Environmental Toxicology and Chemistry

Environmental Toxicology

Organic Selenium, Selenate and Selenite Accumulation by Lake Plankton and the Alga *Chlamydomonas reinhardtii* at Different pH and Sulfate Concentrations

Dominic E. Ponton, Claude Fortin and Landis Hare

Environ Toxicol Chem., **Accepted Article** • DOI: 10.1002/etc.4158

Environmental Toxicology **Environmental Toxicology and Chemistry**
DOI 10.1002/etc.4158

Organic Selenium, Selenate and Selenite Accumulation by Lake Plankton and the Alga *Chlamydomonas reinhardtii* at Different pH and Sulfate Concentrations

Dominic E. Ponton,* Claude Fortin, and Landis Hare

Institut National de la Recherche Scientifique – Centre Eau, Terre et Environnement (INRS-ETE), Université du Québec, Quebec City, Quebec, Canada

* Address correspondence to Dominic.ponton@umontreal.ca

This article contains online-only Supplemental Data

Submitted 21 November 2017; Returned for Revision 10 January 2018; Accepted 18 April 2018

Abstract: Selenium (Se) concentrations measured in lake planktonic food chains (microplankton < 64 µm, copepods and *Chaoborus* larvae) were strongly correlated with the concentrations of dissolved organic Se. These correlations were strengthened slightly by adding the concentrations of dissolved selenate to those of organic Se. To better understand the role of Se species and the influence of water chemistry on Se uptake, we exposed the green alga *Chlamydomonas reinhardtii* to selenite, selenate or selenomethionine at various H^+ ion and sulfate concentrations under controlled laboratory conditions. At low sulfate concentrations, inorganic Se species (selenate >> selenite) were more readily accumulated by this alga than was selenomethionine. However, at higher sulfate concentrations the uptake of selenite was higher than that of selenate while the uptake of selenomethionine remained unchanged. While pH of the exposure water did not influence the uptake of selenate by this alga, the accumulation of selenomethionine and selenite increased with pH because of their relative pH-related speciation. The Se concentrations that we measured in *C. reinhardtii* exposed to selenomethionine were 30 times lower than those that we measured in field-collected microplankton exposed in the same laboratory conditions. This difference is explained by the taxa present in the microplankton samples. Using our laboratory measurements of Se uptake in microplankton and our natural Se concentrations in lakewater allowed us to model Se concentrations in a lake pelagic food chain.

Keywords: Algae, Sulfate, pH, Selenate, Selenite, Organic selenium

INTRODUCTION

Aquatic systems can be contaminated by selenium (Se) from agricultural irrigation, metal mining and refining as well as fossil fuel extraction and combustion (Lemly 2004). Aquatic animals living in Se-contaminated ecosystems can accumulate this element to high concentrations. For example, very high Se concentrations ([Se]) were reported in chironomids (139 µg/g dry weight (dw)) and Se-tolerant mosquitofish (170 µg/g dw) from Kesterson Refuge in the San Joaquin Valley of California (Presser and Ohlendorf 1987). At this and other sites, high [Se] have caused severe deformities in fish and aquatic birds (Presser and Ohlendorf 1987; Lemly 1993). In Canada, recent measurements of Se in yellow perch (*Perca flavescens*) collected from lakes in mining regions of Ontario and Quebec showed [Se] to be high in the muscle (5 to 40 µg/g dw; Ponton and Hare 2015) and liver (8 to 48 µg/g dw; Ponton et al. 2016) of this fish species.

Aquatic animals take up Se mainly from their food (Luoma and Rainbow 2008) and thus their [Se] are determined in large part by those of organisms at lower trophic levels. In the pelagic zone of lakes, [Se] in zooplankton are likely to be determined by those in planktonic algae and bacteria (Stewart et al. 2010) that are in turn controlled by the concentrations of various dissolved Se species (Ponton and Hare 2013). This linkage explains the strong correlation reported between [Se] in lakewater and those in a zooplankton-feeding insect (Croteau et al. 2003; Ponton and Hare 2013).

The chemical speciation of Se in freshwater varies depending on the contamination source as well as the retention time and chemistry of the water body (Bowie 1996; Ponton and Hare 2013). Selenium in natural waters is present in several oxidation states including selenate (Se(VI)), selenite (Se(IV)) and organic Se (Org-Se; Se(-II) to Se(+II); Wallschlager and Feldman

2010). Se(VI) is at higher proportions and concentrations near contamination sources because although its reduction to Se(IV) and Org-Se is possible, its re-oxidation to Se(VI) is thermodynamically improbable under natural conditions (Cutter and Bruland 1984). Org-Se species in water are produced through the biological reduction of inorganic Se species by microorganisms (Cutter 1982). The most common forms of Org-Se in natural waters are selenomethionine (Se-Met) and selenocysteine (Conde and Sanz Alaejos 1997).

Various Se species are reported to differ in their propensity for accumulation in aquatic food chains. In the laboratory, investigators have reported either greater bioaccumulation of Se(VI) than Se(IV) by planktonic green algae (Neumann et al. 2003; Fournier et al. 2006; Simmons and Wallschläger 2011), or no difference in bioaccumulation between these inorganic species in periphyton (Conley et al. 2013), or greater accumulation of Se(IV) compared to Se(VI) (Luoma and Rainbow 2008) by the green alga *C. reinhardtii* (Vriens et al. 2016). Although there are exceptions (Baines et al. 2001), Org-Se is generally thought to be the Se species with the highest accumulation potential (Besser et al. 1993; Fournier et al. 2006; Stewart et al. 2010). In the field, Ponton and Hare (2013) reported that the Org-Se concentrations in lakewater best predicted those in larvae of the phantom midge *Chaoborus*; although adding Se(VI) concentrations raised slightly the strength of the correlations for some lakes. At high sulfate (SO_4) concentrations, the accumulation of inorganic Se species is reported to be reduced in the field (Ponton and Hare 2013) and in the laboratory for both Se(IV) (Morlon et al. 2006) and Se(VI) (Fournier et al. 2010). These observations can be explained by the chemical similarities between inorganic Se and SO_4 ions (Reich and Hondal 2016) that can lead to competition at biological uptake sites and thereby reduce Se uptake. Lakewater pH could also influence the

protonation state of Se(IV) and its bioaccumulation (Riedel and Sanders 1996; Morlon et al. 2006), although this hypothesis remains to be rigorously tested.

The relationship between [Se] in adjacent links of food chains have been characterized using trophic transfer ratios (TTF = $[Se]_{consumer}/[Se]_{prey}$), whereas [Se] at the base of food chains have been described using partition coefficients (k_d) representing the ratio of [Se] in primary producers and those in water (Presser and Luoma 2010). However, k_ds could vary widely among sites due to variations in Se speciation in water, ambient physico-chemical conditions and the occurrence of various types of primary producers. Such variations are not generally considered.

To better understand the influence of Se speciation on Se bioaccumulation by lake planktonic food chains, we compared the accumulation of Se(IV), Se(VI) and Org-Se at various H^+ ion and SO_4 concentrations by the freshwater green alga *Chlamydomonas reinhardtii* in the laboratory. We used this algal species because it is tolerant of high [Se] (Morlon et al. 2005), easy to culture and grows over a wide range in H^+ concentrations (Lustigman et al. 1995). To determine whether our laboratory results for this algal species are representative of Se accumulation by mixed plankton in the field, we also exposed uncontaminated field-collected microplankton (< 64 µm) to various Se species under conditions that were similar to those used for *C. reinhardtii*. Lastly, we used our Se-uptake measurements on field-collected microplankton exposed to Se in the laboratory to predict [Se] in a lake planktonic food chain comprising microplankton, zooplankton (copepods) and their predators, that is, larvae of the phantom midge *Chaoborus*. This predator has been used as a trace element biomonitor in lake plankton in part because it is tolerant to trace elements and thus persists in many lakes impacted by acidic emissions from metal smelters (Hare et al. 2008; Ponton and Hare 2009, 2013).

METHODS

Lake sampling

We collected water, microplankton, crustacean zooplankton and larvae of the phantom midge *Chaoborus* in late May and early June in 2010 and 2011 from 12 to 18 lakes located on the Precambrian Shield in the mining areas of Sudbury (Ontario) and Rouyn-Noranda (Quebec), Canada. Lake locations and information on their Se sources (Figure 1), pH, SO_4 and dissolved organic matter concentrations as well as the different Se species concentrations (Org-Se, Se(IV) and Se(VI)) are given in Supplemental Data (Supp. Data; Table S1). Uncontaminated microplankton was collected from Lake Bedard (47°16´N,71°07´W), which is located 70 kilometers north of Quebec City (Quebec, Canada) on the Precambrian Shield.

Lakewater collection

We used diffusion samplers to collect lakewater by in situ filtration. In the laboratory, the acid-cleaned, Plexiglas or polypropylene, diffusion samplers were filled with ultrapure water and covered with a 0.2 µm pore-size polysulfone membrane (Gelman HT-200) before being sealed individually in plastic bags. In each lake, three diffusion samplers were anchored 1 m below the surface, that is, in the epilimnion where *Chaoborus* larvae feed on zooplankton (Carter and Kwik 1977; Hare and Carter 1987). Equilibration times in lakewater varied depending on the volume of sampler cells, that is, from 3 d (4 mL cells) to 10 d (125 and 250 mL cells; our unpublished data showed that 5 d is sufficient). Water samples were removed immediately upon retrieval of the samplers.

