyanophyte, *A. flos-aquae*, for the low concentration treatment (5 μg Se L^{-1}). However, the diatom, *A. formosa*, showed statistically similar Se enrichment to *A. flos-aquae* for the high selenite concentration treatment (25 μg Se L^{-1}). The chlorophyte, *S. bacillaris*, had the lowest enrichment of selenite for both treatments, although the EFs were statistically similar between *S. bacillaris* and *A. flos-aquae* for the high selenite concentration treatment. Se enrichment was not statistically different among the three algal species for the low concentration selenate treatment (5 μg Se L^{-1}), but the trend was similar to the statistical differences observed at the high selenate concentration (25 μg Se L^{-1}); In the high concentration selenate treatment, *S. bacillaris* and *A. formosa* showed higher Se enrichment than *A. flos-aquae*. In general, interspecific differences in Se enrichment were smaller than expected based on the findings of previous algal studies, with a maximum 3.6-fold difference across three diverse algal taxa characteristic of freshwater ecosystems.

4.2.2 Natural periphyton experiments

Selenium bioconcentration experiments using field-collected periphyton, presented in Chapter 3, were designed to assess the relative influence of different periphyton community composition (as defined by differences in algal assemblage composition) on the bioconcentration of waterborne Se oxyanions in natural periphyton. Five water bodies with different water quality characteristics, all located within the Boreal Plains ecozone in Saskatchewan, Canada, were selected as sampling sites with the intent of producing different communities of periphyton as a result of the differing environmental requirements across algal taxa (Chisolm 1992; Cloen and Dufford 2005; Lowe 1996). For the initial (Year 1) field-collected periphyton experiments, targeted metagenomic analysis of 23S rRNA plastid genes, coupled with relative algal biovolume measurements obtained using light microscopy, showed that periphyton with different algal assemblages had successfully been sampled from the water bodies selected for this study.

Each of the five field-collected biofilms was exposed to environmentally relevant concentrations of selenite or selenate (nominal concentrations of 5 and 25 μg Se L^{-1}) for 8 days under laboratory conditions. The results of initial Se bioconcentration tests revealed that a particular periphyton community (sampled from Site 5) demonstrated high selenite accumulation. It was hypothesized that this was related to a high content of iron oxides, which are known to adsorb selenite (Balistrieri and Chao 1990). This observation lead to a second field collection (Year 2) designed to investigate the relative influence of adsorption processes and biofilm composition (biofilms with or without phototrophic organisms) on the bioconcentration of selenite in Site 5 periphyton. This was accomplished by comparing Se concentrations in natural, untreated periphyton with similar periphyton that had been heat-treated to cease all

biological processes, and biofilms that were grown under similar field conditions but without light, to exclude phototrophs.

Analysis of periphyton-Se concentrations for the different communities, collected during Year 1, showed different trends in Se enrichment for different Se species and different periphyton communities. Overall, selenite was enriched to a higher degree than selenate in periphyton sampled from all five field sites. Periphyton sampled from Site 2 demonstrated the lowest enrichment of selenite, while periphyton sampled from Site 5 demonstrated the highest enrichment of selenite (a 23.6-fold difference in enrichment between Sites 2 and 5 for the 5 μg Se L^{-1} treatment). Differences in selenate enrichment among different periphyton assemblages were small, with a maximum 2.0-fold difference in Se enrichment for both exposure concentrations. Although differences in Se enrichment among periphyton communities were smaller than anticipated and taxon-specific trends in Se enrichment were not easily discernable (especially in light of Year 2 experimental results, discussed below), these results demonstrate differential Se bioconcentration across periphyton communities with fundamentally different algal assemblages.

In the Year 2 experiment, Se enrichment was similar among the different pre-treatments for periphytic biofilms sampled at Site 5. These results indicate that much of the apparent Se bioconcentration observed in periphyton from Site 5 was a result of adsorbate-adsorbent interactions, rather than intracellular uptake, and was likely related to high Fe content in the biofilm. Furthermore, the declining distribution coefficient (K_d) values with increasing ambient selenite concentration observed during this experiment indicate that adsorption site saturation was occurring at the exposure concentrations tested. This has implications for the interpretation of Year 1 data regarding the apparent saturation of selenite uptake, making it difficult to

determine with certainty whether selenite (biological) uptake saturation was occurring in other field-collected periphyton communities. These results also indicate that bulk periphyton-Se concentration may not be useful for predicting the trophic transfer of Se in aquatic ecosystems without supporting Se speciation analysis (indicating adsorbed vs. absorbed Se).

4.2.3 Integration of laboratory and field-based results

Research conducted to meet the objectives outlined in Chapters 2 and 3 of this thesis generated multiple datasets that were integrated to assess the differential accumulation of inorganic Se oxyanions in periphytic algae. Integration of the project results, through the comparison of data derived from laboratory-grown and field-grown periphyton tests, indicated that there were two areas of agreement with regard to the differential bioconcentration of Se oxyanions in periphyton: 1) selenite was generally enriched to a higher degree than selenate when ambient concentrations were similar, and 2) periphyton with different algal assemblage composition can exhibit different degrees of Se enrichment when Se speciation (selenite or selenate) and ambient concentration are similar. Results from the laboratory-grown periphyton experiments, presented in Chapter 2, showed a propensity for the bioconcentration of selenite over selenate across algal species tested, although enrichment of the two Se oxyanions was similar for the species of green algae tested (*S. bacillaris*). In natural, field-collected periphyton (results presented in Chapter 3), selenite was bioconcentrated to a higher degree than selenate across all periphyton communities tested (n=5). These results agree with the consensus that Se(IV), as selenite, is the more available species of inorganic Se in algae (Baines and Fisher 2001; Riedel et al., 1991; Simmons and Wallschlager 2011). The different enrichment of Se observed for periphyton communities with different algal assemblages was apparent across both

laboratory-grown and natural periphyton experiments. Although differences in Se bioconcentration among different periphyton communities was lower than expected (maximum of 3.6-fold difference for laboratory-grown periphyton and a maximum of 23.6-fold difference for natural periphyton), these results provide further support for the hypothesis that mechanisms controlling the accumulation of Se-containing compounds in different types of algae can have different capacities for each Se oxyanion (selenate and selenite).

Complicating the integration of laboratory and field-based data is the disparity between the maximal enrichment of selenite in the periphyton communities tested (cultured single-species vs. natural, multi-species periphyton). In the laboratory-grown periphyton experiments, the maximum enrichment of selenite was 6,050-fold the ambient concentration (*A. flos-aquae*; 5 μg Se L^{-1}). The maximum enrichment of selenite in the field-collected periphyton was 30,027-fold the ambient concentration, before normalization to organic matter content (Site 5; 5 μg Se L^{-1}). However, the Year 2 field-collected periphyton experiment showed that the majority of selenite accumulated by Site 5 periphyton was the result of adsorbent-adsorbate interactions rather than cellular uptake, confounding comparison of the two datasets. If Site 5 is excluded from this comparison, the maximal enrichment of selenite in field-collected periphyton was 7,610-fold before normalizing to carbon content (Site 4; 5 μg Se L^{-1}). This comparison shows much greater agreement between maximal selenite accumulation in laboratory-cultured and field-collected periphyton results, although there are several confounding issues that reduce confidence in the comparison. Firstly, if data from the natural periphyton experiment are normalized to organic matter content (accounting for the incorporation of non-biologically active material in natural periphyton), the maximum enrichment of selenite in periphyton sampled from Site 4 is approximately 2-fold that of the highest enrichment of selenite in laboratory-grown periphyton.

This could either indicate that the natural periphyton sampled from Site 4 has a higher capacity to bioconcentrate selenite, or that sorption processes can play a significant role in the accumulation of selenite in natural periphyton communities according to certain physicochemical parameters, namely available substrate suitability and concentration (of which, natural periphyton may have more). Secondly, the comparison of laboratory- vs. field-derived results is further confounded by the high growth rate of laboratory-cultured, single-species periphyton biofilms relative to the growth rate of the natural, field-collected periphyton, which generally showed no, or low growth during the experimental period. The higher growth rate in laboratory-grown periphyton could have resulted in growth dilution (Hill and Larsen 2005), lowering the apparent concentration of periphyton-Se in those test species. Taken together, these results indicate that it is likely that both internalization and adsorption processes play a role in the accumulation of selenite in natural periphyton, with the magnitude of the latter depending on adsorption substrate suitability and availability.

