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Uptake from Water, Internal Distribution and Bioaccumulation of Selenium in *Scenedesmus obliquus*, *Unio mancus* and *Rattus norvegicus*: Part A

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Abstract The ^{75}Se internal bioavailability was investigated in microalgae, mussels and rats as biological experimental models. The ^{75}Se accumulation from freshwater to microalgae [*Scenedesmus obliquus* (Turpin) Kützing], from freshwater to mussels (*Unio mancus* Lamark) and, finally, per os to rats (*Rattus norvegicus* Berkenhout) was followed using ^{75}Se-labelled selenite looking at ^{75}Se uptake, retention, intracellular distribution and binding with cellular biocomplexes. After exposure to 10, 50 and 500 µg Se L^{-1}, the microalgae showed an inhibitory effect on population growth only at the highest concentration. Mussels exposed to 105 µg Se L^{-1} showed an accumulation of the element with time in all tissues. Intracellularly, Se was present in all subcellular fractions, especially in the cytosol. Rats were treated via oral administration with 5 µg Se rat^{-1}. After 24 h, liver and kidney showed the highest Se concentration.

Keywords Selenium · Microalgae · Mussels · Rats · Water uptake · Bioavailability

The increased availability of selenium is mainly related to industrial and agricultural activities that directly or indirectly are able to mobilize it from natural geological sinks, such as in the combustion of coal and other fossil fuels (Rosen and Liu 2009), or in the application of fertilizers, in which it is present as an impurity (Hopkins et al. 2004). Recent research papers and reviews (Hartikainen 2005; Hamilton 2004; Hopkins et al. 2005; Orr et al. 2006; Alquezar et al. 2007; Navarro-Alarcon and Cabrera-Vique 2008) attributed the uptake of selenium by biota from both water and diet, with a prevailing role of dietary exposure in animals (Chapman et al. 2010; Presser and Luoma 2010). Several papers described Se in aquatic food webs from various viewpoints (Hamilton 2004), but only limited data are available about bioaccumulation and biomagnification

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rates through the food chain considering mammals as the final target (Hamilton 2004; Navarro-Alarcon and Cabrera-Vique 2008; Chapman et al. 2010; Presser and Luoma 2010).

The Se subcellular compartmentalization, or distribution within the cells, depends on its water and food chain availability. Thus, an experimental study was undertaken to compare the metabolic behaviour of ^{75}Se in microalgae, mussels and rats directly exposed to the ionic forms of ^{75}Se. The uptake of Se from freshwater to microalgae [*Scenedesmus obliquus* (Turpin) Kützing], from freshwater to mussels (*Unio mancus* Lamark) and per os to rats (*Rattus norvegicus* Berkenhout) was investigated by the use of ^{75}Se-labelled selenite ions. Moreover, another goal of this paper was to report on selenite ion intracellular distribution and binding with cellular components through the investigation of Se inter-organ distribution and variability. The outline of the experiments for studying uptake and subcellular compartmentalization of selenite in the selected experimental models includes the exposure to ^{75}Se-selenite of: (1) phytoplanktonic microalgae (effect of selenite on the population growth, accumulation by microalgae and its binding with cytosol biocomponents), (2) mussel (accumulation of Se in tissues and its intracellular distribution in the hepatopancreas and mantle, (3) rat (tissue and intracellular Se distribution in liver and kidney). These data constituted the background information for studies on bioaccumulation that was investigated in Sabbioni et al. (2014).

Materials and Methods

^{75}Se radioisotope was purchased from the Radiochemical Center (ORNL, Oak Ridge, TN, USA) as aqueous SeO_3^{2-} (pH 6.7) with specific radioactivity of 37.4 kBq (1.01 μCi) μg^{-1} Se. The radioactive solution was diluted in distilled water or in solutions containing appropriate amounts of selenite to prepare solutions at concentrations ranging from 10 to 500 μg Se L^{-1}. The ^{75}Se-selenite solutions were (1) added to the growth culture media of *S. obliquus*, (2) added to the aquarium water containing mussels (*U. mancus*), or (3) directly administered per os (p.o., oral administration) to rats. ^{75}Se-selenite chemical form was checked by high performance anion chromatography combined with radiometric detection. More than 99.5 % of the ^{75}Se-selenite solution displayed the chromatographic behavior of selenite (4) ions.