Samples (4 mL) for major anions (Cl^-, NO_3^-, SO_4^{2-}) were removed using a pipette with an unused plastic tip that had been rinsed in ultrapure water. These samples were injected into new High Density Polyethylene (HDPE) bottles (4 mL capacity) that had been rinsed with ultrapure

water. Concentrations of anions (Cl^-, NO_3^-, SO_4^{2-}) in lakewater were measured by ion chromatography (Dionex, system ICS-2000; AS-18 column).

The water to be used for Se speciation measurements (a minimum of 30 mL) was stored in acid-washed HDPE or polypropylene bottles, on ice, for a maximum of 10 days to prevent changes in Se speciation. Wang (1994) showed that there were no measurable losses of Se(IV), Se(VI) or selenomethionine following storage of purified tap-water for 15 days at 4 ^oC. Since most bacteria and algae exceed the pore size of the sampler membrane (0.2 µm), they could not have influenced Se speciation in the water samples.

Selenium speciation measurements in lakewater

We measured Se speciation in lakewater by hydride generation of Se(IV) followed by detection using atomic fluorescence spectrometry (HG-AFS; Millennium Excalibur System, PS Analytical) in the laboratory of N. Belzile and Y. Chen at Laurentian University in Sudbury, Ontario. These researchers (Chen et al. 2005a, 2005b) developed the speciation measurement method, which they describe as being simple, precise and not subject to interference due to other elements.

We measured Se(IV) by acidifying a 10 mL subsample of lakewater with HCl to a final concentration of 3.0 M (Cutter 1978). A second subsample (20 mL) was used to determine Org-Se and Se(VI) by acidification with HNO_3 and HCl to a final matrix of 1 and 2%, respectively (volume/volume), and exposure to ultraviolet radiation (wavelength of 300 nm) for 2 h in a quartz tube sealed with Parafilm. Irradiated samples were cooled on ice for 1 h and then acidified to 3.0 M with HCl. This UV treatment creates OH radicals that oxidize organo-Se to Se(IV), and oxidation of Se(IV) to Se(VI) is prevented by HCl. Chen et al. (2005b) describe in detail the photochemical mechanisms involved and show that four types of Org-Se were completely

transformed to Se(IV) using this method. Subtracting the first measurement (Se(IV)) from the second (Se(IV) + Org-Se) gives the Org-Se concentration. The remaining 10 mL of UV-treated sample was acidified to 3.0 M with HCl and submitted to a microwave digestion in closed vials (MicroSYNTH, Milestone; MLSeasyWAVE 3.5.4.0 software) at 110 ^{o}C for 15 min to reduce Se(VI) to Se(IV). These samples were cooled on ice before measuring total Se. Using this method, Chen et al. (2005a) reported 91% recovery of Se(VI) spiked into lakewater. The concentration of Se(VI) is obtained by subtracting Se(IV) + Org-Se (post UV) from the concentration of total Se (post-microwave). Our Org-Se recovery was 100 ± 7% and between 97 and 107% for Se(VI) at a concentration of 0.1 µg/L. Mean values (n = 3) per site that were lower than the detection limit of 21 ng/L were set as half the detection limit, that is, 10.5 ng/L.

Microplankton collection

We used a 2-meter long, 3-cm diameter, plastic tube to collect integrated samples of epilimnetic water in the center of each lake. These samples were immediately filtered through a 64 µm mesh-aperture plastic sieve, to remove macro-zooplankton (see section *Zooplankton collection*), and put in plastic containers. In the laboratory, Lake Bédard subsamples (500 mL glass bottles; 1% Lugol's; n = 3) were taken for the identification and counting of microplankton and bacteria using an inverted microscope (Axiovert, 200; Axio Vision 3.0 software) and a flow cytometer (BD FACSCalibur, BD Biosciences), respectively. Cell volume and surface area were measured according to the methods of Hillebrand et al. (1999). Filtered lakewater (< 0.64 µm; ~40 L) was then centrifuged (Westfalia Separator LWA 205, Centrico Inc.) to concentrate microplankton. The centrifugation process was efficient since algal cell counts in centrifuged samples were two orders of magnitude lower than those prior to centrifugation. Three samples of microplankton per lake (about 2-3 mg dry weight) were placed on acid-washed Teflon sheeting

in an acid-washed 1.5 mL polypropylene centrifuge tube and frozen at -20 ºC until drying, digestion and Se analysis. Field microplankton samples from some lakes had refractory inorganic matter still present after digestion that was removed by centrifugation before analysis. Uncontaminated microplankton from Lake Bédard (for the laboratory experiment) that had been concentrated by flow-through centrifugation was resuspended in about 100 mL of Se-free exposure medium prior to inoculation of the exposure medium.

Zooplankton collection

We collected crustacean zooplankton and *Chaoborus* larvae at night by hauling a 64 µm mesh-aperture plankton net horizontally in the water column of each lake. Crustaceans were separated from *Chaoborus* larvae using a 0.5 mm mesh-aperture sieve and both were held in lakewater at field temperatures for transport to the laboratory.

Chaoborus larvae were sorted according to species (Saether 1972), and five samples per lake were prepared by pooling 10-20 similar sized fourth-instars (Carter and Kwik 1977) per sample. *Chaoborus punctipennis* was the only *Chaoborus* species present in all lakes, with the exception of Lakes Kelly and Rouyn in which *Chaoborus flavicans* was the only species present. Since [Se] in these two species are reported to be similar (Ponton and Hare 2013), we assumed that Se data for the two could be combined.

Planktonic crustaceans held in a plastic bag were subject to a period of anoxia over the first night at 4ºC. This anoxia allowed us to eliminate cladocerans (not tolerant to anoxia) from copepods by flotation, decantation and filtration on a 125 µm mesh-aperture plastic sieve. We verified under a microscope that plankton fractions were composed of at least 90% copepods by volume. For each lake, three samples of copepods (a major prey of *Chaoborus*; Croteau et al. 2003) were placed on acid-washed, preweighed pieces of Teflon sheeting held in acid-washed,

polypropylene, microcentrifuge tubes and frozen at -20 ºC until drying and analysis. The maximum delay between collection and freezing was 1 day, during which time samples were stored at 4 ºC.

Algal cultures

All labware was soaked for 1 day in 15% HNO_3 (volume/volume; Omni Trace Grade) and rinsed five times with demineralized water and two times with ultrapure water (> 18 MΩ cm). An agar culture of *C. reinhardtii* (CPCC 11 wild strain) was obtained from the Canadian Phycological Culture Centre at the University of Waterloo, Waterloo, Ontario. Algae were grown in an axenic modified high-salt medium with an ionic strength of 8.4 mEq/L (Fortin et al. 2004). One-liter Erlenmeyer flasks and the culture medium (without trace elements) were sterilized at 121 ºC for 15 minutes in an autoclave (Sanyo). After sterilization and cooling, sodium hydroxide, trace elements and algae were added using sterilized pipette tips under a sterile Class 100 laminar flow hood. A sterile piece of cotton (USP Sterile Cotton Roll, U.S. Cotton) was put in the opening of each flask to prevent bacterial contamination. Cultures were held in an environmental growth chamber (Conviron, CMP3023) at 20 ± 0.2 ºC under constant, cool-white, fluorescent lighting (100 µE m^{-2} s^{-1}) and flasks rotated at the rate of 50 rotations per minute. We used ~2 L of algae (separated into six 1-L sterile Erlenmeyer flasks) growing exponentially for each experiment. One hour before each experiment, the 2 L of culture was centrifuged (Sorval RC50 Plus, Du Pont) twice at 4,000 x *g* for 5 minutes; algae were rinsed between centrifugations with Se-free exposure medium (see section *Algae and microplankton selenium exposure*) and resuspended in about 100 mL of Se-free exposure medium. This rinsing step allowed the removal of algal exudates that can influence Se speciation. The concentrated algal suspension was counted immediately (Counter Multisizer III, Beckman Coulter).

Algae and microplankton selenium exposure

Algae were exposed to Se at a cellular density of 125,000 cells/mL under the same light and rotation conditions as the stock culture. At the end of the exposure periods, the [Se] in the exposure medium had changed little (data not shown). Reagent-grade ACS salts were used to prepare the exposure medium, the final concentrations of which were: NH_4NO_3 (30 µM), KNO_3 (20 µM), $MgCl_2$ (80 µM), $CaCl_2$ (70 µM) and NaCl (100 µM). This exposure medium represents the average concentrations of major ions in Rouyn-Noranda and Sudbury lakes (unpublished results). Experiments were carried out in 400 mL of exposure medium held in 1 L sterile, polypropylene, Erlenmeyer flasks (in triplicate, except where mentioned).