The finding (presented herein) that the adsorption of selenite to the surface of periphyton may account for much of the apparent Se uptake, under certain circumstances, has important implications for ecological risk assessment (discussed in Chapter 3, section *3.5.3 Selenite adsorption and non-phototrophic uptake experiment*). Although this process has been clearly demonstrated herein, the relative importance of selenite adsorption to different algal species is unclear. To speculate, differences in algal anatomy (i.e., surface area and composition of cell walls) and the composition of extracellular matrices in different periphyton communities could possibly influence selenite adsorption (e.g., sorption site suitability and availability). Different species of algae, as well as young (smaller) versus older (larger) cells, have different surface areas (Hillebrand et al. 1999), which would directly influence available adsorption sites (i.e.,

larger cells should have more available sites). As well, phylogenetically distant algal taxa (e.g., chlorophytes, cyanophytes and diatoms) have vastly different cell wall compositions (Domozych 2011). For example, chlorophyte cell walls generally contain cellulose, cyanophytes have very complex, energetically costly cell walls (bacterial cell walls are so different from typical cell walls that many authors do not refer to them as cell walls, but rather as a "cellular envelope"), and diatoms have cell walls that are largely composed of silica (Domozych 2011). Algal cell walls of varying composition likely differ in the number of available selenite sorption sites (related to cell surface topography and shielding of binding sites) and binding site suitability (selenite binds to positive moieties due to its overall negative charge [Zhang 2008]). Therefore, the influence of surface morphology and chemical composition of different algal cell walls on selenite adsorption needs to be examined on an individual taxon basis, but, as an example, diatoms may possibly adsorb less selenite due to the presence of silicates in their cell walls (silicates are known to compete for sorption site with selenite, and are negatively charged [Balistrieri and Chao 1990]). This hypothesis is complicated by the findings of Riedel and Sanders (1996), who determined that the presence of silicate in solution increased the adsorption of selenite onto heat-killed algal cells, further illustrating the need for careful study of each component of the concepts described above. Additionally, the composition of the extracellular matrix surrounding cells embedded in periphyton is not only complex (extracellular matrices are generally composed of DNA, proteins and polysaccharides in various configurations [IUPAC 2014]), but varies greatly depending on the types of algae present (Domozych 2011); differences in the composition of the extracellular matrix could also influence selenite substrate availability and suitability.

The integration of laboratory and field-derived results has yielded a significant contribution to the body of knowledge surrounding biodynamic modelling of Se freshwater environments. The most important of these contributions is the reduction of uncertainty with regard to Se bioconcentration at the base of the food web (primary producers, here algae). Previous research has indicated that differential Se enrichment in different species of algae (under similar experimental conditions) could vary by as much as several orders of magnitude in marine species (Baines and Fisher 2001). The research presented herein demonstrated a relatively small fold-difference (a maximum of 23.6-fold) across a taxonomically diverse range of freshwater species of algae. Although this difference is much smaller than previously reported differences among algal taxa, the presence of different algal species in Se contaminated systems could still result in a several-fold difference in Se accumulation at the bottom of the food-web. Assuming that there is efficient trophic transfer (i.e., TTF≥1) throughout the food-web, this could still result in several-fold differences in Se bioaccumulation in higher order predators, which has important implication for ecological risk assessment. As such, the integrated results of laboratory and field-derived research also support the need for site-specific characterization of Se bioconcentration in periphyton using complex periphyton communities, while considering the importance of adsorbed versus absorbed selenite, and Se speciation both in water and algae.

4.3 Recommendations for future research

Selenium speciation plays an important role in the bioconcentration of Se in algae, as well as in the bioavailability of Se accumulated by primary producers to higher trophic levels (Simmons and Wallschlager 2005; Wallschlager and Feldmann 2010). The relative importance of selenate vs. selenite contamination in aquatic ecosystems could vary widely, depending on

numerous different physicochemical and biological parameters and processes (discussed throughout this manuscript). For example, selenite appears to accumulate more readily across most species of algae, but this may be of lesser toxicological significance if the selenite remains adsorbed to the exterior of the algal cells (thus, having lower bioavailability) rather than being incorporated and biotransformed into organic selenium compounds. Additionally, in aquatic ecosystems, selenate is typically reduced to selenite in a unidirectional manner, generally resulting in the presence of both oxyanions in systems contaminated with selenate. In order to better understand the process of Se bioconcentration and biotransformation in algae (particularly with regard to adsorbed vs. absorbed selenite), it is recommended that Se speciation be measured in both the dissolved phase and in periphyton for both laboratory- and field-based studies. This would help to illuminate three important aspects of the Se biogeochemical cycle that have been highlighted by the research presented in this thesis: 1) further investigation of the dissimilatory reduction of Se in complex periphyton communities, 2) the potential effects of the release of reduced Se compounds into the water column on Se accumulation in periphyton, and 3) to what extent Se (especially as selenite) is being taken up and biotransformed by algae vs. adsorbed to the surface of the cells. Se speciation analysis in natural periphyton sampled from contaminated sites may provide important insight into links between periphyton-Se concentration and the trophic transfer of Se compounds, potentially allowing for the derivation of periphyton-Se alert or guideline concentrations. It is also recommended that Se speciation analysis be paired with simple food chain experiments (water → algae → invertebrate) to explore links between Se biotransformation (or lack thereof, with regard to adsorbed selenite) in algae and the subsequent effects on bioavailability and trophic transfer of biosynthetic or adsorbed Se compounds. Characterization of selenite adsorption (if any) on the exterior of invertebrates and exploration of

selenite biotransformation processes that may occur in the gut of invertebrate consumers could help ascertain their relative importance (compared to similar processes occurring in algae/periphyton) and provide insight on the issue of linking Se concentration in periphyton with Se accumulated in higher trophic levels. For the purpose of such experiments, it may be most useful to employ a synchrotron-based approach (i.e., X-ray absorption near edge structure) to accurately characterize adsorbed vs. internalized selenium, both in algae and invertebrates. These studies should also consider the effects of selective grazing by invertebrates (Bronmark 1989, 1994). Such an approach could also make use of a non-synchrotron-based method to characterize Se speciation in water, which is available and involves the use of liquid chromatography in tandem with inductively-coupled plasma mass spectrometry (LC/HPLC-ICP-MS) (Donner and Siddique 2018; Kotrebai et al. 2000).

For the purpose of environmental risk assessment, it is recommended that experiments involving Se accumulation in complex, natural periphyton communities focus on lower exposure concentrations than those used for this study to better characterize differences in Se enrichment for periphyton with different algal assemblages. The ambient Se oxyanion concentrations used in this research (5 and 25 μg Se L^{-1}) were specifically selected to obtain tissue residues that were above potential background concentrations and representative of contaminated sites. Although theses concentrations were well within those values previously measured in Se contaminated aquatic environments (up to 100 μg Se L^{-1} [Maher et al. 2010]), lower concentrations must be explored for accurate risk characterization of Se contaminated sites with ambient Se oxyanion concentrations below 5 μg Se L^{-1} and straddling current guidelines for dissolved Se in freshwater, aquatic environments [CCME guideline: 1 μg Se L^{-1} (CCREM 1987; CCME 2007);

Government of BC guideline: 2 μg Se L^{-1} (BC MoE 2014); US EPA guidelines: 3.1 μg/L in lotic waters and 1.5 μg/L in lentic waters (US EPA 2016)].

4.5 Summary and Conclusion

Our understanding of Se oxyanion bioconcentration in periphyton has been further refined by the generation and integration of the laboratory-derived and field-derived results presented herein. Controlled laboratory experiments confirmed that diverse species of algae (belonging to different phyla) have different capacities for the accumulation of waterborne Se oxyanions (selenate and selenite). Selenium bioconcentration/accumulation experiments using field-grown periphyton provided valuable insights into the differential accumulation of Se in periphyton communities with different algal assemblages. More importantly, they served to highlight uncertainty related to the site-specific nature of Se bioconcentration in periphyton and the need to consider other physicochemical parameters outside of total Se content for more accurate characterization and prediction of trophic transfer processes. Overall, the most significant aspect of the results presented in this thesis is the reduction of uncertainty with regard to differential Se enrichment in periphyton. Given that the greatest uncertainty lies in the prediction of Se transfer from the abiotic environment to primary producers, the findings herein will significantly advance biodynamic modelling of Se in periphyton-based aquatic food webs.

REFERENCES

American Public Health Association, American Water Works Association (APHA), Water Pollution Control Federation, & Water Environment Federation. 1998. Standard methods for the examination of water and wastewater 20th ed. Washington, DC: APHA.

Baines SB, Fisher NS, Doblin MA, Cutter GA, Cutter LS, Cole B. 2004. Light dependence of selenium uptake by phytoplankton and implications for predicting selenium incorporation into food webs. Limnol Oceanogr 49(2):566-578.

Baines SB, Fisher NS, Doblin MA, Cutter GA. 2001. Accumulation of dissolved organic selenides by marine phytoplankton. Limnol Oceanogr 46:1936-1944.

Baines SB, Fisher NS. 2001. Interspecific differences in the bioconcentration of selenite by phytoplankton and their ecological implications. Mar Ecol Prog Ser 213:1-12.

Balistrieri LS, Chao TT. 1987. Adsorption of selenium by iron and manganese oxides: environmental implications. Proceedings of the Third Technical Meeting, US Geological Survey Program on Toxic Waste-Ground-Water Contamination. Pensacola FL, USA. pp. E19-E20.

Balistrieri LS, Chao TT. 1990. Adsorption of selenium by amorphous iron hydroxide and manganese dioxide. Geochim Cosmochim Acta 54:739-751.

Bellinger EG, Sigee DC. 2010. A Key to the More Frequently Occurring Freshwater Algae. Freshwater Algae: Identification and Use as Bioindicators. John Wiley and Sons, Ltd. pp 137-244.

Belzile N, Chen YW, Xu R. 2000. Early diagenetic behaviour of selenium in freshwater sediments. Appl Geochem 15:1439-1454.