^{75}Se radioactivity in the wet samples was measured with an automatic γ-counting system Wallac 1480 3″ (Wallac, Perkin Elmer, USA) by integral gamma counting calibrated on an energy range from 50 to 450 keV (main photopeaks of ^{75}Se were 120, 136, 279 and 400 keV). Se concentration in the sample was determined by comparing the detected ^{75}Se radioactivity by ^{75}Se reference standard solutions of known specific radioactivity.

The background level of total Se in microalgae, mussels and rats tissues before their exposure to ^{75}Se was determined by radiochemical neutron activation analysis. Briefly, 50–500 mg of microalgae, mussels or rats tissues were freeze-dried and irradiated for 24 h in sealed ultrapure quartz vials in the HFR nuclear reactor (Petten, The Netherlands) at a thermal flux of 2×10^{14} neutron $cm^{-2}\ s^{-1}$. After 2 weeks decay, the induced ^{75}Se radioisotope was isolated by a radiochemical separation and the ^{75}Se radioactivity determined by computer-based high-resolution γ-ray spectrometry using a Ge detector.

A monospecific log-phase culture of the freshwater green microalgae *S. obliquus* (50 mL) was slightly centrifuged for 5 min. The pellet was resuspended in fresh Algal Assay Procedure (AAP – 1971) culture medium without additions of Na_2-EDTA, centrifuged and resuspended again to obtain a final solution to be exposed to the ^{75}Se-selenite ions. An improved Neubauer counting chamber was used to determine cell concentrations.

Aliquots of *S. obliquus* suspension were inoculated in four Erlenmeyer flasks, each containing 100 mL of AAP culture medium having a starting cell concentration of 10^4 cells mL^{-1} and a final ^{75}Se-selenite concentration of 10, 50 and 500 μg Se L^{-1}. Algae were exposed to Se for 4 days, with accumulated concentrations measured after 2–4 days.

The culture medium was prepared with bi-distilled water (Millipore GS 0.22 μm). Filtered air was introduced through a glass tube down to the bottom of the flask to ensure uniform cell distribution and an appropriate input of CO_2. The cultures were incubated at 25.0 ± 0.5°C and illuminated with two fluorescent lamps (40 W) on a 16:8 light/dark regime. During the test, the pH of the medium in the control flasks was 6.98 ± 0.14. Five mL of each culture were collected daily to determine cell concentration and ^{75}Se uptake. For this purpose, an algal suspension (2.5 mL) was slightly centrifuged for 5 min, the residue per pellet washed with $NaHCO_3$ solution (1.5 mg L^{-1}), centrifuged twice and, finally, monitored for the ^{75}Se content. A calibration curve was obtained by filtration (Millipore GS 0.45 μm) of different volumes of an uncontaminated culture. The filters were weighed after drying (5 h at room temperature in a dissecator) to obtain the algal biomass (mg L^{-1} dry weight).

Aliquots of the residue from the algal culture (cells) incubated with 105 μg Se L^{-1} were ground in a glass homogenizer and centrifuged at 105,000×*g* for 90 min. The biochemical components of the supernatant (cytosol) were fractionated at 4°C by gel filtration on Sephadex G-150 resin (Pharmacia, Uppsala, Sweden). The column (1 × 100 cm) was previously equilibrated with 10 mM

ammonium acetate buffer (pH 7.1) and calibrated by eluting a standard protein solution of known molecular weights. The eluate was spectrophotometrically monitored at 280 nm and the ^{75}Se radioactivity counted in all fractions.