The nominal [Se] of all Se species used was 63 nM (5 µg/L), which was the previous U.S. criterion for the protection of aquatic life in freshwater (US EPA 2016) and approximates [Se] encountered in contaminated water bodies (Conde and Sanz Alaejos 1997; Lemly 2004; Luoma and Rainbow 2008). According to the new criterion, if no fish tissue is available, the 30-day average water [Se] should not exceed 1.2 µg/L more than once every three years (US EPA 2016). The [Se] used was not toxic to *C. reinhardtii* since very high Se(IV) concentrations (50 µM, 4 mg/L) are needed to reduce the growth of *C. reinhardtii* (Morlon et al. 2005) and plants are generally insensitive to Se (Young et al. 2010). The pH of the exposure medium was maintained constant using 5 mM trishydroxymethylaminomethane buffer (TRIS, OmniPur Grade, EM Science), and adjustments were made using hydrochloric acid (HCl, AristarUltra Grade, VWR) to maintain the pH at 7.5 for experiments in which pH was not the studied variable. This buffer was used because its effective pH range is 6.5 to 9.7. Sulfuric acid (ACS grade) was used to obtain the desired SO_4 concentrations. The pH, exposure-time and SO_4

concentrations are different according to each treatment and are then specified in each figure titles.

Following Se exposure, algae were centrifuged two times and rinsed between centrifugations with alkaline (pH 8.5), Se-free, exposure medium to both remove Se adsorbed onto cell membranes and remaining exposure medium. Since inorganic Se is reported to desorb more readily at high pH (Lemly 2004), we rinsed algae with an alkaline solution to remove adsorbed Se. Each algal pellet was removed using a micropipette and placed in an acid-washed 1.5 mL, polypropylene, centrifuge tube and centrifuged (Micromax, Thermo IEC) to remove all remaining water. Pellets were frozen at -20 ^{o}C until drying and analysis.

Selenium concentration measurements in organisms

Frozen organisms were freeze-dried (FTS Systems), weighed using a microbalance (Sartorius M2P PRO 11) and placed in acid-washed, high density, polyethylene bottles where they were digested for 2 days at room temperature in concentrated HNO_3 (Aristar grade; 100 µL per mg sample dry weight) followed by 1 day in concentrated 30% (w/w) hydrogen peroxide (Trace Select Ultra grade; 40 µL per mg sample dry weight); digestate volume was brought up to 1 mL per mg sample dry weight using ultrapure water. Certified reference material (lobster hepatopancreas, TORT-2, National Research Council of Canada) was submitted to the same digestion procedure. Selenium of mass 82 g/mol (^{82}Se) was measured using Inductively Coupled Plasma - Mass Spectrometry (ICP-MS; Thermo Elemental X Series) and interferences with bromine (^{81}Br + ^{1}H) were corrected using a standard curve of several Br concentrations. Selenium in the reference material (5.7 ± 0.1 (SD) µg/g; n = 4) was within the certified range (5.6 ± 0.7 (± 95% confidence interval) µg/g). The ICP-MS detection limit for Se was 0.2 µg/L or 0.2 µg/g (given our digestion procedure) which is ten times higher than the HG-AFS detection

limit used for water analysis. All organism [Se] are reported on a mass and dry weight basis for better general comprehension and water chemistry in molar units.

RESULTS AND DISCUSSION

Selenium concentrations in natural planktonic food chains

Selenium concentrations in the members of planktonic food chains, composed of microplankton (< 64 µm), copepods and *Chaoborus* (Figure 2), were highly correlated with the sum of dissolved Org-Se and Se(VI) concentrations in lakewater (Figure 2A,B,C). No correlations were observed ($P > 0.05$) between Org-Se concentrations alone (that is, without Se(VI) concentrations) and [Se] in copepods or microplankton (Supp. Data, Figure S1). These results suggest that both Org-Se and Se(VI) drive Se accumulation in these planktonic food chains (Figure 2). Of these two Se species, Org-Se concentrations predicted most of the variability in *Chaoborus* [Se] among lakes (Figure 2; Supp. Data, Figure S1; Ponton and Hare 2013). Kelly Lake (Figure 1) differed somewhat from the other lakes in that adding Se(VI) concentrations (solid triangle in Figure 2A,B,C) to Org-Se concentrations (solid square in Figure 2A,B,C) over-predicted [Se] in organisms from this lake. The high SO_4 concentrations in this lake (4 mM) likely competed with those of inorganic Se thereby reducing the accumulation of inorganic Se species at the base of the food chain (Figure 2C; Ponton and Hare 2013; Fournier et al. 2010; Morlon et al. 2006). To explain these trends seen in nature, we measured the influence of Se speciation, SO_4 and H^+ on [Se] in the green alga *Chlamydomonas reinhardtii*.

Influence of sulfate on the uptake of Se species by Chlamydomonas reinhardtii

We measured a [Se] of 225 µg/g (dw) in *C. reinhardtii* exposed to 63 nM (5 µg/L) of Se(VI) for 12 h without the addition of SO_4 (dashed lines in Figure 3). This algal [Se] is very high (k_d = 45,400) and would likely represent a risk to consumers up the food chain. Exposure to

a SO_4 concentration of 1.00 ± 0.06 µM (± SE) reduced this value by 50% (Figure 3A). This SO_4 concentration is 16 times higher than the Se(VI) concentration, which suggests a higher affinity of algal membrane transporters for Se(VI) than for SO_4. This result contrasts with those obtained for the bacterium *Escherichia coli*, which has a higher affinity for SO_4 compared to Se(VI) (Lindblow-Kull et al. 1985; Aguilar-Barajas et al. 2011). *C. reinhardtii* [Se] continued to decrease up to the highest SO_4 treatments (500 compared to 2000 and 4000 µM; inset in Figure 3A). Other studies on green algae (*C. reinhardtii*, *Pseudokirchneriella subcapitata* and *Chlorella* spp.; Fournier et al. 2010; Vriens et al. 2016; Williams et al. 1994; Neumann et al. 2003, respectively) have also reported competition between SO_4 and Se(VI) at shared transporters. The present study is the first to measure the influence of SO_4 on Se(VI) uptake by a freshwater alga over the wide range of SO_4 concentrations encountered in mining regions (30 to 4,230 µM in the present study lakes; Supp. Data, Table S1). These results suggest that the high SO_4 concentrations measured in Lake Rouyn and Kelly Lake reduced Se(VI) accumulation in planktonic organisms from those lakes.

When exposed to 63 nM Se(IV) without added SO_4, [Se] in *C. reinhardtii* were 4 µg/g dw (dashed line in Figure 3B). This [Se] is 56 times lower than that of algae exposed to Se(VI) (225 µg/g dw; Figure 3A), which indicates that the uptake rates for Se(VI) are much higher than those of Se(IV). These results agree with those from a field study in which, at low SO_4 concentrations, Se(IV) was a less important contributor to Se bioaccumulation in lake plankton than was Se(VI) (Ponton and Hare 2013).

In the laboratory, investigators have reported either greater bioaccumulation of Se(VI) than Se(IV) by planktonic green algae (Neumann et al. 2003; Fournier et al. 2006; Simmons and Wallschläger 2011), or little difference in bioaccumulation between these inorganic species

(periphyton: Conley et al. 2013), or greater Se(IV) accumulation compared to Se(VI) (marine phytoplankton: Hu et al. 1997; *C. reinhardtii*: Vriens et al. 2016; book review: Luoma and Rainbow 2008). In the four cases that reported similar or higher bioaccumulation of Se(IV) compared to Se(VI), SO_4 was present in the exposure water. In some of these cases, only the concentration of added SO_4 was considered rather than the total including the initial SO_4 concentration in the exposure medium. In these studies, investigators did not report competitive effects of SO_4 on Se(IV) (Vriens et al. 2016) or found higher Se accumulation when algae were exposed to Se(IV) compared to Se(VI) (Hu et al. 1997; Luoma and Rainbow 2008; Vriens et al. 2016). In fact, *Chlamydomonas reinhardtii* exposed to high SO_4 concentrations ($>10^{-4}$; Figure 3) accumulate less Se when exposed to Se(VI), as opposed to Se(IV), as has been reported in other investigations conducted in seawater or at high SO_4 concentrations (Hu et al. 1997; Luoma and Rainbow 2008; Vriens et al. 2016). These inorganic Se species are likely to be taken up less readily by marine algae because of the very high SO_4 concentrations in seawater (28 mM; Hu et al. 1997) compared to those in fresh waters. For example, SO_4 concentrations in seawater are seven times those measured in Kelly Lake (4 mM) in which the uptake of inorganic Se species is already low compared to lakes with low SO_4 concentrations (Ponton and Hare 2013). Of the two inorganic Se species, Se(IV) would thus contribute more to lake food chain Se when SO_4 concentrations are high, probably because of the high adsorption capacity of Se(IV) (Li et al. 2015) compared to Se(VI) (Chan et al. 2009).

Neither selenomethionine pre-exposure nor the concentrations of either SO_4 or phosphate influenced selenomethionine uptake by *C. reinhardtii* (data not shown). This result suggests that other mechanisms than the ones used for inorganic Se uptake are involved in the uptake of Org-

Se (see section *Temporal patterns of Se uptake by Chlamydomonas reinhardtii in the presence of sulfate*).

Influence of pH on the uptake of Se species by Chlamydomonas reinhardtii in the absence of sulfate

Se(VI) accumulation was not significantly influenced over the pH range that we tested (Figure 4A). Although high H^+ concentrations can alter the surface charge of cells and increase the uptake of Se(VI) anions by terrestrial plants (Kinraide 2003), pH did not influence the uptake of Se(VI) in the present study. Since the acidity constant of Se(VI) (pK_a = 1.9) falls outside of the pH range that we tested (7 to 9), pH induced protonation of Se(VI) could not have influenced uptake of this Se species.

