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# Selenium accumulation and metabolism in algae

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## **Abstract**

Selenium (Se) is an intriguing element because it is metabolically required by a variety of organisms, but it may induce toxicity at high doses. Algae primarily absorb selenium in the form of selenate or selenite using mechanisms similar to those reported in plants. However, while Se is needed by several species of microalgae, the essentiality of this element for plants has not been established yet. The study of Se uptake and accumulation strategies in micro- and macro-algae is of pivotal importance, as they represent potential vectors for Se movement in aquatic environments and Se at high levels may affect their growth causing a reduction in primary production. Some microalgae exhibit the capacity of efficiently converting Se to less harmful volatile compounds as a strategy to cope with Se toxicity. Therefore, they play a crucial role in Se-cycling through the ecosystem. On the other side, micro- or macro-algae enriched in Se may be used in Se biofortification programs aimed to improve Se content in human diet via supplementation of valuable food. Indeed, some organic forms of selenium (selenomethionine and methylselenocysteine) are known to act as anticarcinogenic compounds and exert a broad spectrum of beneficial effects in humans and other mammals. Here, we want to give an overview of the developments in the current understanding of Se uptake, accumulation and metabolism in algae, discussing potential ecotoxicological implications and nutritional aspects.

**Keywords: algae, selenium, uptake, accumulation, metabolism, toxicity**

## 1. Introduction

The element selenium (Se) is regarded as an indispensable micronutrient for many living organisms, including humans, mammals, bacteria and archaea (Novoselov et al. 2002; Rayman, 2012; Mangiapane et al., 2014). It is also required for the growth and metabolic functions of many species of microalgae, at least 33 divided in six phyla (Obata and Shiraiwa, 2005; Araie and Shiraiwa 2009), while for plants its essentiality has not been recognized yet (Schiavon and Pilon-Smits, 2017). In organisms that need Se, this element in the form of the amino acid selenocysteine (SeCys) is incorporated into the catalytic site of essential selenoproteins, which are primarily involved in detoxification and reactive oxygen species (ROS) scavenging processes (Lobanov et al., 2009; Papp et al., 2010; Bulteau and Chavatte, 2015). In humans, some of these proteins play a pivotal role in thyroid metabolism and inadequate dietary Se intakes are responsible for Se deficiency related-health issues, such as Keshan and Kashin–Beck diseases, as well as for high risk of infections and cancer development (Rayman, 2005; Lü et al., 2016). However, Se at high dosages may become toxic to organisms by acting as pro-oxidant, primarily because of its capacity to replace sulfur (S) in proteins, which then lose their correct folding (Van Hoewyk et al. 2008). Excess Se can also deplete the intracellular glutathione (GSH) pool causing ROS accumulation (Van Hoewyk et al. 2008; Grant et al., 2011; Schiavon et al. 2012).

The existence of a narrow window between Se deficiency and toxicity requires a deep study of Se biogeochemistry and cycling in the environment, Se assimilation and biotransformation in organisms like plants and algae, which represent the principal source of Se for humans and other animals.

Although Se concentration are typically low in seawaters and freshwaters (Robberecht and van Grieken, 1982), the global impact of Se on marine photosynthetic organisms is of great interest due to the growing number of water bodies where Se levels pose a concern. For instance, Se concentration as high as 350-400  $\mu\text{g Se L}^{-1}$  has been reported in ponds receiving agricultural drainage waters (Riedel and Sanders, 1996; Conde and Sanz Alaejos, 1997). High levels of Se in waters may results in a significant decrease in primary productivity, as excess Se can impair a variety of processes in algae, particularly growth and photosynthesis (Geoffroy et al., 2007). Accumulation of Se in algae can further negatively impact on the aquatic populations that feed on them, thereby affecting the whole ecosystem (Hartikainen, 2005; Chapman et al., 2010). The incidents at Belews Lake, NC (recipient of power plant coal-fly ash) and Kesterson Reservoir, CA (recipient of agricultural drainage waters) are well-known examples of Se induced-toxicity in animals via food web (Ohlendorf et al., 1990). Additional large-scale cases exist, like the decline of endangered fish in the Colorado River basin (Hamilton, 1999).