Benjamin MM, Leckie JO. 1981. Conceptual model for metal-ligand-surface interactions during adsorption. Environ Sci Technol 15(9):1050-1057.

Besser JM, Canfield TJ, La Point TW. 1993. Bioaccumulation of organic and inorganic selenium in a laboratory food chain. Environ Toxicol 12:57-72.

Besser JM, Huckins JN, Clark RC. 1994. Seperation of selenium species released from Se-exposed algae. Chemosphere 29:771-780.

British Columbia Ministry of Environment (BC MoE). 2014. Ambient Water Quality Guidelines for Selenium Technical Report Update. Victoria, BC, CA: British Columbia Ministry of Environment, Environmental Sustainability and Strategic Policy Division, Water Protection and Sustainability Branch. 257p.

Bronmark, C. 1989. Interactions between epiphytes, macrophytes and freshwater snails: A Review. J. Moll Stud 55:299−311.

Bronmark, C. 1994. Effects of tench and perch on interactions in a freshwater benthic food chain. Ecology 75:1818−1824.

Canadian Council of Ministers of Environment (CCME). 2007. Canadian environmental quality guidelines for the protection of aquatic life. Summary table. Winnipeg, MB, CA: Canadian Council of Ministers of Environment, Task Force on Water Quality Guidelines. 13p. Accessed on-line at http://st-ts.ccme.ca/

Canadian Council of Resource and Environment Ministers (CCREM). 1987. Canadian water quality guidelines. Winnipeg MB, CA: Canadian Council of Resource and Environment Ministers, Task Force on Water Quality Guidelines. 1484p. No longer in publication.

Canton SP, Fairbrother A, Lemly AD, Ohlendorf HM, McDonald LE, MacDonald DD. 2008. Experts workshop on the evaluation and management of selenium in the Elk Valley, British Columbia, Workshop summary report. http://www.env.gov.bc.ca/eirs/epd

Carlson LT, Schwertmann U. 1981. Natural ferrihydrites in surface deposits from Finland and their association with silica. Geochim Cosmochi Acta 45(3), 421-429.

Chisholm SW. 1992. Phytoplankton size. Primary Productivity and biogeochemical cycles in the sea. (eds. Falkowski G, Woodhead AD). Plenum. NY, USA: pp 213-237.

Clarke KR, Gorley RN. 2015. PRIMER v7: User Manual/Tutorial (PRIMER-E, Plymouth)

Clarke KR, Gorley RN, Somerfield PJ, and Warwick RM. 2014. Change in marine communities: an approach to statistical analysis and interpretation, 3rd edition. PRIMER-E, Plymouth.

Cloern JE, Dufford R. 2005. Phytoplankton community ecology: principles applied in San Francisco Bay. Mar Ecol Progr Ser 285:11-28.

Conely JM, Funk DH, Buchwalter DB. 2009. Selenium bioaccumulation and maternal transfer in the mayfly Centroptilum triangulifer. Environ Sci Technol 43:7952-7957.

Conely JM, Funk DH, Hesterberg DH, Hsu LC, Kan J, Liu YT, Buchwalter DB. 2013. Bioconcentration and biotransformation of selenite versus selenate exposed periphyton and subsequent toxicity to the mayfly Centroptilum triangulifer. Environ Sci Technol 47:7965-7973.

Cooke TD, Bruland KW. 1987. Aquatic chemistry of selenium: Evidence of biomethylation. Environ Sci Technol 21:1214-1219.

Cornell RM, Schwertmann U. 1996. The Iron Oxides. VCH, New York. 573p.

Crutchfield JU Jr. 2000. Recovery of a power plant cooling reservoir ecosystem from selenium bioaccumulation. Environ Sci Policy 3:S145-S163.

Cumbie PM, Van Horn, SL. 1978. Selenium accumulation associated with fish mortality and reproductive failure. Proc Ann Conf SE Assoc Fish Wildl Agencies 32:612-624.

Cutter GA, Bruland KW. 1984. The marine biogeochemistry of selenium: A re-evaluation. Limnol Oceanogr 29(6):1179-1192.

Davis JA, James RO, Leckie JO. 1978. Surface ionization and complexation at the oxide/water interface: I. Computation of electrical double layer properties in simple electrolytes. J Colloid Interface Sci 63:480-499.

DeBruyn AMH, Chapman PM. 2007. Selenium toxicity to invertebrates: Will proposed thresholds for toxicity to fish and birds also protect their prey? Environmental Science and Technology 41:1766-1770.

DeForest DK, Brix KV, Elphick JR, Rickwood CJ, deBruyn AMH, Tear LM, Gilron G, Hughes SA, Adams WJ. 2017. Lentic, lotic, and sulphate-dependent waterborne selenium screening guidelines for freshwater systems. Environ Toxicol Chem 36(9):2503-2513.

Dhillon KS, Dhillon SK. 2003. Distribution and management of seleniferous soils. Adv Agron 79:119-184.

Dobbs MG, Cherry DS, Cairns J. 1996. Toxicity and bioaccumulation of selenium to a three-trophic level food chain. Environ Toxicol Chem 15:340–347.

Domingo C, Rodriguez-Clemente R, Blesa M. 1994. Morphological properties of α-FeOOH, γ-FeOOH and Fe3O4 obtained by oxidation of aqueous Fe(II) solutions. J Colloid Interface Sci 165: 244-252.

Domozych DS. 2011. Algal cell walls. eLS doi: 10.1002/9780470015902.a0000315.

Donner MW, Siddique T. 2018. A rapid and sensitive IC-ICP-MS method for determining selenium speciation in natural waters. Can Journal Chem 96:795-802.

Elbrecht V, Leese F. 2015. Can DNA-based ecosystem assessments quantify species abundance? Testing primer bias and biomass—sequence relationships with an innovative metabarcoding protocol. PLoS One 10:7.

Ennis GL, Albright LJ. 1980. Distribution and abundance of periphyton and phytoplankton species in two subarctic Canadian rivers. Can J Bot 60:224-236.

Environment Canada, Environmental Science and Technology Centre, Science Technology Branch. March 2007. Biological Test Method: Growth Inhibition Using a Freshwater Alga. (Report EPS 1/RM/25 Second Ed.). Ottawa, Ontario: Communications Services, Environment Canada.

Fan TWM, Swee TJ, Hinton DE, Higashi RM. 2002. Selenium biotransformation into proteinaceous forms by foodweb organisms of selenium-laden drainage waters in California. Aquat Toxicol 57:65-84.

Fisher NS, Reinfelder JR. 1995. The trophic transfer of metals in marine systems: Metal speciation and bioavailability in aquatic systems. (eds) Tessier A, Turner DR. John Wiley & Sons, Chichester, pp 363–406.

Fisher NS, Wente M. 1993. The release of trace elements by dying marine phytoplankton. Deep-Sea Res 40:671-694.

Foster AL, Brown GE, Parks GA. 2003. X-ray absorption fine structure study of As (V) and Se (IV) sorption complexes on hydrous Mn oxides. Geochim Cosmochim Acta 67:1937-1953.

Fournier E, Adam C, Massabuau JC, Garnier-Laplace J. 2006. Selenium bioaccumulation in Chlamydomonas reinhardtii and subsequent transfer to Corbicula fluminae: role of selenium speciation in bivalve ventilation. Environ Toxicol Chem 25:2692-2699.

Fournier E, Adam-Guillermin C, Potin-Gautier M, Pannier F. 2010. Selenate bioaccumulation and toxicity in Chlamydomonas reinhardtii: Influence of ambient sulphate ion concentration. Aquat Toxicol 97:51-57.

Friesen V, Doig L, Markwart B, Haakensen M, Tissier E, Liber K. (2017). Genetic characterization of periphyton communities associated with selenium bioconcentration and trophic transfer in a simple food chain. Environ Sci Technol 51(13):7532-7541.

Gao S, Tanji KK, Dahlgren RA, Ryu J, Herbel MJ, Higashi RM. 2007. Chemical status of selenium in evaporation basins for disposal of agricultural drainage. Chemosphere 69:585-594.

Guan R, Wang WX. 2004. Dietary assimilation and elimination of Cd, Se, and Zn by Daphnia magna as different metal concentrations. Environ Toxicol Chem 23:2689-2698.

Hamilton SJ. 2003. Review of residue-based selenium toxicity thresholds for freshwater fish. Ecotoxicol Environ Saf 56:201-210.

Heider J, Bock A. 1993. Selenium metabolism in microorganisms. Adv. Physiol. 35:74–107

Herbel MJ, Switzer Blum J, Oremland RS, Borglin SE. 2003. Reduction of elemental selenium to selenide: experiments with anoxic sediments and bacteria that respire Se oxyanions. Geomicrobiol. J. 20:587–602

Hesketh J. 2008. Nutrigenomics and selenium: gene expression patterns, physiological targets, and genetics. Annu Rev Nutr 28:157-177.

Hill WR, Larsen IL. 2005. Growth dilution of metals in microalgal biofilms. Environ Sci Technol 39:1513–1518.

Hillebrand H, Durselen CS, Kirschtel D, Pollingher U, Zohary T. 1999. Biovolume calculation for pelagic and benthic microalgae. J. Phycol 35:403-242.