Specimens of freshwater bivalve mussels (*U. mancus*) were collected near Angera port (Lake Maggiore, Italy). Animals with shell lengths between 55 and 60 mm were selected in order to obtain a group of organisms of similar age. They were acclimatized for 1 week in 10 L Plexiglas tanks that contained unfiltered Lake Maggiore water. During the acclimation period, the mussels were fed on *S. obliquus* ad libitum and the water volume was kept constant at 1.5 L for each mussel. Twelve individuals of *U. mancus* were placed in a 50 L capacity tank with 18 L aerated and filtered lake water (1.5 L mussel^{-1}) (Millipore GS 0.2 μm) containing 105 μg Se L^{-1} as ^{75}Se-selenite. At days 2, 6 and 10 from the initial exposure, four mussels were dissected each time, their tissues washed with $NaHCO_3$ solution (1.5 mg L^{-1}) and monitored for their ^{75}Se content. The ^{75}Se-containing water was changed every 2 days. During the experiment the temperature was 22.0 ± 0.1°C and the conductivity 144.0 ± 1.5 μS. After ^{75}Se counting, hepatopancreas and mantle were pooled and submitted to subcellular fractionation. The uptake of ^{75}Se was assessed in gills, hepatopancreas, foot, mantle, visceral sac residues, adductor muscle and shell.

Subcellular fractions of hepatopancreas and mantle of *U. mancus* were obtained by differential centrifugation, after homogenization in two volumes of 10 mM ammonium acetate buffer (pH 7.1). The following fractions were obtained: nuclei (700×*g* for 10 min), mitochondria (9,000×*g* for 15 min), lysosomes (30,000×*g* for 25 min), microsomes and cytosol (105,000×*g* for 110 min). Each crude fraction was purified by one washing in two volumes of the same buffer followed by centrifugation and the washing volumes were added at each step to the supernatant of the next centrifugation. The ^{75}Se content was measured in all subcellular fractions.

Male albino Sprague–Dawley rats, *R. norvegicus* (350–380 g), were obtained from Harlam Nossan (Milan, Italy). They were kept on a commercial pellet diet and mineral water ad libitum and maintained for 1 week in an animal house with light from 8 a.m. to 8 p.m. under controlled humidity (6.2 ± 10 %) and temperature (23 ± 3°C) conditions, in agreement with the EEC Directive n˙ 86/609.

The background levels of ^{75}Se in food (commercial pellet, Charles River 4RF, Calco, Como, Italy) and mineral water (Fonti S. Bernardo, Garessio, Cuneo, Italy) were 412 ng g^{-1} and 43 ng L^{-1}, respectively. Five rats were kept without food for 24 h, receiving mineral water ad libitum orally. They received 5 μg Se rat^{-1} p.o. as ^{75}Se-selenite by gastric tube under light anaesthesia. The animals were then kept in metabolic cages with free access to food and water for 24 h, at which time they were anaesthetised and sacrificed by heart puncture. The tissues (liver, kidney, spleen, lung, pancreas, testis, femur, heart, epididymis, brain and blood) were dissected out, washed and perfused with a normal saline solution, weighed and the Se radioactivity counted in all tissues. Blood was collected by heparinized syringes and centrifuged at 2,500×*g* for 10 min to separate plasma and red blood cells.

Tissues and some subcellular fractions (nucleus, mitochondria, lysosome, microsome and cytoplasm) of liver and kidney of rats exposed to ^{75}Se-selenite and gel filtration of the corresponding hepatic and renal cytosols were carried out as described above for the mussels.

The significance of differences between average values of different experimental treatments and controls was assessed by the analysis of variance (ANOVA) considering a significance threshold level always set at 5 %. When ANOVA revealed significant differences among treatments, post hoc tests were carried on with Dunnett's method and Tukey's test. Statistical analyses were performed using Microsoft® Excel 2013/XLSTAT©-Pro (Version 7.2, 2003, Addinsoft, Inc., Brooklyn, NY, USA).

