Integrated Environmental Assessment and Management — Volume 00, Number 00—pp. 1–9
Received: 18 December 2023 | Revised: 19 March 2024 | Accepted: 17 April 2024

Environmental Policy & Regulation

The relationship between cellular protein content and selenium accumulation in freshwater microalgae

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EDITOR'S NOTE:

This article is part of the special series "Regulatory Issues Surrounding the Management of Selenium." This series presents a collection of views from North American experts on the aquatic behavior of selenium and the environmental, physiological, and operational factors that need to be considered for Se regulatory frameworks.

Abstract

Variability in the bioconcentration of selenium (Se) by primary producers at the base of the food web results in uncertainty in predictions of bioaccumulation and ecological risk to higher trophic level organisms. Water chemistry, speciation of Se, and periphyton community composition have all been suggested as factors that contribute to variability in bioconcentration by primary producers; however, the role of physiological composition of periphyton species in influencing the bioconcentration of Se has not been previously evaluated. To determine if a relationship exists between algal protein content and Se accumulation, *Parachlorella kessleri*, *Chlorella vulgaris*, and *Raphidocelis subcapitata* were exposed to Se (as selenate) and analyzed for total protein and tissue Se content in the exponential and stationary growth phases. Protein content and Se accumulation in *R. subcapitata* in the stationary phase were also measured under two light intensities. No relationship between cellular protein content and Se accumulation was found for algae in the exponential phase; however, a strong relationship was found in the stationary phase among species and for *R. subcapitata* under differing light intensities. Absolute Se accumulations by *P. kessleri*, *C. vulgaris*, and *R. subcapitata* in the stationary phase were statistically different; however, the concentrations of Se in protein were similar across species. These results suggest that cellular protein content in microalgae influences Se bioconcentration and that algal protein content may improve Se bioaccumulation modeling in food webs. *Integr Environ Assess Manag* 2024;00:1–10. © 2024 The Authors. *Integrated Environmental Assessment and Management* published by Wiley Periodicals LLC on behalf of Society of Environmental Toxicology & Chemistry (SETAC).

KEYWORDS: Bioaccumulation; Cellular protein; Microalgae; Normalization; Selenium

INTRODUCTION

Selenium (Se) is a naturally occurring element in rocks and soils, most notably black shale, phosphate-bearing rocks, and coal (Presser et al., 2004). In aquatic environments, it exists in various forms, most commonly as selenate (SeO_4^-) and selenite (SeO_3^{2-}; Young et al., 2010), but it can also form elemental Se, selenide, and methylated Se (Orr et al., 2006). Mobilization of selenate and selenite into aquatic environments occurs through natural processes (e.g., weather and erosion, volcanic eruptions; Davis et al., 1988; Etteieb et al., 2020); anthropogenic activities such as mining (e.g., phosphate, uranium, coal), power generation, and irrigation are associated with greater mobilization into the environment (Etteieb et al., 2020; Schneider et al., 2015).

Once mobilized, Se has a propensity to bioconcentrate into primary producers and bioaccumulate through the diet in higher trophic levels. Aquatic organisms can have tissue concentrations that are orders of magnitude higher than those in the water column (Ponton et al., 2020). Conversion of inorganic (e.g., selenate and selenite anions) into organic forms is performed by primary producers, where active uptake followed by substitution of sulfur in amino acids creates the amino acids selenocysteine and selenomethionine (Etteieb et al., 2020). The incorporation of Se-containing amino acids into proteins by primary producers promotes its transfer in the food web (Sunde, 1984); Se moves through the trophic levels via the diet and concentrates to levels far higher than those that would result from waterborne uptake alone (Schneider et al., 2015). The primary concern of Se

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Published 24 May 2024 xxxx on wileyonlinelibrary.com/journal/ieam.

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 DOI: 10.1002/ieam.4946 © 2024 The Authors

bioaccumulation in aquatic ecosystems is the potential for effects that can occur in oviparous vertebrates, including reduced growth, embryo–larval developmental effects (teratogenesis), and diminished immunity (Etteieb et al., 2020).

Due to the importance of Se dietary transfer between trophic levels, modeling the relationship between aqueous Se concentrations and the resulting tissue concentrations at higher trophic levels is important from a regulatory and risk management perspective. To that end, Presser and Luoma (2010), and more recently DeForest et al. (2017), have developed models that predict tissue concentrations under different water quality conditions and food web biodynamics. A persistent challenge for these models is variability in Se bioconcentration at the primary producer level (Conly et al., 2013). This is reflected in calculated enrichment factors (EFs; [tissue Se in primary producers]/[Se in water column]) that range from 10^2 to 10^6 (Stewart et al., 2010). In contrast, trophic transfer factors (TTFs) are relatively consistent among taxa: 0.94–3.2 (3.4-fold range) in invertebrates and 0.8–1.7 (2.1-fold range) in fish (Presser & Luoma, 2010). The ecosystem-scale modeling of Se described by Presser and Luoma (2010) emphasizes that empirical observations of Se partitioning from the water to the base of the food web are recommended due to the wide variability in EFs. However, without reliable EF values, modeling concentrations rely on a series of assumptions that can produce erroneous conclusions.

