Factors affecting selenium bioconcentration
at the base of aquatic food webs

by
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B.Sc., University of Victoria, 2008

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Abstract

Selenium is a naturally occurring element and an essential micronutrient for many organisms; however, at high concentrations it can become toxic. Currently, the mechanisms underlying selenium accumulation remain unclear, resulting in uncertainty in the prediction of selenium transfer from water to primary producers at the base of the food web – a process referred to as enrichment. This study assesses how varying concentrations of selenium and sulphate in water affect enrichment. Using reported concentrations of selenium, in water and periphyton collected from three mining regions in British Columbia, Canada, we show that enrichment is inversely related to exposure concentration. The effect of sulphate on enrichment was explored by comparing the fit of multivariate regression models (with and without sulphate) with Akaike’s Information Criterion (AIC). Models without sulphate were significantly better at predicting enrichment than models with sulphate (ΔAICc = 2.29); however, conclusions were limited due to collinearity between selenium and sulphate.

Keywords: selenium; sulphate; enrichment; bioconcentration
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<thead>
<tr>
<th>Acronym</th>
<th>Description</th>
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<tbody>
<tr>
<td>AIC</td>
<td>Akaike Information Criterion – a model selection tool that balances the trade-offs between model simplicity (number of parameters) and wellness of fit and ranks candidate models based on their compromise between these two indicators (Burnham &amp; Anderson 2002)</td>
</tr>
<tr>
<td>BAF</td>
<td>Bioaccumulation Factor</td>
</tr>
<tr>
<td>DL</td>
<td>Detection limit – concentrations of one-half the detection limit (0.5 DL) were assumed for water samples that fell below the detection limit</td>
</tr>
<tr>
<td>EF</td>
<td>Enrichment Function – the concentration of selenium in periphyton divided by the concentration of selenium in water</td>
</tr>
<tr>
<td>S:Se</td>
<td>Sulphur / Selenium concentration ratio</td>
</tr>
<tr>
<td>VIF</td>
<td>Variance Inflation Factor – measures the variance of the estimate (regression coefficient) compared to what is expected if the variable is completely independent from all other predictor variables (Schwartz 2014c)</td>
</tr>
<tr>
<td>WQG</td>
<td>Water Quality Guideline</td>
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### Glossary

<table>
<thead>
<tr>
<th>Term</th>
<th>Definition</th>
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<tbody>
<tr>
<td>Enrichment</td>
<td>The process by which selenium partitions from water to periphyton and enters the aquatic food web</td>
</tr>
<tr>
<td>Periphyton</td>
<td>The assemblage of microbes, microalgae, detritus, and enriched sediment found attached to rocks in aquatic ecosystems (BCMoE 2014)</td>
</tr>
</tbody>
</table>
1.0 Introduction

Selenium is a naturally occurring element and an essential micronutrient for many organisms including humans (Young et al. 2010). In trace amounts, selenium is essential in the production of antioxidants that are necessary for sustaining life (Beckett & Arthur 2005) but at concentrations only slightly greater than essential concentrations selenium can become toxic and cause reproductive, teratogenic, and other adverse effects (Stadtman 1974; Lemly 1993; Luoma & Presser 2009; BCMoE 2014).

Selenium is naturally abundant in sedimentary deposits rich in organic matter, particularly in areas with enriched coal and phosphate deposits (Young et al. 2010; Presser 2013). Under normal conditions selenium is locked up in these deposits and released slowly through natural weathering and volcanic activity; however, anthropogenic activities such as irrigation, mining, and the combustion of fossil fuels can release stored selenium at an accelerated rate (Maher et al. 2010; Winkel et al. 2012).

Selenium can exist in various anionic states of speciation each with a different reactivity, solubility, and bioavailability (Presser & Luoma 2006). Common forms of aqueous selenium are selenate (Se[VI] or SeO$_4^{2-}$), selenite (Se[IV] or SeO$_3^{2-}$), organo-selenide (Se[–II]), and elemental selenium (Se[0]). Selenium typically enters the environment as selenate (a form with relatively low reactivity) and over time is reduced and replaced by selenite and organo-selenide, which are more reactive and more bioavailable (Luoma & Presser 2009). Fast flowing (lotic) waters, such as rivers and streams, have relatively short residence times and therefore typically have higher proportions of selenate. Conversely, slow moving (lentic) environments such as certain estuaries and wetlands generally contain ‘older water’ with a higher proportion of selenite and organo-selenide (Simmons & Wallschläger 2005; Ohlendorf et al. 2011). Site hydrology is thus important to consider in bioaccumulation models, as it can influence selenium speciation and bioavailability.
Traditionally, selenium toxicity is attributed to its competition with sulphur (another essential micronutrient) during protein synthesis (Janz et al. 2010). In high concentrations selenium can replace sulphur during the formation of amino acids. This substitution prevents the formation of di-sulphide bonds that are critical in tertiary folding, and results in malformed proteins that can result in muscular and skeletal deformities in the developing embryos of egg laying vertebrates (Stadtman 1974). Another, more recently proposed mechanism of toxicity is oxidative stress. Selenium is an important component of glutathione (an antioxidant protein). However, an overabundance of selenium shifts the production of glutathione towards an unusable form, resulting in less antioxidants being formed, thereby indirectly increasing the load of reactive oxygen species (Janz et al. 2010). Oviparous (egg laying) vertebrates such as birds, fish, reptiles, and amphibians are particularly susceptible to selenium toxicosis during development as their embryos are unable to excrete excess selenium from the egg (Unrine et al. 2006; Ohlendorf et al. 2011).

Direct uptake of dissolved selenium by animals is slow and negligible compared to dietary uptake of selenium which accounts for more than 95% of tissue selenium (Wang et al. 1996). Thus, it is important to understand selenium enrichment – the process by which selenium partitions from water to periphyton and enters the aquatic food web. The term periphyton is synonymous with biofilm and refers to the assemblage of microbes, microalgae, detritus, and enriched sediment found attached to rocks in aquatic ecosystems (BCMoE 2014). In its dissolved form, selenium has a low bioavailability, but once it is taken up by periphyton its bioavailability increases substantially. Periphyton is the food source for a host of invertebrates, which are in turn fed on by a variety of fish and bird species. In this way selenium works its way up the aquatic food web from water, to periphyton, to invertebrates, and then to fish and birds. Figure 1 (Appendix B) shows a conceptual diagram depicting the relative concentration of selenium across these four trophic levels.

Numerous studies have modelled the bioaccumulation of selenium in aquatic food webs (DuBowy 1989; Peterson & Nebeker 1992; Bowie et al. 1996; Adams et al. 1998; Brix et al. 2005; DeForest et al. 2007; Presser & Luoma 2010; Orr et al. 2012; DeForest et al. 2014). While a great deal of progress has been made towards
understanding its underlying mechanisms, considerable uncertainty remains in the current models – especially when predicting uptake of selenium by organisms at the base of the food web. Enrichment, a term synonymous with bioconcentration, occurs at the base of the food web and describes the process by which periphyton (biofilm, microalgae and bacteria) take-up dissolved selenium from the water. Enrichment is the transfer step where most accumulation occurs (Figure 1 – Appendix B) and is the most difficult transfer step to predict (Stewart et al. 2010). In 2014, the North American Metals Council – Selenium Working Group, identified four factors that affect the accumulation of selenium in periphyton and contribute to the uncertainty in selenium bioaccumulation models (DeForest et al. 2014). These factors included: the magnitude of aqueous exposure concentration; sulphate concentrations which modify selenium uptake; the influence of water chemistry upon enrichment (i.e. selenium speciation); and the variability in uptake among different subgroups of periphyton (e.g. microalgae, detritus, enriched sediment, etc.). These factors were addressed in the report and identified as topics requiring additional investigation.