Hodson PV, Hilton JW. 1983. The nutritional requirements and toxicity to fish of dietary and water-borne selenium. Ecol Bull 35:335−340.

Hodson PV, Reash RJ, Canton PC, Campbell PV, Delos CG, Fairbrother A, Hitt NP, Miller LL, Ohlendorf HM. 2010. Selenium Risk Characterization. Ecological Assessment of Selenium in the Aquatic Environment (eds. Chapman PM, Adams WJ, Brooks ML, Delos CG, Luoma SN, Maher WA, Ohlendorf HM, Presser TS and Shaw DP). Boca Raton, FL: CRC Press. pp 233-256.

Hopper JL, Parker DR, 1999. Plant availability of selenate and selenite as influenced by the competing ions phosphate and sulfate. Plant Soil 210:199-207.

Howard III JH. 1977. Geochemistry of selenium: formation of ferroselite and selenium behaviour in the vicinity of oxidizing sulfide and uranium deposits. Geochimica et Cosmochimica Acta 41:1665-1678.

Hu MH, Yang YP, Martin JM, Yin K, Harrison PJ. 1997. Preferential uptake of Se(IV) over Se(VI) and the production of dissolved organic Se by marine phytoplankton. Mar Environ Res 44:225-231.

International Union of Pure and Applied Chemistry (IUPAC). 2014. Compendium of Chemical Terminology: Gold Book Version 2.3.3. Accessed at: https://goldbook.iupac.org/PDF/goldbook.pdf on November 22, 2016.

Janz DM, DeForest DK, Brooks ML, Chapman PM, Gilron G, Hoff D, Hopkins WA, McIntyre DO, Mebane CA, Palace VP, Skorupa JP, Wayland M. 2010. Selenium Toxicity to Aquatic Organisms. Ecological Assessment of Selenium in the Aquatic Environment (eds. Chapman PM, Adams WJ, Brooks ML, Delos CG, Luoma SN, Maher WA, Ohlendorf HM, Presser TS and Shaw DP). Boca Raton, FL: CRC Press. pp 141-231.

Janz DM, Liber K, Pickering IJ, Wiramanaden CI, Weech SA, Gallego-Gallegos M, Driessnack MK, Franz ED, Goertzen MM, Phibbs J, Tse JJ. 2014. Integrative assessment of selenium speciation, biogeochemistry, and distribution in a northern coldwater ecosystem. IEAM 10(4):543-554.

Kiffney P, Allen K. 1990. The Toxicity and Bioaccumulation of Selenate, Selenite and Seleno-L-Methionine in the Cyanobacterium Anabaena flos-aquae. Arch Environ Contam Toxicol 19:488-494.

Kotrebai M, Birringer M, Tyson JF, Block E, Uden C. 2000. Selenium speciation in enriched and natural samples by HPLC-ICP-MS and HPLC-ESI-MS with perflourinated carboxylic acid ion-pairing agents. Analyst 125:71-78.

Lemly AD. 1993. Taratogenic effects of selenium in natural populations of freshwater fish. Ecotoxicol Environ Saf 26:181-204.

Lemly AD. 1997. Ecosystem recovery following selenium contamination in a freshwater reservoir. Ecotoxicology and Environmental Safety 36:275-281.

Lemly AD. 2004. Aquatic selenium pollution is a global environmental safety issue. Ecotoxicol Environ Saf 59:44-56.

Lide D. 1994. CRC handbook of chemistry and physics: a ready-reference book of chemical and physical data. Boca Raton, FL: CRC Press.

Lindemann T, Prange A, Dannecker W, Neidhart B. 2000. Stability studies of arsenic, selenium antimony and tellurium species in water, urine, fish and soil extracts using HPLC/ICP-MS. Fresenius J Anal Chem 369:214-220.

Lo BP, Elphick JR, Bailey HC, Baker JA, Kennedy CJ. 2015. The effect of sulfate on selenate bioaccumulation in two freshwater primary producers: A duckweed (Lemna minor) and a green alga (Pseudokirchneriella subcapitata). Environ Toxicol Chem. 34:2841–2845.

Lowe RL. 1996. Periphyton patterns in lakes. Algal ecology: freshwater benthic ecosystems. (ed. Stevenson RJ, Bothwell MI, Lowe RL). San Diego, CA: Elsevier. pp 57-76.

Lozupone, C., and R. Knight. 2005. 'UniFrac: a new phylogenetic method for comparing microbial communities', Appl Environ Microbiol, 71: 8228-35.

Luoma SN, Presser TS. 2009. Emerging opportunities in management of selenium contamination. Environmental Science and Technology 43:8483-8487.

Maher WA, Roach A, Doblin M, Fan T, Foster S, Garrett R, Moller G, Oram L, Wallschlager D. 2010. Environmental sources, speciation, and partitioning of selenium. Ecological Assessment of Selenium in the Aquatic Environment (eds. Chapman PM, Adams WJ, Brooks ML, Delos CG, Luoma SN, Maher WA, Ohlendorf HM, Presser TS and Shaw DP). Boca Raton, FL: CRC Press. pp 47–92.

Maier KJ, Ogle RS, Knight AW. 1988. The selenium problem in lentic ecosystems. Lake and Reservoir Management 4(2):155-163.

Martin AJ, Simpson S, Fawcett S, Wiramanaden CIE, Pickering IJ, Belzile N, London J, Wallschlager D. 2011. Biogeochemical mechanisms of selenium exchange between water and sediments in two contrasting lentic environments. Environ Sci Technol 45:2605–2612.

Masscheleyn PH, Patrick WH. 1993. Biogeochemical processes affecting selenium cycling in wetlands. Environ Toxicol Chem 12:2235-2243.

Mayland H. 1994. Selenium in plant and animal nutrition. Selenium in the Environment (eds. Frankenberger WT Jr, Benson S Jr. New York, NY USA. pp 29-45.

Moroder L. 2005. Isoteric replacement of sulfur with other chalcogens in peptides and proteins. J. Peptide Sci. 11:187-214.

Muscatello JR, Janz DM. 2009. Selenium accumulation in aquatic biota downstream of a uranium mining and milling operation. Sci Total Environ 407:1318-1325.

Nriagu JO. 1989. Global cycling of selenium. Occurrence and Distribution of Selenium (ed. Ihnat M). Boca Raton, FL: CRC Press. pp 327-340.

O'Flaherty LM, Phinney HK. 1970. Requirements for the maintenance of growth of Anabaena flos-aquae in culture. J phycol 6:95-97.

Ohlendorf HM, Hothem RL, Bunck CM, Marios KC. 1990. Bioaccumulation of selenium in birds at Kesterson Reservoir, California. Arch Environ Contam Toxicol 19:495-507.

Ohlendorf HM, Kilness AW, Simmons JL, Stroud RK, Hoffman DJ, Moore JF. 1988. Selenium toxicosis in wild aquatic birds. J Toxicol Environ Health 24:67-92.

Ohlendorf HM. 2003. Ecotoxicology of Selenium. Handbook of Ecotoxicology 2nd Ed. (eds. Hoffman DJ, Rattner BA, Burton Jr. GA, Cairns Jr. J.). Boca Raton, FL: CRC Press. pp 465-500.

Oremland RS, Herbel MJ, Blum JS, Langley S, Beveridge TJ, Ajayan PM, Sutto T, Ellis AV, Curran S. 2004. Structural and spectral feature of selenium nanospheres produced by Se-respiring bacteria. Applied and Environmental Microbiology 70(1):52-60.

Oremland RS, Steinberg NA, Presser TS, Miller LG. 1991. In situ bacterial selenate reduction in the agricultural drainage systems of Western Nevada. Applied and Environmental Microbiology 57(2):615-617.

Orr PL, Guiguer KR, Russel CK. 2006. Food chain transfer of selenium in lentic and lotic habitats of a western Canadian watershed. Ecotoxicology and Environmental Safety 63(175-188).

Parida KM, Gorai B, Das NN, Rao SB. 1996. Studies on ferric oxide hydroxides: Adsorption of selenite on different forms of iron oxyhydroxides. J. Colloid Interface Sci 185:355-362.

Ponton DE, Fortin C, Hare L. 2018. Organic selenium, selenate and selenite accumulation by lake plankton and the alga Chlamydomonas reinhardtii at different pH and sulfate concentrations. Environ Toxicol Chem 37(8):2112-2122.

Presser TS, Luoma SN. 2010. A methodology for ecosystem-scale modeling of selenium. IEAM 6(4):685-710.

Presser TS, Swain WC, Tidwell RR, Sefverson RC. 1990. Geologic sources, mobilization, and transport from California coastal ranges to the western San Joaquin Valley: a reconnaissance study. Menlo Park (CA, USA): US Geological Survey. Water-Resources Investigations Report 90-4070.

Presser TS, Sylvester MA, Low WH. 1994. Bioaccumulation of selenium from natural geologic sources in the Western States and its potential consequences. Environ Manage 18:423-436.

Reilly C. 2006. Selenium in food and health. New York, NY:Springer

Renwick, AG. 2006. Toxicology of micronutrients: Adverse effects and uncertainty. J Nutr 136:493S−501S.

