The effect of sulphate on selenium bioaccumulation in two freshwater primary producers and a primary consumer

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Abstract

Site-specific conditions (e.g. presence of sulphate), may be linked to the variability in the uptake of selenium in organisms at the base of a food chain, potentially affecting the risk of adverse effects in higher trophic-level organisms. In this project, the effect of sulphate on selenate bioaccumulation in two primary producers (*Lemna minor* and *Pseudokirchneriella subcapitata*) and a primary consumer (*Daphnia magna*) was explored. When exposed to selenate, all three species exhibited a decrease in selenium tissue concentration with increasing sulphate. When *D. magna* were exposed to sulphate and dietary selenium, sulphate did not affect selenium tissue concentrations. The results were used to develop equations estimating selenium tissue concentrations when exposed to selenate and sulphate. The strong predictive ability of the equations suggests that selenate, sulphate, and dietary selenium (applicable to *D. magna*) are important for describing the relationship between selenate and selenium tissue concentrations.

Keywords: selenate; sulphate; bioaccumulation; *Lemna minor*; *Pseudokirchneriella subcapitata*; *Daphnia magna*

Dedication

This work is dedicated to Patrick, who has been a constant source of support and encouragement.

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Chapter 1.

General Introduction

1.1. Selenium fundamentals

Selenium (Se) is an element in the chalcogen group of the periodic table. It is located between sulfur and tellurium, has the atomic number 34 and an atomic mass of 78.96 (IUPAC, 1988). Selenium is chemically similar to sulphur and both are classified as non-metals, however some allotropes of Se possess properties consistent with metalloids (e.g., acting as a semiconductor) (Young et al., 2010). Discovered by Jöns Jacob Berzelius and Gahn in 1817, the element was described as an impurity during the production of sulfuric acid (Ihnat and Wolf, 1989). The various properties of Se have led to its use in a range of activities and products. Anthropogenic uses of Se include: industrial (e.g., as a colourant for glass and in the production of photovoltaic cells), pharmaceutical (e.g., as an additive to shampoo to treat fungal infections) and nutritional (e.g. as a dietary supplement [USGS 2013]). Geologically, Se is often associated with rocks originating from marine sedimentary basins (Presser et al., 2004). From these rocks, the element is distributed globally through natural (e.g. weathering) and anthropogenic processes (e.g. burning of coal) (Haygarth, 1994). As a result, Se occurs in all environmental compartments and in many materials, in both inorganic and organic forms.

1.1.1. Sources

During the Cretaceous period, significant volcanic activity resulted in the deposition of Se in Cretaceous seas (Presser, 1994). Thus, the highest concentrations of Se are associated with carbon-rich marine shales and phosphate-rich sedimentary rock formed during the Tertiary and Upper Cretaceous periods (McNeal and Balistrieri,

1989; Haygarth, 1994), however Se has also been found in shales that are relatively low in organic carbon (Presser et al., 2004). Weathering of Se-rich rocks has led to naturally elevated Se concentrations in soil and water. Other less significant natural sources of Se include the precipitation of minerals and organic matter, adsorption, chemical or bacterial reduction oxidation and metabolic uptake and release by plant and animals (McNeal and Balistrieri, 1989).

In addition to the natural release of Se, anthropogenic activities may result in elevated concentrations of Se in surface waters and sediments. For example, when Se-laden rock is disturbed by coal, phosphate and uranium mining, surface area that is subject to weathering processes is increased, resulting in increased Se mobilization (Dreher and Finkelman, 1992; Hamilton and Buhl, 2004; Muscatello et al., 2006). Other anthropogenic activities linked to elevated Se in water includes irrigation in agricultural areas with seleniferous soils (Ohlendorf et al., 1986), coal combustion (Wen and Carignan, 2007) and the disposal of coal ash from coal-based power plants (Cherry and Guthrie, 1977).

1.1.2. Speciation

Inorganic Se may exist in various forms; however in aquatic systems, Se is typically present as SeO_4^{2-} (selenate) and SeO_3^{2-} (selenite) (Young et al., 2010). These Se species represent two of the four oxidations states found in the environment: elemental Se (0), selenides (-II), selenites (+IV) and selenates (+VI) (Cutter, 1982). Speciation of Se is dependent on various factors including pH, redox potential, solubility and biological activity (McNeal and Balisterieri, 1989). Selenate is favoured in alkaline, oxic conditions while SeO_3^{2-} more commonly occurs in reducing environments (Yao and Millero, 1995; Barceloux, 1999). In aquatic ecosystems, lotic (flowing) environments are dominated by SeO_4^{2-}, while the reducing conditions of lentic (standing/still) systems result in an increased proportion of (SeO_3^{2-}) (Simmons and Wallschläger, 2005).

Biological reactions tend to be more important than thermodynamic equilibria in explaining the biogeochemical cycling of Se in aquatic environments (Stadtman, 1974). While not predominant in the water column, Se as selenides, are common in biota;

where Se may be reduced to selenides (-II) and incorporated into selenoamino acids (i.e. selenocysteine, selenomethionine) (Sunde, 1997). Organic selenides may be released into the water column though organism death and decay or may be transferred to other organisms via dietary consumption (Lee and Fisher, 1994).

1.1.3. Essentiality

Selenium is a component of selenoproteins, which perform a variety of important functions in most organisms. Selenoproteins contain a UGA codon and a selenocysteine insertion sequence however, other types of selenium containing proteins (referred to as selenium-containing proteins) also exist and occur when selenoaminoacids (i.e. selenomethionine) is non-specifically incorporated into proteins (Young et al., 2010). The first selenoproteins identified were a class of glutathione peroxidase enzymes which serve as antioxidants that protect cells against oxidative damage (Pappas et al., 2008). Other Se-containing proteins are involved in controlling the production of thyroid hormone triiodothyronine, transporting and controlling the synthesis of selenocysteine, muscle metabolism, and catalyzing the reduction of thioredoxin, which regulates the proliferation of normal cells (Brown and Arthur, 2001). While significant research has been conducted to identify and understand the function of selenoproteins, further exploration is required. For example, Brown and Arthur (2001) hypothesized that up to 100 selenoproteins could exist in mammals; however only 30 have been described, of which 15 have an established biological function.

Se has been identified as being essential to the health of organisms in higher trophic levels (e.g. mammals, fish and birds [Mayland, 1994] as well as in organisms at lower levels (e.g. algae [Harrison et al., 1988; Fu et al., 2002] and zooplankton [Cowgill, 1987]). In fish, a deficiency in this element has been linked to muscle degeneration, various histopathologies in cardiac, nerve cord, and liver tissue, reductions in glutathione peroxidase activity and lordosis (Lopez-Albors et al., 1995; Bell et al, 1986; Wang et al., 2013). In terrestrial vertebrates, diseases related to Se-deficiency include alkali disease (in livestock), white muscle disease (in livestock), and Keshan disease (in humans) (Trelease and Beath, 1949; Muth et al.,1958; Whanger, 1989).

While Se is essential in small amounts, it is toxic at higher concentrations. In fact, of all the trace elements, the range between necessity and toxicity is the narrowest for Se, resulting in concern regarding the potential for adverse effects in environments with elevated Se (Luoma and Rainbow, 2008). For example, the range of doses in fish between optimal and toxic dietary intake is approximately seven- to ten-fold (Young et al., 2010).

1.2. Selenium in the environment

A number of government agencies have derived Se water quality guidelines/criteria for the protection of freshwater aquatic life. In Canada, the recommended water quality guideline proposed by the Canadian Council of Ministers of the Environment (formerly Canadian Council of Resource and Environment Ministers) is 1 μg/L in the water column (1987). Provinces and territories however, are permitted to derive their own guideline values. As such, the British Columbia Ministry of Environment (BC MoE) has set a Se water guideline value for the protection of aquatic life of 2 μg/L (2014). In the US, the United States Environmental Protection Agency (US EPA) have proposed a draft water quality criterion of 1.3 μg/L in lentic systems and 4.8 μg/L in lotic aquatic systems (2014). The differences seen in these examples provide an indication of the difficulty in understanding Se movement, fate and risk of adverse effects in aquatic systems.

1.2.1. Bioaccumulation in aquatic ecosystems

In aquatic systems, Se bioaccumulation is an important determinant of toxicity. For example, adverse effects such as decreased larval survival, growth, and teratogenesis in fish have been linked to elevated maternally-derived tissue concentrations of Se (Bennett et al., 1986; Colye et al., 1993; Muscatello et al., 2006). While aquatic animals may be exposed to dissolved Se, dietary exposure is expected to be the primary pathway contributing to Se bioaccumulation in secondary (and higher) consumers (Ohlendorf et al., 1986; Fan et al., 2002; Young et al., 2010). Unlike the transfer of Se from the aquatic compartment to primary producers, the trophic transfer of

Se between higher trophic levels (e.g. fish) is smaller, generally less than a factor of three (Presser and Luoma, 2010). A conceptual model of selenium movement through an aquatic food chain is illustrated in Figure 1.

While aquatic organisms are generally exposed to Se through both water and diet, uptake of dissolved inorganic selenium by animals is relatively slow, and is not expected to contribute significantly to overall Se bioaccumulation (Lemly, 1985; Besser et al., 1993; Wang et al., 1996). Despite having a relatively small direct contribution to bioaccumulation in higher trophic levels, dissolved Se is important to Se uptake at the base of the food web (e.g., into primary producers, micro-organisms). Furthermore, a significant portion of the overall accumulation of Se in aquatic food chains occurs between water and the base of the food web. For example, organisms at the base of the food web can concentrate Se 100 to 10,000 fold over dissolved concentrations in the water column (Luoma and Presser, 2009). The ratio between the concentration of Se in material (e.g. tissue) at the base of the food web and the concentration of Se in water is often described as a distribution coefficient (K_d) or an enrichment function (Stewart et al., 2010).

Amino acids containing Se, are integrated into proteins either non-specifically (as selenomethionine) or specifically into “true” selenoproteins, where selenocysteine is inserted at the active site (Patching and Gardiner, 1999). Seleniferous proteins are transferred from primary producers to primary consumers (i.e. invertebrates, insect larvae) and subsequently to higher trophic level consumers. Selenomethionine, has been identified as the predominant form associated with Se bioaccumulation (Fan et al., 2002). Evidence exists that suggests that this is in part due to excess Se occurring as selenomethionine rather than selenocysteine when plants are exposed to elevated concentrations of Se (Wu, 1998). Organic forms of Se (e.g. selenomethionine, selenocysteine) are bioaccumulated more readily compared to inorganic forms (e.g. SeO_4^{2-} and SeO_3^{2-}) (Heinz et al., 1987; Besser et al., 1993; Fournier et al., 2006; Franz et al., 2011).

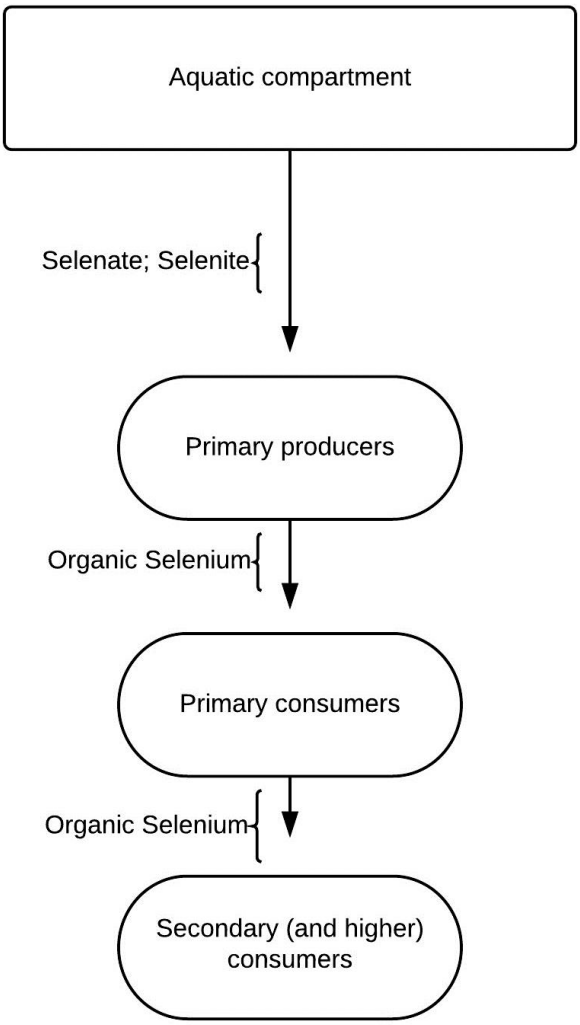

Figure 1-1. A conceptual model of Se movement in an aquatic food chain

1.2.2. Variability in bioaccumulation

A wide range of bioaccumulation rates has been documented among sites, highlighting the difficulty in estimating the bioaccumulation of Se at a particular site. For example, Presser and Luoma (2010) summarized K_d values from 52 field studies and reported K_d estimates ranging from 100 to 300 for freshwater lotic sites and 500 to 2000 for freshwater lentic sites, highlighting how differences between sites can affect Se bioaccumulation. Differences in bioaccumulation rates have also been observed in laboratory studies. For example, Williams et al., (1994) reported Se K_d values ranging from 110 to 1700 in the green alga *Pseudokirchneriella subcapitata* (formerly

Selenastrum capricornutum), and Besser et al., (1993) reported K_d values ranging from 31 to 3100 for another green alga, *Chlamydomonas reinhardtii*. Potential exposure conditions that may also affect Se bioaccumulation are: pH (Riedel and Sanders, 1996), the concentration of PO_4^{3-} (Hopper and Parker, 1999) and the concentration of SO_4^{2-} (Young et al., 2010).

1.2.3. Selenium and sulphate interaction

Sulphate has been shown to reduce the acute toxicity of Se (as SeO_4^{2-}) in several species (Hansen et al., 1993; Williams et al., 1994; Ogle and Knight, 1996; Brix et al., 2001). Similarly, SO_4^{2-} has been shown to reduce Se bioaccumulation (when exposed to SeO_4^{2-}) in several species including algae (Williams et al., 1994; Riedel and Sanders, 1996), aquatic plants (Bailey et al., 1995), and invertebrates (Hansen et al., 1993; Ogle and Knight, 1996).