Despite being the primary drivers for the extent of Se bioaccumulation in the food web, factors that influence Se uptake in particulate material (e.g., phytoplankton, periphyton, detritus, inorganic suspended material, biofilm, sediment, or attached vascular plants) continue to require investigation (DeForest et al., 2017; Presser & Luoma, 2010). Numerous variables have been investigated for their influence on the accumulation of Se in phytoplankton and/or periphyton, including Se water concentration, Se speciation, sulfate concentration, periphyton community composition, dissolved inorganic carbon, and total dissolved phosphorus, among other variables (Gojkovic et al., 2015; Lo et al., 2015; Markwart et al., 2019; Mendes et al., 2022; Oldach et al., 2022; Orr et al., 2006; Sandholm et al., 1973; Schneider et al., 2015).

A factor that has received no attention is the potential role of cellular protein concentration in Se accumulation by microalgae. Phytoplankton rapidly and efficiently accumulates inorganic forms of Se and biotransforms them into selenoamino acids, which are incorporated into proteins (Ponton et al., 2020). Thus, it is hypothesized that protein concentration may partly explain the differences in algal Se uptake that can occur between species under the same exposure conditions. Algal species with higher protein content might be expected to accumulate more Se, similar to other protein components, such as nitrogen and sulfur. If the cellular protein content contributes to Se uptake and bioconcentration at the base of the food web (e.g., algae), it could provide a variable by which to predict EFs more accurately.

The freshwater unicellular Chlorophyta species used in this study (*Parachlorella kessleri*, *Chlorella vulgaris*, *Raphidocelis subcapitata*, and *Tetrasdesmus obliquus*) are representative of green algae that are found ubiquitously in Se contaminated water systems in North America, making up a significant proportion of exposure to higher trophic level organisms (McLarnon-Riches et al., 1998; Simmons & Wallschläger, 2011). Therefore, the aim of this study was to determine if Se accumulation is correlated with cellular protein concentrations in these species and whether culture conditions such as light intensity affect protein concentration and resulting Se accumulation.

METHODS

Exposures were carried out at Nautilus Environmental (Calgary, AB) in a temperature- and photoperiod-controlled environmental chamber. The chemicals used were of reagent grade. Microalgae species were obtained from the Canadian Phycological Culture Centre (CPCC; Waterloo, ON). As per guidance from CPCC, the media for all exposures was a modified recipe for Bold's basal medium (BBM; Stein, 1983). The BBM was modified using $FeCl_3{\cdot}6H_2O$ rather than $FeSO_4{\cdot}7H_2O$ and was composed of: KH_2PO_4, 0.13 mM; $CaCl_2{\cdot}2H_2O$, 0.17 mM; $MgSO_4{\cdot}7H_2O$, 0.30 mM; $NaNO_3$, 2.94 mM; K_2HPO_4, 0.43 mM; NaCl, 0.43 mM; $Na_2EDTA{\cdot}2H_2O$, 0.03 mM; $FeCl_3{\cdot}6H_2O$, 0.59 μM; H_3BO_3, 0.39 mM; $MnCl_2{\cdot}4H_2O$, 9.15 μM; $ZnSO_4{\cdot}7H_2O$, 0.77 nM; $Na_2MoO_4{\cdot}2H_2O$, 1.61 nM; $CuSO_4{\cdot}5H_2O$, 0.32 nM; $Co(NO_3)_2{\cdot}6H_2O$, 0.17 nM.

Aliquots of starter cultures obtained from the CPCC were placed on solid medium slants and stored at 4 ± 2 °C in darkness according to Environment Canada (2007) methods for culturing algae. Axenic cultures of 200 mL were established by inoculating algal cells from the slants into the BBM media. Flasks were placed on an orbital shaker plate and shaken at 100 rpm under continuous 3600–4400 lux cool lighting at 25 ± 2 °C. Cell counts were performed using an L10 Coulter counter (Beckman-Coulter) with previously established cell size thresholds.

The cultures were maintained and evaluated for health (visual assessment of color, cell shape, and integrity) using a compound microscope (Environment Canada, 2007). Cultures were renewed every seven days by transferring 1 mL of culture containing ~3×10^6 cells of each species to a 1-L baffled Erlenmeyer flask containing 200 mL of fresh sterile growth media. Growth curves for each species were generated by performing daily cell counts. The timing of the three growth phases (lag, exponential, and stationary) were identified visually on the resulting growth curve figure as described by Environment Canada (2007).

Experimental design

The first experiment was performed to determine the total protein concentration of each species at each growth phase established during culture. Experiments were conducted in 1 L glass Erlenmeyer test flasks containing 792 mL of modified BBM and 8 mL of sterile culture. Test flasks ($n = 7$) were

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exposed to identical conditions as those stated for culturing, with the exception that flasks were not placed on a shaker plate and instead were continuously aerated by daily rotations of flasks and manual shaking to prevent algae from settling. Protein analysis was performed at each growth phase (lag, exponential, and stationary), which occurred on Days 2, 3, and 7 for *P. kessleri* and *C. vulgaris*, and on Days 3, 7, and 10 for *R. subcapitata* and *T. obliquus*, as confirmed by cell counts. For each growth phase and replicate, 40 mL was subsampled for protein analysis, and a separate 40 mL was subsampled for dry weight.