Riedel GF, Ferrier DP, Sander JG. 1991. Uptake of selenium by freshwater phytoplankton. Water Air Soil Pollut 57(1):23-30.

Riedel GF, Sander JG, Gilmour CC. 1996. Uptake, transformation, and impact of selenium in freshwater phytoplankton and bacterioplankton communities. Aquat Microb Ecol 11:43-51.

Riedel GF, Sanders JG. 1996. The influence of pH and media composition on the uptake of inorganic selenium by Chlamydomonas reinhardtii. Environ Toxicol Chem 15(9):1577-1583.

Saiki MK, Lowe TP. 1987. Selenium in aquatic organisms from subsurface agricultural drainage water, San Joaquin Valley, California. Arch Environ Contam Toxicol 16:657-670.

Sherwood AR, Presting GG. 2007. Universal primers amplify a 23S rDNA plastid marker in eukaryotic algae and cyanobacteria. J. Phycol 43:605−608.

Simmons DBD, Wallschlager D. 2005. A critical review of the biogeochemistry and ecotoxicology of selenium in lotic and lentic environments. Environ Toxicol Chem 24(6):1331-1343.

Simmons DBD, Wallschlager D. 2011. Release of reduced inorganic selenium species into waters by the green fresh water algae Chlorella vulgaris. Environ Sci Technol 45:2165-2171.

Singh R, Paul D, Jain RK. 2006. Biofilms: implications in bioremediation. Trends Microbiol 14:389.

Skorupa JP. 1998. Selenium poisoning of fish and wildlife in nature: lessons from twelve real-world examples. Environmental chemistry of selenium, eds. Frankenberger WT Jr, Enberg RA. New York (NY, USA): Marcel Dekker. pp 315-354.

Smetacek V, Assmy P, Henjes J. 2004. The role of grazing in structuring Southern Ocean pelagic ecosystems and biogeochemical cycles. Antarctic Sci 16:541-558.

Stein, J (Ed.). 1973. Handbook of Phycological methods. Culture methods and growth measurements. Cambridge University Press, Cambridge, London, New York and Melbourne. 448 pp.

Steven B, McCann S, Ward NL. 2012. Pyrosequencing of plastid 23S rRNA genes reveals diverse and dynamic cyanobacterial and algal populations in two eutrophic lakes. FEMS Microbiol Ecol 82:607−15.

Stevenson RJ. 1996. An Introduction to Algal Ecology in Freshwater Benthic Habitats. Algal ecology: freshwater benthic ecosystems. (ed. Stevenson RJ, Bothwell MI, Lowe RL). San Diego, CA: Elsevier. pp 3-30.

Stewart R, Grosell M, Buchwalter D, Fisher N, Samuel L, Mathews T, Orr P, Wang WX. 2010. Bioaccumulation and Trophic transfer of Selenium. Ecological Assessment of Selenium in the Aquatic Environment (eds. Chapman PM, Adams WJ, Brooks ML, Delos CG, Luoma SN, Maher WA, Ohlendorf HM, Presser TS and Shaw DP). Boca Raton, FL: CRC Press. pp 93-139.

Stolz JF, Basu P, Santini JM, Oremland RS. 2006. Arsenic and selenium in microbial metabolism. Annu Rev Microbiol 60:107-130.

Stolz JF, Oremland RS. 1996. Bacterial respiration of arsenic and selenium. FEMS Microbiology Reviews 23:615-627.

Tarkowska-Kukuryk M, Mieczan T. 2012. Effect of substrate on periphyton communities and relationships among food web components in shallow hypertrophic lake. J Limnol 72(2):279-290.

Terry N, Zayed AM, De Souza MP, Tarun AS. 2000. Selenium in higher plants. Ann Rev Plant Physiol Plant Mol Biol 51:401-432.

Tessier E, Amouroux D, Abril G; Lemaire E; Donard O. 2002. Formation and volatilization of alkly-iodidesand-selenides in macrotidal estuaries. Biogeochemistry 59(1):183-206

United States Environmental Protection Agency (US EPA). 1999. Rapid bioassessment protocols for use in wadeable streams and rivers – periphyton, ebenthic macroinvertebrates, and fish 2nd ed. (EPA Publication No. 841-B-99-002). Washington, DC: US EPA.

United States Environmental Protection Agency (US EPA). 2016. Aquatic life ambient water quality criterion for selenium – freshwater. (EPA Publication No. 822-R-16-006). Washington, DC: US EPA.

Vandermeulen JH, Foda A. 1988. Cycling of selenite and selenate in marine phytoplankton. J Mar Biol 98:115-123.

Wallschlager D, Feldmann J. 2010. Formation, occurrence, significance, and analysis of organoselenium and organotellurium compounds in the environment. Met Ions Life Sci 7:319–364.

Wang T, Wang J, Burken JG, Ban H, Ladwig K. 2007. The leaching characteristics of selenium from coal fly ashes. J Environ Qual 36:1322-1328.

Weeks ME. 1932. The discovery of the elements. VI. Tellurium and selenium. J. Chem. Educ. 9(3):474. DOI: 10.1021/ed009p474

Wessjohann LA, Schneider A, Abbas M, Brandy W. 2007. Review: selenium in chemistry and biochemistry in comparison to sulfur. The Journal of Biological Chemistry 388:997-1006.

Williams MJ, Ogle RS, Knight AW, Burau RG. 1994. Effects of sulfate on selenate uptake and toxicity in the green alga Selenastrum capricornutum. Arch Environ Contam Toxicol 27:449–453.

Wiramanaden CIE, Liber K, Pickering IJ. 2010. Selenium speciation in whole sediment using X-ray absorption spectroscopy and micro X-ray fluorescence imaging. Environ Sci Technol 44:5389–5394.

Yang SI, Lawrence JR, Swerhone GDW, Pickering IJ. 2011. Biotransformation of selenium and arsenic in multi-species biofilm. Environmental Chemistry 8:543-551.

Young TF, Keith F, Adams WJ, Besser J, Hopkins WD, Jolley D, McNaughton E, Presser TD, Shaw DP, Unrine J. 2010. What You Need to Know About Selenium. Ecological Assessment of Selenium in the Aquatic Environment (eds. Chapman PM, Adams WJ, Brooks ML, Delos CG, Luoma SN, Maher WA, Ohlendorf HM, Presser TS and Shaw DP). Boca Raton, FL: CRC Press. pp 7-45.

Yu RQ, Wang WX. 2004. Biological uptake of Cd, Se(IV) and Zn by Chlamydomonas reinhardtii in response to different phosphate and nitrate additions. Aquat Microb Ecol 35:163-173.

Zhang L, Wang WX. 2007. Size-dependence of subcellular metal distribution in prey in influencing the trophic transfer of metals in marine fish. Limnol Oceanogr 51:2008-2017.

Zhang N, Lin LS, Gang D. 2008. Adsorptive selenite removal from water using iron-coated GAC adsorbents. Water Res 42:3809-3816.

Zhang YQ, Moore JN, Frankenberger WT Jr. 1999. Speciation of soluble selenium in agricultural drainage waters and aqueous soil-sediment extracts using hydride generation atomic absorption spectrometry. Environ Sci Technol 33:1652-1656.

Zhang YQ, Zahir ZA, Frankenberger WT Jr. 2004. Fate of colloidal-particulate elemental selenium in aquatic systems. J Environ Qual 33:1652-1656.

APPENDIX A

SUPPLEMENTARY INFORMATION FROM CHAPTERS 2 AND 3

LIST OF APPENDIX TABLES

LIST OF APPENDIX FIGURES

Table A.1: Nutrient list and nominal concentrations in test water.

Macronutrient	Concentration (mg L^{-1})	Element	Concentration (mg L^{-1})
$NaNO_3$	15.94	N	2.63
$MgCl_2 \bullet 6H_2O$	6.25	Mg	1.65
$CaCl_2 \bullet 2H_2O$	2.76	Ca	0.75
$MgSO_4 \bullet 7H_2O$	9.19	S	1.20
K_2HPO_4	0.65	P	0.12
		K	0.293
$NaHCO_3$	9.38	Na	6.88
		C	1.34

Micronutrient	Concentration (µg L^{-1})	Element	Concentration (µg L^{-1})
H_3BO_3	115.95	B	20.27
$MnCl_2 \bullet 4H_2O$	259.76	Mn	72.11
$ZnCl_2$	2.05	Zn	0.98
$CoCl_2 \bullet 6H_2O$	0.89	Co	0.22
$CuCl_2 \bullet 2H_2O$	0.008	Cu	0.003
$Na_2MoO_4 \bullet 2H_2O$	4.54	Mo	1.8
$FeCl_3 \bullet 6\,H_2O$	100	Fe	20.7
$Na_2EDTA \bullet 2\,H_2O$	46.9	—	—

Reference: Environment Canada, Environmental Science and Technology Centre, Science Technology Branch. March 2007. Biological Test Method: Growth Inhibition Using a Freshwater Alga. (Report EPS 1/RM/25 Second Ed.). Ottawa, Ontario: Communications Services, Environment Canada.

Table A.2: Mean (±SD) mass per area and composition of periphyton sampled from 5 different water bodies (sites 1 – 5) (*n*=5). Both natural and heat-killed periphyton pre-treatments were used to calculate Site 5 – Yr 2 parameters. Insufficient material was available for all analyses for Site 5 – year 2 shade cloth periphyton pre-treatment.