Results and Discussion

The growth data of *S. obliquus* populations exposed to 10, 50, 500 μg Se L^{-1} for 4 days and the relative negative controls are shown in Table 1. No significant inhibitory or stimulating effect on algal cell growth was observed compared to the controls ($p < 0.05$). Only at day 1 and 500 μg Se L^{-1} exposure, the inhibitory effect was significantly different from the control ($p < 0.05$). At 500 μg Se L^{-1}, growth inhibition decreased at day 2 and disappeared at days 3 and 4.

The incorporation of ^{75}Se in the microalgae (natural Se content <2 ng Se g^{-1} wet weight as determined by NAA) exposed to 10, 50 and 500 μg Se L^{-1} is displayed in Fig. 1 as the mean of at least three replicates per treatment presenting a relative SD of <15 %. The uptake of Se was directly dependent on the concentration, the highest values being found at 500 μg Se L^{-1}.

The *S. obliquus* populations were able to concentrate selenite and to metabolize the incorporated element forming biocomplexes of different molecular weights. The selenium bioconcentration factors ($BCF = [Se]_{biomass}/[Se]_{water}$) in microalgae were summarized in Table 2 considering the 2- and 4-days scenarios. After 2 days of exposure, the highest BCF of 1,400 was observed at 10 μg Se L^{-1}, whereas after 4 days of exposure at 50 and 500 μg Se L^{-1} the selenium BCFs were 2,600 and 2,400, respectively.

Table 1 Effects of Se-selenite on the growth of *S. obliquus* populations

Exposure (ng ^{75}Se mL^{-1})	Algal growth (cell number 10^2 mL^{-1} ± SD)				
	0 day	1 day	2 days	3 days	4 days
Control	1.12 ± 0.26	8.91 ± 2.32	60.12 ± 9.12	113.72 ± 12.34	200.24 ± 16.63
10	1.13 ± 0.27	9.53 ± 3.14	63.37 ± 5.74	136.75 ± 17.15	237.71 ± 8.54
50	1.11 ± 0.25	10.44 ± 2.91	64.36 ± 8.71	119.91 ± 11.83	214.77 ± 9.23
100	1.10 ± 0.22	9.73 ± 3.42	62.63 ± 7.94	128.54 ± 16.15	221.48 ± 12.82
500	1.14 ± 0.21	5.21 ± 2.53*	50.81 ± 7.59	151.57 ± 11.42	216.55 ± 16.86

* Significantly different from the control ($p < 0.05$)

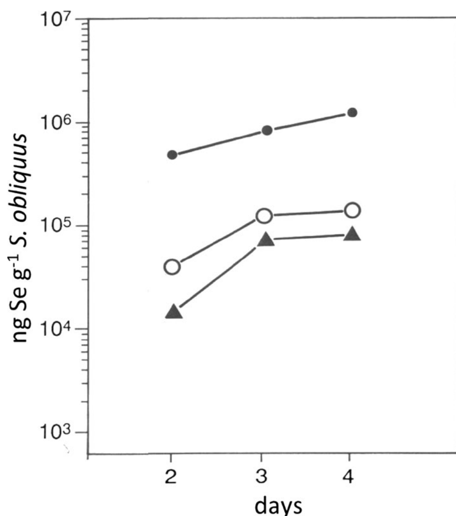

Fig. 1 Bioaccumulation curves in *S. obliquus* exposed to increasing concentrations [10 (*black triangle*), 50 (*empty circle*) and 500 (*black circle*) µg L^{-1}] of selenium as ^{75}Se-selenite (n = 3–4 per treatment with a SD < 15 %)