As identified by DeForest et al. (2014), the magnitude of the aqueous selenium concentration influences selenium accumulation in periphyton. However, despite this concept being widely accepted, it is often not applied in selenium bioaccumulation models. When modelling the bioaccumulation of selenium, the first step in the food chain is typically expressed by an Enrichment Factor (EF or Kd), which is characterized as a ratio between the concentration of selenium in periphyton and the concentration of selenium in water (Presser & Luoma 2010; Stewart et al. 2010). The EF indicates the amount of selenium that is biologically available and is expressed mathematically as:

$$EF = \frac{[Se]_{\text{periphyton}}}{[Se]_{\text{water}}}$$  (1)

Selenium bioaccumulation is often modeled using a ratio-based approach that uses either a single ratio bioaccumulation factor (DuBowy 1989) or combines a series of stepwise bioaccumulation (or trophic transfer) factors, representing successive steps in the food chain (Presser & Luoma 2010). This approach calculates a concentration ratio that approximates the accumulation of selenium from one trophic level to the next, but it inherently assumes that the concentration ratio remains constant over varying exposure
concentrations. For example, at the base of the food web ratio-based models assume that the ratio $\frac{\text{[Se]}}{\text{[Se]}}$ remains constant despite varying concentrations of selenium in water. Thus changing the concentration of selenium in water by a factor of $x$ is assumed to change the concentration of selenium in periphyton also by a factor of $x$. However, this assumption ignores the observations made by regression-based models that have compared EF to the concentration of selenium in water and found that enrichment is not proportional across exposure concentrations (DeForest et al. 2007; Orr et al. 2012; DeForest et al. 2014).

Antagonism is common among metals and metalloids (Gailer 2007) and antagonistic relationships with selenium have been described with elements and compounds such as mercury (Cuvin-Aral & Furness 1991; Yang et al. 2008), arsenic (Moxon 1938; Levander 1977), phosphate (Hopper & Parker 1999), and sulphate (Hurd-Karrer 1934; Shrift 1954; Hansen et al. 1993; Williams et al. 1994; Riedel & Sanders 1996; Fournier et al. 2010; Lo 2014). Of these, sulphate interference is the most applicable to the bioaccumulation of selenate in lotic environments (DeForest et al. 2014). Competitive antagonism between selenium and sulphate analogues during cellular absorption is well accepted (Hurd-Karrer 1934; Shrift 1954), and laboratory experiments show that an increase in the concentration of aqueous sulphate decreases the bioavailability of selenium (Williams et al. 1994; Fournier et al. 2010) and reduces its acute toxicity to some organisms (Brix et al. 2001; Fournier et al. 2010). Based on these observations there has been a push to include ambient sulphate concentrations in bioaccumulation models when deriving selenium water quality guidelines (USEPA 2004; DeForest et al. 2014). However, the existing evidence for an effect of sulphate on selenium bioaccumulation and toxicity is equivocal (deBruyn & Chapman 2007), and a review by Skorupa (1998) concluded that selenium-sulphate antagonism is a lab-based phenomenon not supported by field data. Consequently, the effect of sulphate on the bioaccumulation of selenium remains inconclusive and further investigation, especially of field data, is required.

The present study seeks to increase understanding of selenium enrichment in the field, using data collected from three mining regions of British Columbia, Canada. The specific objectives are to investigate whether there is an inverse relationship
between selenium enrichment and exposure concentration; and to explore the effect of aqueous sulphate on enrichment at the base of the lotic food web. We predict that including ambient sulphate in bioconcentration models will help predict the enrichment of selenium.

2.0 Methodology

2.1. Data collection

Selenium concentrations in water and periphyton were assembled from three mining regions in British Columbia (BC), Canada – see map (Figure 2 – Appendix B). Data were compiled from twenty-one peer-reviewed and open access reports and six private water databases. These sources represent eighteen years (1996 – 2013) of environmental monitoring data collected from the Elk Valley coal mines in southeast BC (McDonald & Strosher 1998; EVS 2005; Minnow et al 2010; Teck 2011; Golder 2013a; Teck 2013; Teck 2014); the Peace River Coal zone in northeast BC (Golder 2007a, 2007b; Golder 2009a, 2009b; Golder 2010a, 2010b; Golder 2011; Golder 2012; Golder 2013b); and Kemess Mine in northwest BC (Hatfield 2007; Hatfield 2008; Hatfield et al 2008; Hatfield 2009; Hatfield 2010). Supplementary water data from the Elk Valley, Brule Mine, Willow Creek Mine, Wolverine Mine, Trend Mine and Northgate water databases were made available through Golder Associates (see Appendix E for more information).

One hundred and ten samples (Elk Valley = 68; Peace River Coal = 33; Kemess = 9) were collected from sampling stations selected to represent a combination of exposed and reference sites in order to monitor the concentration of selenium in various media over time. All samples were from lotic (fast flowing) water bodies, except six samples from the Kemess region that were from mixed (lentic/lotic) systems.
Concentrations of selenium in periphyton were paired with concentrations of selenium in water that were collected from the same sampling location during the same year (i.e. co-located samples). The Enrichment Factor (EF) – the concentration of selenium in periphyton divided by the concentration of selenium in water (Equation 1) – was calculated from non-transformed data as an indicator of enrichment (i.e. bioconcentration) at the base of the food chain. All data (selenium concentrations of water and periphyton, sulphate concentrations in water, and EFs) were log\textsubscript{10} transformed prior to analysis.

2.2. Water samples

Whenever monthly water data were available, the geometric mean of monthly averages was calculated to generate an annual average concentration in µg/L (total selenium). When periphyton was collected from an area without long-term water data, a single water sample was collected concurrently with the periphyton sample and used to estimate the annual average. The accuracy of using a single measurement rather than an annual average was assessed using a paired t-test on a subset of data that contained both values. There was no evidence of a difference between single values and annual averages ($t = -1.67$, $p = 0.102$, $n = 38$). Concentrations of one-half the detection limit (0.5 DL) were assumed for water samples that fell below the detection limit. To test the sensitivity of this assumption we also analyzed the data assuming that concentrations below the detection limits were equal to the full detection limit (1 DL), 10% of the detection limit (0.1 DL), and zero. Changing the detection limits did not affect our major conclusions (see Appendix C for details).

2.3. Periphyton samples

Most periphyton values were measured as a single composite sample and recorded in mg/kg dry weight (dw). When multiple periphyton samples were available for
a given site and year, the geometric mean of the composites was used to avoid pseudo-replication.

2.4. Statistical analysis

2.4.1. Selenium enrichment

Prior to testing the relationship between selenium enrichment and exposure concentration an Analysis of Covariance (ANCOVA) was used to assess whether or not the regional data could be combined. Three variables were used in the ANCOVA: the concentration of selenium in water as the predictor variable, the concentration of selenium in periphyton as the response variable, and region as the categorical variable.

For each region the slope between the \( \log_{10} \) concentration of selenium in water and the \( \log_{10} \) concentration of selenium in periphyton was determined using single-factor regression analysis. The slope of each relationship was compared to a slope of zero (a slope indicating no relationship) and also compared to a slope of one (a slope indicating a constant distribution coefficient across exposure concentrations).

2.4.2. Effect of sulphate on enrichment

The effect of sulphate ions on the uptake of selenium from water to periphyton was investigated in two ways. The relationship between enrichment, the concentration of selenium in water, and the sulphate-selenium molar ratio was explored graphically (Figure 5 – Appendix B), and using the data available a range of single and multivariate regression models were compared to assess the significance of including sulphate as a model parameter. Table 1 (Appendix A) lists the parameters used in each regression model.

Data, for the concentration of sulphate in water, were available for 52 of 68 (76%) co-located water samples in the Elk Valley, and 19 of 33 (58%) samples in the Peace
River region. Concentrations of sulphate in water were unavailable for Kemess so the region was dropped from further analysis. All available data were pooled for a total sample size of 71.

As a metric to measure the competitive uptake of sulphate, a *sulphur*-selenium ratio (S:Se) was calculated for each water sample using equation 2. Where both the concentration of selenium and sulphate are in the same units and a conversion factor of 0.334 (based off the molecular weight of sulphur / sulphate) is used to account for the relative fraction of sulphur in sulphate.

\[
S:Se = \frac{[S_{\text{aq}}] \times 0.334}{[Se]} \tag{2}
\]

The S:Se ratio was preferred over using the total sulphate concentration as it better reflects the likelihood of sulphur ions interfering with the uptake of selenium. For example, for a given concentration of total sulphate, we would expect more inhibition of selenium in water with a large S:Se and less inhibition of selenium in water with a small S:Se. In Figure 5 (Appendix B), \(\log_{10}\) EF is plotted against the \(\log_{10}\) concentration of selenium in water (x) and the \(\log_{10}\) S:Se ratio (y). Using the R-package ‘ggplot2’, \(\log_{10}\) EF was scaled by size so that large circles represent water samples with high EFs and small circles represent water samples with low EFs. In this way, trends between variables could be visually assessed that could not easily be analyzed due to their collinearity.