In contrast to Se(VI), pH related changes in the speciation of Se(IV) influenced Se uptake by *C. reinhardtii*. We measured a significant ($P < 0.05$) pH effect on Se accumulation by *C. reinhardtii* (Figure 4B) following 12 h of exposure to 63 nM Se(IV). In contrast, two other studies on this algal species reported no clear influence of pH on Se(IV) accumulation (Morlon et al. 2006 (pH 5-8); Riedel and Sanders 1996 (pH 5-9)). The pH effect that we measured can be explained by the fact that Se(IV) speciation changes markedly as pH increases from 7 to 9, since the protonated form ($HSeO_3^-$) is progressively replaced by the deprotonated form (SeO_3^{2-}) up to pH 8.4 (pK_a) at which point ~50% of each Se(IV) species is present (thermodynamic calculation using MINEQL+; Schecher and McAvoy 1992). Our results suggest that this alga's anionic transporters may have a much higher affinity for the deprotonated form of Se(IV), that is SeO_3^{2-}, such that concentrations of this Se(IV) species are highly correlated with those of *C. reinhardtii* [Se] (Figure 4B) at a constant total Se(IV) of 63 nM. Greater bioavailability of the deprotonated Se(IV) species is consistent with the fact that it has a higher ionic charge (-2) than the protonated

species (-1) and thus has greater electrostatic attraction to anionic transporters in biological membranes. At pH 9, Se(IV) accumulation was five times higher than at pH 7 (Figure 4B). This phenomenon can be important in natural systems where Se is present mainly as Se(IV), as well as where pHs are high, such as in oceans (pH 8.2) or downstream from water bodies such as Kelly Lake and Lake Rouyn that have been treated with lime to neutralize acidic effluents (Lemly 2004; Ponton and Hare 2013). Although the Se(IV) concentration was constant in our experiment, in nature, Se(IV) concentrations could increase with increasing pH because of its tendency to adsorb to particles at acidic pH (Lemly 2004; Ponton and Hare 2013), which would also favor Se uptake of SeO_3^{2-}. Those results, and the evidence that at high SO_4 concentrations, Se(IV) uptake is more important than Se(VI), suggest that in Lake Rouyn (high SO_4 concentrations and high pH), Se(IV) uptake could be favored over that of Se(VI).

C. reinhardtii accumulated significantly more Se ($P < 0.05$) when exposed for 12 h to selenomethionine at either pH 8.5 or 9.0 compared to that at pH 7.0 or 7.5 (Figure 4C). To observe if this pH-related effect was, as for Se(IV), a consequence of the charge of the Se species, we calculated the net Se-Met charge at different pHs. Se-Met uptake was highly correlated with the charge of Se-Met, which changed as a function of the pH (Figure 4C). Se-Met has two pK_as, that is, 1.6 and 9.5 (ChemAxon website). At natural pHs from 4 to 7, the charge of the molecule is neutral but gradually becomes more negative as the pH increases from 7 to 10 (Supp. Data, Figure S2). Again, as for Se(IV), higher pH could favor the uptake of Se-Met in nature.

Temporal patterns of Se uptake by Chlamydomonas reinhardtii in the presence of sulfate

When exposed to selenomethionine for 4 days (Figure 5), [Se] in *C. reinhardtii* were about the same (4 µg/g) as when this alga was exposed to Se(IV) (without SO_4 at pH 7.5; Figure

3B). It took 30 h for the alga to reach a steady state in its [Se] when exposed to selenomethionine, whereas it took only 12 h to reach a steady state when exposed to the two inorganic species (Figure 5). Note that the apparent decline in algal [Se] when the alga was exposed to Se(VI) (between 12 and 48 h) was likely caused by dilution due to algal growth. The inorganic and Org-Se species also differed because there was an initial lag in the uptake of the selenomethionine whereas this was not the case for the inorganic species (Figure 5). After two days, the algae exposed to Org-Se had [Se] about three times higher than those exposed to the inorganic Se species (Figure 5), which is explained in part by the fact that SO_4 (100 µM) in the exposure medium reduced the accumulation of the inorganic Se species (as shown in Figure 3) but not that of Org-Se. Fournier et al. (2006) reported similar results for the same algal species and the same SO_4 concentrations (100 µM). In their study, selenomethionine uptake after 1 h was much greater than with Se(VI) (16×) and Se(IV) (100×) at their lowest aqueous concentrations tested (2530 nM). To explain the lag in Se accumulation by the alga when exposed to selenomethionine we hypothesize that *C. reinhardtii* used a non-specific deaminase to use the methionine from the Se-Met as a source of nitrogen (Munoz-Blanco et al. 1990). Munoz-Blanco et al. (1990) showed that between 20 and 48 h, there was an important decline in L-methionine concentrations in the *C. reinhardtii* growth medium and that algal growth was proportional to this decline. We also observed a rise of *C. reinhardtii* [Se] during a similar time period (20 to 48 h; Figure 5). Se-Met deamination could have resulted in the formation of Se(IV) that would have been absorbed. In fact, the [Se] of *C. reinhardtii* exposed to Se-Met after 30 h is similar to that when this alga is exposed to Se(IV) without SO_4 (Figure 3).

Microplankton selenium accumulation in the laboratory

There was a substantial difference in the absolute [Se] measured in cultured *C. reinhardtii* exposed to selenomethionine (4 µg/g dw at 63 nM; Figures 4C and 5) and those measured in field microplankton (30 µg/g dw) where ambient Org-Se concentrations were lower than 10 nM (Figure 2C). To investigate this large difference, we exposed microplankton (< 64 µm) collected from an uncontaminated lake to selenomethionine under the same exposure regime that we used for *C. reinhardtii*. As expected from our field measurements, the steady state microplankton [Se] (Figure 6) was ~30 times higher than that attained by *C. reinhardtii* (Figures 4 and 5). This result suggests that the difference we measured between *C. reinhardtii* [Se] in the laboratory and those in field microplankton are not explained by a difference in the species of organic Se to which algae are exposed (selenomethionine rather than selenocysteine) but by the types of organisms that make up field microplankton community.

To explain this difference between algae in the laboratory and field-collected microplankton, we identified the algae in our samples of field microplankton and found that 80% of the biovolume was composed of Chrysophyceae, Dinophyceae, Euglenophyceae and Cryptophyceae (Supp. Data, Figure S3), whereas only 10% were green algae (Chlorophyceae, of which *C. reinhardtii* is a part). Baines and Fisher (2001) reported that some of the algal groups that were important in our microplankton samples (Dinophyceae and Cryptophyceae) accumulate higher [Se] in the laboratory than do many others. Another second major difference between the laboratory algal cultures and field microplankton was that bacteria were absent in the former whereas in our field samples they were more numerically abundant than were algae, that is, 15,500 ± 3,580 bacterial cells/µL compared to 2.5 algae cells/µL (n = 3). Bacteria are reported to explain from 34 to 67% of the Se uptake in natural samples exposed to Se(IV) (Baines et al.

2004). When expressed as cell volumes, there was no significant difference between bacterial and algal biovolumes in our field microplankton ($1.6\times10^6 \pm 0.4\times10^6$ $\mu m^3/\mu L$ and 1×10^6 $\mu m^3/\mu L$). However, in terms of surface area, bacteria in our microplankton samples greatly exceeded algae (30,000 ± 7000 versus 1,150 $\mu m^2/\mu L$, respectively), which suggests that the total number of bacterial uptake sites may be much higher in number than those of algae. This factor may explain the difference in Se accumulation between field microplankton samples and *C. reinhardtii*. We conclude that Se uptake in nature varies among taxa, since organic Se uptake by the green alga *C. reinhardtii* is 30 times lower than that of microplankton composed of several algal types and bacteria exposed to the same Se-Met concentration (63 nM).

Field-collected microplankton took up less Se (35 µg/g dw; Figure 7) than did *C. reinhardtii* (225 µg/g) when exposed to 63 nM Se(VI) (without added SO_4). The lower uptake of Se by microplankton than by algae when exposed to Se(VI) without sulfate also suggested that a low proportion of green algae was present in the lake microplankton. A SO_4 concentration of 0.2 µM ($10^{-6.7}$ M) reduced microplankton Se(VI) accumulation by 50% (Figure 7), whereas the comparable value for *C. reinhardtii* was 1 µM, which suggests a higher affinity of Se(VI) for this alga's Se transporters than for those of lake microplankton. At the highest SO_4 concentration (4 mM) tested, the microplankton [Se] (~5 µg/g) was higher than those measured in *C. reinhardtii* exposed to Se(VI) (< 0.2 µg/g) (Figure 3A).

When exposed to Se(IV) for 12 h in the laboratory (without added SO_4), the microplankton [Se] was 51 µg/g (Figure 7) which was much higher than that of *C. reinhardtii* (4 µg/g). In contrast to our results obtained with *C. reinhardtii*, we measured no significant competitive effect of SO_4 on Se(IV) accumulation by lake microplankton (Figure 7). Those

results with natural microplankton suggest again that Se(IV) uptake is less influenced by SO_4 concentrations than Se(VI) uptake is.