A hypothesis for the interaction between SeO_4^{2-} and SO_4^{2-} suggests that there is direct competition between SeO_4^{2-} and SO_4^{2-} for binding sites on cell surfaces, as a result of the structural similarity of the two anions (Shrift, 1973). This was based partly on research conducted by Shrift (1954) using *Chlorella vulgaris*. In this study, it was reported that different concentrations of SeO_4^{2-} and SO_4^{2-} yielded the same bioaccumulation rate when their molar ratio was kept constant. Research conducted by Leggett and Epstein (1956) suggest that SO_4^{2-} is actively taken up by cells via a carrier-sulphate complex (permease). Therefore, Shrift (1973) hypothesized that Se and SO_4^{2-} compete for the same permease and that affinity for the anions (SeO_4^{2-} or SO_4^{2-}) was determined by their molar ratio. However, this hypothesis was not supported by results reported by Williams et al., (1994), showing that the maintenance of the same molar ratio between two treatment groups, did not yield similar Se uptake rates in the green algae *S. capricornutum*.

A possible explanation for the discrepancy between the results reported by Shrift and Williams et al. is that while the anions may compete for the same active sites, the permease may have different affinities for each anion when present at concentrations below saturation. A hypothesis to further develop this suggestion is the possible

existence of two permease systems. Results from studies investigating the uptake of SO_4^{2-} in *Scenedesmus* sp. and *Hydrodictyon reticulatum*, suggest that inorganic SO_4^{2-} could be accumulated by two systems, one mechanism unique to SO_4^{2-} and another that is more generalized (Kylin, 1967; Roybova et al., 1982).

The environmental relevance of the interaction between SeO_4^{2-} and SO_4^{2-} lies in their frequent association. Due to the physical similarity between Se and S, S can be replaced by Se in sulphide minerals and they often co-occur in volcanic deposits (Luttrell, 1959). Thus, anthropogenic activities that mobilize Se from geologic deposits also tend to concurrently mobilize SO_4^{2-} (Gates et al., 2009). Since elevated concentrations of SeO_4^{2-} and SO_4^{2-} often co-occur, and SO_4^{2-} directly affects the bioaccumulation and toxicity of SeO_4^{2-}, the interactions between these anions may contribute significantly to the variability that is seen in bioaccumulation rates at different sites.

1.2.4. Toxicity

Elevated concentrations of Se in freshwater systems have been linked to various adverse effects at the level of tissues to the population level. The first major reported case of Se toxicity in a freshwater environment occurred at Belews Lake, NC, where 4 years of effluent discharge from a coal-fired power plant increased the Se concentration in the lake, resulting in the loss of 26 of 29 resident fish species (Lemly, 1985). In a nearby less-contaminated sub-basin (3-4 µg/L Se), sublethal effects included edema and lesions in ovarian tissue (Sorensen et al., 1984). Selenium concentrations in the water decreased to < 5 µg/L by 1985, following reduction of effluent discharge, at which time 21 fish species were reported in the lake and, 5 years following cessation of effluent discharge, 26 fish species were observed in the lake (Barwick and Harrell, 1997). Other examples of Se contamination resulting in adverse effects in freshwater environments include declines in the Hyco Lake fishery, NC, which also received coal fry ash effluent (Crutchfield, 2000), decreased spotted sandpiper hatchability (Harding et al., 2005) and fish reproductive failure (Rudolph et al., 2008; Nautilus Environmental and Interior Reforestation, 2009) in the Elk Valley, BC, due to contamination from local coal mining

operations (Harding et al., 2005; Elk Valley Se Task Force, 2008), and fish deformities due to Se release from uranium mining (Pyle et al., 2001; Muscatello et al., 2006).

1.2.5. Mode of toxic action

While the mechanisms behind Se toxicity have not been conclusively established, three main hypotheses have been put forward. The first and most commonly reported hypothesis involves the nonspecific substitution of sulphur with Se in amino acids due to the physical similarities between the two elements. With regard to oviparous vertebrates, the most Se-sensitive species, Se-substituted proteins are maternally transferred and believed to cause teratogenesis and pathological alternations in the organs and tissues of progeny (Lemly, 2002; Sorensen, 1991). In these organisms, it has been theorized that selenomethionine and selenocysteine substitute for methionine and cysteine in proteins, resulting in improperly folded, malfunctional proteins during embryonic development (Diplock and Hoekstra, 1976; Reddy and Massaro, 1983; Sunde, 1984). In such situations, selenoamino acids may prevent the formation of disulfide bonds that are found in functional proteins (e.g. forming cysteine) (Lemly, 2002).

A second hypothesis focuses on the relationship between Se and oxidative stress. As an oxidative catalyst, Se can continuously oxidize thiols and reduce oxygen, resulting in the production of superoxides (free radicals) (Xu et al., 1991), which can bind to enzymes and proteins, inhibiting their function and resulting in oxidative stress (Palace et al., 2004). The link between Se exposure and oxidative stress has been reported in both fish and birds (Palace et al., 2004; Hoffman et al., 1989).

The third proposed mechanism of toxicity suggests that an excess of selenocysteine may inhibit selenium metabolism by disrupting selenium methylation, resulting in an accumulation of hydrogen selenide (Sayato et al., 1997; Spallholz et al., 2002). Hydrogen selenide is a highly toxic form of selenium and has been linked to hepatotoxicity and also shown to induce toxic DNA breaks in yeast cells (Sayato et al., 1997; Peyroche et al., 2012). However, research on this hypothesis remains limited and

efforts to understand the cellular mechanism of toxicity remains focused on the nonspecific integration of Se for S in proteins and the role of oxidative stress.

1.3. Overview of research

Despite the evidence that SO_4^{2-} interacts significantly with the toxicity and bioaccumulation of Se, the effect of SO_4^{2-} on Se bioaccumulation in freshwater systems with elevated concentrations of SO_4^{2-} has not been well described. In fact, it has been suggested that SO_4^{2-} does not affect the distribution and bioaccumulation of Se (Rudd et al., 1980; Messer et al., 1989), although the conclusions of these authors were based on results obtained from lentic and laboratory based lentic-like systems (e.g. closed-system microcosms) which may be dominated by SeO_3^{2-}. Regardless, further research on the relationship between SeO_4^{2-} and SO_4^{2-} under conditions relevant to SeO_4^{2-}-dominated systems is needed.

In order to better understand the effect of SO_4^{2-} on Se bioaccumulation in lotic (SeO_4^{2-} dominated) systems, laboratory-based exposures of species at the primary producer and consumer level can provide an efficient means to evaluate potential interactions. This research should focus on trophic levels that comprise the largest proportion of Se uptake and transfer in freshwater food chains. Areas of interest that would improve the environmental relevance of laboratory-based exposures characterizing SeO_4^{2-} and SO_4^{2-} interaction include evaluating differences between species, exposure duration, an expanded range of SeO_4^{2-} and SO_4^{2-} exposure concentrations, and the impact of multiple exposure routes.

Evaluating the effect of SO_4^{2-} on the bioaccumulation of Se in freshwater primary producers has focused predominantly on green algae (e.g. *C. reinhardtii* and *P. subcapitata*). Therefore, for a greater understanding of the impact of SO_4^{2-} in primary producers, it is important to expand research to a species from a separate family to compare potential differences in uptake. Another exposure condition that requires further development is the use of a wider range of Se and SO_4^{2-} concentrations. Typically, the concentrations of SO_4^{2-} used for exposures have been have been < 100 mg/L (Williams et al, 1994; Riedel et al., 1996; Fournier et al., 2010), whereas most

SO_4^{2-} concentrations in western Canadian river waters are below 580 mg/L but may be as high as 3040 mg/L (Environment Canada, 1984 as cited by Health Canada, 1994), suggesting that an expanded dataset covering a larger range of SO_4^{2-} is needed.

The majority of previous research on the effect of SO_4^{2-} on Se tissue concentration in invertebrates following exposure to SeO_4^{2-} has focused on short exposure durations, which may not result in effects similar to those following chronic exposures to Se. Furthermore, acute exposures provide insufficient time to allow for reproduction which serves as an elimination route of Se for adult daphnids (Lam and Wang, 2006). Assessing the impact of SO_4^{2-} on Se accumulation in the food chain should mimic the environment to the extent that is possible in the laboratory. For example, exposures could consider an organism's multiple (e.g. aqueous and dietary) exposure routes, which may be particularly relevant to primary consumers. It has been estimated that up to 40% of Se tissue concentration in *D. magna* was linked to uptake of aqueous (dissolved) Se (Tsui and Wang, 2007), therefore suggesting that Se uptake form the aqueous compartment may play a significant role in Se tissue concentrations in some species.

To explore these data gaps, two studies were conducted. The first examined the effect of SO_4^{2-} on Se tissue concentrations in an aquatic macrophyte (*Lemna minor*) and a green alga (*P. subcapitata*). Laboratory experiments were carried out with *L. minor*, and an existing dataset for *P. subcapitata* was evaluated (data provided by Josh Baker, Nautilus Environmental). This work was designed to determine if potential differences in Se uptake between different primary producers existed, as well as expand existing knowledge of SeO_4^{2-} and SO_4^{2-} interactions over a larger range of SO_4^{2-} concentrations. In a second study, the effects of SO_4^{2-} on Se tissue concentrations were assessed to determine if they were preserved across two trophic levels. In this case, the freshwater cladoceran, *D. magna*, was exposed to a range of dietary Se (using algae that were grown in a selenium-enriched media) as well as different combinations of dissolved Se and SO_4^{2-} for 21 days, encompassing both dietary and water-borne exposure routes.

The results from both studies were then used to develop equations that described the relationship between SeO_4^{2-}, SO_4^{2-} and Se tissue concentrations.

Together, these studies expand upon existing research by increasing the environmental relevance of the results, as well as exploring a means of mathematically describing the relationship between SeO_4^{2-} and SO_4^{2-} on the uptake and trophic transfer of Se.

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Chapter 2.

The effect of sulphate on selenate bioaccumulation in two freshwater primary producers: a duckweed, *Lemna minor*, and a green alga, *Pseudokirchneriella subcapitata*

2.1. Abstract

Predicting selenium bioaccumulation is complicated because site-specific conditions, including sulphate concentration, affect the bioconcentration of inorganic selenium into the food web. Selenium tissue concentrations were measured in a duckweed, *Lemna minor,* and a green alga, *Pseudokirchneriella subcapitata*, following exposure to selenate and sulphate. Selenium accumulation differed between species, and sulphate reduced selenium uptake in both species indicating that the anion is an important factor in modifying selenium tissue concentration in primary producers.

2.2. Introduction

Selenium (Se) is a trace element and important micronutrient necessary for key biological functions. However, the range of body burdens that differentiate proper physiological function and Se-induced toxicity is narrow. Se occurs naturally in geological formations (e.g., black shale, phosphate rocks, coal) (Maher et al., 2010), is widely distributed, and can be mobilized through natural processes such as the weathering of rocks and soils. Mobilization may also occur through anthropogenic processes including coal, phosphate and uranium mining, coal combustion, and agricultural irrigation (Dreher and Finkelman, 1992; Hamilton and Buhl, 2004; Muscatello

et al., 2006; Outridge et al., 1999) resulting in elevated concentrations of Se in aquatic environments.

Inorganic Se is present in aquatic environments primarily in oxidation states (IV) and (VI) as selenite (SeO_3^{2-}) and selenate (SeO_4^{2-}), respectively. Typically, SeO_4^{2-} is the dominant Se species in lotic (flowing) environments, while reducing conditions often associated with lentic (still) systems leads to increased occurrence of SeO_3^{2-} (Simmons and Wallschlager, 2005). These anions rarely reach concentrations that directly cause toxicity to aquatic organisms; deleterious effects generally occur in higher trophic levels following accumulation and transfer of Se through the food web.

A significant portion of the incorporation of inorganic Se into the food web occurs in lower trophic levels, where microorganisms and primary producers (e.g., algae and plants) actively accumulate inorganic Se (Orr et al., 2012) and convert it into organic forms, primarily as the seleno-amino acids selenomethionine and selenocysteine which are incorporated into proteins (Wrench, 1978; Sunde, 1997). Estimates put bioconcentration rates of inorganic Se from water into primary producers at 10^2 to 10^6-fold higher than the trophic transfer rates of organic Se between higher trophic levels in aquatic food webs (Baines and Fisher 2001). Once Se is integrated into a food web, organisms in higher trophic levels may accumulate Se to concentrations that can cause adverse effects.

Several cases of Se-related toxicity have been documented in the field, including the disappearance of 12 of 16 fish species in Belews Lake, NC, USA, which was linked to Se-containing discharge from a coal-fired power plant (Lemly, 1985). In addition, agricultural drainage water containing Se resulted in the loss of resident fish species, and in reproductive effects in birds in the Kesterson National Wildlife Refuge (CA, USA) (Ohlendorf et al., 1986). Adverse effects associated with Se exposure include embryo toxicity and teratogenicity (following the maternal transfer of Se) (Gillespie and Baumann, 1986; Holm et al. 2005, Muscatello et al., 2006, Rudolph et al., 2008), the induction of oxidative stress (Palace et al., 2004), as well as histopathological changes (Sorensen et al., 1984).

The relationship between inorganic Se concentrations in water and tissues in exposed organisms is not well understood, part of which is a result of the significant variability in the uptake of inorganic Se from water into primary producers (typically referred to as the distribution rate [K_d] or enrichment function [EF]) between sites. For example, K_d values from 52 field studies ranged from 107 (San Diego Creek, CA, USA) to 3,044 (Hyco Reservoir, NC, USA), reflecting a 30-fold difference between freshwater sites (summarized by Presser and Luoma, 2010). In contrast, the transfer of Se between higher trophic levels in freshwater environments exhibits much lower variability, with trophic transfer factors (TTFs) for invertebrates ranging from 0.94 to 3.2 (a 3.4-fold range), and 0.8 to 1.7 (a 2.1-fold range) in fish (Presser and Luoma, 2010).