A second experiment was performed to determine if Se exposure altered the total protein content of the four algal species in the exponential and stationary phases. Test flasks ($n = 3$) contained 792 mL of modified BBM amended to 40 µg/L Se (added as Na_2SeO_4) before inoculation of algae into the test flasks. A negative control with modified BBM without amended Se was also tested. The conditions for this experiment were the same as those described in the first experiment. At exponential and stationary growth phases for each species (confirmed by cell counts), 40 mL was subsampled for protein analysis, and a separate 40 mL was subsampled for dry weight.

In a third experiment, the relationship between total protein content and tissue Se in the exponential and stationary phases was examined. Se exposure with modified BBM amended to 40 µg/L Se (added as Na_2SeO_4) was performed in triplicate with *P. kessleri*, *C. vulgaris*, and *R. subcapitata*. The experimental conditions were identical to those described above. At exponential and stationary growth phases of each species, 40 mL of the algal culture were subsampled for each of protein analysis, dry weight, and tissue Se analysis.

In the fourth and final experiment, the cellular protein content of *R. subcapitata* was manipulated under varying light intensities to examine Se accumulation in the stationary phase. *Raphidocelis subcapitata* was cultured in triplicate under two light conditions (high range at 7890–11 960 lux and medium range at 3600–4400 lux cool white). The medium-range (MR) lighting was as specified by Environment Canada (2007), and high-range (HR) lighting was chosen based on the optimal range suggested for *Chlorella* sp. (He et al., 2015). Before algal inoculation, Se was added (as Na_2SeO_4) to a final concentration of 40 µg/L. The stationary phase was confirmed by cell counting as above. Subsamples of 40 mL were collected for each of protein analysis, dry weight, and tissue Se analysis at the stationary phase for all replicates.

Dry weight

For algal dry weight determinations, a 40 mL well-mixed subsample of the algal culture was placed into preweighed and dried aluminum weigh pans and placed in a 60 °C oven for 48 h.

Protein extraction and analysis

For protein extraction, a combination of sonication methods described by Meijer and Wijffels (1998) and Lee et al. (2017) were used: 40 mL aliquots of algal cultures were placed into 50 mL plastic Falcon tubes and centrifuged at 3500 *g* for 15 min. The supernatant was decanted without disturbing the pellet, and the algae were then washed with deionized reverse osmosis water. The centrifugation and washing steps were performed three times. The final algal pellet was resuspended in 0.08 M KCl to and cooled to 4 ± 2 °C for 30 min before protein extraction. The resuspended pellet was placed on ice, and then cells were lysed via sonication at 8 W (RMS) with a frequency of 20 kHz for 15 s. The tube was placed on ice for a minimum of 2 min before the sonication cycle was repeated twice more, for a total sonication time of 45 s. After cell lysis, samples were centrifuged at 3500 *g* for 10 min and the supernatant was used in the protein assay. This method provided consistent results with sufficient percent recovery, and effective lysis of the cells was confirmed microscopically.

The Bradford microplate method (Bradford, 1976) was performed using a commercially available Quick Start kit (Bio-Rad) for total protein content analysis. In this method, 150 µL of sample was added to the wells followed by 150 µL of Bradford reagent. The sample and reagent were incubated at room temperature for 5 min. The microplate was read using a UV spectrophotometer microplate reader (Bio-Rad) at 595 nm. A standard curve using bovine serum albumin (Barbarino & Lourenço, 2005) and a blank control were run on each plate to convert UV absorbance data to concentration.

Tissue Se concentration

Next, 40 mL aliquots of samples taken from cultures were pipetted into 50 mL centrifuge tubes and centrifuged at 3500 *g* for 15 min. The supernatant was decanted without disturbing the pellet, which was then washed with deionized reverse osmosis water. The centrifugation and washing steps were repeated twice. The pellets were then frozen at −20 °C and shipped on ice to TrichAnalytics for analysis of total Se by laser ablation-inductively coupled plasma-mass spectrometry (LA-ICP-MS).