To predict the uptake of selenium in periphyton, multiple regression models were generated from all possible combinations of the variables aqueous selenium, aqueous sulphate, and region. To be biologically relevant, only regression models containing aqueous selenium were considered, and as recommended standard practice (O’Brien 2007) models with variance inflation factors (VIFs) greater than 10 were dropped from analysis. The remaining eight regression models (Table 1) were compared using Akaike’s Information Criterion (AIC).

AIC is a model selection tool that balances the trade-offs between model simplicity (number of parameters) and wellness of fit and ranks candidate models based
on their compromise between these two indicators (Burnham & Anderson 2002; Crawley 2005). AIC is particularly useful when comparing multivariate linear regressions, and it can be advantageous over traditional hypothesis testing as it gives consistent results independent of the order in which models are computed (Burnham & Anderson 2002). AICc, a type of AIC analysis specifically tailored to dealing with small sample sizes, was used to account for any bias introduced by the sample-size. More information regarding AIC and AICc is included in Appendix D.

All graphing and statistical analyses (T-test, ANCOVA, Regression, and AICc) were completed using R Studio (version 0.98).

### 3.0 Results and Discussion

#### 3.1. Regional differences in enrichment

Results of the ANCOVA, used to assess whether or not regional selenium data could be combined, found no evidence that slopes differ by region ($F = 0.56; p = 0.58$), but strong evidence for a difference among regional intercepts ($F = 14.43, p < 0.001$). Thus the selenium data for each region was analyzed individually. Intercepts are highest for Kemess and lowest for Peace River Coal (Table 2 – Appendix A). The actual intercept is not biologically relevant, as no uptake will occur when the concentration of selenium in water is zero; however the intercepts suggest that for a given concentration of selenium in water, the concentration of selenium in periphyton is naturally highest in the Kemess area, followed by the Elk Valley, and then Peace River Coal. From among these three regions, mean enrichment ranged from 477 (Elk Valley) to 1,494 (Peace River Coal). This variability is in agreement with Presser & Luoma (2010) whom report regional variation in selenium enrichment ranging from 107 to 21,500.
Variation in hydrogeology and water chemistry may account for some of the regional differences in enrichment. In the Elk Valley and Peace River Coal, all samples were collected from lotic sites whereas six of the nine Kemess samples were collected from mixed (lentic/lotic) water bodies. EFs are typically higher in lentic than lotic systems, as lentic waters are more likely to contain selenite and organo-selenide, which are more reactive and bioavailable than selenate (Adams et al. 2000; Simmons & Wallischläger 2005; Orr et al. 2012). Even a slight increase in selenite or organo-selenide would explain the higher enrichment observed in the Kemess area.

Selenium concentrations in water were recorded in units of µg total selenium/L, but the composition of selenium species in each sample was unknown. Selenate is the most common form of selenium found in lotic environments, as lotic systems are rich in oxygen and have short residence times that restrict the mobilization of selenate to more reduced forms (Simmons & Wallischläger 2005; Presser 2013). Thus we assumed that all aqueous selenium, collected from lotic environments, was in the form selenate. We encourage future studies to collect selenium speciation data, as knowing the composition of selenium species may increase the predictive power of selenium bioaccumulation models and increase their ability to generalize across regions of varying water chemistry.

3.2. Enrichment from water to periphyton

In all regions, there is a positive log-log relationship between the concentration of selenium in water (µg/L) and the concentration of selenium in periphyton (mg/kg dw) (Figure 3 – Appendix B). The coefficients that describe these relationships are recorded in Table 2 (appendix A). These results are similar to the results of studies by Luoma & Presser (2009), Chapman et al. (2010), Presser & Luoma (2010), Orr et al. (2012), and BCMoE (2014) who also conclude that the concentration of selenium in water has a positive effect on the concentration of selenium in periphyton.

Regression slopes (Table 2 – Appendix A) range from 0.19 (Elk Valley) to 0.28 (Kemess) but do not differ significantly among regions ($F = 0.56; p = 0.58$). Both the concentration of selenium in water ($x$) and the concentration of selenium in periphyton
(y) are log_{10}-transformed, so a change in x by one percent corresponds to a change in y by $\beta_i$ percent – where $\beta_i$ is the slope coefficient of regression. Thus for the Elk Valley, a 1% change in $[\text{Se}]_{\text{water}}$ will increase $[\text{Se}]_{\text{periphyton}}$ by 0.19%; for Kemess, a 1% change in $[\text{Se}]_{\text{water}}$ will increase $[\text{Se}]_{\text{periphyton}}$ by 0.28%; and for Peace River Coal, a 1% change in $[\text{Se}]_{\text{water}}$ will increase $[\text{Se}]_{\text{periphyton}}$ by 0.25%.

The coefficient of determination ($r^2$) indicates the percentage of variance in the response variable y that is explained by x, and denotes how well the calculated regression line fits the data (Schwartz 2014a). $r^2$ is highest for Kemess ($r^2 = 0.59$), followed by the Elk Valley ($r^2 = 0.31$), followed by Peace River Coal ($r^2 = 0.14$) (Table 2 – Appendix A). Thus in the Kemess area, $[\text{Se}]_{\text{water}}$ explains 59% of the variation in $[\text{Se}]_{\text{periphyton}}$ – implying a relatively strong fit; in the Elk Valley $[\text{Se}]_{\text{water}}$ explains 31% of the variation in $[\text{Se}]_{\text{periphyton}}$ – implying a medium fit; and in the Peace River Coal region $[\text{Se}]_{\text{water}}$ explains 14% of the variation in $[\text{Se}]_{\text{periphyton}}$ – implying a weak fit.

The regression slopes in Table 2 (Appendix A) are estimates of the Enrichment Function. When they are compared to a slope of zero (i.e. no relationship), they are highly significant in the Elk Valley ($p < 0.0001$) and moderately significant in the Kemess and Peace River Coal regions ($p < 0.016$ and $p < 0.034$ respectively). Regression slopes in all regions are significantly less than one ($p < 0.0001$); and thus an increase in $[\text{Se}]_{\text{water}}$ results in an increase in $[\text{Se}]_{\text{periphyton}}$, but the magnitude of change decreases as the concentration of selenium in water increases. Figure 3 (Appendix B) shows the log_{10}-log_{10} relationship between selenium concentrations of co-located water and periphyton samples that were collected from each region.

When enrichment factors are plotted against the concentration of selenium in water, it is evident that enrichment decreases as the exposure concentration increases. Figure 4 (Appendix B) shows the regression of EF against the concentration of selenium in water, compared to a line with an intercept equalling the geometric mean EF and a slope of zero (i.e. the expected slope if enrichment was proportional across exposure concentrations). In this case, there is autocorrelation between the variables (because the concentration of selenium in water occurs both in the denominator of EF and in the x-axis) and thus regression analysis was not performed. However, plotting these data in
this configuration is an effective way to visualize the fact that EF is not constant over exposure concentrations; rather, it is proportionally greater at low exposure concentrations and proportionally lower at high exposure concentrations.

The regression lines from Figure 3 (Appendix B), and their corresponding coefficients from Table 1 (Appendix A), show strong evidence that for regions dominated by selenate, selenium enrichment is not proportional across aqueous selenium concentrations. These conclusions are in agreement with previous observations in the literature. For example, McGeer et al. (2003) shows that for a wide range of metals and metalloids, bioaccumulation (in aquatic biota ranging from algae to invertebrates to fish) is inversely related to exposure concentration. DeForest et al. (2007) confirmed this trend with selenium and showed that both bioconcentration and bioaccumulation, in a variety of freshwater invertebrates and fish, follow the same inverse relationship with the highest uptake occurring at low exposure concentrations and the lowest uptake occurring at high exposure concentrations. Fournier et al. (2006) examined uptake of three different selenium species, by the fresh water alga Chlamydomonas reinhardtii, and found that uptake of selenite was linear for concentrations up to 2000 µg/L but that uptake of selenate and organo-selenide decreased as the concentration of selenium in water increased. They attribute this decrease to a saturation of selenium transport systems and suggest a saturation point of ~1000 µg/L for selenate and 100 µg/L for selenomethionine. In most of our water samples the concentration of selenium was less than 1000 µg/L, however saturation kinetics may still be a mechanism underlying the inverse relationship that we observed between selenium enrichment and exposure concentration.