In aquatic ecosystems, Se-compounds could income from both natural and anthropogenic sources, such as irrigation of agricultural lands, coal mining and combustion (Fournier et al., 2010). Once absorbed, Se can induce a variety of physiological effects in algae depending on its chemical form, algal accumulation capacity and species (Wheeler et al., 1982; Dazhi et al., 2003; Abdel-Hamid and Skulberg, 2006; Pastierova et al., 2009; Umisová et al., 2009). Microalgae requiring Se for their metabolism incorporate this element in a number of essential selenoproteins that are involved in antioxidant defense systems. Some of them can also volatilize Se in the form of dimethyldiselenide (DDSe) (Vriens et al., 2016), the

same compound that is produced by Se hyperaccumulator plants (Galeas et al., 2007).

The further sections will give an overview of Se uptake, accumulation and metabolism in algae. The environmental and toxicological impact of Se accumulation in algae will be discussed, as well as some nutritional aspects of using Se-enriched algae in human nutrition.

## 2. Mechanisms of selenium uptake

Selenium uptake in algal cells is critical to its transfer in the food web and effects in aquatic ecosystems. Algae can take up selenium as either selenite (SeIV,  $\text{Se}_2\text{O}_3$ ) or selenate (SeVI,  $\text{Se}_2\text{O}_4$ ), which are the dominant soluble forms of Se in aquatic bodies (Cutter et al., 1984; Plant et al., 2004), while organic selenides (selenoamino acids, selenoproteins and methylselenides) are only present at very low concentrations (Fan et al., 2002). Selenate compounds are normally higher soluble and more bioavailable than selenite compounds to marine and freshwater algae (Plant et al. 2004; Chapman et al. 2010), because selenite tends to be strongly adsorbed to mineral and organic-matter surfaces (Chapman et al. 2010; Vriens et al., 2016). The relative abundance of Se compounds can vary depending on several factors, principally the pH and redox state of water (Geering et al., 1968; Riedel and Sanders 1996; Tuzen and Sari 2010). Selenate, for instance, is more stable under oxidizing conditions, while selenite is generally abundant in slightly oxidizing environments (Geering et al., 1968).

The pH influences the degree of protonation of Se ionic forms in water determining the formation of more biologically active Se compounds that can promote Se uptake by algae. Selenic acid ( $\text{H}_2\text{SeO}_4$ ) is almost completely dissociated over a pH range of 5 to 9, thereby the uptake of selenate by algae can be considered nearly constant under this condition (Riedel and Sanders, 1996). Conversely, selenous acid ( $\text{H}_2\text{SeO}_3$ ) is a weak acid and undergoes significant changes in its chemical form depending on the pH (Riedel and Sanders, 1996; Morlon et al., 2006) and only when the pH is lower than 7, it dissociates in water and algae quickly absorb Se in the form of selenite (Riedel and Sanders, 1996).

The water pH can also modify the overall algal surface charge and the activity and properties of Se membrane transporters, which require a specific pH for their optimal efficiency. For instance, in the unicellular green alga *Chlamydomonas reinhardtii* the optimum uptake rates for selenate and selenite are attained at pH values near 8 and lower than 5, respectively (Riedel and Sanders, 1996).

To date, no transporter with specificity only for Se has been identified in algae as in other photosynthetic organisms, and intake of Se oxyanions is likely mediated by proteins that do not discriminate between Se and other chemically similar anions (Sors and Ellis, 2005; Umysová et al., 2009; Schiavon and Pilon-Smits, 2017). Therefore, Se competes with other nutrients for transport in these organisms. Also, Se can reduce the uptake of metals like mercury (Hg) and cadmium (Cd), to which Se binds with extraordinarily high affinity, thereby mitigating the toxic effects exerted by these metals on organisms that absorb them (Berry and Ralston, 2008; Lin et al., 2012; Zhao et al., 2014).

The presence and water concentration of competing ionic constituents like sulfate, phosphate and silicate, can affect Se uptake capacity in algae (Lo et al., 2015; Vriens et al., 2016). Selenate in particular, shares high chemical similarity with sulfate and both ions compete for the binding sites of sulfate permeases to enter inside algal cells, as it happens in plants (Cabannes et al., 2011; Schiavon et al., 2015; Schiavon and Pilon-Smits, 2017). The reduction of selenate transport and accumulation in algae by ambient sulfate concentration has been widely reported (Williams et al., 1994; Fournier et al., 2010; Simmons and Emery 2011; Lo et al., 2015; Vriens et al., 2016). As an example, a 55% decrease of selenate uptake was observed in *C. reinhardtii* grown with 600  $\mu\text{M}$  sulfate compared to control experiments where sulfate was given at 150  $\mu\text{M}$  (Vriens et al., 2016). On the other side, increasing periods of Se exposure decreased sulfur (S) content in the same microalga (Vriens et al., 2016).