These data highlight the difficulty in predicting the bioaccumulation of Se in the environment, as site-specific conditions can appreciably alter Se uptake from water to the lowest trophic level. Sulphate (SO_4^{2-}) has been identified as a potential contributor to site-specific differences in Se accumulation. In laboratory studies, decreased bioaccumulation of SeO_4^{2-} by algal species is associated with increased SO_4^{2-}. For example, an increase in SO_4^{2-} from 9.9 to 99 mg/L resulted in a decrease in the bioaccumulation of SeO_4^{2-} by several fold in *Selenastrum capricornutum* (subsequently renamed *Pseudokirchneriella subcapitata*) (Williams et al., 1994). A similar effect of SO_4^{2-} has been reported for *Chlamydomonas reinhardtii* when exposed to waters containing between 0.8 and 96 mg/L SO_4^{2-} (Riedel and Sanders, 1996; Fournier et al, 2010).

These studies demonstrate the importance of SO_4^{2-} in modifying the accumulation of SeO_4^{2-} in algae. However, the SO_4^{2-} concentrations used in these studies do not encompass a realistic range of environmentally-relevant SO_4^{2-} concentrations. Aquatic environments vary considerably in SO_4^{2-} concentration; for example, sites in Western Canada have been reported to range from 1 to 3040 mg/L SO_4^{2-} (Health Canada, 1994), and may also be influenced by anthropogenic activities. For example, SO_4^{2-} concentrations ranging from 67 to 211 mg/L have been found in pit lakes formed by open pit coal mining (Miller et al., 2013) and SO_4^{2-} concentrations between 258 and 1630 mg/L have been reported in four small lakes downstream of effluent discharge from a uranium milling operation (Wiramanaden et al., 2010).

Furthermore, SO_4^{2-} and Se concentrations in surface and groundwater are often positively correlated (Gate et al., 2009; Morrison et al., 2012), suggesting that elevated concentrations of Se may co-occur with higher concentrations of SO_4^{2-}.

Ultimately, the characterization of the relationship between SO_4^{2-} and Se accumulation will aid in the development of a model for Se uptake that reflects local SO_4^{2-} concentrations. The objective of this study was to determine the effect of SO_4^{2-} concentrations on the Se tissue concentration in an aquatic macrophyte (duckweed, *Lemna minor*) and a unicellular green alga (*P. subcapitata*) when exposed to SeO_4^{2-}. These species are commonly used in laboratory investigations, and can be used as models for two different taxa of primary producers to compare differences in accumulation and interactions between Se and SO_4^{2-}.

2.3. Methods

Exposures were conducted in temperature- and photoperiod-controlled environmental chambers at the Nautilus Environmental laboratory (Burnaby, BC, Canada). All chemicals used were of reagent grade.

Procedures used for *L. minor* followed general guidance provided by Environment Canada (2007a) for conducting toxicity tests with this species, with the exception that a higher density of fronds was used in the present study to ensure that sufficient tissue would be available to measure Se accumulation. Four treatment waters were used in exposures: 1) deionized water, 2) moderately hard water, which contained approximately 81 mg/L SO_4^{2-} (USEPA, 2001), 3) moderately hard water supplemented with an additional 80 mg/L SO_4^{2-} (as sodium sulphate), and 4) moderately hard water supplemented with an additional 240 mg/L SO_4^{2-}. Nutrients were added to water in each treatment group as described by Environmental Canada (2007a), which introduced an additional 57 mg/L SO_4^{2-} to each water treatment. Sodium selenate was added to each of these water types to achieve nominal Se concentrations of 5, 10, 20 and 40 µg/L, resulting in 16 treatments overall.

The exposure was initiated with seven, three-frond *L. minor* plants in 200-mL glass beakers containing 150 mL of treatment solution. Plants used in exposures were obtained from an in-house laboratory culture that originated from a culture (CPCC #490) obtained from the Canadian Phycological Culture Centre (CPCC) (Waterloo, ON, Canada). Three replicates were used for each treatment group. Exposures were static and conducted at 25±2 °C, under continuous fluorescent light with an intensity of 4000 to 5600 lux. After 7 d of exposure, *L. minor* were removed from the exposure media, rinsed in de-ionized water, blotted dry and frozen prior to analysis for tissue Se concentrations.

Exposures conducted using *P. subcapitata* followed procedures modified from methods published by Environment Canada (2007b) for culturing this species. Water treatments consisted of deionized water and deionized water amended with two concentrations of sulphate (added as calcium and magnesium sulphates using a 1:1mass ratio of Ca:Mg) Nutrients were added to the exposure waters as described by Environment Canada (2007b) which resulted in approximately 5 mg/L of SO_4^{2-} being supplemented into each water type. This resulted in three SO_4^{2-} concentrations being evaluated (5, 155 and 396 mg/L). Sodium selenate was added to each of these three water types, achieving concentrations of 10, 18, 32, 56, and 100 µg Se/L, and resulting in 15 treatments overall.

Exposures were conducted in 250-mL glass Erlenmeyer flasks containing 100 mL of treatment solution. Three replicates were used per treatment and the exposures were performed under static conditions with continuous aeration. The exposure temperature was 24±2 °C and continuous fluorescent light with an intensity of 3600 to 4400 lux was provided. Exposures were initiated by the addition of 1 mL of an in-house algal culture, which was in a logarithmic growth phase, containing approximately 3×10^6 cells/mL of *P. subcapitata* (strain UTCC #37), originally obtained from the CPCC. The exposure duration was 7 d, after which the contents of each flask were centrifuged at 939 *xg* for 20 min. The solution was decanted, and the pellet of algal cells was resuspended in deionized water buffered with 15 mg/L sodium bicarbonate. The centrifugation step was repeated and the algal pellet was frozen prior to chemical analysis.

Exposure solutions were sampled at the start of the exposures and SO_4^{2-} and Se concentrations were measured by ALS Environmental Ltd (Burnaby, BC, Canada) using ion chromatography and inductively coupled plasma-optical emission spectrophotometry (ICP-OES), respectively. Se was expected to remain predominantly in the form of SeO_4^{2-} over the 7 day exposure period as a preliminary exposure using these methods found that only 0.2% of SeO_4^{2-} was converted to SeO_3^{2-} (unpublished). Total Se was measured in *L. minor* fronds and *P. subcapitata* cells by Applied Speciation (Bothell, WA, USA) using inductively coupled plasma dynamic reaction cell mass spectrometry (ICP-DRC-MS). Moisture content was measured in sub-samples of *L. minor* and *P. subcapitata* and used to convert Se tissue concentrations from a wet to dry weight basis. Moisture content was measured by drying a pre-weighed subsample in a convection oven maintained at 60 °C for a minimum of 16 h; samples were then cooled and re-weighed. All tissue concentrations are reported here on a dry weight basis.

The data were analyzed using the statistical software JMP (JMP, version 10). Analyses were performed based on measured concentrations of Se and SO_4^{2-}. A two way analysis of variance (ANOVA) was used to evaluate the effects of Se and SO_4^{2-} concentrations on tissue concentrations of Se, as well as to identify whether there was an interaction between the two variables. Once the significance of Se and SO_4^{2-} effects were established, multiple linear regression was then used to express tissue concentrations of Se as a function of water concentrations of Se and SO_4^{2-}. Regression equations were calculated using both non-transformed and log-transformed values for Se and SO_4^{2-}, and the combination that provided the strongest R-square value was used. For both *L. minor* and *P. subcapitata*, this resulted in log transformation of the SO_4^{2-} data, but not the Se data.

2.4. Results

Lemna minor was exposed to four concentrations of SeO_4^{2-} in each of four water types containing a range of SO_4^{2-} concentrations (Table 2-1) and measured for tissue Se concentrations after 7 d. At the end of the exposure period, all plants appeared healthy and all treatments saw an increase in frond count by a minimum of 10-fold. Tissue concentrations of Se increased with increasing concentrations of aqueous Se (Figure

2-1). Increasing SO_4^{2-} reduced the accumulation of Se in plant tissue. For example, a concentration of 5 µg/L dissolved Se resulted in final tissue concentrations of 1.40±0.13 and 0.33±0.09 mg/kg when exposed to 51 and 335 mg/L SO_4^{2-}, respectively. Similarly, at a Se concentration of 40 µg/L, tissue concentrations were 3.77±1.34 and 1.94±0.11 mg/kg Se at the same SO_4^{2-} concentrations. The highest tissue concentration observed was 10.2 mg/kg Se when *L.* minor were exposed to the highest concentration of Se (37 µg/L) and the lowest concentration of SO_4^{2-} (51 mg/L).

Lemna minor			*Pseudokirchneriella subcapitata*		
Nominal Se (µg/L)	Measured Se (µg/L)	Measured SO_4^{2-} (mg/L)	Nominal Se (µg/L)	Measured Se (µg/L)	Measured SO_4^{2-} (mg/L)
5	4.5	51	10	10	5
10	9	51	18	18	5
20	18.5	51	32	30	5
40	37.1	51	56	54	5
5	3.9	132	100	91	5
10	9.2	132	10	9	155
20	19.8	132	18	18	155
40	39	132	32	31	155
5	4.2	220	56	56	155
10	9.1	220	100	97	155
20	18.3	220	10	10	396
40	39.3	220	18	18	396
5	4.7	335	32	31	396
10	9.3	335	56	55	396
20	19.6	335	100	102	396
40	38.5	335			

Table 2-1. Comparison of nominal and measured selenium and sulphate exposure concentrations for *Lemna minor* and *Pseudokirchneriella subcapitata*. Measured values represent single samples collected at beginning of exposure.

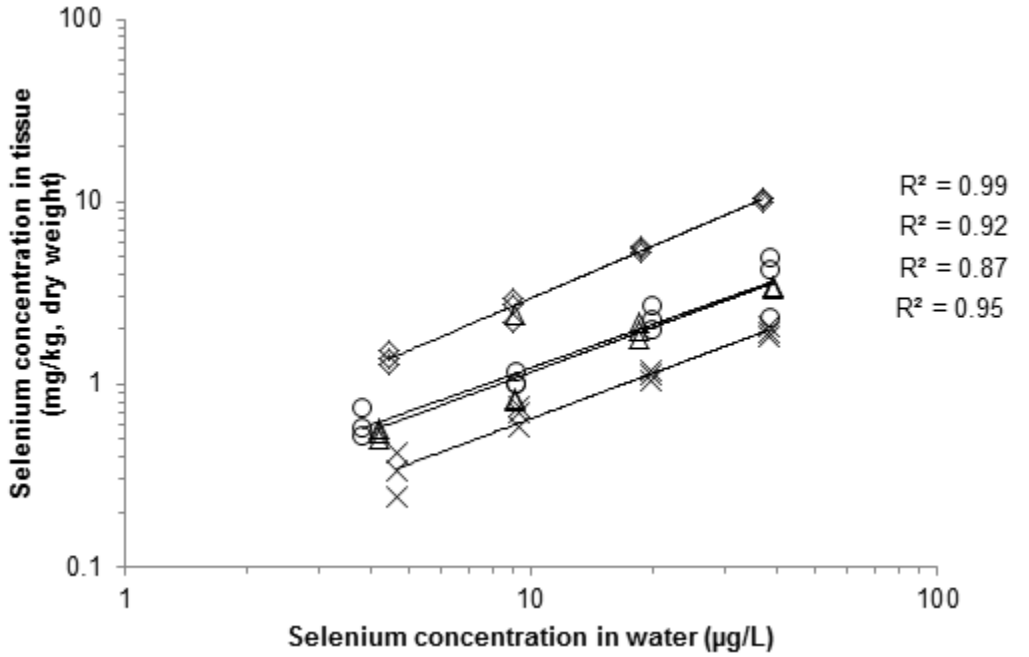

Figure 2-1. *Lemna minor* tissue selenium concentration following exposure to selenate at different concentrations of sulphate. Each line represents a single concentration of sulphate with 4 different selenium concentrations and 3 replicates per concentration. Sulphate treatments were: 51 mg/L (diamond), 132 mg/L (circle), 220 mg/L (triangle) and 335 mg/L (x) (n=48).

A two-way ANOVA, showed that both Se and SO_4^{2-} exhibited a significant effect on Se tissue concentrations (Se: $F(3,32) = 196.77$, $p < 0.0001$ and SO_4^{2-}: $F(3,32) = 179.12$, $p < 0.0001$). This analysis also detected a significant interaction between Se and SO_4^{2-} concentrations ($F(9,32) = 31.69$, $p < 0.0001$). These 3 terms (Se, SO_4^{2-} and an interaction estimate) were incorporated into a multiple linear regression model. Parameter estimates derived from this analysis were used to formulate an equation (1) used to make predictive estimates of Se tissue concentrations in *L. minor* as a function of water Se and SO_4^{2-} concentrations. When predicted Se tissue concentrations were compared against the measured Se tissue concentrations using linear regression, the R^2 value was 0.92, suggesting that under these exposure conditions, Se tissue concentration can be predicted on the basis of aqueous concentrations of Se and SO_4^{2-} (Figure 2-2).

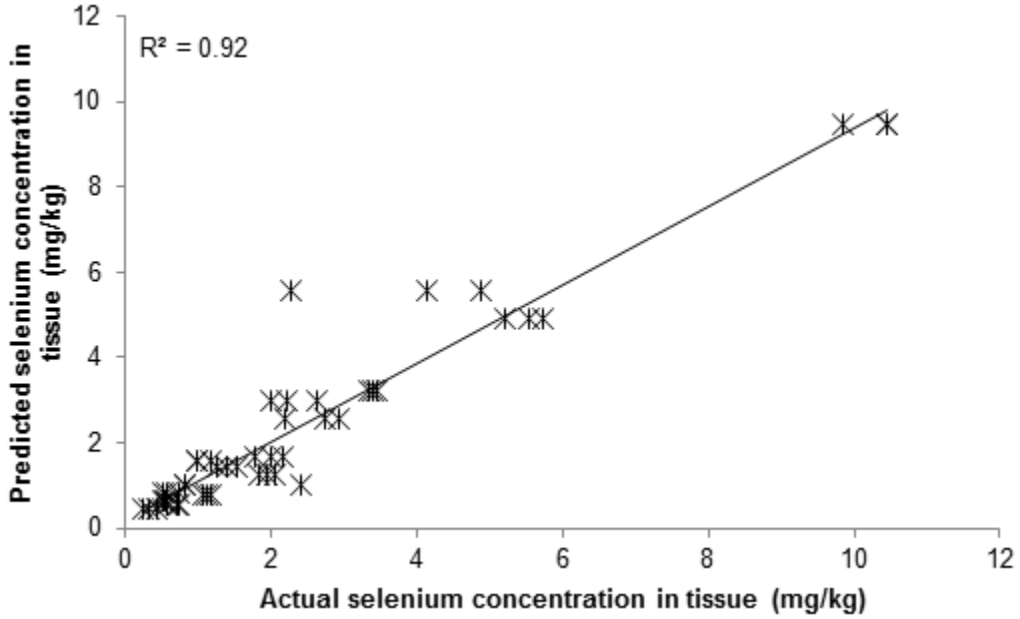

Figure 2-2. Actual vs. predicted tissue concentration of selenium in *Lemna minor* tissue based on exposure to selenate in the presence of sulphate (n=48).