Field-site	Mass/Area (mg cm^{-2})	% Organic Material	% Inorganic material	% Iron Content
Site 1	0.06 ± 0.01	76.5 ± 7.2	23.5±7.2	0.37 ± 0.05
Site 2	0.60 ± 0.09	50.1 ± 3	49.9±3	0.44 ± 0.2
Site 3	0.24 ± 0.07	43.0 ± 5.5	57±5.5	1.78 ± 0.64
Site 4	0.04 ± 0.01	59.5 ± 1.9	40.5±1.9	2.27 ± 0.78
Site 5	0.35 ± 0.22	39.2 ± 12.2	60.8±12.2	6.46 ± 2.99
Site 5 Yr 2	0.19 ± 0.07	35.6 ± 14.1	64.4 ± 14.1	9.91 ± 1.91
Site 5 Yr 2 SC	0.13 ± 0.06	-	-	14.1 ± 7.3

Table A. 3: Mean (±SD) water quality, light intensity and dissolved Se measurements for Se oxyanion bioconcentration tests assessing differential Se(IV) and Se(VI) uptake in periphyton sampled from 5 different water bodies (Sites 1 – 5). Mean and SD were calculated using all treatment replicates from water collected/measurements taken on all sampling days.

Site Tested	Treatment	DO (mg L^{-1})	Temp. (°C)	Conductivity (µS cm^{-1})	pH	Alkalinity (mg $CaCO_3$ L^{-1})	Hardness (mg $CaCO_3$ L^{-1})	PAR (µmol m^{-2} s)	Water Se (µg Se L^{-1})	CV for Water Se (%)
Site 1	Control	8.7 ± 0.1	19.1 ± 0.2	69 ± 1	7.3 ± 0.1	19 ± 1	6 ± 2	19 ± 2	0.04 ± 0.02	-
	5 µg Se(IV)/L	8.7 ± 0.1	19 ± 0.1	68 ± 0	7.4 ± 0.1	19 ± 1	5 ± 1	19 ± 2	4.60 ± 0.37	8.1
	25 µg Se(IV)/L	8.7 ± 0.2	19 ± 0.1	68 ± 0	7.4 ± 0.1	18 ± 2	5 ± 1	21 ± 1	23.40 ± 1.40	6.0
	5 µg Se(VI)/L	8.8 ± 0.2	19.1 ± 0.2	68 ± 0	7.3 ± 0.1	20 ± 1	4 ± 1	20 ± 3	4.92 ± 0.30	6.1
	25 µg Se(VI)/L	8.8 ± 0.1	19 ± 0.1	68 ± 0	7.4 ± 0.1	19 ± 1	5 ± 1	21 ± 2	24.21 ± 1.06	4.4
Site 2	Control	8.7 ± 0.1	19.8 ± 0.3	79 ± 12	7.5 ± 0.2	24 ± 5	5 ± 1	20 ± 2	0.04 ± 0.01	-
	5 µg Se(IV)/L	8.7 ± 0.1	20 ± 0.6	76 ± 7	7.5 ± 0.1	23 ± 3	5 ± 1	21 ± 2	4.50 ± 0.29	6.4
	25 µg Se(IV)/L	8.7 ± 0.1	19.7 ± 0.3	78 ± 8	7.6 ± 0.1	23 ± 4	6 ± 1	20 ± 2	22.95 ± 0.73	3.2
	5 µg Se(VI)/L	8.7 ± 0.1	19.9 ± 0.3	77 ± 8	7.6 ± 0.1	22 ± 3	5 ± 1	20 ± 2	4.64 ± 0.16	3.5
	25 µg Se(VI)/L	8.8 ± 0.1	19.9 ± 0.2	78 ± 10	7.6 ± 0.1	22 ± 4	6 ± 1	21 ± 1	22.94 ± 0.57	2.5
Site 3	Control	8.5 ± 0.2	20.1 ± 0.2	72 ± 2	7.5 ± 0.1	21 ± 2	7 ± 2	23 ± 1	<LoD	-
	5 µg Se(IV)/L	8.6 ± 0.1	19.7 ± 0.3	72 ± 1	7.5 ± 0.0	21 ± 1	7 ± 1	20 ± 2	4.33 ± 0.32	7.3
	25 µg Se(IV)/L	8.6 ± 0.1	19.8 ± 0.3	72 ± 1	7.5 ± 0.0	21 ± 2	7 ± 1	20 ± 2	23.14 ± 1.02	4.4
	5 µg Se(VI)/L	8.6 ± 0.1	19.9 ± 0.2	71 ± 3	7.5 ± 0.1	20 ± 1	7 ± 1	21 ± 3	4.65 ± 0.22	4.7
	25 µg Se(VI)/L	8.5 ± 0.1	20.3 ± 0.5	70 ± 3	7.5 ± 0.1	20 ± 1	6 ± 1	20 ± 3	24.31 ± 1.63	6.7
Site 4	Control	8.5 ± 0.3	19.4 ± 0.4	74 ± 2	7.5 ± 0.1	20 ± 4	7 ± 1	20 ± 2	<LoD	-
	5 µg Se(IV)/L	8.7 ± 0.2	19.3 ± 0.5	73 ± 1	7.5 ± 0.0	22 ± 2	7 ± 1	20 ± 3	4.45 ± 0.17	3.9
	25 µg Se(IV)/L	8.7 ± 0.3	19.3 ± 0.6	72 ± 2	7.5 ± 0.1	20 ± 2	6 ± 1	20 ± 2	22.81 ± 0.67	2.9
	5 µg Se(VI)/L	8.6 ± 0.1	19.3 ± 0.4	73 ± 1	7.5 ± 0	22 ± 1	7 ± 1	20 ± 3	4.81 ± 0.28	5.9
	25 µg Se(VI)/L	8.7 ± 0.2	19.3 ± 0.5	73 ± 1	7.5 ± 0	22 ± 2	7 ± 2	21 ± 2	23.55 ± 0.7	3.0
Site 5	Control	8.8 ± 0.2	19.6 ± 0.3	70 ± 3	7.5 ± 0.1	20 ± 1	7 ± 1	19 ± 3	<LoD	-
	5 µg Se(IV)/L	8.6 ± 0.2	19.6 ± 0.1	70 ± 2	7.5 ± 0.1	20 ± 1	6 ± 1	20 ± 4	4.02 ± 0.47	11.6
	25 µg Se(IV)/L	8.7 ± 0.1	19.6 ± 0.2	70 ± 2	7.5 ± 0.1	20 ± 2	5 ± 1	18 ± 3	21.58 ± 1.56	7.2
	5 µg Se(VI)/L	8.8 ± 0.1	19.6 ± 0.2	68 ± 1	7.5 ± 0.1	19 ± 2	5 ± 1	19 ± 3	4.65 ± 0.11	2.5
	25 µg Se(VI)/L	8.7 ± 0.1	19.8 ± 0.2	71 ± 3	7.5 ± 0.1	20 ± 2	6 ± 1	21 ± 2	23.11 ± 0.47	2.0

*LoD for water Se: 0.018 - 0.053 µg Se L^{-}

Table A.4**:** Mean (±SD) water quality, light intensity and dissolved Se measurements for Se accumulation tests assessing differential Se(IV) accumulation in pre-treated periphyton/substrate. Mean and SD were calculated using all treatment replicates from water collected/measurements taken on all sampling days.

Periphyton Treatment	Se Treatment	DO ($mg\ L^{-1}$)	Temp. (°C)	Conductivity ($\mu S\ cm^{-1}$)	pH	Alkalinity ($mg\ CaCO_3\ L^{-1}$)	Hardness ($mg\ CaCO_3\ L^{-1}$)	PAR ($\mu mol\ m^{-2}\ s$)	Water Se ($\mu g\ Se\ L^{-1}$)	CV for Water Se (%)
Natural	Control	8.6 ± 0.2	19.3 ± 0.6	70 ± 1	7.5 ± 0.0	23 ± 2	6 ± 1	17 ± 2	<LoD	-
	5 µg Se(IV)/L	8.6 ± 0.1	19.3 ± 0.5	69 ± 1	7.5 ± 0.1	24 ± 1	7 ± 1	19 ± 2	4.26 ± 0.42	9.9
	25 µg Se(IV)/L	8.6 ± 0.1	19.4 ± 0.6	70 ± 0	7.5 ± 0.0	24 ± 2	7 ± 1	20 ± 1	21.25 ± 2.04	9.6
Heat Killed	Control	8.7 ± 0.1	18.9 ± 0.3	70 ± 2	7.5 ± 0.0	20 ± 3	6 ± 1	<LoD	<LoD	-
	5 µg Se(IV)/L	8.8 ± 0.1	18.7 ± 0.3	70 ± 1	7.5 ± 0.0	21 ± 2	6 ± 1	<LoD	4.27 ± 0.54	12.6
	25 µg Se(IV)/L	8.8 ± 0.1	18.7 ± 0.4	70 ± 1	7.5 ± 0.0	21 ± 2	6 ± 1	<LoD	21.21 ± 2.56	12.1
Shade Cloth	Control	8.7 ± 0.1	19.1 ± 0.5	69 ± 1	7.5 ± 0.0	23 ± 2	6 ± 1	<LoD	<LoD	-
	5 µg Se(IV)/L	8.7 ± 0.1	19.2 ± 0.5	68 ± 1	7.5 ± 0.1	24 ± 2	6 ± 1	<LoD	4.38 ± 0.20	4.6
	25 µg Se(IV)/L	8.6 ± 0.1	19.2 ± 0.5	69 ± 1	7.5 ± 0.0	24 ± 2	7 ± 1	<LoD	22.56 ± 0.64	2.8
FeO(OH)	Control	8.8 ± 0.1	18.8 ± 0.4	69 ± 1	7.5 ± 0.1	22 ± 2	6±1	<LoD	<LoD	-
	5 µg Se(IV)/L	8.8 ± 0.1	18.7 ± 0.4	69 ± 1	7.5 ± 0.0	22 ± 1	6±1	<LoD	2.28 ± 1.75	76.8
	25 µg Se(IV)/L	8.8 ± 0.1	18.7 ± 0.5	70 ± 1	7.5 ± 0.0	21 ± 2	7±1	<LoD	13.37 ± 7.51	56.2