Most higher organisms have evolved cellular transport systems that actively transport micronutrients across biological membranes, but which eventually saturate following Michaelis-Menten kinetics (Stewart et al. 2010). A similar system for selenium would allow organisms to actively take up selenium when environmental concentrations are low and reduce uptake when environmental concentrations are high. While saturation kinetics helps to explain why enrichment changes over exposure concentration, it remains unclear whether it is the only mechanism regulating selenium
uptake or if another mechanism, such as competitive inhibition with sulphate, may also be at play.

Despite the inverse relationship between exposure concentration and the enrichment factor, that is consistently observed in the literature, ratio-based selenium models continue to assume proportionality and use fixed distribution coefficients (Presser & Luoma 2010; Presser 2013). Ratio-based bioconcentration models are ideal for predicting passively partitioning chemicals such as lipophilic organic compounds, but they can be limited when predicting the uptake of metals and metalloids. A single ratio does not consider the complex physiology that has evolved in many organisms to deal with the uptake, storage, and elimination of trace elements such as selenium.

A consequence of assuming a fixed distribution coefficient is that when the concentration of selenium in water is below a certain concentration value, enrichment will be underestimated, and when the concentration of selenium in water is above that value, enrichment will be overestimated. The specific value, at which underestimation or overestimation of enrichment will occur, will vary regionally according to the site-specific relationship between EF and the concentration of selenium in water. However, if the regression equation between EF and the concentration of selenium in water is known, the site-specific value can be determined by setting ‘y’ equal to log₁₀ EF and solving for [Se]Water (Equation 3). Thus in the Elk Valley, which has a mean EF of 477, the value at which underestimation or overestimation will occur is 5.82 µg/L. In Figure 4 (Appendix B), the geometric mean EF is depicted by the grey dotted line, and the associated value is shown by the red dotted line. If EF is assumed to be constant across exposure concentrations, it will be underestimated when the concentration of selenium in water is less than ~6 µg/L and overestimated when the concentration of selenium in water is greater than ~6 µg/L (Figure 4 – Appendix B). Using the method described above for Kemess, enrichment will be underestimated or overestimated at a value of 11.2 µg/L (Figure 4 – Appendix B); and for Peace River Coal enrichment will be underestimated or overestimated at a value of 0.817 µg/L (Figure 4 – Appendix B)

\[
\log(\text{EF}) = \beta_0 + \beta_1 \times \log([\text{Se}]_{\text{Water}})
\]  

(3)
Inaccurately predicting the enrichment of selenium may have environmental or economic consequences. At low concentrations, under-predicting enrichment may not be a problem since low concentrations of selenium are typically safe for higher vertebrates, and at higher (potentially hazardous) concentrations enrichment will be overestimated – adding an extra degree of environmental conservatism. However, from an economic perspective, overestimating selenium enrichment may result in unnecessary restrictions that cause economic loss. Also, at concentrations close to the Water Quality Guidelines (WQG), inaccurate predictions may lead to an incorrect assessment of risk.

In British Columbia, the selenium WQG is 2 µg/L (BCMoE 2014). Table 3 (Appendix A), reports the concentration of selenium in periphyton when the concentration of selenium in water is equal to the BC WQG and enrichment is either assumed constant or variable over exposure concentration. At 2 µg/L, assuming constant enrichment will underestimate the concentration of selenium in periphyton by by 1.33 mg/kg in the Elk Valley – 52% less than is estimated when exposure concentration is allowed to vary (Table 3 – Appendix A and Figure 3 – Appendix B). Similarly, the concentration of selenium in periphyton will be underestimated by 3.07 mg/kg (71%) in the Kemess region, and overestimated by 1.46 mg/kg (95%) in the Peace River Coal region. Thus, modeling EF as a fixed ratio, rather than as a variable function, increases the risk of underestimating or overestimating enrichment – and subsequently, risk.

Assuming a fixed EF ratio may have implications for risk management, especially when EFs are used to estimate the tissue concentration of selenium in higher trophic levels or when they are used to back-calculate WQGs from acceptable selenium tissue concentrations. As is demonstrated above, assuming a fixed EF will underestimate the concentration of selenium in periphyton in two of our three regions (Elk Valley and Kemess) at the current provincial WQGs. When developing WQGs, it is critical that resource managers minimize the chance of false negatives (type II errors) if they are to protect ecosystems. Thus, it is preferable to overestimate risk and err on the side of caution, than it is to underestimate risk and incorrectly assume that a guideline is safe.
DeForest et al. (2007) compared theoretical tissue concentrations, extrapolated from the chronic aquatic WQG using either a ratio-based or regression based bioaccumulation factor (BAF), against a tissue threshold for selenium proposed by Heinz et al. (1989). They found that estimates of the ratio-based BAF (calculated as the geometric mean) surpassed the tissue threshold whereas estimates of the regression based BAF fell just below the same threshold. From these findings, DeForest et al. (2007) concluded that it is important to account for exposure concentration when modeling the uptake of selenium, and that risk can be overestimated when using fixed ratio-based BAFs.

In the absence of more complete data a single ratio-based EF can provide an estimate of bioaccumulation but the limitations of assuming a fixed EF must be explicitly considered and addressed. Regression models, which capture the inverse relationship between EF and exposure concentration, are superior to ratio-based models at predicting selenium bioaccumulation, so whenever possible they should be used to estimate enrichment at the base of the food web.

### 3.3. Effect of ambient sulphate concentrations on the enrichment of selenium

#### 3.3.1. Model selection with $\text{AIC}_c$

Comparison of eight linear regression models with $\text{AIC}_c$ revealed model $\text{I}$ ($\log[\text{Se}]_p = \beta_0 + \beta_1 * \log[\text{Se}]_w + \beta_2 * \text{Region}$) as the best of the candidate models for predicting the concentration of selenium in periphyton (Table 3 – Appendix A). Thus the concentration of selenium in water and region, but not the concentration of sulphate in water, are important factors for predicting the concentration of selenium in periphyton. Model $\text{I}$ has the lowest AIC ($\text{AIC}_I = 2.73$) and is the only model with a $\Delta \text{AIC} < 2$ ($\Delta_I = 0$); making it the only candidate model with strong support. Model $\text{I}$ also has the largest Akaike weight ($w_I = 0.51$) meaning there is a 51% probability that model $\text{I}$ is the best candidate model for predicting the concentration of selenium in periphyton.
Model$_2$ \( (log[Se]_p = \beta_0 + \beta_1 \times log[Se]_w + \beta_2 \times Region + \beta_3 \times log[Se]_w \times Region) \) has the second lowest AIC (AIC$_2 = 4.85$) and is thus ranked as the second best model. The log-likelihood of model$_2$ is slightly higher than model$_1$ (LL = 3.04 versus 2.94) indicating a better fit, however model$_2$ is less parsimonious (it contains one extra parameter – i.e. the interaction term between the [Se]$_{water}$ and region). When both parsimony and wellness of fit are accounted for there is only weak evidence that model$_2$ is the best model \( (\Delta_2 = 2.12 > 2) \). Akaike weights give an 18% probability of model$_2$ being the best model \( (w_2 = 0.18) \); and the evidence ratio \( (w_1/w_2) \) between the top two models is 2.83, indicating that model$_1$ is 2.83 times more likely than model$_2$ to predict the concentration of selenium in periphyton.

Model$_3$ \( (log[Se]_p = \beta_0 + \beta_1 \times log[Se]_w + \beta_2 \times log[SO_4]_w + \beta_3 \times Region) \) is the best candidate model that includes a term for sulphate (AIC$_3 = 5.02$). Model$_3$ is comparable to model$_2$, but has slightly less evidence supporting it and thus has a lower overall ranking (Table 3 – Appendix A). Model$_3$ has a lower log-likelihood than model$_2$ \( (LL_3 = 2.95 \text{ versus } LL_2 = 3.04) \), a higher \( \Delta \text{AIC} \) \( (\Delta_3 = 2.29 \text{ versus } \Delta_2 = 2.12) \), and a lower Akaike weight \( (w_3 = 0.16 \text{ versus } w_2 = 0.18) \). In comparison to the top model, the evidence ratio \( (w_1/w_3) \) indicates that model$_1$ is 3.12 times more likely than model$_3$ to be the best model for predicting the concentration of selenium in periphyton.