P. subcapitata were exposed to three concentrations of SO_4^{2-} and five concentrations of Se, as SeO_4^{2-}, for 7 d (Table 2-1). At the end of exposure, algal cells appeared healthy and cell density increased approximately 60-fold in any treatments. Algal tissue Se concentrations increased with water Se concentrations in each water type and the relationship exhibited a strong correlation with R^2 values ranging from 0.74 to 0.93 (Figure 2-3). As observed with *L. minor*, increasing SO_4^{2-} concentrations reduced the accumulation of Se. This observation was confirmed using a two-way ANOVA where both aqueous Se and SO_4^{2-} concentrations, as well as their interaction, were shown to significantly affect tissue concentrations (Se $F(4,30) = 28.60$, $p < 0.0001$, SO_4^{2-} $F(2,30) = 98.94$, $p < 0.0001$, and interaction $F(8,30) = 14.07$, $p = < 0.0001$). Parameter estimates from a multiple linear regression analysis were used to produce an equation (2) predicting Se tissue concentrations in *P. subcapitata* as a function of aqueous Se and $SO4^{2-}$. When predicted Se tissue values were compared to measured concentrations using linear regression, the relationship between predicted and measured tissue concentration was strong ($R^2 = 0.93$) (Figure 2-4). These results suggest that similar to *L. minor*, Se and SO_4^{2-} concentrations could be used to effectively

predict Se tissue concentrations within the range of concentrations used in this study. All measured Se and SO_4^{2-} can be found in the Appendix.

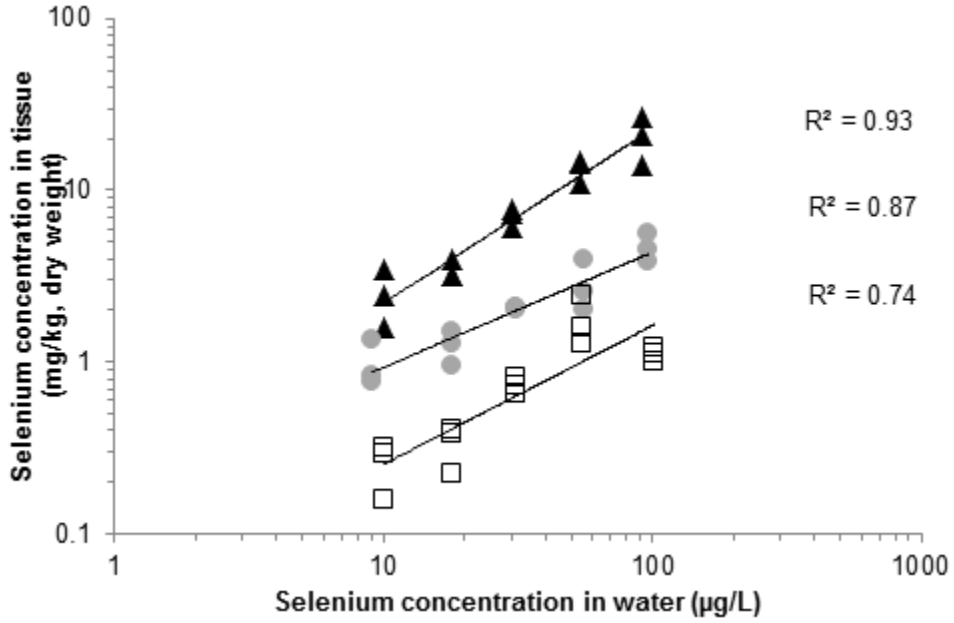

Figure 2-3. *Pseudokirchneriella subcapitata* bioaccumulation of selenium from exposure to selenate at different concentrations of sulphate (n=45). Sulphate concentrations were: 5 mg/L (triangle), 155 mg/L (circle), and 396 mg/L (square).

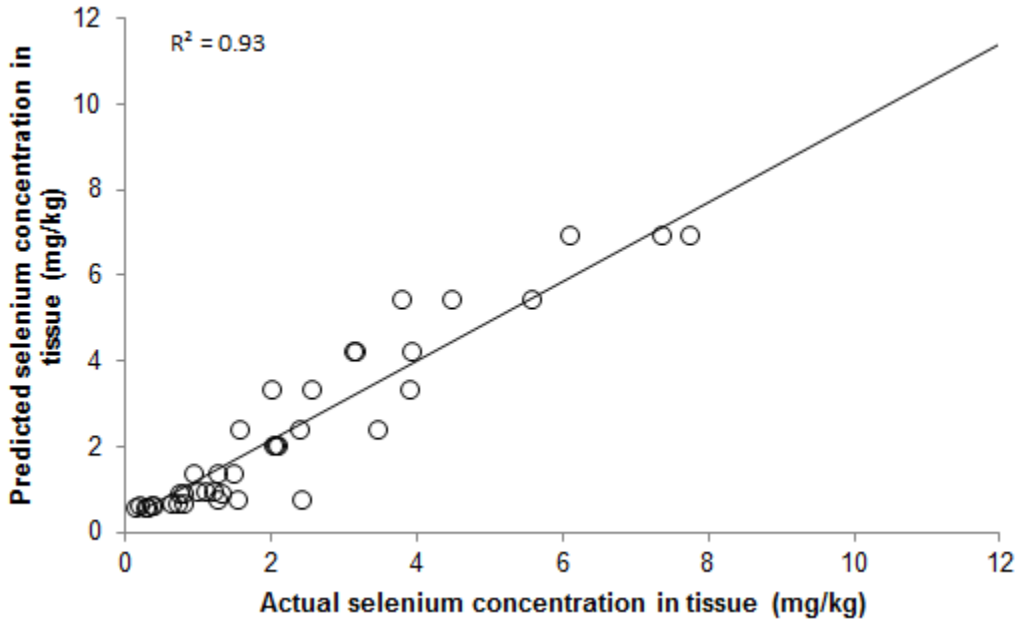

Figure 2-4. Actual vs. predicted tissue concentrations of selenium in *Pseudokirchneriella subcapitata* based on exposure to selenate in the presence of sulphate (n =45).

Equations describing selenium tissue concentrations of *Lemna minor* (1) and *Pseudokirchneriella subcapitata* (2) when exposed to selenate and sulphate.

(1) $Selenium_{tissue}$

$$= 10.81 + 0.12\,Selenate_{water} - 4.826 logSulphate_{water} - [0.271(Selenate_{water} - 17.734) \times (logSulphate_{water} - 2.173)]$$

(2) $Selenium_{tissue}$

$$= 8.958 + 0.094\,Selenate_{water} - 4.713 logSulphate_{water} - [0.117(Selenate_{water} - 42) \times (logSulphate_{water} - 1.829)]$$

Units: Selenium $_{tissue}$- mg/kg Se dry weight; Selenate $_{water}$ – µg/L Se; Sulphate $_{water}$- mg/L SO_4^{2-}

2.5. Discussion

The results of the present study show that SO_4^{2-} concentration has a significant modulatory effect on Se tissue concentration of primary producers at SO_4^{2-} concentrations over twice the amount previously evaluated. This provides a greater understanding of SeO_4^{2-} and SO_4^{2-} interactions for freshwater systems where SO_4^{2-} concentrations exceed 100 mg/L. The exposure experiments also demonstrated that *L. minor* exhibited greater accumulation of Se compared to *P. subcapitata*; however, based on the predicted Se tissue equations, species differences decreased with increasing SO_4^{2-}. For example, when Se ranged from 1 to 10 μg/L, and SO_4^{2-} ranged from 1 to 100 mg/L, predicted concentrations were 1.1 to 3.9-fold higher in *L. minor* than *P. subcapitata*, with the largest predicted differences occurring at 1 mg/L SO_4^{2-}.

Statistical analyses found SeO_4^{2-}, SO_4^{2-} and an interaction term between the two anions exerted statistically significant effects on Se tissue concentration in both species tested. The significance of the interaction term implies that the effect of SO_4^{2-} on Se tissue concentration is not constant across concentrations of dissolved SeO_4^{2-} and SO_4^{2-} tested and therefore a modifier (the interaction term) is required to more accurately describe the relationship between the anions and Se tissue concentration. Also, in a study using *P. subcapitata*, treatments with different concentrations but the same S:Se molar ratio resulted in statistically different uptake of SeO_4^{2-} [20]. Collectively, these results suggest that over the concentrations tested, direct competition between SeO_4^{2-} and SO_4^{2-} cannot fully explain the antagonistic relationship between these two anions and Se tissue concentration.

Williams and co-workers (1994) reported that Se was accumulated by *P. subcapitata* at a higher rate than observed in the present study. For example, concentrations in algal cells reported by that study were 1.4 to 8.2 higher than predicted by equation (2) generated in the present study. Despite the difference in overall Se tissue concentrations, the effect of SO_4^{2-} on SeO_4^{2-} uptake by *P. subcapitata* was largely consistent with previous research. Higher accumulation rates were also reported in *C. reinhardtii* exposed to 60 mg/L SO_4^{2-} and 10, 100 and 1000 µg/L Se (Besser et al., 1993); Se tissue concentrations reported were under-predicted by a factor of 2.8 to 3.7

by equation (2), although measurements were lower than predicted after 24 h. These differences suggest that comparisons of Se accumulation between studies should consider both species and exposure duration differences.

The results presented here show that SO_4^{2-} has a significant effect on the accumulation of Se (as SeO_4^{2-}) by primary producers. Differences between species tissue Se concentration could be attributed to inherent morphological differences. *P. subcapitata* is a unicellular green algae, 40 to 60 μm^3 in size (Environment Canada, 2007b), compared to the much larger *L. minor*, which are generally 2-to 4-mm long and consist of ≥1 fronds and a single root and (Environment Canada, 2007a). These physical differences suggest that available surface area (and likely the number of uptake sites) could lead to differences between the species' ability to accumulate Se.

Moreover, the equations derived herein provide a useful predictor of tissue Se concentrations for these species as a function of Se and SO_4^{2-} concentrations. Caution must be used in their application to other organism species and waters with different chemical constituents as Se species (e.g. selenite) and chemistry characteristics (e.g. presence of phosphate) may also influence Se tissue concentrations (Maier and Knight, 1994; Hopper and Parker, 1999). Therefore, while it is expected that the general relationships between aqueous Se, SO_4^{2-} and accumulation are likely to describe a significant proportion of variability that is observed between sites, the variability in Se tissue concentration reported here and when compared with results by other investigators, demonstrate the importance of not overgeneralizing results from a particular study and focus instead on site specific assessments.

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Chapter 3.

The effect of sulphate on the bioaccumulation of selenate and dietary selenium in the water flea, *Daphnia magna*

3.1. Abstract

One of the difficulties in estimating selenium's potential for adverse effects in higher trophic level organisms is characterizing the variability in selenium bioconcentration at the base of food webs. Site-specific conditions, such as the presence of competitive anions (e.g. sulphate), may explain some of this variability. Reduction in selenium tissue concentration with sulphate exposure has been demonstrated with green algae; however, the relevance of this relationship to the accumulation of selenium in primary consumers requires further exploration. In this study, the water flea *D. magna* was exposed to dietary selenium (using Se-exposed *Pseudokirchneriella subcapitata*) and dissolved selenate and sulphate ranging from 10 to 40 μg/L and 80-320 mg/L, respectively. The green alga, *P. subcapitata*, was exposed to the same selenate and sulphate treatments prior to being used to feed *D. magna*. The 21-d exposure resulted in *D. magna* selenium tissue concentrations ranging from <0.045 to 0.67 mg/kg dry weight when exposed to dietary selenium and 0.15 to 1.65 mg/kg when exposed to both dietary and dissolved selenium. Results demonstrated that effects of sulphate (decreased selenium tissue concentration) in algae were reflected in *D. magna* selenium tissue concentrations. In addition, aqueous sulphate did not affect the dietary uptake of selenium in daphnids, however, sulphate significantly reduced the uptake of dissolved selenate. The results were used to develop a predictive equation to describe daphnid selenium tissue concentration when exposed to selenate, dietary selenium and sulphate. The strong relationship between predicted and measured tissues

selenium values suggest that under the range of exposure concentrations, all three factors need to be considered when assessing potential selenium tissue concentrations in *D. magna*.

3.2. Introduction

Adverse effects due to selenium (Se) exposure in freshwater environments have typically been documented in organisms of higher trophic levels (e.g., fish and birds). Effects including decreases in population size due to reproductive failure and teratogenesis or mortality of progeny have been linked primarily to dietary uptake and the subsequent maternal transfer of Se to offspring (Gillespie and Bauman, 1986). A significant portion of Se accumulation in the food web occurs as a result of high bioconcentration factors between the water compartment and the primary trophic levels (bioconcentration rates are typically >100-fold for primary producers) since trophic transfer factors of 3.2 or less for freshwater invertebrates and less than 3 for fish have been reported (Presser and Luoma, 2010). Therefore, knowledge regarding the dynamics of food-web transfer of Se to higher levels of the food chain in aquatic environments is needed and requires an understanding of accumulation of Se at lower trophic levels.