Statistical analyses

Statistical analyses were performed using Rstudio (Rstudio Team, v. 2021.2.382). For the first experiment, a two-way analysis of variance (ANOVA) followed by Tukey's post hoc method for pairwise multiple comparisons was used to analyze for differences between the total cellular protein content between the four algae species at each of the three growth phases. If a significant interaction was detected, a one-way ANOVA was performed followed by Tukey's post hoc test to determine the significance of one factor. The untransformed data met the assumption for normality; however, they did not meet the assumption of equal variance. A log transformation of protein concentrations (normalized to dry weight) was necessary to meet the assumptions of ANOVA. For the second experiment, *t*-tests were performed to determine whether protein content differed in control algae and those exposed to 40 µg/L Se in

 DOI: 10.1002/ieam.4946 © 2024 The Authors

the four algae species at the exponential and stationary growth phases. For the third experiment, linear regression analyses were performed to determine if a relationship existed between total protein content and tissue Se for *C. vulgaris*, *R. subcapitata*, and *P. kessleri* at either the exponential or stationary growth phases. Linear regression was performed to determine if there was a relationship between total protein content and tissue Se for *R. subcapitata* across light regimes. A Chow test was used to determine if the coefficients of regression were significantly different than each other. Significance was established at $\alpha = 0.05$ for all statistical analyses.

RESULTS

Growth curves for *P. kessleri*, *C. vulgaris*, *R. subcapitata*, and *T. obliquus* are shown in Figure 1 and initiation and length of each of the growth phases are shown in Table 1.

All four species of algae were evaluated for total protein content (μg protein/mg dry weight) at each phase (lag, exponential, stationary) in the absence of Se (Figure 2). Total cellular protein content on a dry weight basis increased in each growth phase but not to the same extent; the highest levels of protein were observed in the stationary phase for all species. A two-way ANOVA revealed that species, growth phase, and the interaction between the two factors were significant for total protein content (species: $F[3, 84] = 49.43$, $p < 0.0001$; growth phase: $F[2, 84] = 480.00$, $p < 0.0001$; and interaction: $F[6, 84] = 37.60$, $p < 0.0001$). A one-way ANOVA and Tukey's post hoc test for multiple pairwise comparisons indicated that there were significant differences ($p < 0.05$) between species during each phase (Figure 2). The smallest increase in protein content was in *T. obliquus*, which increased 3.6-fold between the lag and stationary phases, followed by *C. vulgaris* (4.4-fold) and *R. subcapitata* (8.9-fold), and the greatest increase occurred in *P. kessleri* (25.1-fold). The greatest increases generally occurred between the lag phase and the exponential phase, except for *P. kessleri*.

Parachlorella kessleri, *C. vulgaris*, *R. subcapitata*, and *T. obliquus* were examined for total protein content after exposure to 40 μg/L Se (as Na_2SeO_4) in the exponential and stationary phases (Figure 3). No significant differences (*t*-tests, $p > 0.05$) were observed in protein content between controls and Se-exposed groups.

The relationship between protein content and tissue Se accumulation was examined in *P. kessleri*, *C. vulgaris*, and