Estimation of Se concentrations in field plankton from modeled parameters

We used our laboratory data to estimate [Se] in indigenous planktonic organisms. Because Se accumulation by microplankton better represented field values than did that of *C. reinhardtii*, we used the data from our microplankton Se exposures in the laboratory as input to the equation below (modified from Presser and Luoma 2010). Steady state [Se] in microplankton or particulate organic matter ($[Se]_{microplankton}$; µg/g) can be expressed as:

$$[Se]_{microplankton} = k_{d\,(IV)}[Se(IV)] + \frac{(5.4 + 0.63e^{(-0.43[SO_4])})[Se(VI)]}{63} + k_{d\,(Org\text{-}Se)}[Org - Se] \quad (1)$$

where $k_{d(IV)}$ and $k_{d(Org\text{-}Se)}$ are partition coefficient constants (L/Kg) obtained from our measurements of uptake by microplankton of dissolved Se(IV) and Org-Se, respectively. The k_d for these two Se species were obtained by dividing the steady state microplankton [Se] exposed to Se(IV) (Figure 7; mean = 0.633 x 10^6 nmol/Kg dw) or to selenomethionine (Figure 6; 1.38 x 10^6 nmol/Kg) by the dissolved [Se] in the exposure medium (63 nM). The calculated k_d for Se(IV) ($k_{d(IV)}$) was 1.01 x 10^4 L/Kg whereas the k_d for selenomethionine ($k_{d(Org\text{-}Se)}$) was 2.19 x 10^4 L/Kg. In the Se(VI) term of equation 1 (second term), the k_d ($k_{d(VI)}$) was replaced by the equation for the curve presented in Figure 7 (which includes the competitive effect of SO_4 on Se(VI) uptake) divided by the dissolved [Se] (63 nM). These high k_ds (especially for Org-Se) obtained from our laboratory experiments are similar to the one calculated from Figure 2C (3.4 x 10^4 ± 1.3 x 10^4 L/Kg; ± SD; n = 12) and those reported for sites in California (Salton Sea Estuary, 1.74 x 10^4 L/Kg; Newport Bay, 1.89 x 10^4 L/Kg; San Francisco Bay, 2.15 x 10^4 L/Kg), and in Xiamen

Bay, Fujian Province, China and are among the highest cited in the literature review of Presser and Luoma (2010).

Using equation 1, along with the estimated values of the partition coefficient constants (equation 1), the concentrations of the three Se species and SO_4 concentrations in lakewater (Ponton and Hare 2013; Supp. Data, Table S1), we estimated field microplankton [Se] from each lake. To estimate copepod [Se], we multiplied the estimated values obtained for microplankton by the trophic transfer factor ($TTF_{copepods} = [Se]_{copepods}/[Se]_{microplankton}$) of 1.0 ± 0.7 (± SD; n = 12; Supp. Data, Figure S4A). Note that this TTF value for copepods is similar to that reported by Presser and Luoma (2010) for marine copepods (TTF of 1.35). We then multiplied the estimated copepod [Se] by the TTF of *Chaoborus* ($[Se]_{Chaoborus}/[Se]_{copepods}$), which is 0.6 ± 0.2 (±SD; n = 13; Supp. Data, Figure S4B), to obtain the estimated *Chaoborus* [Se] from each lake. This TTF value for *Chaoborus* is somewhat lower than those for other predators (0.8 and 1.5; Presser and Luoma 2010). These consumer-prey relationships were highly significant at $P < 0.01$ (Supp. Data, Figure S4).

Estimated *Chaoborus* [Se] are in good agreement with those measured in the field, as indicated by the similarity of the regression line to the 1 to 1 line in Figure 8. Note that this model takes into account many independent measurements that could have large uncertainties and therefore careful application and interpretation are needed. Comparing the contributions of the three Se species to the predicted microplankton [Se] (equation 1), and extrapolating up the food chain to *Chaoborus* larvae, suggests that the majority (74 ± 19 %) of the Se accumulated by the food chain is explained by the dissolved Org-Se concentrations in lakewater. Our model predicted that dissolved Se(VI) concentrations do not explain any (0 ± 1 %) of the Se accumulated by the food chain leading to *Chaoborus*. The main reason are that Se(VI)

concentrations are very low in our lakes (Supp. Data, Table S1), microplankton accumulated less Se when exposed to Se(VI) than Se(IV) and Se(VI) uptake only was influenced by SO_4 in the laboratory (Figure 7). In contrast, Se(IV) explains 26 ± 19 % of the predicted *Chaoborus* [Se].

Figure 8 shows that model predictions are improved by considering Se(IV) along with Org-Se accumulation, especially for the highly contaminated lakes Rouyn (2010 and 2011) and Kelly (2011). This result appears to contradict the trend in Figure 2 where considering Se(VI) and not Se(IV) improved the prediction of Se accumulation in nature. This apparent contradiction could be the result of a difference in plankton community composition between the lakes located in mining areas and the microplankton we collected from Lake Bedard as well as the fact that in Lake Rouyn (high pH and high SO_4 concentrations), Se(IV) uptake could have been higher than Se(VI) uptake, as suggested by our laboratory results.

CONCLUSION

The results of our study suggest that caution should be used when extrapolating from measurements of Se uptake by single algal species to the complex phytoplankton communities present in the field. Furthermore, the influences of SO_4 and pH on Se uptake by algae and bacteria need to be considered. Overall, organic Se appears to be the main Se species responsible for Se uptake by plankton in oligotrophic Canadian Shield lakes impacted by mining activities. Of the two inorganic Se species, Se(IV) uptake will be favored over Se(VI) in either high pH or high SO_4 environments. At low SO_4 concentrations and low pH, Se(VI) uptake could be more important than Se(IV) uptake for the same dissolved [Se].

Supplemental Data—The Supplemental Data are available on the Wiley Online Library at DOI: 10.1002/etc.xxxx.

Acknowledgment—We thank A. Crémazy for assistance on algae exposures, A. Przytulska-Bartosiewicz for algae identification and Y.W. Chen and N. Belzile for their help with Se speciation measurements. We are also thankful to M. Rosabal, N. Fabien-Ouellet and A. Clerqc for their assistance in the field. The present study was supported by the Natural Sciences and Engineering Research Council of Canada.

Data Availability—Electronic supplemental data are available on the Wiley Online Library. Please contact the corresponding author (Dominic.ponton@umontreal.ca) for unpublished data access.

REFERENCES

Aguilar-Barajas E, Díaz-Pérez C, Ramírez-Díaz MI, Riveros-Rosas H, Cervantes C. 2011. Bacterial transport of sulfate, molybdate, and related oxyanions. *BioMetals* 24:687–707.

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

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

Baines SB, Fisher NS, Doblin MA, Cutter GA. 2001. Uptake of dissolved organic selenide by marine phytoplankton. *Limnol. Oceanogr.* 46:1936–1944.

Besser JM, Canfield TJ, Lapoint TW. 1993. Bioaccumulation of organic and inorganic selenium in a laboratory food chain. *Environ. Toxicol. Chem.* 12:57–72.

Bowie GL, Sanders JG, Riedel GF, Gilmour CC, Breitburg DL, Cutter GA, Porcella DB. 1996. Assessing selenium cycling and accumulation in aquatic ecosystems. *Water, Air and Soil Pollution* 90:93–104.

Carter JCH, Kwik JK. 1977. Instar succession, vertical distribution, and interspecific competition among four species of *Chaoborus*. *J. Fish. Res. Board Can.* 34:113–118.

Chan YT, Kuan WH, Chen TY, Wang MK. 2009. Adsorption mechanism of selenate and selenite on the binary oxide systems. *Water Res* 43:4412–4420.

Chen YW, Zhou M, Tong J, Belzile N. 2005a. Application of photochemical reactions of Se in natural waters by hydride generation atomic fluorescence spectrometry. *Anal. Chim. Acta* 545:142–148.

Chen YW, Zhou XL, Tong J, Truong Y, Belzile N. 2005b. Photochemical behavior of inorganic and organic selenium compounds in various aqueous solutions. *Anal. Chim. Acta* 545:149–157.

Conde JE, Sanz Alaejos M. 1997. Selenium concentrations in natural and environmental waters. *Chem. Rev.* 97:1979–2003.

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

Croteau MN, Hare L, Marcoux P. 2003. Feeding patterns of migratory and non-migratory fourth instar larvae of two coexisting *Chaoborus* species in an acidic and metal contaminated lake: Importance of prey ingestion rate in predicting metal bioaccumulation. *Arch. Hydrobiol. 158:*57–74.

Cutter GA. 1978. Species determination of selenium in natural waters. *Anal. Chim. Acta* 98:59–66.

Cutter GA. 1982. Selenium in reducing waters. *Science* 217:829–831.

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

Fortin C, Dutel L, Garnier-Laplace J. 2004. Uranium complexation and uptake by a green alga in relation to chemical speciation: The importance of the free uranyl ion. *Environ. Toxicol. Chem.* 23:974−981.

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

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

Hare L, Carter JCH. 1987. Zooplankton populations and the diets of 3 *Chaoborus* species (Diptera, Chaoboridae) in a tropical lake. *Fresh. Biol*. 17:275–290.