Characterizing Se accumulation in aquatic food chains is confounded by differences in speciation and other modifying factors between aquatic systems. For example, lotic systems tend to be dominated by selenate (SeO_4^{2-}), whereas lentic systems have reducing conditions that may result in occurrence of both selenite (SeO_3^{2-}) and SeO_4^{2-} (Simmons and Wallschläger, 2005). Se speciation is important in understanding bioaccumulation since Se species are accumulated at different rates. For example, greater Se accumulation has been observed in organisms with exposure to SeO_3^{2-} compared to SeO_4^{2-} (Maier and Knight, 1994).

Water chemistry may also modify uptake rates of both SeO_4^{2-} and SeO_3^{2-}. An antagonistic relationship between SO_4^{2-} and uptake of Se (as SeO_4^{2-}) has been observed in a number of species at the primary trophic level. For example, a decrease in Se uptake with increasing SO_4^{2-} was observed with the freshwater alga *Pseudokirchneriella*

subcapitata (formerly named *Selenastrum capricornutum*) in concentrations of Se ranging from 10 to 100 µg/L and 5 to 396 mg/L SO_4^{2-} (Williams et al., 1994; Lo et al., 2014). Reduced SeO_4^{2-} uptake due to increasing SO_4^{2-} has also been reported in the algae *Chlamydomonas reinhardtii* (Reidel and Sanders, 1996) and the aquatic macrophytes *Ruppia maritima* (Bailey et al., 1995) and *Lemna minor* (Lo et al., 2014).

A small number of studies have provided evidence that the inhibitory effect of SO_4^{2-} on Se accumulation also occurs in freshwater invertebrates. Hansen et al. (1993) investigated the effect of SO_4^{2-} on the bioconcentration of SeO_4^{2-} by the cladoceran *Daphnia magna* and reported that increasing SO_4^{2-} from 13 to 207 mg/L significantly reduced Se accumulation in a 48-h exposure. Ogle and Knight (1996) also reported a significant effect of SO_4^{2-} on tissue Se concentrations in 72-h exposures of this species across a range of SO_4^{2-} concentrations from 10.2 mg/L to 325 mg/L. Although dietary accumulation of selenium is considered to be the predominant pathway of accumulation in most higher organisms, waterborne inorganic SeO_4^{2-} has been shown to contribute significantly to accumulation of selenium in *D. magna* (Tsui and Wang, 2007). Furthermore, assessments of potential accumulation of Se from inorganic and dietary sources should encompass longer periods of exposure to more accurately reflect what may occur over a chronic exposure period; for example, Besser et al., (1993) reported a continued increase in Se tissue concentrations until the end of the exposure period (14-d) in daphnids exposed to dietary Se.

In order to understand the reason for differences in bioaccumulation rates between sites, there is a need to describe the factors that affect bioaccumulation of selenium into the base of the food-web. Thus, the objective of this study was to determine the effect of SO_4^{2-} on SeO_4^{2-} uptake across species in the first two trophic levels. This study was divided into two phases: the first in which the green alga, *P. subcapitata*, was exposed to SeO_4^{2-} and SO_4^{2-}, and a second in which these algal cells were used as a diet for the water flea, *D. magna*, and this diet was provided with and without dissolved SeO_4^{2-} under different concentrations of aqueous SO_4^{2-}. The study incorporated a 21-d exposure period that incorporated a significantly longer timeframe than previously used for daphnid Se accumulation studies (48-h to 14-d).

3.3. Methods

The first phase of the experiment involved exposing *P. subcapitata* to various combinations of Se (as SeO_4^{2-}) and SO_4^{2-}. Treatments groups of *P. subcapitata* were exposed for 7 d using procedures modified from Environment Canada guidelines for culturing algae (2007). Water treatments consisted of moderately hard water (USEPA 2002) amended with sodium sulphate to achieve nominal concentrations of 80, 160 and 320 mg/L SO_4^{2-}. Sodium selenate was then added to each of these waters to obtain nominal Se concentrations of 10, 20 and 40 µg/L, resulting in 9 different experimental treatments. Nutrients that were required for growth, as described by Environment Canada (2007), were added to all treatments, although the addition rate was increased by four-fold to increase the overall cell yield. Addition of nutrients added approximately 20 mg/L sulphate to each exposure solution, resulting in nominal addition rates of 100, 180 and 340 mg/L sulphate.

A laboratory culture of *P. subcapitata* (strain UTCC #37, originally obtained from the Canadian Phycological Culture Centre CPCC, Waterloo, ON, Canada) that was reproducing in a logarithmic growth phase was added to 1-L glass Erlenmeyer flasks to provide approximately 3 x 10^6 cells/mL in 1 L of exposure solution. The exposure temperature was 25 °C and the algae were grown under continuous fluorescent light at 3600 to 4400 lux. Exposures were terminated after 7 d. Cell densities were determined by counting algal cells using a microscope and hemacytometer (APHA et al., 2005). Following density determinations, algae were centrifuged at 939 *xg* for 10 min and the overlying solution decanted. The algal pellet was re-suspended in deionized water containing 15 mg/L sodium bicarbonate to provide buffering. The algae were then rinsed twice more following the same procedures. Algal cells were pooled by treatment and stored in the dark at 4 °C. To produce sufficient selenized algae to be used as a dietary source of Se for *D. magna*, *P. subcapitata* exposures were conducted in duplicate, on two separate occasions, following which all cells were pooled by treatment. After pooling, samples of *P. subcapitata* were analyzed for Se concentration.

The second phase of this study involved exposing *D. magna* to dietary Se using the *P. subcapitata* cells that were grown in the 9 water types described above. In this

exposure, *D. magna* were separated into two treatment groups; the first group was provided Se via diet only (*D. magna* $_{Diet}$), and a second group were exposed to both a selenized diet as well as dissolved SeO_4^{2-} (*D. magna* $_{Diet + Water}$) using the same waterborne Se and SO_4^{2-} treatments (i.e., 10, 20, and 40 µg/L Se, and 80, 160 and 320 mg/L SO_4) that were used in preparation of the algal cell diet. Thus, there were 18 exposures treatments for *D. magna*, in addition to a control.

D. magna were exposed in triplicate in 1-L glass beakers containing 1 L of solution containing 20, <72-h old neonates in each container. Neonates were collected from a laboratory culture, (originally purchased from Aquatic BioSystems, Fort Collins, CO, USA). The *daphnia* were fed selenized *P. subcapitata* daily at a rate of 2.1 x 10^4 cells per test vessel. In addition, YTC Daphnid Feed Mixture (Aquatic BioSystems, Fort Collins, CO, USA) was provided on day 0, 7 and 14 of the exposure to provide supplemental nutrition at a dose of 1.5 mL per 1-L of exposure solution. Exposure solutions were renewed 3 times per week. The exposure duration was 21 d; on day 20, the solutions were renewed and the *Daphnia* were not fed to allow for a 24-h depuration period. Exposures were conducted at 20±2 °C with a 16 h light: 8 h dark photoperiod under cool white fluorescent lights at a light intensity range of 400 to 800 lux. Observations regarding mortality were recorded daily. At experiment termination, *Daphnia* were rinsed in deionized water, blotted dry and frozen at -10 °C prior to chemical analysis.

To confirm treatment concentrations, total Se and SO_4^{2-} were measured in exposure solutions at the initiation of algal exposures, and at the beginning, middle, and at end of the *Daphnia* exposures. These analyses were conducted by ALS Environmental (Burnaby, BC, Canada) using the turbidimetric method to measure SO_4^{2-} and inductively coupled plasma-optical emission spectrophotometry (ICP-MS) to measure Se. Measurements of tissue concentrations of Se were performed by Applied Speciation (Bothell, WA, USA) using inductively coupled plasma dynamic reaction cell mass spectrometry (ICP-DRC-MS). The moisture content of tissue samples (pooled by species), was determined by drying a measured amount of tissue at 60 °C for 16 h, and cooling and weighing the dried samples. Measurements of moisture content were used

to convert Se tissue concentrations from a wet to dry-weight basis; all tissue concentrations reported here are provided on a dry weight basis.

Statistical analysis of the data used a combination of multiple linear regression and one-way and two-way analysis of variance (ANOVA). Statistical analyses were performed on the basis of measured concentrations using the statistical software JMP (SAS, 2007). To evaluate the effect of Se on organism health (as represented by algal cell density and daphnid survival), one-way ANOVAs were used. Due to the lack of replicate measurements, multiple linear regression was used to assess the effect of SO_4^{2-} on *P. subcapitata* Se tissue concentration.

Analysis of the *D. magna* results was directed at identifying relationships between SO_4^{2-} and the accumulation of Se from aqueous and dietary sources. Specifically the following were assessed: 1) the effect of SO_4^{2-} on Se tissue concentrations when daphnia were exposed to dietary Se; 2) whether Se contribution from diet and water to Se tissue concentration was independent of one another; and 3) the effect of SO_4^{2-} on Se tissue concentrations when daphnia were exposed to dissolved Se. Following determination of these factors, results from statistical analyses were used to derive an equation to predict the effect of SO_4^{2-} and SeO_4^{2-} on *D. magna* tissue concentration.

Multiple linear regression was used to assess the effect of SO_4^{2-} on Se tissue concentrations in the *D. magna* $_{\text{Diet Se}}$ treatment group. Dietary Se concentration and SO_4^{2-} concentration were treated as independent variables, with *Daphnia* tissue Se concentrations as the dependent variable. Once it was determined that SO_4^{2-} did not affect tissue concentrations in the *D. magna* $_{\text{Diet,}}$ treatment group, a single variable linear regression model was used to describe tissue Se concentrations resulting from dietary exposure. Prior to assessing whether Se contribution from diet and water to Se tissue concentration where independent of each other, it was necessary to estimate the Se tissue concentration attributable to exposure to SeO_4^{2-} in water. These estimated tissue concentrations were labeled *D. magna* $_{\text{Water}}$. To derive these estimates, the differences between Se tissue measurements from the *D. magna* $_{\text{Diet + Water}}$ treatment group and the tissue concentrations associated with the corresponding *D. magna* $_{\text{Diet}}$ treatment groups

were determined. To assess the independence of Se accumulation between sources, an uptake term was derived by dividing the estimated tissue concentrations associated with *D. magna* $_{\text{Water}}$ by aqueous concentrations of SeO_4^{2-}. Under the assumption of independence, this uptake term would not be affected by dietary Se. One-way ANOVAs were then used to determine if there was any relationship between dietary treatment on uptake; this analysis was performed separately for each SO_4^{2-} exposure concentration. After establishing the independence of uptake from exposure route, linear regression was used to evaluate the estimated *D. magna* Se tissue concentrations resulting from waterborne Se as a function of dissolved Se and SO_4^{2-}. For all linear regression analyses, SO_4^{2-} concentrations were log-transformed prior to analysis because this provided a better model fit (lower root-mean-square-error).

After determining the effects of SO_4^{2-} on Se tissue concentrations, an equation was formulated to describe (predict) the expected Se tissue concentration in *D. magna* following exposure to dietary and dissolved Se while also being exposed to SO_4^{2-}. The predictive equation followed the general format outlined in equation (1), and then used parameter estimates from the linear regression analyses (previously used to assess the significance of SO_4^{2-} on tissue concentration) to substitute into each component of the equation. Results from the predictive equation were compared against measured Se tissue concentrations to determine whether *D. magna* tissue concentrations could be accurately predicted by SeO_4^{2-} , SO_4^{2-} and dietary Se.

(1) $$Daphnia\ tissue\ Se = Tissue\ Se_{\ from\ water} + Tissue\ Se_{\ from\ diet}$$

3.4. Results

The purpose of the first phase of experiments was to confirm the antagonistic effect of SO_4^{2-} on the accumulation of Se by *P. subcapitata* when exposed to SeO_4^{2-}, and to provide a source of algae that could be used to feed *D. magna* for the evaluation of Se trophic transfer. At the end of the experiment, algal cells appeared healthy and no significant differences in average algal density were observed between treatments ($F_{(9,26)}=1.23$, $p = 0.32$), indicating that Se treatment did not have an adverse effect on the cultured algae. Measured concentrations of Se and SO_4^{2-} were within 10% of expected

concentrations (Table 3-1). One SO_4^{2-} measurement from the 292 mg/L treatment was excluded due to deviating > 15% from two other measurements which were sampled at the same time from the same batch of water.

Pseudokirchneriella subcapitata exposure		*Daphnia magna* exposure	
Selenium (µg/L)	Sulphate (mg/L)	Selenium (µg/L)	Sulphate (mg/L)
9.2 ± 0.4	102.5 ± 5.9	<0.01	77.1 ± 0.88
18.2 ± 1.3	102.5 ± 5.9	<0.01	159.5 ± 0.5
33.9 ± 0.1	102.5 ± 5.9	<0.01	292.7 ± 1.9
9.0 ± 1.0	184.0 ± 6.3	9.2 ± 0.82	77.1 ± 0.88
17.4 ± 0.5	184.0 ± 6.3	17.8 ± 1.59	77.1 ± 0.88
37.0 ± 5.4	184.0 ± 6.3	31.8 ± 2.49	77.1 ± 0.88
8.9 ± 1.3	309.7 ± 5.4	8.4 ± 0.25	159.5 ± 0.5
18.0 ± 1.1	309.7 ± 5.4	17.6 ± 1.51	159.5 ± 0.5
39.8 ± 4.3	309.7 ± 5.4	31.8 ± 3.89	159.5 ± 0.5
--	--	8.4 ± 0.52	292.7 ± 1.9
--	--	17.6 ± 1.17	292.7 ± 1.9
--	--	35.4 ± 0.84	292.7 ± 1.9

Table 3-1. Selenium and sulphate concentrations (mean ± SD) measured in exposure water for *Pseudokirchneriella subcapitata* and *Daphnia magna*. For *P. subcapitata*, selenium n=2 and sulphate n=6. For *D. magna*, selenium n=3 and sulphate n=6, with the exception of the 292 mg/L treatment, where an outlier was removed (n=5).