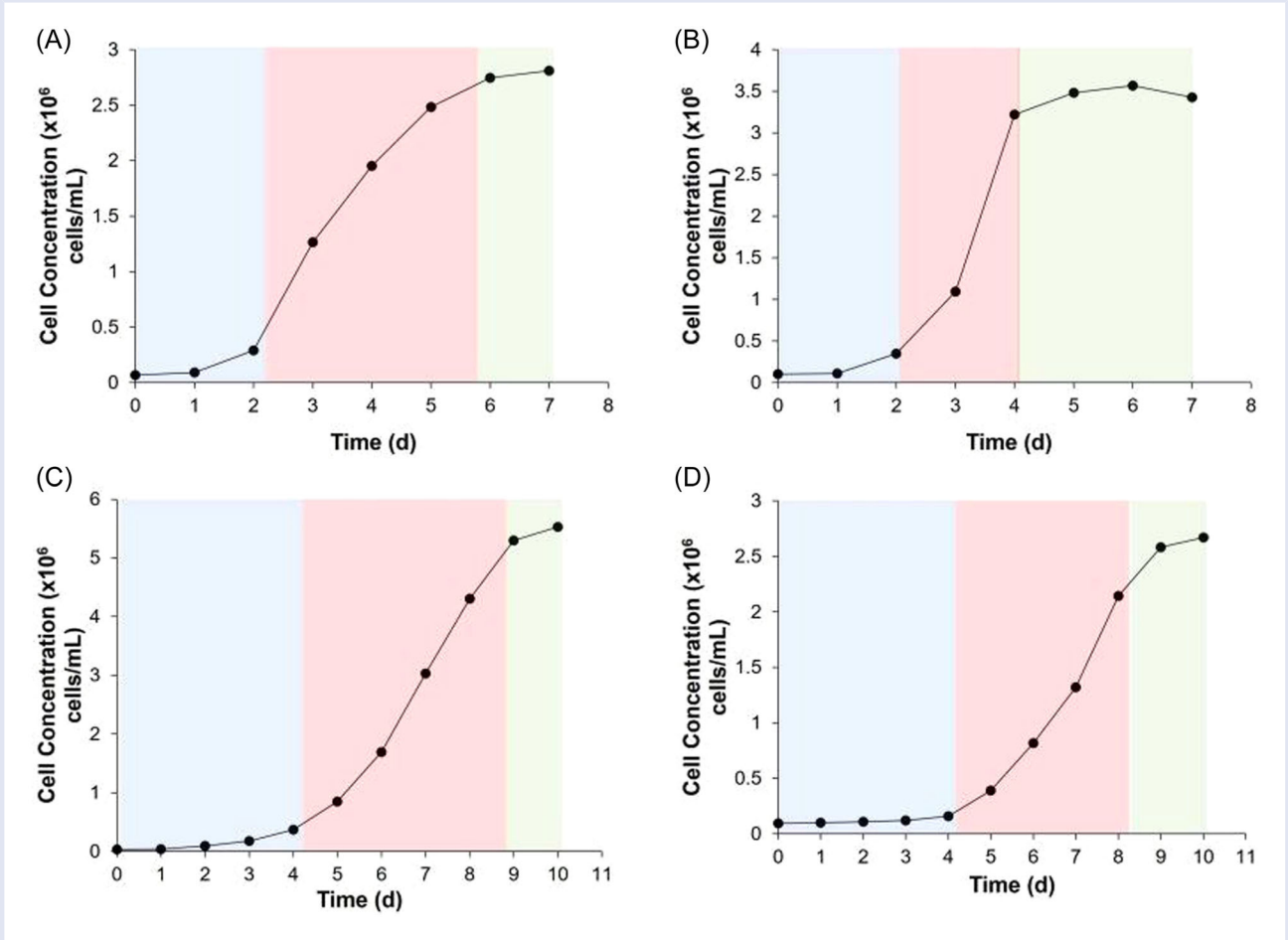


FIGURE 1 Daily cell counts (cells/mL) of *Parachlorella kessleri* (A), *Chlorella vulgaris* (B), *Raphidocelis subcapitata* (C), and *Tetradesmus obliquus* (D) grown in 200 mL modified Bold's basal medium (BBM) at 3600–4400 lux. The different phases are indicated for each species by background color: blue = lag phase, red = exponential phase, green = stationary phase. Each point is the cell count taken from a single flask ($n = 1$) over an incubation period of 7–10 days

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TABLE 1 Durations in days of lag, exponential, and stationary phases of growth as determined from population growth curves

Species	Lag phase	Exponential phase	Stationary phase
Parachlorella kessleri	0–2	3–5	6–7
Chlorella vulgaris	0–2	3–4	5–7
Raphidocelis subcapitata	0–4	5–8	9–10
Tetradesmus obliquus	0–4	5–8	9–10

R. subcapitata exposed to 40 µg/L Se in the exponential phase (Figure 4). There was no relationship identified between total protein content and tissue Se (R^2 value of 0.02; $p > 0.05$). In the stationary phase, the same exposures were used for *P. kessleri*, *C. vulgaris*, and *R. subcapitata* (Experiment 3) as well as two additional treatments for *R. subcapitata* (Experiment 4): One exposed to MR lighting (3600–4400 cool lux) and the other to HR lighting (7890–11 960 cool lux) were examined for a relationship between total protein content and tissue Se concentrations (Figure 5). A strong relationship between total protein content and tissue Se was found among different species in the stationary phase ($R^2 = 0.95$, $p < 0.0001$). A strong relationship between total protein content and tissue Se was also revealed under different light intensities in *R. subcapitata* ($R^2 = 0.83$, $p < 0.05$). The regression coefficients from both experiments in the stationary phase did not differ significantly (Chow test [$F(2,11) = 2.2$, $p = 0.16$).

Because both total protein content and tissue Se were calculated based on dry weight, Se was then calculated based on weight of protein (Table 2). When the Se content is normalized to total protein content in the stationary phase (Experiment 3), the mean Se concentration for *P. kessleri*, *C. vulgaris*, and *R. subcapitata* ranged from 39 to 43 µg Se/g protein; no significant difference was detected between protein normalized Se concentrations among species ($F(4,4) = 0.916$, $p = 0.533$).

DISCUSSION

The results presented here demonstrate that total protein content differed among species and changed between growth phases. In the exponential growth phase, protein content differed significantly between *P. kessleri*, *C. vulgaris*, and *R. subcapitata*, whereas *T. obliquus* had a protein content similar to *P. kessleri*. In the stationary phase, protein content differed significantly between species with *P. kessleri* > *C. vulgaris* > *R. subcapitata* > *T. obliquus*. Differences in cell structure can influence the effectiveness of cell lysis methods and protein extraction. For example, López et al. (2010) reported that cell robustness can reduce cell disruption, and cell lysis may also be influenced by the formation of algal colonies; for instance, *Tetradesmus* is considered pleomorphic and forms coenobia, which have been reported to reduce effectiveness of cell lysis and protein recovery (Oliveria et al., 2021). Consequently, experiments were not continued with *T. obliquus* beyond the initial trials, and the results for protein content presented for