Hare L, Tessier A, Croteau MN. 2008. A biomonitor for tracking changes in the availability of lakewater cadmium over space and time. *Hum. Ecol. Risk Assess.* 14:229–242.

Hillebrand H, Dürselen C, Kirschtel D, Pollingher U, Zohary T. 1999. Biovolume calculation for pelagic and benthic microalgae. *J. Phycol.* 35:403–424.

Hu M, Yang Y, Martin J, Yin K, Harrison PJ. 1997. Preferential uptake of Se(IV) over Se(VI) and the production of dissolved organic Se by marine phytoplankton. *Mar. Environ. Res.* 44:225–231.

Kinraide TB. 2003. The controlling influence of cell-surface electrical potential on the uptake and toxicity of selenate (SeO_4^{2-}) *Physiol. Plant.* 117:64–71.

Lemly AD. 1993. Teratogenic effects of selenium in natural fish populations of freshwater fish. *Ecotoxicol. Environ Saf.* 26:181–204.

Lemly DA. 2004. Aquatic selenium pollution is a global environmental safety issue. *Ecotoxicol Environ. Saf.* 59:44–56.

Li Z, Man N, Wang S, Liang D, Liu J. 2015. Selenite adsorption and desorption in main Chinese soils with their characteristics and physicochemical properties. J Soils Sediments 15:1150–1158.

Lindblow-Kull C, Kull FJ, Shrift A. 1985. Single transporter for sulfate, selenate, and selenite in *Escherichia coli* K-12. *J. Bacteriol.* 163:1267–1269.

Luoma SN, Rainbow PS. 2008. Selenium: dietary exposure, trophic transfer and food web effects. In: Luoma SN, Rainbow PS, Eds, *Metal contamination in aquatic environments: science and lateral management*. Cambridge University Press, Cambridge, U.K. pp 327–351.

Lustigman B, Lee LH, Weiss-Magasic C. 1995. Effects of cobalt and pH on the growth of *Chlamydomonas reinhardtii*. *Bull. Environ. Contam. Toxicol.* 55:65−72.

Morlon H, Fortin C, Floriani M, Adam C, Garnier-Laplace J, Boudou J. 2005. Toxicity of selenite in the unicellular green alga *Chlamydomonas reinhardtii*: Comparison between effects at the population and sub-cellular level. *Aquat. Toxicol.* 73:65–78.

Morlon H, Fortin C, Adam C, Garnier-Laplace J. 2006. Selenite transport and its inhibition in the unicellular green alga *Chlamydomonas reinhardtii*. *Environ. Toxicol. Chem.* 25:1408–1417.

Munoz-Blanco J, Hidalgo-Martinez J, Cardenas J. 1990. Extracellular deamination of L-amino acids by *Chlamydomonas reinhardtii* cells. *Planta* 182:194 –198.

Neumann PM, De Souza MP, Pickering IJ, Terry N. 2003. Rapid microalgal metabolism of selenate to volatile dimethylselenide. *Plant Cell* 26:897–905.

Ponton DE, Caron A, Hare L, Campbell PGC. 2016. Hepatic oxidative stress and metal subcellular partitioning are affected by selenium exposure in wild yellow perch (*Perca flavescens*). *Environ. Poll.* 214:608–617.

Ponton DE, Hare L. 2009. Assessment of nickel contamination in lakes using the phantom midge *Chaoborus* as a biomonitor. *Environ. Sci. Technol.* 43:6529–6534.

Ponton DE, Hare L. 2015. Feeding behavior and selenium concentrations in yellow perch (*Perca flavescens*). *Environ. Sci. Technol.* 49:7633–7640.

Ponton DE, Hare L. 2013. Relating selenium concentrations in a planktivore to selenium speciation in lakewater. *Environ. Poll.* 176:254–260.

Presser TS, Luoma SN. 2010. A methodology for ecosystem-scale modeling of selenium. *Int. Environ. Assess. Manag.* 6:685–710.

Presser TS, Ohlendorf HM. 1987. Biogeochemical cycling of selenium in the San Joaquin Valley, California, USA. *Environ. Manag.* 11:805–821.

Reich HJ, Hondal RJ. 2016. Why nature chose selenium. *ACS Chem. Biol.* 11:821−841.

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

Saether OA. 1972. Nearctic and Palaearctic *Chaoborus* (Diptera: Chaoboridae). In: Das Zooplankton der Binnengewässer; Schweizerbart'sche Verlagsbuchhandlung. Stuttgart, Germany, pp 257–304.

Schecher WD, McAvoy DC. 1992. MINEQL+: A software environment for chemical equilibrium modeling. *Comput. Environ. Urban* 16:65–76.

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

Stewart AR, Grosell M, Buchwalter D, Fisher N, Luoma SN, Mathews T, Orr P, Wang WX. 2010. Bioaccumulation and trophic transfer of selenium. In: Chapman PM, Adams WJ, Brooks ML, Delos CG, Luoma SN, Maher WA, Ohlendorf HM, Presser TS, Shaw DP, Eds, Ecological assessment of selenium in the aquatic environment. CRC Press 2010, New York, USA, pp 93–139.

US EPA. 2016. Aquatic life ambient water quality criterion for selenium – freshwater. 2016. United States Environmental Protection Agency. Office of Water, Office of Science and Technology, Washington, D.C. EPA 822-R-16-006, 807 p.

Vriens B, Behra R, Voegelin A, Zupanic A, Winkel LHE. 2016. Selenium uptake and methylation by the microalga *Chlamydomonas reinhardtii*. *Environ. Sci. Technol.* 50:711–720.

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

Wang D. 1994. Loss of selenium in water samples at natural levels during storage. *Microchim. Acta* 116:33–39.

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

Young TF, Finley K, Adams W, Besser J, Hopkins WA, Jolley D, McNaughton E, Presser TS, Patrick-Shaw D, Unrine J. 2010. What you need to know about selenium. In: Chapman PM, Adams WJ, Brooks ML, Delos CG, Luoma SN, Maher WA, Ohlendorf HM, Presser TS, Shaw DP, Eds, Ecological assessment of selenium in the aquatic environment. CRC Press 2010, New York, USA, pp 7–36.

Figure 1. Map showing the location of Rouyn-Noranda (Quebec) and Sudbury (Ontario), Canada (stars), and our study lakes in these regions. The locations of major smelters are represented by chimneys.

Figure 2. Relationships between Se concentrations (± SD; μg/g dry weight; n = 3-5) in field-collected A: *Chaoborus* larvae (redrawn from Ponton and Hare 2013)); B: copepods; C: microplankton (< 64 µm) and the concentrations of dissolved organic selenium (Org-Se) plus Se(VI) (nM; n = 3). Triangles represents water Org-Se + Se(VI) concentrations and squares are only water Org-Se concentrations. Data for Kelly Lake (solid symbols) were not included in the regression. Regressions are significant at P = or < 0.001.

Figure 3. Selenium concentrations in *Chlamydomonas reinhardtii* ($[Se]_{C.\ reinhardtii}$; µg/g dry weight) exposed for 12 h at a pH of 7.5 to 63 nM of either A: Se(VI) (means ± SD; n = 3) or B: Se(IV) (n = 1) and at various concentrations of sulfate (log $[SO_4]$; M). Dashed lines are *C. reinhardtii* Se concentrations in treatments without added sulfate. Insert in panel A is Se uptake at the highest sulfate concentrations. Curves of best fit have $r^2 > 0.99$ and $P < 0.001$.

Figure 4. Mean (± SD, n = 3) selenium concentrations in *Chlamydomonas reinhardtii* ($[Se]_{C.\ reinhardtii}$; µg/g dry weight) exposed at various pHs (sulfate absent) for 12 h to 63 nM of either A: Se(VI), B: Se(IV) or C: selenomethionine. In B, the concentrations of the deprotonated Se(IV) species (SeO_3^{2-}, as calculated using MINEQL+) were varied by changing the pH of the exposure solution (treatment pH is given above data points; the two dominant Se(IV) species are SeO_3^{2-} and $HSeO_3^-$; pK_a = 8.4). In C, the selenomethionine negative charge is obtained from Supp. Data Figure S2 and calculated from the pK_as of 1.6 and 9.5 (treatment pH is given above data points). Best fit curves have $r^2 > 0.99$ and $P < 0.002$.

Figure 5. Selenium concentrations in *Chlamydomonas reinhardtii* ($[Se]_{C.\ reinhardtii}$; µg/g dry weight) exposed to 63 nM of either selenomethionine (solid squares), Se(IV) (open circles) or Se(VI) (open triangles) for up to 100 h at a sulfate concentration of 100 µM and a pH of 7.5. Values are means ± SD (n = 3) for selenomethionine (48, 72, 96 h), whereas those for Se(VI) and Se(IV) are single samples. Both curves of best fit have r^2 = 0.99 and P = 0.01.

Figure 6. Mean (± SD, n = 3) selenium concentrations of field-collected microplankton (< 64 µm; $[Se]_{microplankton}$; µg/g dry weight) exposed for 0, 12 or 24 h to 63 nM of selenomethionine at a sulfate concentration of 100 µM.

Figure 7. Selenium concentrations in field-collected microplankton (< 64 µm; $[Se]_{microplankton}$; µg/g dry weight; n = 1) exposed, at pH 7.5, to 63 nM Se(VI) (closed circles) or Se(IV) (open triangles) at various sulfate concentrations.