Models 4 through 8 have high \( \Delta \text{AICs} \) and low Akaike weights (Table 3 – Appendix A). There is no evidence supporting these other models.

The variance inflation factor (VIF) of each model parameter is listed in Table 4 (Appendix A). The VIF measures the variance of the estimate (regression coefficient) compared to what is expected if the variable is completely independent from all other predictor variables (Schwartz 2014c). A low VIF (around one) indicates that the predictor variable is not collinear with any other predictors, and a high VIF (around 10) indicates a high degree of collinearity.

All variables in multivariate models should have VIFs less than 10 (Schwartz 2014c) – although some authors recommend this cut off to be as low as three (Zuur et al. 2010). In our analysis, all models containing a term for sulphate had VIFs > 7 for aqueous selenium, aqueous sulphate, and all interaction terms involving these two
variables (Table 4 – Appendix A). Of the linear models tested, model₃ is the only one containing sulphate with a $\Delta$AIC close to two ($\Delta_3 = 2.29$) – in other words, it is the only model with sulphate that has weak evidence of support. However, model₃ has a high VIF for aqueous selenium (7.17) and a high VIF for sulphate (7.01), indicating considerable collinearity. This collinearity between selenium and sulphate water concentrations makes it difficult to identify the independent affect of each variable on the concentration of selenium in periphyton. The limitations and consequences of using AIC with collinear data are discussed in section 3.3.5.

3.3.2. Sulphur-selenium ratio

In order to assess the relationship between collinear variables, the concentration of selenium in water, the S:Se concentration ratio, and the EF were plotted against each other for each region (Figure 5 – Appendix B). In the Elk Valley, EF decreases (circles get smaller) as the concentration of selenium in water increases; this inverse trend between enrichment and exposure concentration was noted earlier. Also, small EFs are associated with low S:Se concentration ratios and large EFs are associated with high S:Se concentration ratios. This appears to be opposite to what we would expect if sulphate inhibition were a major mechanism affecting EF. If competition with sulphate ions reduces the enrichment of selenium, we would expect more accumulation (i.e. larger EFs) at low S:Se ratios when there are fewer sulphate ions (relative to selenium) competing for uptake. Conversely, we expect less accumulation (i.e. smaller EFs) at higher S:Se ratios when there are relatively more sulphate ions competing for uptake. However, in the Elk Valley we see the opposite – a decrease in EF with a decrease in S:Se; this suggests that sulphate inhibition is either not occurring, or that other processes, such as collinearity or autocorrelation, are overshadowing it. For Peace River Coal, concentrations of selenium in water range from 0.236 µg/L to 5.47 µg/L. There is a slight inverse relationship between EF and the concentration in water, but no detectable relationship between EF and S:Se. (Figure 5 – Appendix B). Combined, the observations in the Elk Valley and Peace River Coal suggest that EF is more likely to decrease in response to an increase in exposure concentration than to an increase in the S:Se concentration ratio. However autocorrelation between the S:Se ratio, EF and the concentration of selenium in water, increases the apparent relationship between
variables, and collinearity between the concentration of sulphate in water and the concentration of selenium in water confound these results. More data is needed, across a broader range of concentrations and S:Se ratios, to better test these observations.

The observation above is contrary to the common acceptance, maintained by laboratory studies, that selenium and sulphate are antagonistic with one another and that high concentrations of sulphate in water reduces selenium bioavailability and bioaccumulation (Simmons & Wallschlager 2005). For example, Hansen et al. (1993) evaluated the effect of varying sulphate concentrations on the bioaccumulation of selenium, in the invertebrates Chironomus decorus and Daphnia magna. The authors exposed C. decorus and D. magna, over a period of 48 hours, to selenate concentrations of 5.92 mg/L and 0.71 mg/L respectively, and selenium-sulphur molar ratios (Se:S) ranging from 1:0 to 1:480. They concluded that increasing sulphate ion concentrations significantly decreased the bioaccumulation of selenium in both species. In a similar study, Williams et al. (1994) found that increased sulphate concentrations were linked to significantly lower selenate uptake and increased growth in the green alga Selenastrum capricornutum. Their results showed that at the low selenium treatment (10 µg/L), increasing sulphate from 3.3 to 33 mg/L caused a greater than four-fold decrease in algal accumulation; while at the high selenium treatment (100 µg/L) the same increase in sulphate resulted in a 14-fold decrease in algae accumulation. These results suggest that as the concentration of selenium in water increases, sulphate becomes more effective at inhibiting selenium uptake. Yet another study (Fournier et al. 2010), found that selenium bioaccumulation in the unicellular green alga Chlamydomonas reinhardtii was ten times higher when ambient sulphate concentrations were low (8 µmol/L) compared to when they were high (80 µmol/L). The authors also assert that toxicity in C. reinhardtii, is directly related to the concentration of sulphate in water, which competes with selenium for uptake.

Following from these studies, some researchers believe that the ‘mediating effect’ of sulphate ions should be considered when deriving WQG. In the US, the WQG for selenium is 5 µg/L, which was derived by the US EPA from data with low ambient sulphate concentrations. If sulphate buffers selenium bioaccumulation then 5 µg/L may be overly conservative (USEPA 2004; DeForest et al. 2014).
However, Hansen et al. (1993) and Williams et al. (1994) explicitly, and Fournier et al. (2010) implicitly, acknowledge the limitations of extrapolating their findings to real world environments. For example, Hansen et al. (1993) concludes that “The effect of sulphate on the accumulation of Se through biomagnification is still unknown” and “it does not appear that we have sufficient evidence to justify the consideration of sulphate as a factor in the regulation of Se in aquatic environments.”

In 1997, the U.S. Fish and Wildlife Service reviewed the literature on selenium-sulphate antagonism and found no evidence that increasing sulphate significantly reduces the bioconcentration of selenium in the field (White 1997; Skorupa 1998). In 1978, Birkner analyzed water, sediment, and tissue selenium samples from thirty lentic sites in the US and found no evidence of ambient sulphate influencing selenium bioaccumulation – despite dissolved sulphate ranging from 5 to 9611 mg/L across his sites. In Birkner’s study, no correlation was found between aqueous selenium and sulphate concentrations. Another bioaccumulation study, conducted by the Fish and Wildlife Service over the 1980’s and 90’s analyzed a paucity of field data looking for selenium sulphate antagonism in real aquatic environments. Their data, collected from agricultural evaporation ponds, expanded Birkner’s (1978) work and included sulphate concentrations ranging from 5 to 100,000 mg/L. They still did not find significant evidence of selenium sulphate interference (White 1997). Skorupa (1998) observed that 7 out of 12 of the major toxic events involving selenium occurred in areas with high ambient sulphate; however, despite these sites being dominated by selenate, they were lentic environments where bioconcentration is typically greater due to the presence of at least some selenite. Neither White (1997) nor Skorupa (1998) specifically report on whether or not their studies were affected by collinearity between the concentrations of selenium and sulphate in water.

White (1997) attributes the discrepancy between laboratory and field studies to the “lab-to-field-dilemma” (Landis & Yu 1995), where overly simplistic laboratory methods do not extrapolate to real world environments. However, understanding the components of bioaccumulation that differ between lab and field studies may yield a more satisfying answer. Some of these differences include the duration of the study, the limitations of extrapolating from lentic to lotic systems, the limitations of field data, the
potential collinearity between selenium and sulphate, and the inverse relationship between enrichment and exposure concentrations that was described above.

3.3.3. Study duration

The laboratory studies cited above all measured the effects of sulphate on selenium bioaccumulation over an acute timespan between 48 (Hansen et al. 1993) and 96 (Williams et al. 1994; Fournier et al. 2010) hours. Thus they did not account for chronic exposures or long-term selenium cycling, which are inherent properties of field studies. Over time, selenate is transformed into other forms of environmental selenium (such as selenite or organo-selenide), which are not inhibited by sulphate and which are more bioavailable. Selenite and organo-selenide have a much greater propensity to bioaccumulate than selenate (Orr et al. 2012; BCMoE 2014) and even a small amount of these selenium species in the environment could mask the sulphate inhibition observed in bench-top studies. The field studies by Birkner (1978), White (1997), and Skorupa (1998) are predominantly from lentic regions, and thus would all have a greater proportion of selenite than would typically be found in lotic waters; therefore extreme care must be taken when extrapolating their findings to lotic environments.