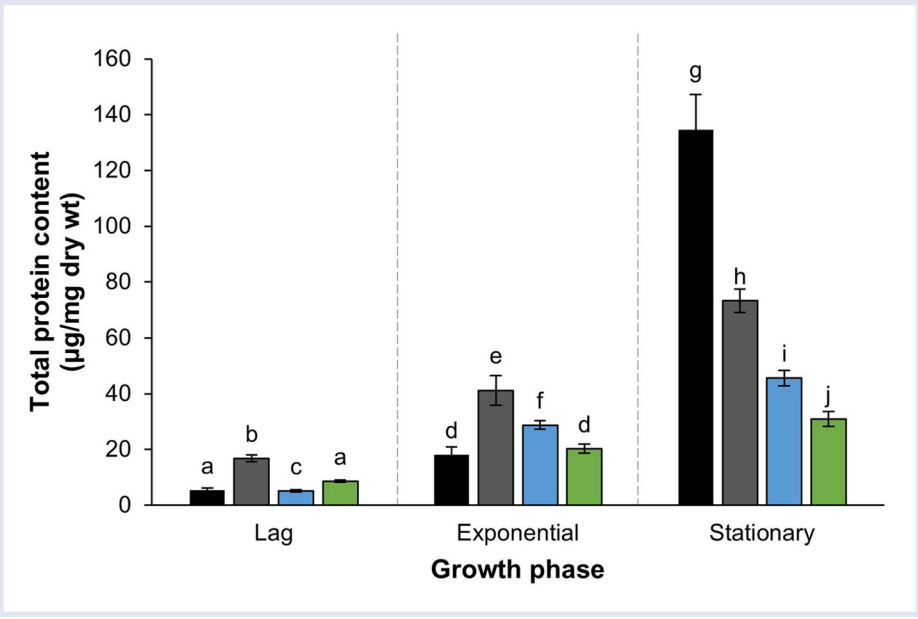


FIGURE 2 Total protein content of *Parachlorella kessleri* (■), *Chlorella vulgaris* (■), *Raphidocelis subcapitata* (■), and *Tetradesmus obliquus* (■) at the lag, exponential, and stationary phases of the algae growth cycle. Values are means ± SEM (standard error of the mean), $n = 7$. Different letters denote significance of protein content within a given phase

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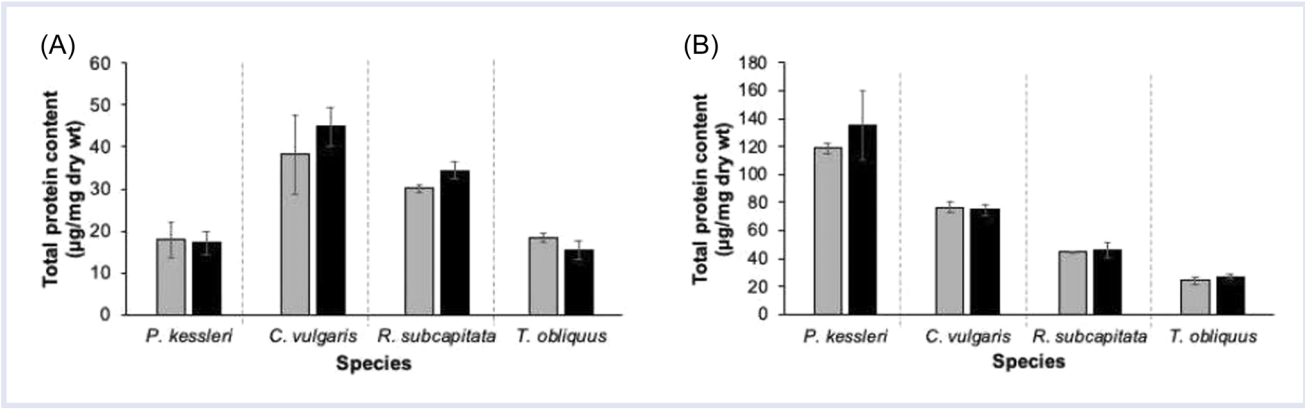


FIGURE 3 Total protein content of *Parachlorella kessleri*, *Chlorella vulgaris*, *Raphidocelis subcapitata*, and *Tetradesmus obliquus* at the exponential (A) and stationary (B) phases of the algae growth cycle with 0 (negative control;) or 40 µg/L Se ( ). Values are means ± SEM (standard error of the mean), $n = 3$. Different letters denote significance

this species may be underestimated; cell lysis was considered effective for the other three species.

For all four species, the protein concentration was greatest in the stationary phase. Lourenço et al. (2004) also reported that cellular composition (proportion of protein, lipid, and carbohydrate) changed with phases of growth of several marine microalgae species; however, these authors reported that the highest cellular protein concentration occurred in the exponential growth phase rather than the stationary phase.

The results presented here for *R. subcapitata* demonstrate that differences in light intensity altered algal cellular composition, with increasing intensity associated with higher protein concentration. Nzayisenga et al. (2020) also examined the changes in cellular composition of freshwater microalgae in relation to increased lighting intensity; a negative relationship between lipid and protein content was observed among species with increased lipid content resulting from high lighting intensities of >110 µE/m^2/s, whereas a higher protein content was observed at lower light intensities. He et al. (2015) reported that the protein content of *Chlorella* increased with increasing light intensity to 10 800 lux before declining after photoinhibition occurred at 21 000 lux.