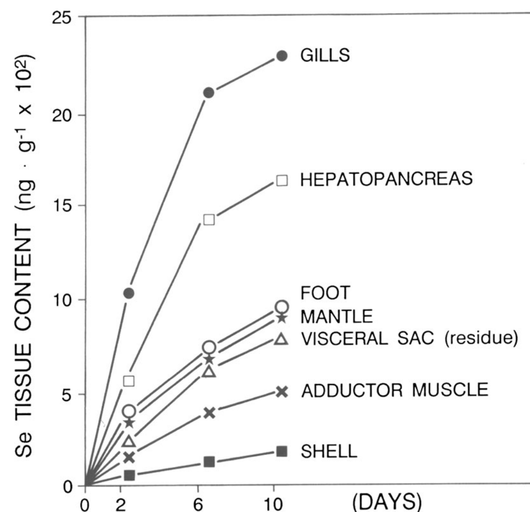

Fig. 2 Uptake and tissue distribution of selenium by *U. mancus* exposed to 105 µg ^{75}Se L^{-1} as ^{75}Se-selenite (n = 9)

Table 2 Bioconcentration factors in *S. obliquus* exposed for 2 and 4 days to 10, 50 and 500 µg ^{75}Se L^{-1} water

^{75}Se concentration (ng mL^{-1})	BCF[a]	
	2 days	4 days
10	1,400	–
50	800	2,600
500	1,000	2,400

[a] (ng ^{75}Se g^{-1} *S. obliquus*) × (ng ^{75}Se mL^{-1} water)$^{-1}$

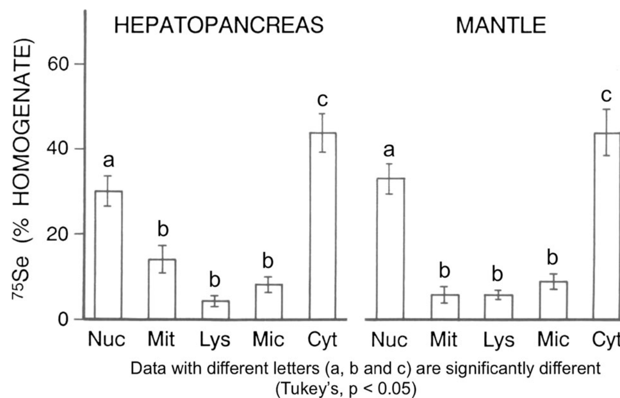

Fig. 3 Intracellular distribution of Se in hepatopancreas and mantle of *U. mancus* exposed to 105 µg ^{75}Se L^{-1} as ^{75}Se-selenite. The results, expressed as the percentage of the total ^{75}Se in the homogenate, are the mean of individual tissues from four animals and the relative SD. *Nuc* nucleus, *Mit* mitochondria, *Lys* lysosome, *Mic* microsome and *Cyt* cytoplasm

Se uptake and incorporation in various tissues of *U. mancus* exposed to 105 µg ^{75}Se L^{-1} for 2, 6 and 10 days are shown in Fig. 2 as the mean values of nine animals. Se accumulation occurred in all of the tested tissue. Particularly, after 10 days of exposure, the gill and hepatopancreas showed the highest levels of Se with 2.33 and 1.64 µg Se g^{-1} wet weight, respectively. Figure 3 shows the subcellular distribution (i.e. nucleus, mitochondria, lysosome, microsome and cytoplasm) of ^{75}Se after 10 days of exposure at 105 µg L^{-1} in hepatopancreas and mantle samples from four mussels. ^{75}Se was found in all subcellular fractions. More than 40 %

Table 3 Bioconcentration factors in tissues of *U. mancus* exposed for 2 and 10 days to 105 μg Se L^{-1} as ^{75}Se-selenite

Tissue	BCF[a]	
	2 days	4 days
Gills	10	22
Hepatopancreas	6	16
Mantle	4	9
Foot	4	9
Visceral sac (residue)	2	8
Adductor muscle	2	5
Soft tissue (total)	4	0
Shell	1	2

[a] (ng ^{75}Se g^{-1} *U. mancus*) × (ng ^{75}Se mL^{-1} water)$^{-1}$

Table 4 The distribution of selenium in rat tissues 24 h after p.o. administration of 5 μg ^{75}Se-selenite rat^{-1} (n = 5)