3.3.4. Data limitations

One limitation of using pre-collected field-data is the inability to manipulate environmental conditions to yield specific experimental treatments. In our study, S:Se concentration ratios ranged from 771 to 18,643 with a geometric mean of 3,454. 90% (64 of 71) of the water samples had selenium concentrations less than 100 µg/L, and only three samples had selenium concentrations greater than 500 µg/L – all of which were associated with sulphate concentrations greater than 1000 mg/L. Consequently our power for predicting high-selenium low-sulphate situations is limited. In a bench top study examining multiple treatment combinations of selenium and sulphate, Ogle and Knight (1996) observed sulphate interference but their results were heavily influenced by a single treatment – representing the highest concentration of selenate (500 µg/L) and the lowest concentration of sulphate (0 mg/L). The signal generated by this treatment was key to their conclusions and highlighted the importance
of systematically testing multiple treatment groups. It is possible we did not observe sulphate antagonism because our dataset was missing the treatment combinations (i.e. high selenium low sulphate) that would trigger this signal, however White (1997) analyzed selenium-sulphate interference over a larger range of sulphate concentrations and still did not find evidence of interference. Nevertheless, systematically collecting data that covers a broader range of selenium and sulphate water concentrations and ratios is recommended for future field studies.

### 3.3.5. Collinearity between selenium and sulphate

In multiple linear regressions, regression coefficients give insight into the marginal contribution of each parameter to the outcome of the model. However, when two variables are collinear, one can mask the relative contribution of the other and this can lead to problems interpreting model results (Schwartz 2014c). The high VIFs in models containing sulphate indicated collinearity between selenium and sulphate water concentrations. This collinearity is one reason we do not have any high-selenium low-sulphate water samples. As the concentration of selenium in water increased, the concentration of sulphate in water also increased, and this makes it difficult to identify the independent effect of each variable on the EF.

It is important to note that the results of our AIC are confounded by the collinearity between selenium and sulphate in water (Figure 6 – Appendix B). Since aqueous selenium and sulphate are collinear, the marginal contribution of adding sulphate to a model that already contains a term for selenium in water will be low – regardless of the true relationship between sulphate and enrichment. In addition, to be biologically relevant, all models had to include a term for selenium in water, precluding an independent analysis of the effect of sulphate alone. The results of our AIC indicate that including the concentration of sulphate in water does not help explain enrichment. However, given the above stated limitations, it is not possible with this analysis to tell whether sulphate truly has no effect, or if the effect of sulphate is simply masked by its collinearity with the concentration of selenium in water.
Lab studies can escape the perils of collinearity by setting up specific experimental treatments, however these treatments may not always reflect the real world. Selenium and sulphate are naturally present in the sediment commonly targeted for coal mining, and as waste rock produced by the mining process weathers both elements leach into the aquatic environment (Hauer & Sexton 2013). Thus, determining the independent affect of either element on enrichment is difficult as both are correlated to the 'mining effort'. Future studies considering other geographical regions and other types of mining, such as phosphate or uranium, would help to clarify whether or not collinearity between selenium and sulphate water concentrations is a phenomenon specific to coal mining regions in British Columbia, or whether it can be expected in other areas as well.

3.3.6. Inverse relationship between enrichment and exposure concentration

Most studies examining the effect of sulphate on the bioaccumulation of selenium do not consider the inverse relationship between the concentration of selenium in water and the EF. Since the concentrations of selenium and sulphate in water are highly correlated ($r^2 = 0.85$ and 0.75 for the Elk Valley and PRC regions respectively (Figure 6 – Appendix B)), caution is recommended when examining the effect of either variable on its own. When exposure concentration is excluded from the analysis it can easily appear that increasing sulphate concentrations cause a reduction in EF; however, the effect of exposure concentration and the collinearity between aqueous selenium and sulphate must explicitly be considered when assessing the effect of sulphate on EF in the field.

4.0 Conclusions

The results from this study show that selenium enrichment is inversely related to exposure concentration and that regression-based models outperform ratio-based
models at predicting the enrichment of selenium from water to periphyton. However our prediction, that regression models which account for ambient sulphate are better at predicting the enrichment of selenium, was not supported by our data. Collinearity between the concentrations of selenium and sulphate in water limited our resolution of a clear mechanistic relationship.

Despite compelling evidence from lab-based studies, the effect of sulphate on selenium bioaccumulation in real world environments remains unclear, and thus we caution against using high levels of ambient sulphate as justification for raising selenium water quality guidelines until further information is known. Additional studies, especially well designed field studies that take into account relevant environment concentrations of selenium and sulphate, and that consider selenium speciation, long-term selenium cycling, and the inverse relationship between enrichment and exposure concentration, are required to better understand the effect of sulphate on selenium accumulation in aquatic environments.
References


Appendix A.

Tables

Table 1. Multiple linear regression models compared with \( \text{AIC}_c \)

<table>
<thead>
<tr>
<th>#</th>
<th>A priori model</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>( \log[\text{Se}]_p = \beta_0 ) (Intercept only model)</td>
</tr>
<tr>
<td>2</td>
<td>( \log[\text{Se}]_p = \beta_0 + \beta_1 \times \log[\text{Se}]_w )</td>
</tr>
<tr>
<td>3</td>
<td>( \log[\text{Se}]_p = \beta_0 + \beta_1 \times \log[\text{Se}]_w + \beta_2 \times \log[\text{SO}_4]_w )</td>
</tr>
<tr>
<td>4</td>
<td>( \log[\text{Se}]_p = \beta_0 + \beta_1 \times \log[\text{Se}]_w + \beta_2 \times \text{Region} )</td>
</tr>
<tr>
<td>5</td>
<td>( \log[\text{Se}]_p = \beta_0 + \beta_1 \times \log[\text{Se}]_w + \beta_2 \times \log[\text{SO}_4]_w + \beta_3 \times \text{Region} )</td>
</tr>
<tr>
<td>6</td>
<td>( \log[\text{Se}]_p = \beta_0 + \beta_1 \times \log[\text{Se}]_w + \beta_2 \times \text{Region} + \beta_3 \times \log[\text{Se}]_w + \beta_3 \times \text{Region} )</td>
</tr>
<tr>
<td>7</td>
<td>( \log[\text{Se}]_p = \beta_0 + \beta_1 \times \log[\text{Se}]_w + \beta_2 \times \log[\text{SO}_4]_w + \beta_3 \times \text{Region} + \beta_4 \times \log[\text{Se}]_w \times \text{Region} )</td>
</tr>
<tr>
<td>8</td>
<td>( \log[\text{Se}]_p = \beta_0 + \beta_1 \times \log[\text{Se}]_w + \beta_2 \times \log[\text{SO}_4]_w + \beta_3 \times \text{Region} + \beta_4 \times \log[\text{SO}_4]_w \times \text{Region} )</td>
</tr>
</tbody>
</table>

Models predicting the concentration of selenium in periphyton \([\text{Se}]_p\). Candidate models represent combinations of the variables \([\text{Se}]_w, [\text{SO}_4]_w\), and \text{Region}, that are biologically relevant (i.e. contain \([\text{Se}]_w\)) and have VIFs < 10.