With respect to selenite transport, contrasting results have been described in *C. reinhardtii*. Morlon et al. (2006) observed the inhibition of selenite influx by sulfate concentrations ranging from 50  $\mu\text{M}$  to 5 mM, but not by phosphate ions. In other studies, selenite transport was unchanged under increasing sulfate concentration in the growth medium, whereas it was strongly depleted by high phosphate levels (Riedel and Sanders, 1996; Wang and Dei, 2001; Yu and Wang, 2004; Vriens et al., 2016). Differences in results were probably due to different exposure periods to selenite and/or algae acclimation to phosphate-depleted media prior the experiments.

Selenite is usually more quickly absorbed and accumulated by microalgae compared to selenate (Boisson et al., 1995; Umisová et al. 2009; Vriens et al., 2016). Kinetic analysis of Se uptake supports the existence of various systems that mediate the transport of selenite and selenate across cell membranes in these organisms. In *C. reinhardtii*, a rapidly saturated system for selenite transport at nanomolar ion concentrations and non-specific uptake mechanisms that dominate at high selenite concentrations (mM) have been proposed (Morlon et al., 2006), whereas for selenate a saturable transport system has been hypothesized, as Se uptake rates were decreased by high selenate concentrations (Morlon et al., 2006). In a recent study, selenate uptake in *C. reinhardtii* treated with Se concentrations within 0-100  $\mu\text{M}$  showed a  $V_{\text{max}}$  of 73 ( $\mu\text{mol}\cdot\text{cell}^{-1}\cdot\text{h}^{-1}$ ) and a  $K_{\text{m}}$  of 89  $\mu\text{M}$  (Vriens et al., 2016). Previously, a  $K_{\text{m}}$  equal to 12  $\mu\text{M}$  was reported in the same microalga (Fournier et al., 2006).

Selenium uptake properties were also investigated in deep in the coccolithophorid *Emiliania huxleyi*, which consumes selenite as the main source of Se, with an optimum ion concentration of 1 nM (Obata et al., 2004; Araie et al., 2011). The use of a [ $^{75}\text{Se}$ ]selenite radiotracer technique allowed the identification of two main uptake mechanisms in this microalga: a high-affinity, ATP-dependent and promptly saturable transport system ( $K_{\text{m}}=29.8$  nM), which is active within a few minutes after Se exposure, and a low-affinity passive transport system linearly related to selenite external concentration and dominating upon long term periods of Se exposure (Obata et al., 2004; Araie et al., 2011).

In macroalgae, Se uptake has not been deeply investigated as compared to microalgae. However, recent studies suggest that saturable systems for selenite and selenate uptake may exist in these organisms, as increasing ambient concentrations of either ion reduced Se accumulation in thalli of the green macroalga *Ulva australis* (Schiavon et al., 2016). Interestingly, high ambient sulfur concentrations did not

decrease selenate accumulation in *Ulva* spp. (Schiavon et al., 2012), thereby suggesting that *Ulva* spp. might have a S independent mechanisms involved in selenate uptake (Schiavon et al., 2012).

### 3. Selenium metabolism and selenoproteins

Because of its chemical similarity with S, once absorbed by algae Se can enter the S reductive assimilation pathway and be converted to selenide, which is further used as a substrate for the synthesis of the Se-amino acids selenocysteine (SeCys) and selenomethionine (SeMet) (Neumann et al., 2003). Some microalgae can efficiently methylate these amino acids and produce volatile compounds, and/or accumulate Se in the form of Se<sup>0</sup> (Neumann et al., 2003; Vriens et al., 2016) (Figure 1). Intriguingly, microalgae that require Se for their metabolism hold mechanisms that control the specific incorporation of the Se-amino acid selenocysteine (SeCys) into the catalytic site of essential selenoproteins, as reported in humans and mammals (Hatfield et al., 2002; Novoselov et al. 2002).