Exposure to Se did not affect protein concentrations in the test species in the exponential and stationary phases, indicating that the presence of elevated Se concentration did not stimulate significant protein production in the cells. Accumulated Se was not affected by protein content of *P. kessleri*, *C. vulgaris*, and *R. subcapitata* in the exponential phase; however, a strong relationship was present in the stationary phase, suggesting that the accumulation of Se by these green algae is indeed affected by the protein concentration of the cells, at least under the more stable conditions associated with the stationary phase. A strong relationship between protein concentration and Se accumulation also occurred for *R. subcapitata* in the stationary phase when the total protein content was varied as a result of culturing under different light intensities.

Consistent with the higher protein concentration in the stationary phase, the Se concentration in each species was

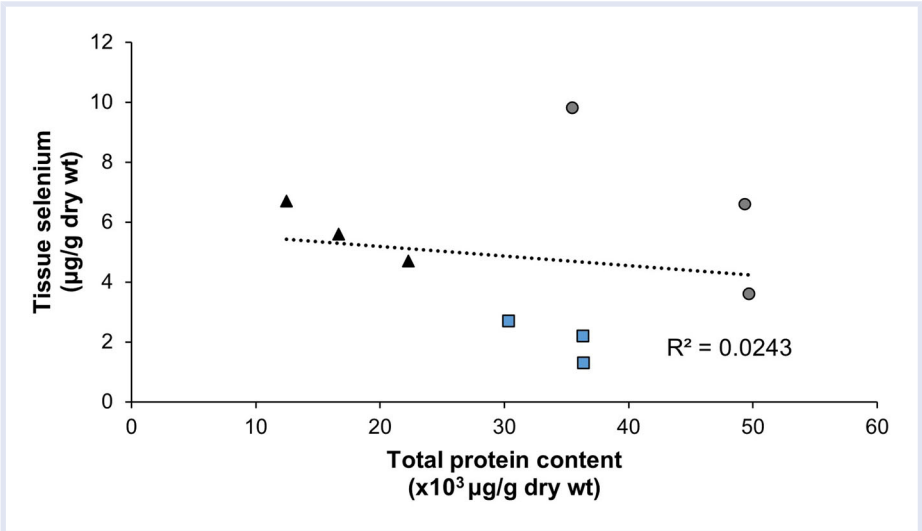


FIGURE 4 Algal uptake of selenium (Se) from exposure to 40 µg/L Se as a function of total protein content during the exponential growth phase. Algal species were: *Parachlorella kessleri* ( ), *Chlorella vulgaris* ( ), and *Raphidocelis subcapitata* ( ). Relationship analyzed by linear regression ($p > 0.05$)

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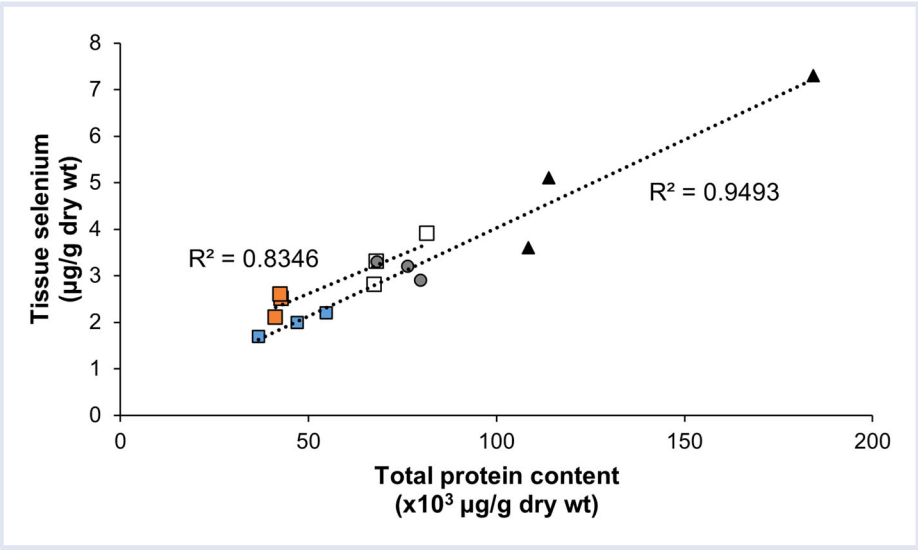


FIGURE 5 Algal uptake of selenium (Se) from exposure to 40 µg/L Se as a function of total protein content during the stationary growth phase at different light levels. Two experiments are presented. In the first, algal species and respective treatments were: *Parachlorella kessleri* at medium range (MR) lighting (3600–4400 lux; ▲), *Chlorella vulgaris* at medium range lighting (3600–4400 lux; ●), and *Raphidocelis subcapitata* at MR lighting (3600–4400 lux; ■). In the second, treatments were: *Raphidocelis subcapitata* at MR lighting (3600–4400 lux; ■) and *Raphidocelis subcapitata* at high range (HR) lighting (7890–11 960; □). Relationships analyzed by linear regression ($p < 0.05$)

also higher in the stationary phase than in the exponential phase. Expressed on the basis of mass of protein, Se did not differ significantly among species in the stationary phase, further supporting the relationship between protein content and tissue Se in this phase. In fact, the concentration of Se on a mass of protein basis was remarkably similar between species.