Tissue	Se content (mean ± SD)	
	% dose	ng g^{-1}
Liver	20.7 ± 3.60	126 ± 4.10
Kidney	3.64 ± 1.42	97.9 ± 5.30
Spleen	0.73 ± 0.21	26.6 ± 2.50
Lung	0.87 ± 0.01	23.9 ± 0.90
Pancreas	0.97 ± 0.01	20.1 ± 1.15
Testis	0.83 ± 0.02	14.2 ± 0.10
Femur	0.21 ± 0.02	13.5 ± 4.10
Heart	0.32 ± 0.02	13.2 ± 1.40
Epididymis	0.10 ± 0.01	8.44 ± 0.23
Brain	0.11 ± 0.01	4.42 ± 0.51
Blood	–	262 ± 0.20

of the ^{75}Se present in the total homogenate was in the soluble cytoplasmatic fraction and about 30 % in the nuclear fractions of both tissues.

Mussels exposed to ^{75}Se-spiked water (105 μg Se L^{-1}) showed a time dependent accumulation of Se with preferential incorporation into gills and hepatopancreas tissues, as indicated by the relative BCFs summarized in Table 3. At different exposure times, the uptake was of the order of 0.07 % of the total dose. After 10 days of exposure, the highest Se concentration was present in gill tissue, which is in agreement with their active role in interacting and accumulating ionic species of trace elements in filter feeding organisms. In these tissues, 50 %–60 % of the element was associated with cellular organelles and the remaining was found in the cytosol (Fig. 3).

The estimated BCFs in mussels ranged from 1 to 10 and from 2 to 22 after 2 and 4 days (Table 3), respectively. These values were significantly lower than those for *S. obliquus*, which may be due to their different metabolism and trophic role within the selected models.

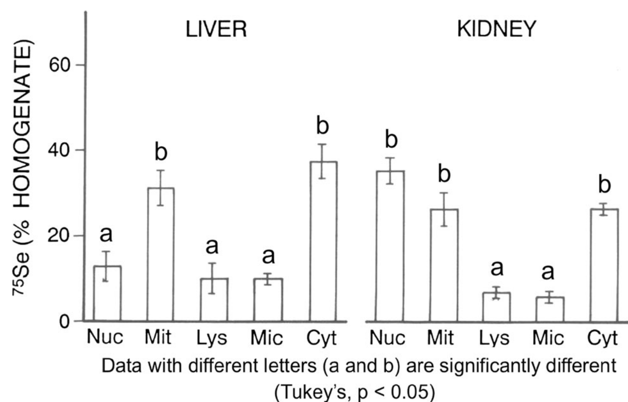

Fig. 4 Intracellular distribution of Se in liver and kidney of rats 24 h after p.o. administration of 5 μg ^{75}Se rat^{-1} as ^{75}Se-selenite. The results, expressed as the percentage of the total ^{75}Se in the homogenate, are the mean values from five animals individual tissues accompanied by the relative SD. *Nuc* nucleus, *Mit* mitochondria, *Lys* lysosome, *Mic* microsome and *Cyt* cytoplasm

The Se distribution in rat tissues and blood after p.o. administration of 5 μg Se rat^{-1} as ^{75}Se-selenite to five animals was reported in Table 4. Liver and kidney showed the highest amount of radioactivity expressed as % dose or as ng g^{-1} wet weight. Other tissues contained <1 % of the administered dose. Amongst those, the calculated Se content ranged from about 26.6 ng g^{-1} (spleen) down to 4.42 ng g^{-1} (brain).

The intracellular distribution of ^{75}Se in rat liver and kidney is summarized in Fig. 4. In the liver, the nuclear fraction contained about 13 % and the cytosol about 37 % of the Se present in the homogenate, whereas in the kidney the nuclear content was higher (35 %) and the cytosol content lower (26 %). In both tissues, considerable amounts of ^{75}Se (about 30 %) were found in the mitochondrial fraction.