Algal Se tissue concentrations increased with increasing concentrations of SeO_4^{2-} and uptake decreased with increasing concentrations of SO_4^{2-} (Figure 3-1). Se tissue concentrations ranged from below the detection limit (0.14 mg/kg) to 1.5 mg/kg. The highest algal tissue Se concentration corresponded to the exposure with the highest Se and the lowest sulphate concentrations (Table 3-2). A multiple linear regression analysis confirmed that dissolved Se, SO_4^{2-} and an interaction term between the two parameters were significantly related to algal Se tissue concentration (p= 0.004, p=0.002, p=0.022, respectively). The significance of an interaction term between the two anions implies

that the effect of SO_4^{2-} is not constant across SeO_4^{2-} concentrations and an additional modifying term (interaction) is required to describe the change.

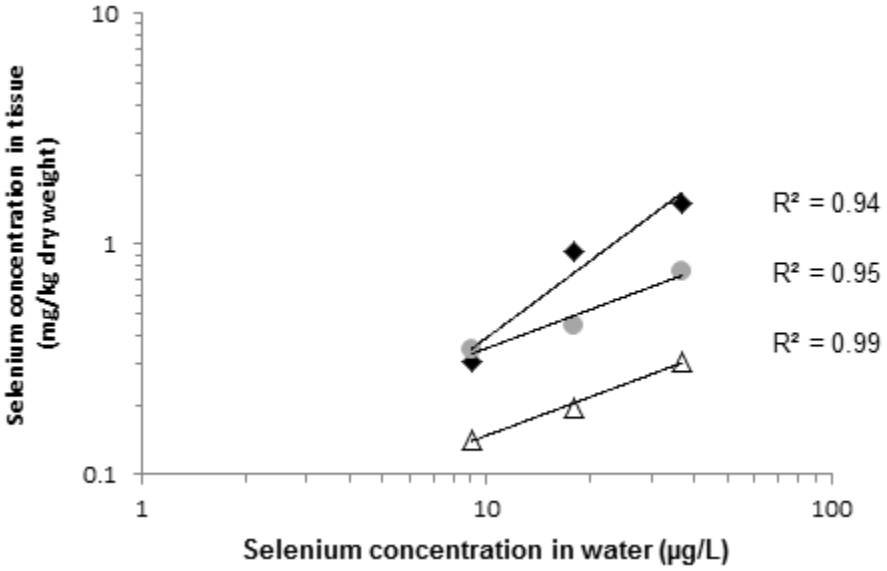

Figure 3-1. *Pseudokirchneriella subcapitata* selenium tissue concentration following exposure to dissolved selenate and sulphate. Each line represents a sulphate concentration, with 3 concentrations of selenate. Sulphate treatments were 103 mg/L (diamond), 184 mg/L (circle) and 310 mg/L (triangle). Algal tissue was pooled by treatment (n=9).

The second phase of the experiment was performed to determine if SO_4^{2-} affected the trophic transfer of Se to a second trophic level, represented by *D. magna*. During the 21-d exposure, daphnid treatment groups (*D. magna* $_{Diet}$ and *D. magna* $_{Diet + Water}$) were exposed to dissolved SO_4^{2-} and either dietary Se (selenium enriched algae) or a combination of dietary and dissolved Se. Mean survival of *D. magna* was 82 ± 13% across treatment groups, and was not affected by Se tissue concentration in either of the treatment groups (p = 0.32 and p = 0.14, respectively).

In the *D. magna* $_{Diet}$ treatment group, tissue Se concentrations at the termination of the experiment ranged from below detection limits (i.e., <0.045 mg/kg) to 0.67 mg/kg (Table 3-2). Increasing Se tissue concentrations with increasing dietary Se was observed, however tissue concentrations were unaffected by SO_4^{2-} concentrations. The statistical significance of these relationships were confirmed using linear regression (p <0.001 and p=0.15, respectively). In the *D. magna* $_{Diet + Water}$ treatment group, the

measured *Daphnia* tissue Se concentrations ranged from 0.15 to 1.65 mg/kg, indicating that the presence of dissolved SeO_4^{2-} in water resulted in increased concentrations of tissue Se relative to tissue concentrations in daphnia in the diet-only exposure group (Table 3-2).

Nominal sulphate (mg/L)	Nominal selenate (µg/L)	Measured *Pseudokirchneriella subcapitata* Tissue selenium (mg/kg)	Measured *Daphnia magna* tissue selenium (mg/kg)	
			Dietary selenium	Dietary selenium and selenate
81	10	0.31	0.23 ± 0.01	0.61 ± 0.10
81	20	0.94	0.58 ± 0.01	1.02 ± 0.13
81	40	1.50	0.67 ± 0.09	1.65 ± 0.16
162	10	0.35	0.14 ± 0.05	0.31 ± 0.08
162	20	0.44	0.21 ± 0.03	0.56 ± 0.08
162	40	0.77	0.34 ± 0.01	1.01 ± 0.04
324	10	<0.14	<0.045	0.15 ± 0.03
324	20	0.20	0.09 ± 0.05	0.40 ± 0.08
324	40	0.31	0.24 ± 0.04	0.69 ± 0.06

Table 3-2. Tissue selenium concentrations measured in *Pseudokirchneriella subcapitata* and *Daphnia magna* following exposure to selenium and sulphate. *P. subcapitata* tissue selenium concentrations were measured on a singular pooled sample. *D. magna* tissue selenium concentrations are represented as mean ± SD.

Evaluations of the relationship between the uptake rate of Se (via exposure to SeO_4^{2-}) and exposure to dietary Se yielded results indicating that uptake rate did not vary significantly across dietary Se concentrations. This suggests that contributions to Se tissue concentrations from Se in water (as SeO_4^{2-}) and diet do not influence one another ($p = 0.09$, 0.31 and 0.92 at SO_4^{2-} concentrations of 77, 160 and 293 mg/L, respectively).

Results based on estimated Se tissue concentrations (representing uptake from dissolved SeO_4^{2-} only), suggest that exposure to increasing concentrations of SeO_4^{2-} result in an increase in *D. magna* Se tissue concentrations (Figure 3-2). Also, increasing SO_4^{2-} concentrations was found to decrease estimated Se tissue concentrations. The

significance of SeO_4^{2-} and SO_4^{2-} on Se tissue concentration were confirmed using linear regression (p=<0.001 and p=<0.001, respectively). Furthermore, the effect of SO_4^{2-} was not consistent across the exposure concentration range and therefore a modifier (interaction term) was required to more accurately describe the relationship between SeO_4^{2-} and SO_4^{2-} (p=0.003). All measured Se and SO_4^{2-} can be found in the Appendix.

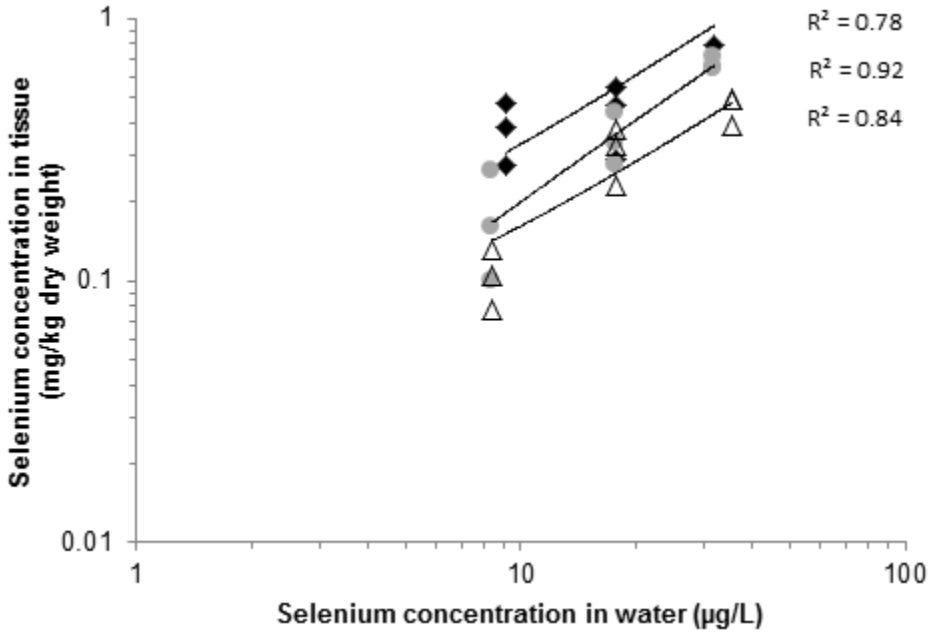

Figure 3-2. Estimated *Daphnia magna* selenium tissue concentrations resulting from exposure to selenate at 3 concentrations of sulphate. Data points represent singular measured concentrations at sulphate concentrations of 77 mg/L (diamond), 160 mg/L (circle) and 292 mg/L (triangle) sulphate (n=27).

Having established the independence of uptake of Se from dietary and SeO_4^{2-}, a predictive equation was formulated with two main elements: the first to describe Se tissue concentration attributed to exposure to Se (as SeO_4^{2-}) in water and the second to describe the contribution of Se from diet (Equation 1). The parameter estimates as determined from linear regression analyses used to quantify these relationships are shown in Equation (2). Note that due to findings regarding significance, SO_4^{2-} was integrated into the terms related to Se contribution from the water compartment only.

(2) $Daphnia\ tissue\ Se =$

$$[1.24 + 0.02Se_w - 0.56logSO_4{}^{2-} - 0.03(Se_w - 19.79)(logSO_4{}^{2-} - 2.19)] + [0.029 + 0.46Se_d]$$

Daphnia tissue Se: mg/kg dry weight; Se_w = Se in water, µg/L; Se_d = Se in diet, mg/kg dry weight

A comparison between predicted *D. magna* Se tissue concentrations (estimated from equation 2) and measured values is illustrated in Figure 3-3. The R^2 (0.95) indicated a high degree of reliability, suggesting that under the exposure conditions used, Se tissue concentrations in *Daphnia* can be estimated based on concentrations of SeO_4^{2-}, SO_4^{2-} and dietary Se. Of note is that if the predictive equation is deconstructed to contain only the water or dietary exposure components, the predictive ability of the equation is reduced (R^2: 0.79 and 0.56, respectively). This implies that *D. magna* Se tissue concentrations depend on both the dietary uptake of Se and the uptake of waterborne SeO_4^{2-}.

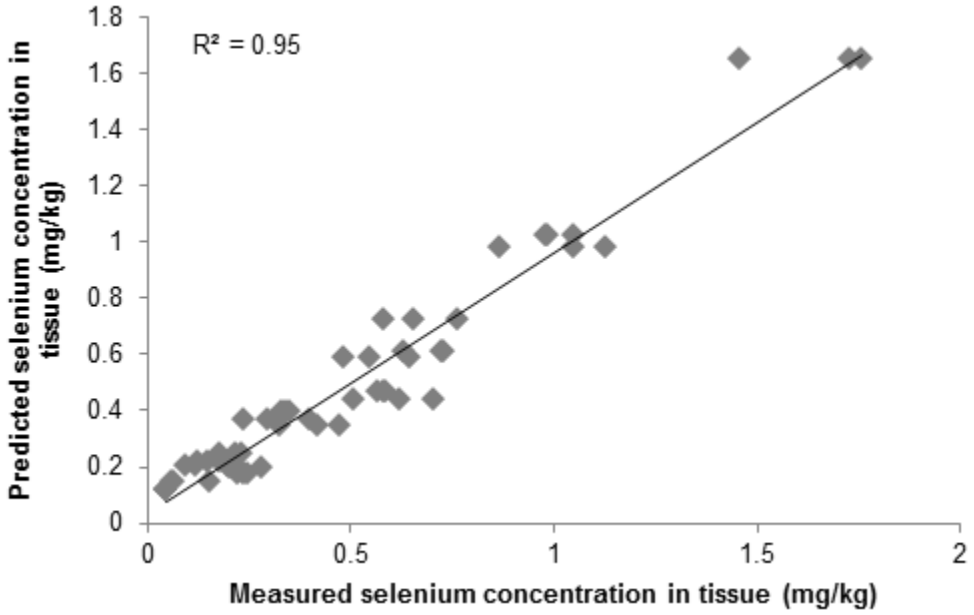

Figure 3-3. Actual vs. predicted tissue concentration of selenium in *Daphnia magna* tissue based on exposure to selenate, sulphate and dietary selenium (n=54).

3.5. Discussion

In this study, *P. subcapitata* and *D. magna* were exposed to three concentrations of SO_4^{2-} and SeO_4^{2-} to determine the effect of waterborne SO_4^{2-} concentrations on Se tissue concentrations in organisms at the primary producer and consumer levels. While previous studies (e.g. Williams et al., 1994; Ogle and Knight, 1996; Hansen, 1993) have examined the effect of SO_4^{2-} on dissolved SeO_4^{2-} on each species individually, the relationship between trophic levels and multiple Se exposure routes had not been previously evaluated. The results of this study confirmed that SO_4^{2-} concentration in water affects Se tissue concentrations by reducing the accumulation of Se from aqueous SeO_4^{2-}, but does not affect the dietary uptake of Se. This suggests that the effects of SO_4^{2-} on Se tissue concentrations of freshwater primary producers would be reflected in the tissue concentrations of primary consumers when one of the sources of Se is aqueous SeO_4^{2-}.

The results for *P. subcapitata* indicated that algal cell density did not significantly differ between treatments, which is consistent with results from Williams et al. (1994), who also did not observe statistically significant differences in algal growth with the same species exposed to 10 and 100 µg/L Se at a SO_4^{2-} concentration of 99 mg/L. Also, SO_4^{2-} was shown to reduce uptake of SeO_4^{2-}, which is consistent with results presented by Williams et al. (1994) and Lo et al. (2014), however, the Se tissue concentrations measured in this study are lower than reported by those authors. At similar exposure concentrations, Se tissue concentrations in this study differed by a factor of approximately 2.9 compared to results reported by Lo et al. and a factor of 12 for results reported by Williams et al. This variability in Se tissue concentration could be a reflection of differences between nutrient type and concentration used in the different exposures. Regarding nutrient concentration, the current and Lo et al., (2014) exposures followed nutrient addition as described by Environment Canada methods for culturing and testing *P. subcapitata* (2007). The difference between these two exposures however, was that the current exposure quadrupled the concentration of nutrients to increase algal cell yield. This increase in nutrient concentration may have resulted in growth biodilution, where rapid reproduction of algal cells may decrease the overall concentration of contaminant per cell (Pickardt et al., 2002).