Figure 8. Predicted selenium concentrations in *Chaoborus* larvae (µg/g dry weight) from 16 lakes as a function of predicted values (from equation 1) based on selenium concentrations in lakewater (Ponton and Hare, 2013) and on laboratory measurements of Se uptake by field-collected microplankton. Broken line is 1:1 relationship. Closed symbols are estimations considering dissolved organic selenium (Org-Se) concentrations only and open symbols are estimations with both dissolved organic Se and Se(IV) concentrations.

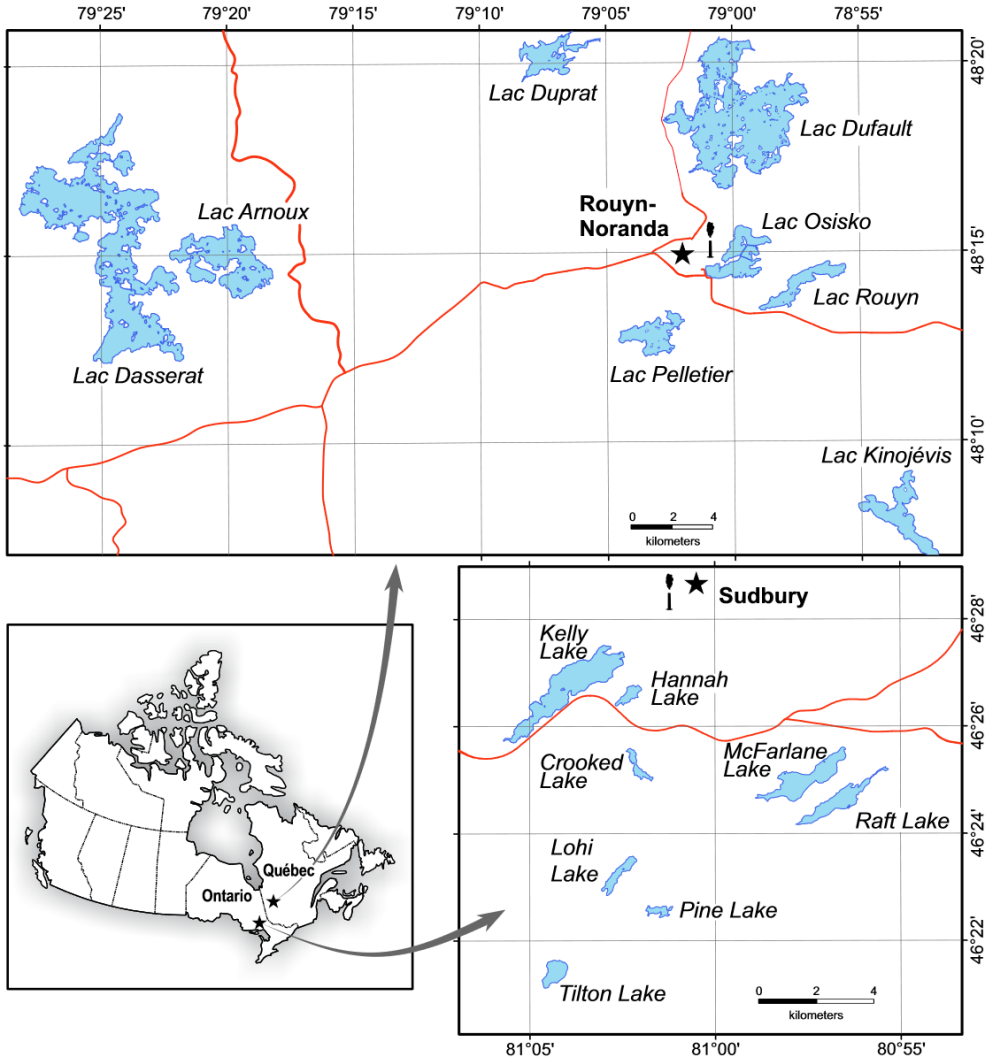