Limit of Detection for: water Se: 0.105 - 0.185 $\mu g\ Se\ L^{-1}$; PAR: 1 μmol photons $m^{-2}\ s$

Table A.5**:** Mean (±SD) periphyton Se, before and after normalization to organic matter content, for Se oxyanion bioconcentration tests assessing differential Se(IV) and Se(VI) uptake in periphyton sampled from 5 different water bodies (Sites 1 – 5) and exposed under similar conditions.

	Periphyton Se - Day 8 (µg g^{-1} d.w.)					Organic Matter Normalized Periphyton Se - Day 8 (µg Se g^{-1} OM d.w)				
Treatment	Site 1	Site 2	Site 3	Site 4	Site 5	Site 1	Site 2	Site 3	Site 4	Site 5
Control	0.8 ± 0.5	0.3 ± 0.1	0.3 ± 0.0	0.8 ± 0.2	1.0 ± 0.6	1.0 ± 0.6	0.6 ± 0.2	0.6 ± 0.1	1.4 ± 0.4	2.4 ± 1.4
5 µg/L selenite	33.1 ± 1.6	7.3 ± 0.4	22.6 ± 9.3	33.7 ± 5.6	119.1 ± 71.8	43.2 ± 2.1	14.5 ± 0.9	43.1 ± 4.6	56.6 ± 9.4	222.3 ± 69.2
25 µg/L selenite	46.9 ± 21.3	16.8 ± 1.6	49.7 ± 13.1	65.7 ± 23.4	222.5 ± 92.5	65.0 ± 9.1	33.4 ± 3.2	127.3 ± 18.7	126.8 ± 15.4	567.7 ± 236.1
5 µg/L selenate	2.7 ± 0.4	3.2 ± 0.3	2.2 ± 0.1	3.2 ± 0.4	2.8 ± 0.7	3.5 ± 0.6	6.4 ± 0.6	5.1 ± 0.3	5.4 ± 0.7	7.1 ± 1.9
25 µg/L selenate	7.5 ± 0.8	8.6 ± 1.2	6.9 ± 0.9	9.5 ± 3	3.9 ± 2.2	9.8 ± 1.1	17.1 ± 2.4	16.1 ± 2.1	19.1 ± 3.7	10 ± 5.6

Table A.6: Mean (±SD) enrichment function (EF), before and after normalization to organic matter content, for Se oxyanion bioconcentration tests assessing differential Se(IV) and Se(VI) uptake in periphyton sampled from 5 different water bodies (sites 1 – 5) and exposed under similar conditions.

	Enrichment Function - Day 8					% Organic Matter Normalized Enrichment Function - Day 8				
Treatment	Site 1	Site 2	Site 3	Site 4	Site 5	Site 1	Site 2	Site 3	Site 4	Site 5
5 µg/L selenite	7306 ± 347	1625 ± 106	4270 ± 472	7610 ± 1214	30027 ± 18931	9551 ± 453	3244 ± 211	9933 ± 1100	12790 ± 2040	76599 ± 48293
25 µg/L selenite	2134 ± 303	734 ± 70	2376 ± 343	3307 ± 403	10504 ± 4711	2790 ± 395	1465 ± 139	5525 ± 798	5564 ± 675	26796 ± 12017
5 µg/L selenate	558 ± 98	696 ± 67	472 ± 31	663 ± 92	597 ± 158	730 ± 128	1390 ± 133	1098 ± 72	1115 ± 154	1523 ± 404
25 µg/L selenate	312 ± 35	372 ± 56	290 ± 39	484 ± 92	170 ± 96	408 ± 46	743 ± 112	675 ± 90	814 ± 154	433 ± 245

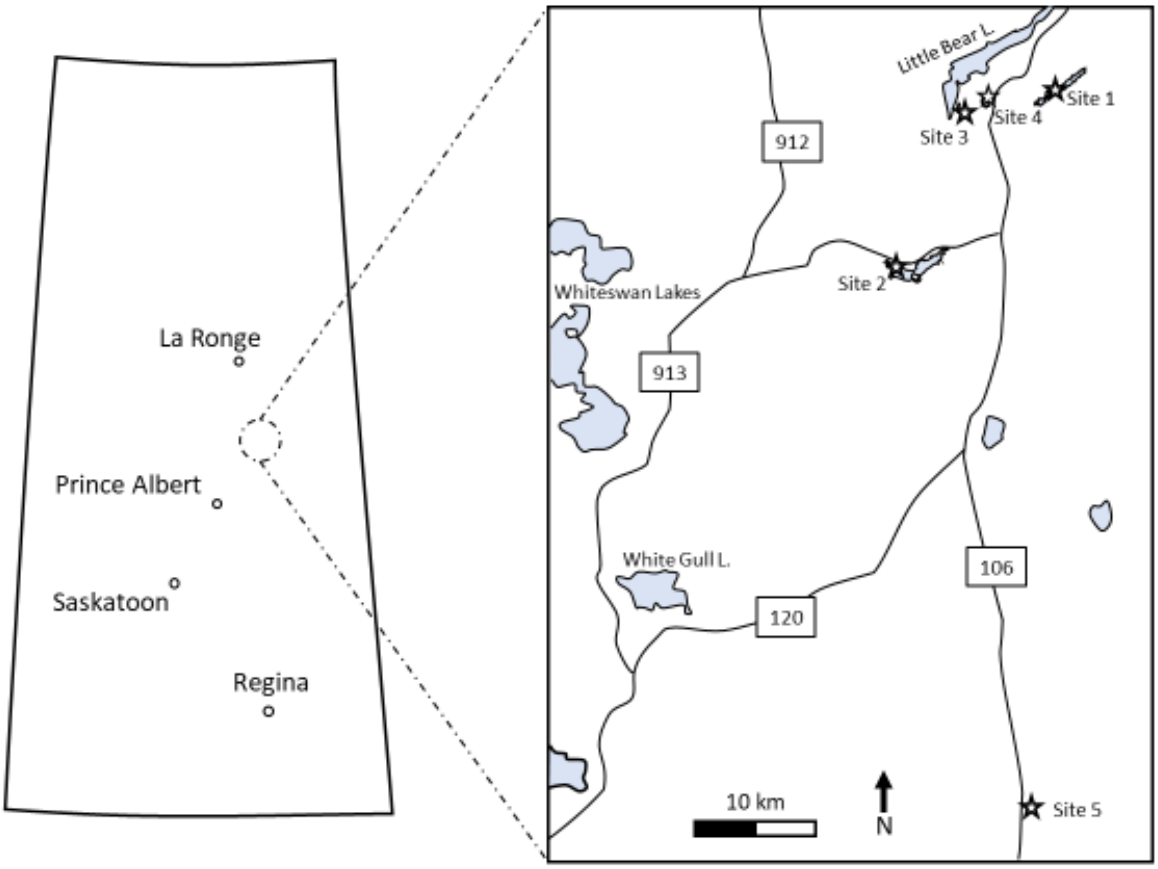

Figure A.1: Map of periphyton collection sites located in northern Saskatchewan, Canada.

APPENDIX B

ADDITIONAL METHODS AND RATIONALE FROM CHAPTER 3

B.1 Additional rationale for normalizing periphyton-Se and EF to organic matter content

Natural periphyton can have a significant inorganic component as a result of the incorporation of silt, sediment and mineral precipitates into the biofilm. Inorganic material in periphyton can bias the measurement of tissue metal concentrations by increasing the weight of the sample while not participating in biological uptake. The presence of inorganic material may be the reason that periphyton sampled from some Se impacted freshwater ecosystems were found to be less enriched in Se than phytoplankton sampled from the same waters (Lemly 1985; Muscatello and Janz 2009; Muscatello et al. 2008). Ash mass, the portion of sample remaining after incineration during ash-free dry mass measurement, is a good indicator of the inorganic material accumulated in periphyton (Stevenson 1996). Normalizing periphyton Se and EFs to OM content as a proxy for algae content allows for a more accurate comparison of Se accumulation across periphyton communities with different ratios of organic to inorganic materials. Alternatively, periphyton-Se could have been normalized to photo pigment content, buy this method is more complicated due to differences in photo pigment production (e.g., only cyanobacteria produce Chl *b*) and would involve the use of HPLC to fully characterize the pigments produced by each periphyton biofilm.