Differences between algal Se tissue concentration reported in this study and those by Williams et al. (1994) are not likely attributed to growth dilution since nutrient concentrations in this study were low by comparison. Williams et al. reared *P. subcapitata* in modified Woods Hole medium (Nichols 1973) which contains higher concentrations of nutrients (approximately 210-7000 times greater, depending on the nutrient) as well as vitamins (B12, B1 and biotin) which are not part of the mixture recommended by Environment Canada (2007). It is difficult to evaluate how the use of vastly different nutrient media would affect algal cells (e.g. growth, cell size, Se uptake) without conducting direct comparisons. However, one specific difference between nutrient media that may be relevant to Se uptake is the difference in manganese (Mn) concentration, which was higher in the Woods Hole medium by a factor of 110. Manganese deficiency has been shown to reduce protein content in *Scenedesmus intermedius* (Adam and Issa, 2000). Therefore, potential differences in algal protein content due to Mn availability could be correlated to Se uptake and tissue concentration via formation of Se amino acids, selenoproteins and selenium containing proteins.

The second phase of this study exposed *D. magna* to algae reared in selenium enriched media, as well as the similar concentrations of SO_4^{2-} and Se in exposure water. Survival of *D. magna* in the exposure treatments averaged 82 %, with no evidence of a dose response, suggesting that the accumulated Se in tissue was not sufficient to cause adverse effects on survival. These results are consistent with those reported by Boyum and Brooks (1988), who reported *D. magna* survival of between 50% and 70%, after being fed selenized algae and exposed to SeO_4^{2-} ranging from 50 to 500 μg/L for 28 days.

Results from the *D. magna* $_{Diet}$ treatment group (organisms provided Se via diet only) demonstrated that SO_4^{2-} did not affect Se tissue concentrations when exposed to a range of Se dietary concentrations. This result suggests that the proportion of SeO_4^{2-} present in algal cells is not significant relative to other forms of Se. It is understood that algal cells use Se to form selenocysteine and that excess Se may be integrated into the amino acid selenomethionine (Wu et al. 1998). While the proportion of total tissue Se existing as organo-Se is undetermined for *P. subcapitata*, studies using *Scenedesmus quadricauda* and *Chlorella sp.* and have suggested that selenomethionine may make up

24-41% of total Se content (Umysova et al., 2009; Neumann et al., 2003) and that up to 85% of total Se may in an organic form (Uymysova et al., 2009). Therefore, if a large portion of tissue Se is present as organic Se, then the effect of SO_4^{2-} on Se uptake from diet would be minimal since the interaction between SO_4^{2-} and Se occurs when Se is present as SeO_4^{2-}.

Evaluations regarding the relationship between *D. magna* Se tissue concentration and two exposure routes found that contributions of Se from each source to overall Se tissue concentration were independent of one another. This differs from the observations reported by Boyum and Brooks (1988), who reported a decrease in tissue concentration of radiolabeled Se when fed selenized algae and thus concluded that *D. magna* decreased uptake of dissolved Se when fed dietary Se. Possible reasons for the discrepancy in findings could be related to the viability of algal cells. Regarding algal cell viability, the authors reported that following exposure to Se, algae was filtered, frozen and resuspended in solution prior to feeding daphnids. The authors did not provide details regarding freezing methodology (e.g. temperature, duration) therefore it is difficult to assess algal cell viability at time of exposure to *D. magna*.

Depending on cryopreservation techniques, *C. reinhardtii* cells may be viable following thawing (Taylor and Fletcher, 1999). If algal cells in the Boyum and Brooks exposure were viable when added to the *D. magna* exposure vessels, it is possible that the algal cells took up radiolabeled Se (as SeO_4^{2-}), which subsequently could have been consumed by daphnids thereby confounding the exposure routes of Se. Conversely, if the algal cells did not remain intact during the freeze-thaw process, it is possible that SeO_4^{2-} that had not yet been converted to organic forms could have been released into the exposure solution thereby providing non-radiolabeled SeO_4^{2-} available for uptake by *D. magna*.

Results from the *D. magna* exposures demonstrated reduced Se tissue concentrations with increasing SO_4^{2-} when exposed to SeO_4^{2-}, which was also previously reported by Ogle and Knight (1996) and Hansen et al (1993). Collectively, these studies suggest that this mechanism occurs across a range of SO_4^{2-} concentrations of at least 10 to 325 mg/L. However, at similar exposure concentrations, Se tissue concentrations

reported by Ogle and Knight were higher than those reported here by a factor of 4. One potential source for this difference in accumulation is the experimental duration employed in these studies. Ogle and Knight exposed the organisms for 72-h, whereas the organisms in this study were exposed for 20-d. During the 20-d period, the daphnids reproduced, which likely resulted in the transfer of Se into young, thereby reducing the Se-load in the adult daphnids. Maternal transfer efficiency of Se in each brood of offspring has been reported to range from 0.1% to 10% (Lam and Wang, 2006). These contrasting results suggest that laboratory assessments of Se accumulation in primary consumers should encompass potential effects of maternal transfer on tissue concentration.

This study also found that tissue Se concentration of *D. magna* provided both dietary and dissolved Se were an average of 2.9 times higher than in the dietary exposure alone, indicating that approximately 62% of the selenium burden in *D. magna* was derived from the water column, compared with only 38% being derived from the diet. The contribution of Se from water (when present as SeO_4^{2-}) was higher than the suggested theoretical estimate of 20-40% (Tsui and Wang, 2007). It is worth noting however, that contributions of Se from each source compartment are dependent on the concentrations of Se as well as assimilation or uptake efficiencies. While Se dietary assimilation efficiency of *Daphnia sp.* are approximately 20-30% (Guan and Wang, 2004; Yu and Wang, 2004), overall uptake of Se could be small if dietary Se concentrations are low. Therefore, the importance of SeO_4^{2-} on *D. magna* Se tissue concentration should be further explored using higher dietary concentrations (>1.5 mg/kg Se, dry weight) to determine if the contribution of Se from water remains prominent.

Application of the predictive equation derived here is limited by the data used in its derivation. When applied to the data by Ogle and Knight (1996) and Besser et al. (1993), the estimated *Daphnia* tissue concentrations were underestimated in most exposure concentrations of Se and SO_4^{2-}. The underestimation is primarily attributable to the lower Se accumulation observed in this study (and on which the predictive equation is based) compared to those reported by Ogle and Knight (1996) and Besser et al. (1993). Besser et al. (1993) reported daphnid Se tissue concentration of 1.1 mg/kg

(dry weight) after 14-d with daily feeding using algae which was higher than the concentrations measured in the current study (under similar exposure concentrations), by a factor of 4.7. The most likely explanation for this difference is the high Se in the algae (*Chlamydomonas reinhardtii*) used by Besser et al., which contained approximately 4 mg/kg Se dry weight after 24 hours exposure compared to 0.31 mg/kg Se dry weight in *P. subcapitata* in this current study. These differences between studies emphasize that the equation presented here is not a general predictive equation of tissue Se. Instead, the equation highlights that overall Se tissue concentration in *D. magna* can be described by SeO_4^{2-}, SO_4^{2-} and dietary Se when exposed to SeO_4^{2-} in water.

The results of these studies demonstrated that SO_4^{2-} significantly affects the uptake of dissolved Se in both *P. subcapitata* and *D. magna*. Furthermore, contributions of Se to *D. magna* Se tissue concentration from inorganic (as SeO_4^{2-}) and dietary sources were independent of one another. Ultimately, these exposures suggest that in the exposure range employed, SO_4^{2-} has a significant effect on overall uptake of Se in aquatic food chains in which SeO_4^{2-} predominates.

3.6. References

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Chapter 4.

General discussion and conclusion

4.1. Summary of exposures

The focus of this research was to explore the effect of SO_4^{2-} on the accumulation and transfer of Se at lower trophic levels using two separate studies. Specific topics of interest included:

- An evaluation of species related differences in uptake by primary producers (*L. minor* and *P. subcapitata*);
- An evaluation of effects of SO_4^{2-} on uptake of Se across a wide range of SO_4^{2-} conditions;
- Evaluation of an SeO_4^{2-} exposure to an invertebrate (*D. magna*) using a exposure duration that included reproduction and the potential for maternal excretion of Se; and
- exposure of an invertebrate to dietary and waterborne Se .

The first study focused on the measuring the effect of SO_4^{2-} on uptake of Se in two primary producers, a duckweed, *L. minor* and a green alga, *P. subcapitata*. For duckweed exposed to SO_4^{2-} concentrations ranging from 50 to 325 mg/L and Se concentrations from 10 to 40 µg/L, the results demonstrated that to SeO_4^{2-} and SO_4^{2-} had a significant effect on Se tissue concentration. Also, the effect of SO_4^{2-} was shown to change with exposure concentrations, therefore a modifying term (that accounted for this change) was required to more accurately describe the relationship of these anions and Se tissue concentration. These same terms were also found to be significant for *P.*

subcapitata when exposed to SO_4^{2-} concentrations between 5 to 500 mg/L and Se concentrations between 10 and 100 µg/L. These results demonstrated that the effect of SO_4^{2-} on Se tissue concentration (when exposed to SeO_4^{2-}) is significant at concentrations higher than previously tested (approximately 100mg/L). This is important, as SO_4^{2-} concentrations can range widely from 1-3040 mg/L (Health Canada, 1994), however, it should be mentioned that in BC, the highest SO_4^{2-} guideline for the protection of aquatic life is 429 mg/L (Ministry of Environment, 2013a). The *L. minor* results demonstrated that the effect of SO_4^{2-} on SeO_4^{2-} uptake is relevant to non-algal species of freshwater primary producers, which had been the focus of prior research.

Results from the first study also indicated that the uptake of Se in *L. minor* was higher than *P. subcapitata*, which could be a reflection of physical differences between species. The species vary dramatically in size and structure (single cell compared to multiple structures including frond(s) and root). Also, *L. minor* is a floating macrophyte capable of taking up nutrients from both root and frond tissues (Cedergreen and Madsen, 2002), suggesting that the larger size and surface area of this species (compared to *P. subcapitata*) may be related to a higher uptake of Se.

The second study evaluated whether the effect of SO_4^{2-} on Se tissue concentration was conserved across the first and second trophic levels, using a green alga (*P. subcapitata*) and a water flea (*D. magna*). During this study, *D. magna* were exposed to dietary Se (using algae that had been grown in a selenium enriched media), and to dissolved inorganic Se (as SeO_4^{2-}). Previous research on SeO_4^{2-} /SO_4^{2-} interaction in *D. magna* was conducted using short aqueous (SeO_4^{2-}) exposures thus the potential chronic and trophic transfer effects were unknown. The results demonstrated that SO_4^{2-} did not affect the tissue concentration of Se when exposed to dietary Se alone; however, SO_4^{2-} did significantly affect accumulation due to waterborne SeO_4^{2-} exposure. Both dietary Se and uptake of SeO_4^{2-} were found to significantly contribute to Se tissue concentration. Lastly, the study demonstrated that reductive effect of SO_4^{2-} on Se tissue concentration in algae was reflected in *D. magna* when Se is present as aqueous SeO_4^{2-}.

Predictive equations were derived in both studies to describe the relationship between Se and SO_4^{2-} on Se tissue concentration using the parameter estimates from linear regression analyses. For *L. minor* and *P. subcapitata*, the equations consisted of terms for SeO_4^{2-}, SO_4^{2-} and an interaction between the two parameters. As there were two sources of Se for *D. magna* (dietary Se and SeO_4^{2-}), the equation consisted of parameter estimates derived from separate linear regression analyses, one for each Se source. For all three species exposed in this research, comparisons of predicted versus measured values demonstrated that under the exposure conditions used, a large portion of Se tissue concentrations were related to SeO_4^{2-}, SO_4^{2-} and dietary Se (in the case of *D. magna*). Furthermore, the effect of SO_4^{2-} was found to change with exposure concentration, therefore a modifying term representing this changing relationship was required to more accurately predict Se tissue concentration. The changing effect of SO_4^{2-} while inferable from results of previous studies, had not been explicitly identified as being a significant component to describing the relationship between SeO_4^{2-} and SO_4^{2-} on Se tissue concentration.

Collectively, the three exposures indicated that over the exposure ranges of SeO_4^{2-} and SO_4^{2-}, tissue concentrations of Se decrease as a function of increasing SO_4^{2-} concentrations. To compare differences in Se uptake between species, K_d values were calculated (tissue concentration in μg/kg divided by water concentration in μg/L) for each species and plotted as a function of SO_4^{2-} (Figure 4-1). These data suggest that there are species differences in uptake rates of Se from water, and also imply that primary producers take up SeO_4^{2-} more actively than primary consumers (as represented by *D. magna*), which has been documented in field studies (Presser and Luoma, 2010). A potential explanation for the low uptake of Se in *D. magna* could be related to the multiple routes of excretion available to the organism (e.g. molting, production of neonates, water and feces) (Guan and Wang, 2004), thus potentially allowing the organism to shed Se more efficiently than primary producers.