Table 2. Regional regression coefficients for the relationship between selenium concentrations in water and periphyton (log-log scale)

<table>
<thead>
<tr>
<th>Region</th>
<th>Slope*</th>
<th>Intercept*</th>
<th>( r^2 )</th>
<th>( n )</th>
<th>( F )</th>
<th>( p )-value</th>
<th>( T )†</th>
<th>( p )-value†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elk Valley</td>
<td>0.19 A</td>
<td>0.3 a</td>
<td>0.31</td>
<td>68</td>
<td>29.8</td>
<td>&lt; 0.0001</td>
<td>-23.5</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Kemess</td>
<td>0.28 A</td>
<td>0.55 b</td>
<td>0.59</td>
<td>9</td>
<td>9.96</td>
<td>0.016</td>
<td>-8.17</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Peace River Coal</td>
<td>0.25 A</td>
<td>0.11 c</td>
<td>0.14</td>
<td>33</td>
<td>4.9</td>
<td>0.034</td>
<td>-6.6</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

* Values in the column with the same letter are not significantly different \((p > 0.05)\)
† \( T \)-statistic (and associated \( p \)-value) when regression is compared to a slope of one
<table>
<thead>
<tr>
<th>Region</th>
<th>Variable</th>
<th>Constant</th>
<th>Difference (mg/kg dw)</th>
<th>% Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elk Valley</td>
<td>2.28</td>
<td>0.954</td>
<td>-1.33</td>
<td>-58</td>
</tr>
<tr>
<td>Kemess</td>
<td>4.3</td>
<td>1.23</td>
<td>-3.07</td>
<td>-71.4</td>
</tr>
<tr>
<td>Peace River Coal</td>
<td>1.53</td>
<td>2.99</td>
<td>1.46</td>
<td>95.4</td>
</tr>
</tbody>
</table>

Variable and constant enrichment measured at BC’s selenium WQG (i.e. 2 µg/L); Difference calculated as constant – variable enrichment; % Change calculated as ((constant – variable) / variable *100)

<table>
<thead>
<tr>
<th>#</th>
<th>Model</th>
<th>K</th>
<th>LL</th>
<th>AICc</th>
<th>Δi</th>
<th>wi</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>$\log[Se]<em>P = \beta</em>\theta + \beta_1 * \log[Se]_W + \beta_2 * Region$</td>
<td>4</td>
<td>2.94</td>
<td>2.73</td>
<td>0</td>
<td>0.51</td>
</tr>
<tr>
<td>2</td>
<td>$\log[Se]<em>P = \beta</em>\theta + \beta_1 * \log[Se]_W + \beta_2 * Region + \beta_3 * \log[Se]_W * Region$</td>
<td>5</td>
<td>3.04</td>
<td>4.85</td>
<td>2.12</td>
<td>0.18</td>
</tr>
<tr>
<td>3</td>
<td>$\log[Se]<em>P = \beta</em>\theta + \beta_1 * \log[Se]_W + \beta_2 * \log[SO_4]_W + \beta_3 * Region$</td>
<td>5</td>
<td>2.95</td>
<td>5.02</td>
<td>2.29</td>
<td>0.16</td>
</tr>
<tr>
<td>4</td>
<td>$\log[Se]<em>P = \beta</em>\theta + \beta_1 * \log[Se]_W + \beta_2 * \log[SO_4]_W + \beta_3 * Region + \beta_4 * \log[Se]_W * Region$</td>
<td>6</td>
<td>3.04</td>
<td>7.23</td>
<td>4.5</td>
<td>0.05</td>
</tr>
<tr>
<td>5</td>
<td>$\log[Se]<em>P = \beta</em>\theta + \beta_1 * \log[Se]_W + \beta_2 * \log[SO_4]_W + \beta_3 * Region + \beta_4 * \log[SO_4]_W * Region$</td>
<td>6</td>
<td>3</td>
<td>7.32</td>
<td>4.59</td>
<td>0.05</td>
</tr>
<tr>
<td>6</td>
<td>$\log[Se]<em>P = \beta</em>\theta + \beta_1 * \log[Se]_W$</td>
<td>3</td>
<td>-1.04</td>
<td>8.44</td>
<td>5.71</td>
<td>0.03</td>
</tr>
<tr>
<td>7</td>
<td>$\log[Se]<em>P = \beta</em>\theta + \beta_1 * \log[Se]_W + \beta_2 * \log[SO_4]_W$</td>
<td>4</td>
<td>-1.01</td>
<td>10.6</td>
<td>7.91</td>
<td>0.01</td>
</tr>
<tr>
<td>8</td>
<td>$\log[Se]<em>P = \beta</em>\theta$</td>
<td>2</td>
<td>-19.7</td>
<td>43.7</td>
<td>40.9</td>
<td>0</td>
</tr>
</tbody>
</table>

$[Se]_P$ is the concentration of selenium in periphyton (mg/kg dw), $[Se]_W$ is the concentration of selenium in water (µg/L), $[SO_4]_W$ is the concentration of sulphate in water (mg/L), and Region is the location where samples were collected.
Table 5. Variance Inflation Factors for each model parameter used in AICc

<table>
<thead>
<tr>
<th>Model #</th>
<th>$[Se]_{Water}$</th>
<th>$[SO_4]_{Water}$</th>
<th>Region</th>
<th>$[Se]_{Water}: Region$</th>
<th>$[SO_4]_{Water}: Region$</th>
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</thead>
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<tr>
<td>1</td>
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<td>-</td>
<td>1.22</td>
<td>-</td>
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<td>1.23</td>
<td>1.10</td>
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<tr>
<td>3</td>
<td>7.17</td>
<td>7.01</td>
<td>1.22</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>7.28</td>
<td>7.45</td>
<td>1.23</td>
<td>1.17</td>
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<tr>
<td>5</td>
<td>8.00</td>
<td>8.62</td>
<td>7.71</td>
<td>-</td>
<td>7.16</td>
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<td>7</td>
<td>7.01</td>
<td>7.01</td>
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<td>8*</td>
<td>-</td>
<td>-</td>
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<td>-</td>
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</tbody>
</table>

* Models 6 and 8 only have a single term, thus VIFs are not applicable.
Appendix B.

Figures

Figure 1. Relative concentration of selenium across four trophic levels in an aquatic food chain

The Enrichment Function (EF) between water and periphyton (range: $10^2 - 10^6$); the Trophic Transfer Function (TTF_{Prey}) between periphyton and invertebrates (range: 0.6 – 23); and the Trophic Transfer Function (TTF_{Pred}) between predatory fish (or birds) and their invertebrate prey (range: 1 – 3) (Stewart et al. 2010). Note: diagram is not to scale.
Figure 2. Study area showing the Elk Valley, Kemess, and Peace River Coal mining regions of British Columbia, Canada
Figure 3. Relationship between concentrations of selenium in co-located water and periphyton samples

Shading shows 95% CI’s and dotted lines show the expected relationship if the geometric mean EF remains proportional over exposure concentrations.
Figure 4. Relationship between EF and the concentration of selenium in water

Shading shows 95% CI’s; dotted grey line is the geometric mean EF; dotted red line is the threshold at which EF will either be underestimated or overestimated if EF is assumed as a fixed distribution coefficient.
Figure 5. Relationship between the concentration of selenium in water, the sulphur-selenium ratio, and EF
Figure 6. **Collinearity between selenium and sulphate water concentrations**

$[\text{Se}]_{\text{water}}$ and $[\text{SO}_4]_{\text{water}}$ in the Elk Valley (red) and PRC (blue) regions. Size of circles represents the $\log_{10} [\text{Se}]_{\text{periphyton}}$.
Appendix C.

Assumptions

Statistical assumptions

Prior to analyzing the data, the assumptions of homoscedasticity, normality, leverage, and x-axis attenuation were assessed and deemed satisfactory. Diagnostic plots of each test are shown below in Figure 6.

![Diagnostic plots](image)

Figure 7. Diagnostic plots of pooled data were tested for the assumptions of: A) Homoscedasticity: residuals plot shows a random scatter above and below the line of fit; B) normality: Q-Q plot looks reasonable; and C) leverage: no points are exerting unreasonable leverage in Cook’s distance test.

Outliers

Three periphyton samples and two water samples had abnormally high concentrations of selenium and were flagged as potential outliers. The three periphyton samples were collected from Peace River Coal at stations B-2, B-3, and G-2 (Golder 2005); and the two water samples were collected from the Elk Valley at stations ELUCA (McDonald & Strosher 1998) and ER3 (Teck 2013 Lentic Study). All five outliers, which were removed prior to analysis, are clearly indicated in Appendix E: Supporting Information.
Detection limits

The following section shows the sensitivity analysis for choosing detection limits (DL) at full DL, 0.5 DL, 0.1 DL, and zero. Table 5 shows how various DLs affect the slope of enrichment, and Table 6 shows how various DLs affect the results of AIC<sub>c</sub>. Our final analysis assumed 0.5 DL for any values below the DL.