The microalgae exposure to selenite suggests that the element in such form is well tolerated by the microalgae, apparently without significant inhibitory or stimulatory effects on their growth. This is coherent with the findings of Morlon et al. (2005), suggesting that Se direct toxicity on the unicellular alga *Chlamydomonas reinhardtii* (P.A.Dangeard) is not likely to take place at concentrations usually found in the environment. Moreover, this is line with Chapman et al. (2010), who reported that primary producers generally concentrate between 10^2- and 10^6-fold the Se water concentration and that the enrichment factor is species-specific. According to Chapman et al. (2010), chlorophytes tend to accumulate 0.1 mg kg^{-1} starting from water spiked with about 0.001 mg Se kg^{-1}. Voecke et al. (1980) highlighted for the same species stimulatory effects at concentrations of 10 and 50 μg Se L^{-1} as selenite (VI), while inhibition was found at 500 μg Se L^{-1}. The cause of this effect has not been established yet, even though it may

be due to the different Se oxidation state or directly related to the exposure conditions such as the composition of culture media.

The rat exposure to selenite provided information on absorption, distribution and retention of ionic ^{75}Se. After ingestion of ^{75}Se, rat liver and kidney were the main target tissues (Table 4). Indeed, the same was found also for some fish and bird species (Hamilton 2004) presenting, in particular, the liver as the main target organ, even though the exposure routes were mainly from diet. The ^{75}Se association with many biocomponents in the cytosol of rat liver and kidney is consistent with its ability to incorporate into proteins (Fan et al. 2002).

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References

Alquezar R, Markich SJ, Twining JR (2007) Uptake and loss of dissolved ^{109}Cd and ^{75}Se in estuarine macroinvertebrates. Chemosphere 67:1202–1210

Chapman PM, Adams WJ, Brooks ML et al (2010) Ecological assessment of selenium in the aquatic environment. SETAC Press, Pensacola

Fan TWM, The SJ, Hinton DE, Higashi RM (2002) Selenium biotransformation into proteinaceous forms by food-web organisms of selenium laden drainage waters in California. Aquat Toxicol 57:65–84

Hamilton SJ (2004) Review of selenium toxicity in the aquatic food chain. Sci Total Environ 326:1–31

Hartikainen H (2005) Biogeochemistry of selenium and its impact on food chain quality and human health. J Trace Elem Med Biol 18:309–318

Hopkins WA, Staub BP, Baionno JA, Jackson BP, Roe JH, Ford NB (2004) Trophic and maternal transfer of selenium in brown house snakes (*Lamprophis fuliginous*). Ecotoxicol Environ Saf 58:285–293

Hopkins WA, Staub BP, Baionno JA, Jackson BP, Talen LG (2005) Transfer of selenium from prey to predators in a simulated terrestrial food chain. Environ Pollut 134:447–456

Morlon H, Fortin C, Floriani M et al (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

Navarro-Alarcon M, Cabrera-Vique C (2008) Selenium in food and the human body: a review. Sci Total Environ 400:115–141

Orr PL, Guiguer KR, Russel CK (2006) Food chain transfer of selenium in lentic and lotic habitats of a western Canadian watershed. Ecotox Environ Saf 63:175–188

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

Rosen BP, Liu Z (2009) Transport pathways for arsenic and selenium: a mini-review. Environ Int 35:512–515

Sabbioni E, Polettini AE, Fortaner S, Farina M, Groppi F, Manenti S, Libralato G (2014) Uptake from water, internal distribution and bioaccumulation of selenium in *Scenedesmus obliquus*, *Unio mancus* and *Rattus norvegicus*: part B. Bull Environ Contam Toxicol. doi:10.1007/s00128-014-1406-3

Voecke RW, Sears KL, O'Toole JJ, Widman RB (1980) Growth responses of selected freshwater algae to trace elements and scrubber ash slurry generated by coal-fired power plants. Water Res 14:141–150