Because accumulated Se is incorporated primarily into proteins (i.e., as selenoamino acids), it is perhaps not surprising that Se concentration might vary as a function of protein concentration among primary producers. For example, the concentration of nitrogen has commonly been used as a proxy measurement to estimate protein concentrations in plants because nitrogen occurs primarily in protein (Krul, 2019). Thus, because nitrogen is accumulated in a manner that is correlated with the concentration of protein in the cells, it follows that other constituents of proteins, such as Se, might also be higher in species that have higher protein, because they will tend to accumulate those constituents to a greater degree. Moreover, plants or algae that produce tissues that are high in carbohydrate or lipid essentially dilute the protein with those other constituents, thus diluting the protein-associated Se on a mass basis.

If this relationship holds across species, normalization of Se to protein concentration might be useful for predicting Se uptake by primary producers. From a monitoring standpoint, this might also serve to reduce the high degree of variability that is typical when measuring Se in the periphyton, by measuring Se per milligram of protein, rather than per milligram of dry weight. This would be analogous to expressing polychlorinated biphenyls (PCBs) normalized to tissue lipid content (Hebert & Keenleyside, 1995), which has been useful in modeling PCBs in food webs (Thomann & Connolly, 1984) as well as establishing lipophilic contaminant guidelines (Arblaster et al., 2014; Schlechtriem et al., 2012; Webster et al., 2013).

The extent to which differences in protein concentration might help explain other food web characteristics, such as TTF values between primary producers and invertebrates and between invertebrates and fish, remains to be established. However, it is notable that differences in protein concentration appear to be largely consistent with the TTF values

TABLE 2 Concentration (mean ± SEM, standard error of the mean) of protein and selenium in algae exposed to 40 µg Se/L as selenate in the stationary phase

Species	Protein (µg/g dry weight)	Selenium (µg/g dry weight)	Selenium (µg/g protein)
Parachlorella kessleri	13.55 ± 2.44	5.33 ± 1.07	39.2 ± 3.4
Chlorella vulgaris	7.48 ± 0.34	3.13 ± 0.12	42.2 ± 3.5
Raphidocelis subcapitata	4.61 ± 0.52	1.97 ± 0.14	43.0 ± 1.8
R. subcapitata medium light	4.23 ± 0.05	2.40 ± 0.15	56.7 ± 3.0
R. subcapitata high light	7.25 ± 0.46	3.33 ± 0.32	45.9 ± 2.2

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that occur between these trophic levels, because both protein concentration and Se concentration generally increase proportionally up the food chain. For example, the TTF from the periphyton to invertebrates is, on average, approximately twofold (Presser & Luoma, 2010), and invertebrates have protein concentrations that are approximately double that observed in the periphyton. Similarly, TTF values from invertebrates to fish tissue demonstrate a smaller increase in Se between these trophic levels (i.e., TTF of ~1.1; Presser & Luoma, 2010), which is consistent with the smaller increase in protein concentration from invertebrates to fish. A variety of other factors are likely to influence the transfer of protein-associated Se among trophic levels, including dietary assimilation efficiency, metabolic use of protein as a source of energy, as well as mechanisms of excretion and elimination; however, in the same manner that nitrogen concentration increases across trophic levels as a result of incorporation of protein from prey, this may also partly provide a mechanistic explanation of the increasing Se that occurs.

This study identified a strong relationship between total protein concentration and Se accumulation for three species of green algae and, to the best of our knowledge, reflects the first time this potential interaction has been investigated. However, before these findings can be interpreted more broadly, additional research will be needed to assess whether this relationship holds across other species of primary producers, any relationship that this might have with TTF values between trophic levels, and whether other forms of Se such as selenite produce similar results. These future studies can improve the accuracy of predicted EFs and TTFs and thus improve the models for risk assessments of contaminated areas.

AUTHOR CONTRIBUTION

Courtney Bogstie: Conceptualization; data curation; formal analysis; investigation; methodology; project administration; resources; software; validation; visualization; writing—original draft. **Melanie Gallant:** Conceptualization; methodology; supervision; validation; visualization; writing—review and editing. **James R. Elphick:** Conceptualization; funding acquisition; supervision; visualization; writing—review and editing. **Christopher Kennedy:** Supervision; writing—review and editing.

ACKNOWLEDGMENT

There are no funders to report.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

All data, including any associated metadata, analytical results, and statistical analyses, are available from corresponding author Courtney Bogstie (courtney_bogstie@atkinsrealis.com) and can also be located in the supplemental data section of the Master of Environmental Toxicology Thesis (Simon Fraser University) titled "The relationship between cellular protein content and selenium accumulation in freshwater micro-algae" located at https://summit.sfu.ca/item/35804.

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 DOI: 10.1002/ieam.4946 © 2024 The Authors