Table 6: Regression slopes measuring relationship between the concentration of selenium in water and the concentration of selenium in periphyton

<table>
<thead>
<tr>
<th>Region</th>
<th>DL</th>
<th>Slope</th>
<th>Intercept</th>
<th>r²</th>
<th>n</th>
<th>F</th>
<th>p-value</th>
<th>T&lt;sup&gt;†&lt;/sup&gt;</th>
<th>p-value&lt;sup&gt;†&lt;/sup&gt;</th>
<th>Note</th>
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<tbody>
<tr>
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<td>0.31</td>
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<td>-23.2</td>
<td>&lt;0.0001</td>
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<td>0.3</td>
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<td>68</td>
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<td>&lt;0.0001</td>
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<tr>
<td></td>
<td>0.1</td>
<td>0.18</td>
<td>0.31</td>
<td>0.31</td>
<td>68</td>
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<td>&lt;0.0001</td>
<td>-24.6</td>
<td>&lt;0.0001</td>
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<td>0</td>
<td>0.19</td>
<td>0.3</td>
<td>0.31</td>
<td>67</td>
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<td>&lt;0.0001</td>
<td>-23</td>
<td>&lt;0.0001</td>
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<td>Kemess</td>
<td>Full</td>
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<td>0.6</td>
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<td>10.4</td>
<td>0.015</td>
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<td>0.0003</td>
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<td>0.59</td>
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<td>9.96</td>
<td>0.016</td>
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<tr>
<td></td>
<td>0.1</td>
<td>0.18</td>
<td>0.71</td>
<td>0.57</td>
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<td>0.019</td>
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<td>0.062*</td>
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<td>Full</td>
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<td>33</td>
<td>7.8</td>
<td>0.009</td>
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<td>0.1</td>
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<td>0.1</td>
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<td>0.078*</td>
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</table>

<sup>†</sup> Test statistic and p-value when slope is compared to a slope of one
<sup>*</sup> The choice of DL resulted in these values being different
<sup>1</sup> Changing the detection limit does not affect any of the results in the Elk Valley
<sup>2</sup> Slope changes significantly. However, assuming values below the detection limit were equal to zero eliminated 3 values from the analysis. This reduced Kemess’s sample size from 9 to 6, which has very low power
<sup>3</sup> Slope is no longer significantly different from zero but still is significantly different from one
Table 7: The effect of various detection limits on model selection using AICc

<table>
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<tr>
<th>DL</th>
<th>Model</th>
<th>K</th>
<th>LL</th>
<th>AICc</th>
<th>Δi</th>
<th>w_i</th>
<th>Note</th>
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<td>Full</td>
<td>( \log[Se]_p = \beta_0 + \beta_1 \times \log[Se]_t + \beta_2 \times \text{Region} )</td>
<td>4</td>
<td>3.03</td>
<td>2.55</td>
<td>0</td>
<td>0.48</td>
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<tr>
<td></td>
<td>( \log[Se]_p = \beta_0 + \beta_1 \times \log[Se]_t + \beta_2 \times \text{Region} + \beta_3 \times \log[Se]_t \times \text{Region} )</td>
<td>5</td>
<td>3.4</td>
<td>4.12</td>
<td>1.56*</td>
<td>0.22</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>( \log[Se]_p = \beta_0 + \beta_1 \times \log[Se]_t + \beta_2 \times \log[SO_4]_t + \beta_3 \times \text{Region} )</td>
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<td>4.83</td>
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<td>( \log[Se]_p = \beta_0 + \beta_1 \times \log[Se]_t + \beta_2 \times \log[SO_4]_t + \beta_3 \times \text{Region} )</td>
<td>5</td>
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<td>2.25 0.16</td>
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<td>6</td>
<td>1.67</td>
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<td>4.63 0.05</td>
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<td>43.07</td>
<td>37.67 0</td>
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</table>

4 Model includes an interaction term, which we know from testing a larger dataset is not important. Order of models is still the same and \( \Delta \text{AIC}_c \) of the best model with sulfate is still > 2.

5 DL used for analysis

6 Model order has changed, but top model remains the same and \( \Delta \text{AIC}_c \) of second best model is still > 2.
Appendix D.

Introduction to Akaike Information Criterion (AIC)

Information-theoretic approaches using Akaike Information Criterion (AIC) is a method of statistical computing that differs from frequentist statistics in the way that it tests hypothesis (Burnham & Anderson 2001). AIC calculates the probability of the hypothesis given the data, whereas frequentist statistics calculates the probability of the data given the hypothesis. For example, the $p$-value in frequentist statistics is defined as the probability of getting your results (or more extreme results) given that the null hypothesis is true (Schwartz 2014b). In other words, it calculates the probability of the data (the test-statistic), given the (null) hypothesis. AIC on the other hand, calculates the likelihood of multiple competing hypotheses given a particular dataset. For example, it can compare a number of competing models (multiple hypotheses) and calculate the likelihood of each model given the data. Instead of calculating a $p$-value and either supporting or rejecting a single hypothesis, AIC gives evidence supporting a ‘best’ inference – given the data and a set of a priori models (Burnham & Anderson 2001). First proposed in 1973 (Akaike) AIC has become a common tool in wildlife biology and ecology for elucidating relationships between multiple predictor and response variables (Symonds & Moussalli 2011).

AIC measures the relative support for competing models, by balancing the trade-offs between model simplicity and model fit (Burnham and Anderson 2001; Crawley 2005). Model simplicity (or parsimony) is based off the number of model parameters (i.e. intercept, slope coefficients and variance term); all else equal, a model with fewer parameters will have a lower AIC than a model with more parameters. Model fit is determined by calculating the maximum log-likelihood of the model fitting the data; for an equal number of parameters, a better fitting model will have a lower AIC than a worse fitting model. AIC combines parsimony and fit into a single value that can be compared and ranked against other candidate models. A low AIC indicates a better ranking than a high AIC, so the model with the lowest AIC (minAIC) is selected as the best candidate model given the empirical data at hand (Burnham & Anderson 2001).

AIC is calculated using Equation 4, where $\ln$ is the natural logarithm, $\text{Likelihood}$ is the probability of the model fitting the data, and $K$ is the number of parameters (i.e. the intercept, number of slope parameters, and the residual variance).

$$
AIC = -2\ln(\text{Likelihood}) + 2K
$$

(AIC) is a form of AIC that accounts for the bias introduced at smaller sample sizes (Burnham & Anderson 2001). $AIC_c$ is typically used when $n < 40$, but it can also be used with larger sample sizes. When $n$ is large $AIC_c$ will give the same results as AIC, because the extra term on the right of equation 5 approaches zero as the size of the dataset ($n$) increases relative to the parameters ($K$) (Burnham & Anderson 2001).

$$
AIC_c = -2\ln(\text{Likelihood}) + 2K + \frac{2K(K+1)}{n-K-1}
$$
Delta AIC (ΔAIC or Δ_i) and Akaike weights (w_i) are two indicators used to compare candidate models (Mazerolle 2004). ΔAIC (Equation 6) is a relative measure of each model and is calculated as the difference between each model (AIC_i) and the best model (minAIC). Support for each model decreases as ΔAIC increases, and as a rule of thumb, a ΔAIC > 2 suggests weak evidence for the model (Burnham & Anderson 2001). Thus if the ΔAIC of the second best ranking model is > 2 there is little evidence to support it as a competing model – and thus strong evidence to support the top model only.

\[ \Delta AIC = \Delta_i = AIC_i - \text{minAIC} \]  

(6)

Akaike weights (w_i) report the probability of each model being the best among candidate models. Akaike weights (Equation 7) are derived from ΔAIC, but scaled from zero and one. Akaike weights can also be used to calculate evidence ratios (Equation 8), which are in turn used to compare the relative strength between models.

\[ \text{Akaike weight} = w_i = \frac{\exp(-\Delta_i/2)}{\sum_{j=1}^{R} \exp(-\Delta_j/2)} \]  

(7)

\[ \text{Evidence ratio} = \frac{w_j}{w_i} \]  

(8)

Strengths and Limitations of AIC

One of AIC's largest strengths is as a tool for model selection particularly with non-nested models, or when more than three models are compared (Mazerolle 2004). In traditional hypothesis testing the order in which models are tested is made subjectively, whereas in AIC models are compared simultaneously (Burnham & Anderson 2001). Thus when selecting between non-nested linear-regressions models, AIC can be advantageous over hypothesis testing as it gives results that are independent of the order in which models are compared. Another advantage of AIC is that it supplies several lines of evidence (e.g. ranking of models, Akaike weights, and evidence ratios) rather than just a dichotomy of 'reject or not'.

A limitation of AIC is that it is only a relative ranking restricted to the set of candidate models chosen a priori. Thus, AIC values can never be compared across data sets. Additionally, there will always be a 'best' model even if all of the models are a poor fit for the data. Discretion and experience must direct which models are biologically relevant to compare.
Appendix E.

Supporting Information

A summary of raw data is available upon request.