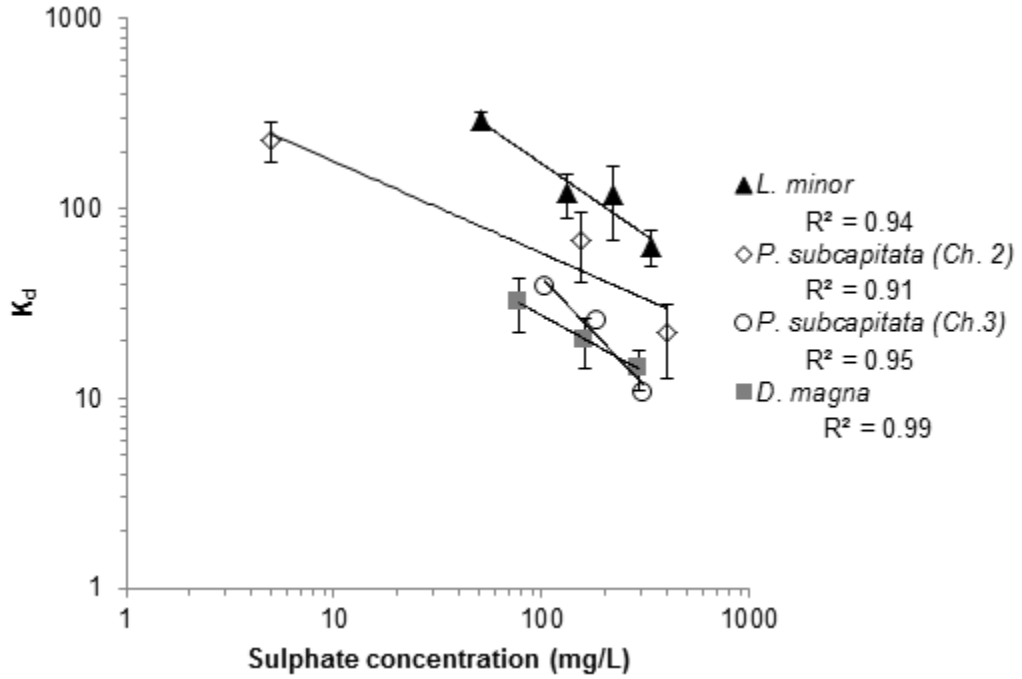

Figure 4-1. Partitioning coefficient (K_d) (mean ± SD) as a function of sulphate for *Lemna minor* (triangle), *Pseudokirchneriella subcapitata* (diamond-chapter 2; circle-chapter 3), and *Daphnia magna* (square) (n=15, 12, 9, 9, respectively).

4.2. Future directions

4.2.1. Laboratory based research

While the studies conducted here have expanded the scope of knowledge associated with Se and SO_4^{2-} and Se tissue concentration, continued research is warranted. As discussed in Chapters 2 and 3, uptake of Se by *P. subcapitata* differed between exposures as well as with results reported by other authors. Considering the exposures used the same algal species, the variability in accumulation suggests that differences in accumulation may be attributable to differences in exposure conditions. With regard to the data presented here, the primary difference between the exposures presented in Chapters 2 and 3 relates to the amount of nutrient that was added. Specifically, four times the quantity of nutrients was added to the algae being grown as diet for *D. magna*, compared with the initial exposures with this species described in section 2, and this higher nutrient addition rate corresponded to lower uptake rates.

Nutrient media composition, exposure duration or light intensity may have contributed to laboratory-based differences in Se accumulation observed relative to other authors.

One strategy for addressing concerns regarding species of environmental relevance and confounding effects of nutrient media would be to evaluate effects of uptake using a species that does not require supplemental nutrients. For example, the freshwater moss, *Fontinalis antipyretica* has been successfully exposed without the addition of nutrients in a range of water types including dechlorinated water (hardness 15 mg/L as $CaCO_3$) (Elphick et al., 2011) and well water (hardness 105 mg/L as $CaCO_3$) (Davies, 2007). This species has a wide geographical distribution and has been previously investigated for Se uptake from water in various sites located in Russia (Gapeeva et al., 2010), Spain (Vazquez et al., 2007) and Slovenia (Mechora et al., 20120). Therefore, use of this species for exploring SeO_4^{2-} and SO_4^{2-} interaction would not only remove possible effects due to nutrient addition (as in the case of *L. minor* and *P. subcapitata*) but also provide exposure conditions that may better approximate aqueous systems low in nutrient content. Exposures using this moss would also provide additional data to determine whether larger, non-algal primary producers take up SeO_4^{2-} at an increased rate.

Based on the existing knowledge about Se transfer in a food chain, it is expected that dietary Se is the primary means by which Se will enter and accumulate in higher trophic level organisms. While the results from this study suggest that decreases in Se uptake at primary trophic level will be reflected in primary consumers, additional studies are warranted to confirm that this effect will also be transferred to the secondary consumers and higher. Besser et al. (1993), conducted a laboratory food chain study using *Chlamydomonas reinhardti*i (a green alga), *D. magna* and *Lepomis macrochirus* (a fish). The research focused on differences in accumulation of organic and inorganic Se. Exposures were conducted as separate steps and contact between species occurred only when one species was being fed to another. A similar approach could be used to evaluate bioaccumulation in a food chain at varying concentrations of SO_4^{2-} and SeO_4^{2-}.

4.2.2. Model development

This research demonstrated Se tissue concentration at the lower trophic levels could be estimated based on exposure levels of SeO_4^{2-} and SO_4^{2-}; however, the predictive equations were based on data collected from controlled laboratory studies. Therefore, comparisons between Se tissue concentrations derived from laboratory studies against concentration of organisms measured in the field are warranted. In this exercise, field data should originate from SeO_4^{2-} dominated systems with measured SeO_4^{2-} and SO_4^{2-} concentrations. This comparison could serve as a method of validation and establishing environmental relevance of this methodology for predicting Se accumulation.

Following validation, integration of SO_4^{2-} into Se models could be explored. Various authors have already suggested techniques for modeling Se bioaccumulation in aqueous systems (Presser and Luoma, 2010, Orr et al., 2012). Typically, these biodynamic models are derived using field data from an existing site or by combining data from several field studies. Presser and Luoma (2010) developed a methodology for modeling Se in an ecosystem which approached Se modelling in several steps. For the step that requires selection of a K_d value (to estimate transfer from the water compartment to the base of the food chain), the authors suggested choosing a coefficient based on site-specific data and if unavailable, choosing a generalized number based on data from similar sites or to use a default K_d of 1000. Presser and Luoma also noted that a non-site specific K_d is frequently the largest source of uncertainty in the model. Consequently, the methods used in this project could be adapted to assess the effect of SO_4^{2-} on SeO_4^{2-} uptake in site water, thereby estimated a K_d value that could be used as a model input.

4.3. Relevance to water quality guidelines

4.3.1. Generic guidelines

Once the effect of SO_4^{2-} on SeO_4^{2-} uptake is sufficiently characterized and validated using environment concentrations, this relationship could be incorporated in Se

water quality guidelines derivation. In British Columbia, the Ministry of Environment acknowledges that toxicity of some contaminants may be influence by different environmental conditions. For example, SO_4^{2-} water quality guidelines for the protection of aquatic life are based on different water hardness categories, from very soft (0-30 mg/L) to very hard water (181-250 mg/L) (BC Ministry of Environment, 2013a). Similarly, the water quality guideline for zinc (and a number of other metals) is also dependent on hardness and can be calculated using a modifying equation provided by the BC Ministry of Environment (1999). Ultimately, SO_4^{2-} may be recognized as a modifying factor of Se bioaccumulation and, therefore, toxicity, and integrated into Se water quality guidelines/criteria, resulting in generating protection values adapted to various site conditions. However, this modification would likely need to be limited to sites that are dominated by SeO_4^{2-}.

4.3.2. Site-specific guidelines

There are several issues preventing the integrating of SO_4^{2-} as a modifier in a generic water quality guideline. The focus of this work on SO_4^{2-} and Se interactions does not account for systems where SeO_3^{2-} is present, systems where sediment and sediment dwelling organisms may play a significant role in Se bioaccumulation, or when more complex food webs are present. Despite these data gaps, the relationships presented here for Se and SO_4^{2-} may be suitable on a site-specific basis, where concerns related to these data gaps can be properly addressed.

In British Columbia, when water quality guidelines are either over- or under-protective at a site, they may be modified as site specific water quality objectives (Ministry of Environment, 2013b) on the basis of characteristics of the site. One strategy is to use a water effects ratio, where toxicity testing is conducted using the contaminant of interest in laboratory water and site water. Results from the two types of water are compared and if toxicity is different in site water, the ratio between toxicity endpoints are integrated into derivation of a water quality objective. For selenium, in which bioaccumulation, is the primary concern, an analogous laboratory based approach may be useful, in which the effect of site water on uptake of selenium could be measured. The methodologies used in this research could be adapted for use as a water effect

ratio-like strategy for assessments of Se bioaccumulation, where Se bioaccumulation is compared in laboratory water and site water (containing relevant concentrations of Se and SO_4^{2-}). An advantage of this laboratory-based technique is that it may be used for sites where direct measurements in the field are not feasible (e.g., in cases where a mine site is under development) as existing site water could be amended with an anticipated range of SO_4^{2-} concentrations, simulating site conditions. While further research into the feasibility of such an approach is warranted, this strategy highlights that research into the effect of SO_4^{2-} on Se accumulation has practical application in freshwater systems impacted by Se-related industrial activities.

4.4. References

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Appendix.

Measured exposure and organism tissue concentrations

Table A-1. Measured concentrations from *Lemna minor* exposure

Selenium (ug/L)	**Sulphate (mg/L)**	***L. minor* Selenium (mg/kg dw)**
4.5	50.8	1.27
4.5	50.8	1.40
4.5	50.8	1.52
9.0	50.8	2.75
9.0	50.8	2.20
9.0	50.8	2.92
18.5	50.8	5.73
18.5	50.8	5.55
18.5	50.8	5.22
37.1	50.8	9.84
37.1	50.8	10.46
37.1	50.8	10.45
3.8	132	0.73
3.8	132	0.52
3.8	132	0.56
9.2	132	0.99
9.2	132	1.17
9.2	132	0.99
19.8	132	2.22
19.8	132	2.64
19.8	132	1.99
39.0	132	2.27
39.0	132	4.15
39.0	132	4.89

Table A-1. Measured concentrations from *Lemna minor* exposure continued

Selenium (ug/L)	**Sulphate (mg/L)**	***L. minor* Selenium (mg/kg dw)**
4.2	220	0.51
4.2	220	0.54
4.2	220	0.56
9.1	220	2.40
9.1	220	0.82
9.1	220	0.83
18.3	220	1.79
18.3	220	2.17
18.3	220	1.99
39.3	220	3.39
39.3	220	3.35
39.3	220	3.4
4.7	335	0.24
4.7	335	0.34
4.7	335	0.42
9.3	335	0.71
9.3	335	0.75
9.3	335	0.58
19.6	335	1.19
19.6	335	1.06
19.6	335	1.13
38.5	335	1.84
38.5	335	2.05
38.5	335	1.93

Table A-2. Measured concentrations from *Pseudokirchneriella subcapitata* exposure (chapter 2)

Selenium (ug/L)	**Sulphate (mg/L)**	***P. subcapitata* Selenium (mg/kg dw)**
10	5	1.58
10	5	2.42
10	5	3.48
18	5	3.94
18	5	3.15
18	5	3.17
30	5	7.77
30	5	7.38
30	5	6.12
54	5	14.28
54	5	10.87
54	5	14.64
91	5	26.44
91	5	14.10
91	5	20.73
9	155	1.35
9	155	0.82
9	155	0.76
18	155	1.28
18	155	1.51
18	155	0.95
31	155	2.09
31	155	2.04
31	155	2.10
56	155	2.02
56	155	2.56
56	155	3.92
97	155	4.51
97	155	3.82
97	155	5.60

Table A-2. Measured concentrations from *Pseudokirchneriella subcapitata* exposure (chapter 2) continued

Selenium (ug/L)	**Sulphate (mg/L)**	***P. subcapitata* Selenium (mg/kg dw)**
10	396	0.29
10	396	0.32
10	396	0.16
18	396	0.40
18	396	0.38
18	396	0.22
31	396	0.73
31	396	0.81
31	396	0.65
55	396	1.57
55	396	1.29
55	396	2.45
102	396	1.13
102	396	1.22
102	396	1.00

Table A-3. Measured concentrations from *Pseudokirchneriella subcapitata* (chapter 3)

Selenate (µg/L)	**Sulphate (mg/L)**	***P. subcapitata* tissue selenium (mg/kg, dw)**
9.0	102.6	0.31
17.9	102.6	0.94
36.9	102.6	1.50
9.0	184.3	0.35
17.9	184.3	0.44
36.9	184.3	0.77
9.0	309.7	0.14
17.9	309.7	0.20
36.9	309.7	0.31

Table A-4. Measured concentrations from *Daphnia magna* exposure

Selenium (ug/L)	**Sulphate (mg/L)**	***D. magna* Selenium (mg/kg dw)**
<0.0001	77.1	0.24
<0.0001	77.1	0.22
<0.0001	77.1	0.25
<0.0001	77.1	0.59
<0.0001	77.1	0.57
<0.0001	77.1	0.58
<0.0001	77.1	0.76
<0.0001	77.1	0.58
<0.0001	77.1	0.66
<0.0001	159.5	0.20
<0.0001	159.5	0.12
<0.0001	159.5	0.10
<0.0001	159.5	0.23
<0.0001	159.5	0.22
<0.0001	159.5	0.18
<0.0001	159.5	0.33
<0.0001	159.5	0.34
<0.0001	159.5	0.35
<0.0001	292.7	<0.05
<0.0001	292.7	<0.05
<0.0001	292.7	<0.05
<0.0001	292.7	0.06
<0.0001	292.7	0.15
<0.0001	292.7	0.07
<0.0001	292.7	0.20
<0.0001	292.7	0.22
<0.0001	292.7	0.28

Note: < signifies the value is less than

Table A-4. Measured concentrations from *Daphnia magna* exposure continued

Selenium (ug/L)	**Sulphate (mg/L)**	***D. magna* Selenium (mg/kg dw)**
9.2	77.1	0.51
9.2	77.1	0.62
9.2	77.1	0.71
17.8	77.1	1.05
17.8	77.1	1.13
17.8	77.1	0.87
31.8	77.1	1.76
31.8	77.1	1.73
31.8	77.1	1.46
8.4	159.5	0.30
8.4	159.5	0.40
8.4	159.5	0.24
17.6	159.5	0.55
17.6	159.5	0.65
17.6	159.5	0.48
31.8	159.5	0.98
31.8	159.5	1.05
31.8	159.5	0.99
8.4	292.7	0.15
8.4	292.7	0.12
8.4	292.7	0.18
17.6	292.7	0.32
17.6	292.7	0.47
17.6	292.7	0.42
35.4	292.7	0.63
35.4	292.7	0.72
35.4	292.7	0.73