Figure 1; Ponton et al. 2018

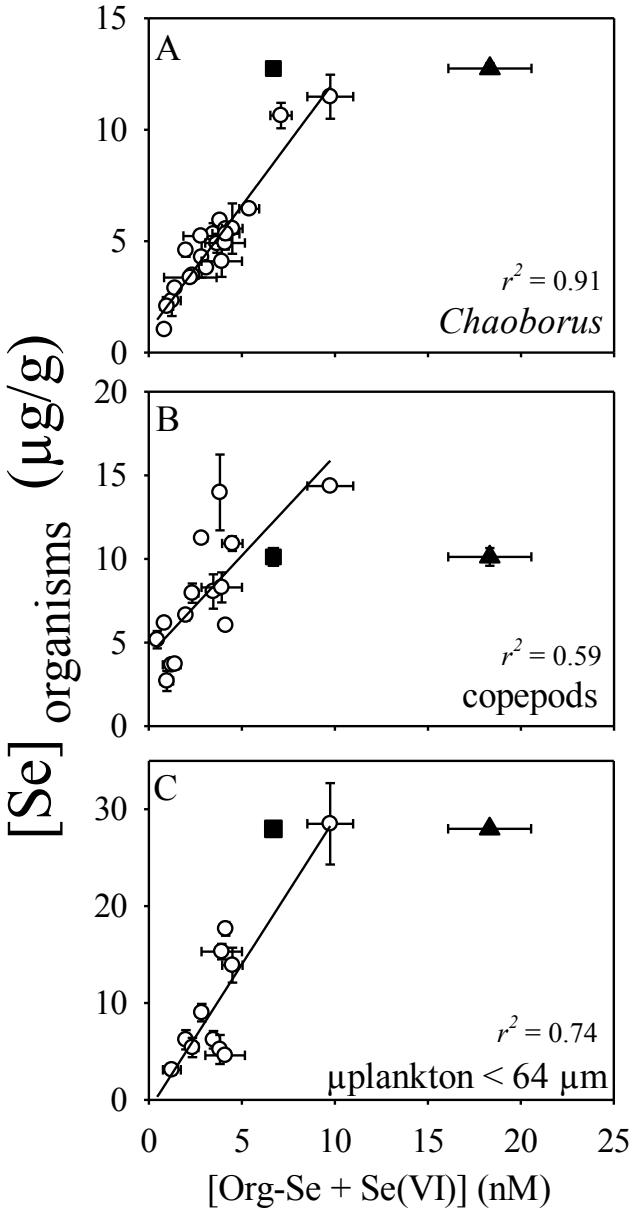

Figure 2: Ponton et al. 2018

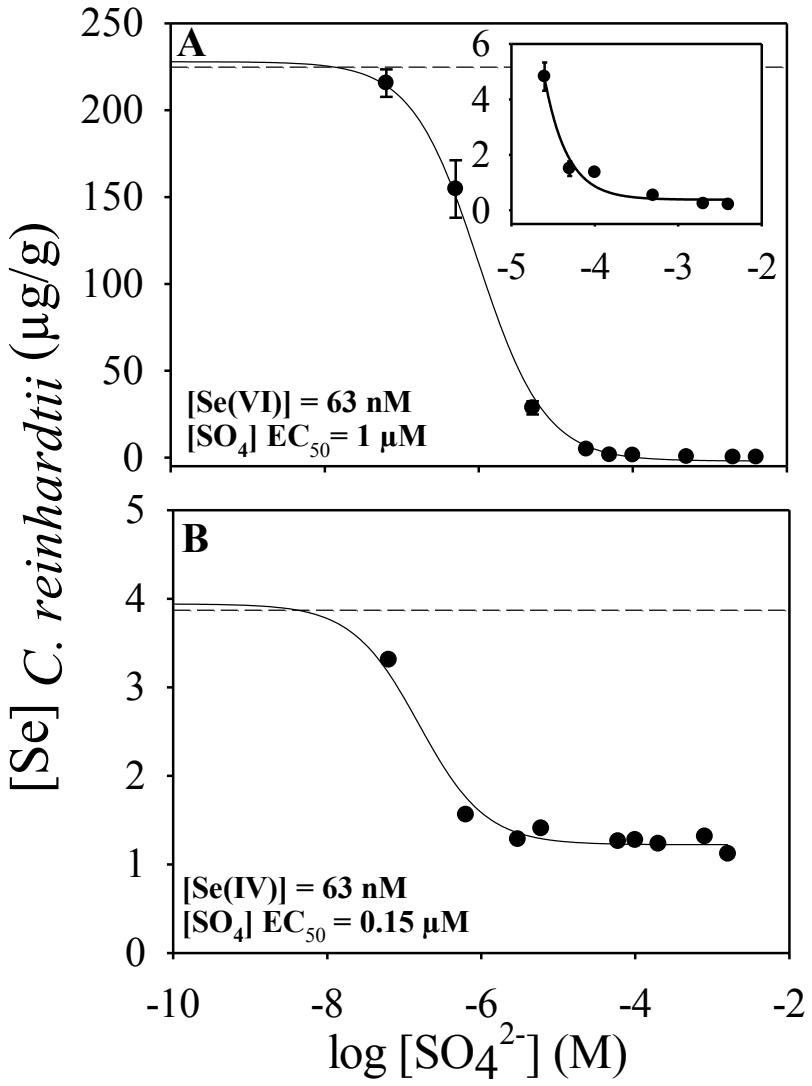

Figure 3; Ponton et al. 2018

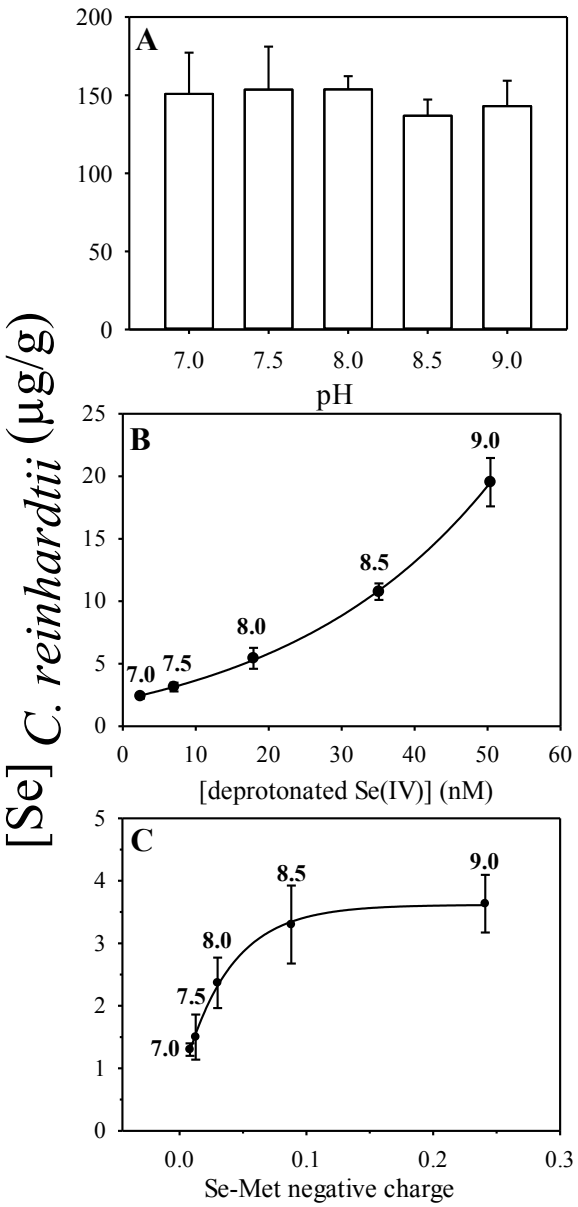

Figure 4; Ponton et al. 2018

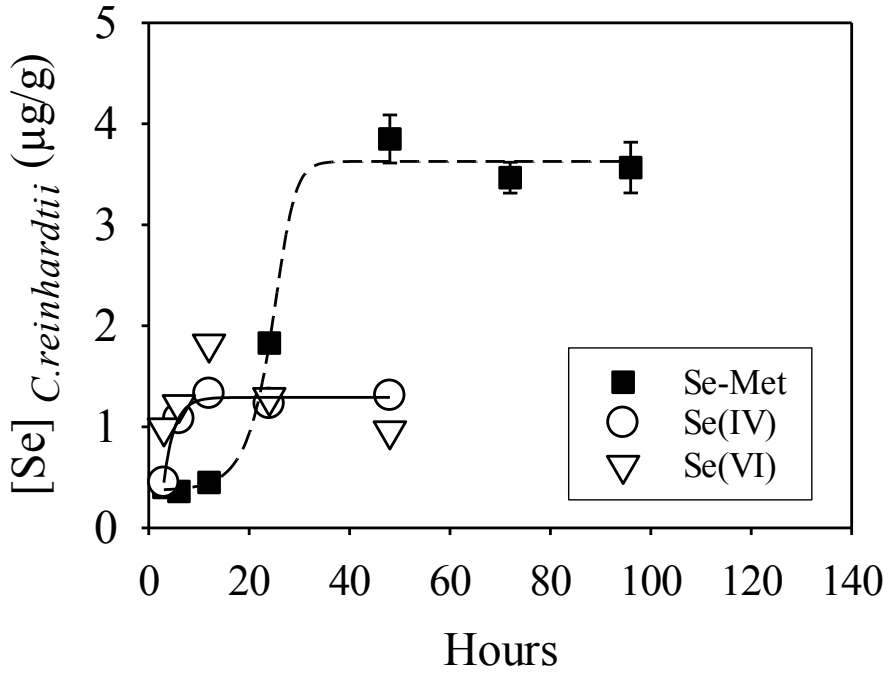

Figure 5; Ponton et al., 2018

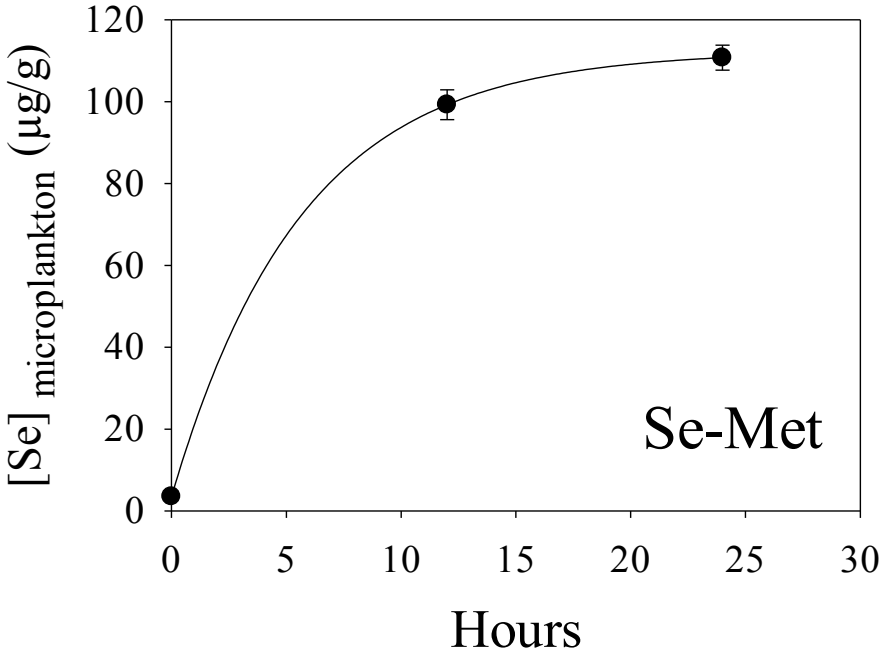

Figure 6; Ponton et al. 2018

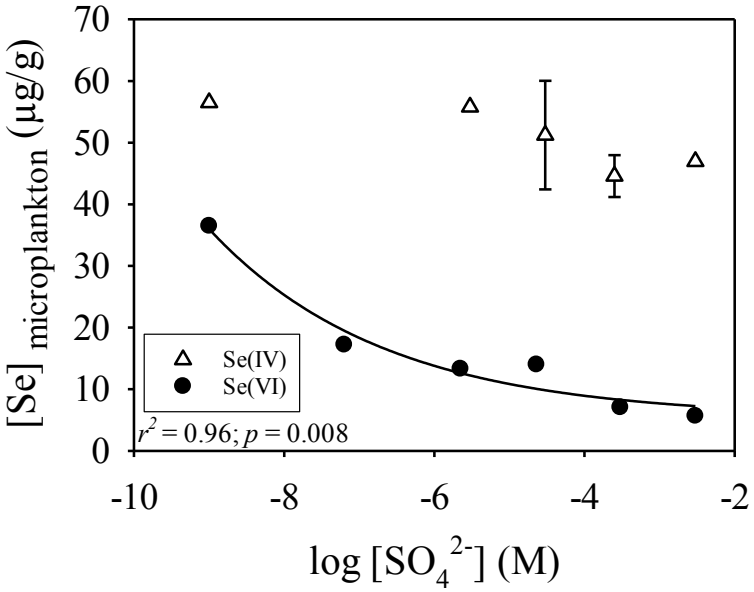

Figure 7; Ponton et al. 2018

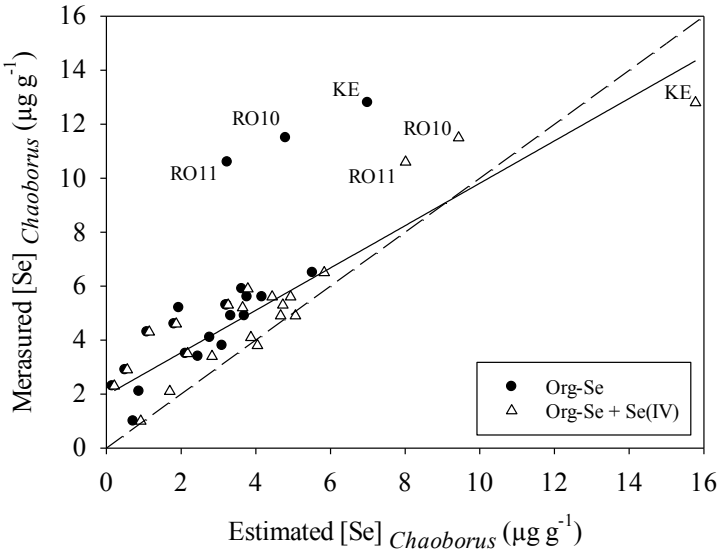

Figure 8; Ponton et al. 2018