B.2 Additional DNA extraction, sequencing and data analysis methods

DNA was extracted from each sample using the MoBIO PowerSoil DNA Isolation Kit as per the manufacturer's instructions, except that a second Inhibitor Removal Technology precipitation step was performed. Targeted DNA sequencing was performed by Contango Strategies Ltd. (Saskatoon, SK) to identify cyanobacteria and eukaryotic algae via polymerase chain reaction (PCR) amplification of the plastid 23S rRNA gene (Sherwood and Presting 2007;

Steven et al. 2012). Library preparation and sequencing was performed as per the manufacturer's instructions for MiSeq v3 paired-end 300 bp sequencing (Illumina). After sequencing, the forward and reverse reads were merged using PANDAseq (Masella et al. 2012). All sequences were then filtered and reads that were considered to be low quality and discarded if they did not meet the following criteria: average quality greater than Q30, longer than 350 bp, and exact match to the forward primer. Additionally, if the read had any N (unknown) base, it was discarded. The forward and reverse primers were then removed from each sequence. Bioinformatics pipelines consisting of internally developed scripts and selected QIIME scripts (Caporaso et al. 2010; Edgar 2010) were used to process the reads. Similar sequences were clustered into Operational Taxonomic Units (OTUs) using a 97% identity threshold, and the *pick_de_novo_otus.py script.* All OTU's with less than 10 representative sequences across all samples were discarded. Taxonomic classification of the OTU's was performed using the SILVA database release 123.

B.3 Additional rationale for the assessment of periphyton composition using metagenomic and microscopy methods

Although each of these methods have limitations, these limitations can be moderated by using the two methods in tandem. For statistical comparison of algal communities, targeted gene sequencing is better at detecting small or rare algal species and has a lower rate of researcher introduced error (gene sequence identification and enumeration); however, there is currently a lack of consensus on the efficacy of species abundance and biomass estimates using DNA metabarcoding techniques (Elbrecht and Leese 2015). Although the accuracy of traditional light microscopy methods can be limited by researcher experience, there are standardized methods for

algal identification and determination of cell abundance and biovolume (Bellinger and Sigee 2010; Hillebrand et al. 1999; US EPA 1999). Unfortunately, our analysis using plastid 23S rRNA gene sequencing was limited by the small number of reference sequence entries currently in databases for this gene (SILVA database release 123; Quast et al. 2013; Yilmaz et al. 2011). This limited DNA-based classification to sequence-defined OTUs, rather than matching gene sequences previously catalogued genera or species. Ideally, DNA metabarcoding techniques would be also used for biomass and abundance estimates, however, DNA-based methods are currently unable to accurately characterize the effect of primer efficiencies across taxa in complex assemblages (Elbrecht et al. 2017; Elbrecht & Leese 2015; Pinol et al. 2014). This reduces confidence in estimates of abundance and biomass based solely on targeted genomic sequencing, as well as the likelihood that some taxa present in the sample were prevented from amplification by PCR. In addition, the number of gene copies among and even within individual taxa is highly variably. For example, prokaryotes can have up to fifteen copies of rRNA genes in a single genome (Klappenbach et al. 2001). Copy numbers for ribosomal RNA genes in plants (eukaryotes) are also highly variable because chloroplasts contain multiple copies of DNA, often numbering into the thousands (Miyamura et al. 1986; Oldenburg et al. 2006; Shaver et al. 2008). Gene copy numbers in algal chloroplasts can also vary depending on the cell cycle, with replication occurring during the S phase (Kabeya and Miyagishima 2013). This could skew the total number of reads for the 23S rRNA gene sequence toward species with more copies or result in the incorrect classification of an organism into more than one OTU if gene sequences vary enough between copies (set at a 97% similarity threshold for this study). Methodological weaknesses aside, plastid 23S rRNA targeted metagenomic analysis provided a strong statistical basis for differentiating complex algal assemblages based on the interspecific genetic diversity of

this gene (Steven et al. 2012) while light microscopy analysis provided the best estimate of the relative biomass (by way of biovolume) for the algal species identified.

REFERENCES

Bellinger EG, Sigee DC. 2010. A Key to the More Frequently Occurring Freshwater Algae. Freshwater Algae: Identification and Use as Bioindicators. John Wiley and Sons, Ltd. pp 137-244.

Caporaso, JG, Kuczynski J, Stombaugh J, Bittinger, K, Bushman FD, Costello EK, Fiere, N, Pena AG, Goodrich JK, Gordon JI, Huttley GA, Kelley ST, Knights D, Koenig JE, Ley RE, Lozupone CA, McDonald D, Muegge BD, Pirrung M, Reeder J, Sevinsky JR, Turnbaugh PJ, Walters WA, Widmann J, Yatsunenko T, Zaneveld J, Knight R. 2010. QIIME allows analysis of high-throughput community sequencing data. Nat Methods 7:335–6.

Edgar RC. 2010. Search and clustering orders of magnitude faster than BLAST. Bioinformatics. 26:2460-1.

Elbrecht V, Leese F. 2015. Can DNA-based ecosystem assessments quantify species abundance? Testing primer bias and biomass—sequence relationships with an innovative metabarcoding protocol. PLoS One 10:7.

Elbrecht V, Vamos EE, Meissner K, Aroviita J, Leese F. 2017. Assessing strengths and weaknesses of DNA metabarcoding-based macroinvertebrate identification for routine stream monitoring. Methods Ecol Evol. 8(10):1265-1275.

Hillebrand H, Durselen CS, Kirschtel D, Pollingher U, Zohary T. 1999. Biovolume calculation for pelagic and benthic microalgae. J. Phycol 35:403-242.

Kabeya Y, Miyagishima S. 2013. Chloroplast DNA Replication Is Regulated by the Redox State Independently of Chloroplast Division in *Chlamydomonas reinhardtii*. Plant Physiol 161(4):2102–2112

Klappenbach JA, Saxman PR, Cole JR, Schmidt TM. 2001. rrndb: the Ribosomal RNA Operon Copy Number Database. Nucleic Acids Res 29:181–184

Lemly AD. 1985. Toxicology of selenium in a freshwater reservoir: implications for environmental hazard evaluation and safety. Ecotoxicol Environ Saf 10:314-338.

Masella AP, Bartram AK, Truszkowski JM, Brown DG, Neufeld JD. 2012. PANDAseq: PAired-eND Assembler for Illumina sequences. BMC Bioinformatics. 13:31.

Miyamura S, Nagata T, Kuroiwa T. 1986. Quantitative fluorescence microscopy on dynamic changes of plastid nucleoids during wheat development. Protoplasma 133:66–72.

Muscatello JR, Belknap AM, Janz DM. 2008. Accumulation of selenium in aquatic systems downstream of a uranium mining operation in northern Saskatchewan, Canada. Environ Pollut 156:387–93.

Muscatello JR, Janz DM. 2009. Selenium accumulation in aquatic biota downstream of a uranium mining and milling operation. Sci Total Environ 407:1318-1325.

Oldenburg DJ, Rowan BA, Zhao L, Walcher CL, Schleh M, Bendich AJ. 2006. Loss or retention of chloroplast DNA in maize seedlings is affected by both light and genotype. Planta 225(1):41-55.

Pinol J, Mir G, Gomez-Polo P, Agusti N. 2014. Universal and blocking primer mismatches limit the use of high-throughput DNA sequencing for the quantitative metabarcoding of arthropods. Mol Ecol Resour 1–12.

Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, Piplies J, Glockner FO. 2013. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Res 41(database issue):D590-6.

Shaver JM, Oldenburg DJ, Bendich AJ. 2008. The structure of chloroplast DNA molecules and the effects of light on the amount of chloroplast DNA during development in Medicago truncatula. Plant Physiol 146(3):1064-74.

Sherwood AR, Presting GG. 2007. Universal primers amplify a 23S rDNA plastid marker in eukaryotic algae and cyanobacteria. J Phycol 43:605−608.

Steven B, McCann S, Ward NL. 2012. Pyrosequencing of plastid 23S rRNA genes reveals diverse and dynamic cyanobacterial and algal populations in two eutrophic lakes. FEMS Microbiol Ecol 82:607−15.

Stevenson RJ. 1996. An Introduction to Algal Ecology in Freshwater Benthic Habitats. Algal ecology: freshwater benthic ecosystems. (ed. Stevenson RJ, Bothwell MI, Lowe RL). San Diego, CA: Elsevier. p. 3-30.

United States Environmental Protection Agency (US EPA). 1999. Rapid bioassessment protocols for use in wadeable streams and rivers – periphyton, ebenthic macroinvertebrates, and fish 2nd ed. (EPA Publication No. 841-B-99-002). Washington, DC: US EPA.

Yilmaz P, Kottmann R, Pruesse E, Quast C, Glockner FO. 2011. Analysis of 23S rRNA genes in metagenomes - A case study from the Global Ocean Sampling Expedition. Syst Appl Microbiol 34:462–469.