In humans, 25 genes for selenoproteins have been identified so far, and selenoproteins biosynthesis occurs via cotranslational insertion of SeCys by the recoding of a UGA codon, which usually functions as a stop signal (Driscoll and Copeland, 2003; Bulteau and Chavatte, 2015). The main players in this process are the selenocysteine insertion sequence (SECIS) present in the 3' untranslated region (UTR) of all selenoproteins, which is necessary to direct accurate UGA recoding as selenocysteine, and the selenocysteine-tRNA([Ser]Sec), which has an anticodon complementary to the UGA codon (Labunskyy et al., 2014; Bulteau and Chavatte, 2015). In *C. reinhardtii*, genes containing SECIS elements are relatively similar, but not identical, to those described in animals. They were shown to drive the synthesis of selenoproteins in mammalian cells, in support of the assumption that Sec insertion systems in photosynthetic organisms and animals share a common origin (Noselov et al., 2002). Evolutionary analyses have further indicated that selenoproteins in *C. reinhardtii* and animals evolved early and that the environment has played an important role in selenoproteome evolution. Selenoproteomes were likely independently lost in plants, yeasts and some animals due to an unknown environmental factor (Noselov et al., 2002; Lobanov et al., 2007). With respect to photosynthetic organisms, it seems that aquatic life has supported Se metabolism, whereas terrestrial habitats led to a dramatic reduction in the use of Se (Lobanov et al., 2007).

Several approaches, including bioinformatics and labeling techniques using <sup>75</sup>Se, allowed the identification of selenoproteins in various microalgae species, such as *C. reinhardtii* (Noselov et al., 2002; Grossman et al., 2007); *Ostreococcus* (Prasinophyceae) (Lobanov et al., 2007; Palenik et al., 2007), *Cyanidioschyzon* (Cyanidiaceae) (Maruyama et al., 2004), *Emiliana huxleyi* (Haptophytes) (Araie et al., 2008), and *Thalassiosira pseudonana* (Price and Harrison, 1988).

*C. reinhardtii* exposed to selenite produces a SeCys-containing phospholipid hydroperoxide glutathione peroxidase and a selenoprotein W homologs as major selenoproteins (Noselov et al., 2002). A methionine-S-sulfoxide reductase has been also identified specific to this microalga, as well as a selenocysteyl-tRNA that recognizes the Sec codon UGA (Noselov et al., 2002).

A Sec-containing glutathione peroxidase has been detected in *T. pseudonana* (Price and Harrison, 1988), while in *E. huxleyi*, six selenoproteins (EhSEP1–EhSEP6) have been discovered so far. Among these, the most abundant selenoprotein EhSEP2 works as a protein-disulfide isomerase (PDI)-like protein (Obata and Shiraiwa, 2005). PDI proteins catalyze the formation, reduction, and isomerization of disulfide bonds to control changes in conformation and folding of the proteins in the endoplasmic reticulum. The second most abundant Se-protein detected in this microalga, EhSEP1, has been classified as thioredoxin reductase (TR) 1 (Araie et al., 2008), which reduces thioredoxin and regulates enzyme activity by oxidizing or reducing the disulfide bonds in a number of enzymes. The induction of TR activity by Se has been further reported in the green alga *Scenedesmus quadricauda* (Umysová et al., 2009).

Some microalgae, like *Chlorella* sp. and *C. reinhardtii*, display high capacity to synthesize the Se volatile compounds dimethyldiselenide (DMDSe) and/or dimethylselenide (DMSe) upon either selenate or selenite supply (Neumann et al., 2003; Vriens et al., 2016). This discovery is of great importance because it indicates a crucial role for microalgae in Se-cycling through the ecosystem. In *Chlorella* sp., rates of Se volatilization were orders of magnitude higher than those normally measured in plants and were inhibited by sulfate (Neumann et al., 2003). In *C. reinhardtii*, Se methylation was showed to be very efficient (up to 89% of intracellular Se) and was strongly related to Se accumulation in cells (Vriens et al., 2016). Interestingly, in this microalga, DMDSe was found to be prevalent over DMSe, as observed in Se hyperaccumulator plants (Tagmount et al., 2002; Sors et al., 2005). Plant species that hyperaccumulate Se utilize the enzyme SeCys methyltransferase (SMT) to convert SeCys to methyl-SeCys (MeSeCys), thus preventing or reducing SeCys misincorporation into the protein structures (Neuhierl and Böck, 1996; LeDuc et al., 2004; Sors et al., 2005). The methylated SeCys can be further converted to the volatile less toxic compound DMDSe (Terry et al., 2000; Sors et al., 2005). In the case of Se-non hyperaccumulators, Se volatilization produces DMSe using SeMet as a substrate (Schiavon and Pilon-Smits, 2017).

In macroalgae, no essential selenoproteins have been identified so far, but Se volatilization has been observed (de Souza et al., 2002). The metabolic fate of Se in these organisms needs more investigation, as the identification of Se-metabolites produced by macroalgae may be of crucial importance to shed light on Se utilization strategies by these organisms.

#### **4. Intracellular selenium accumulation, toxicity and defense mechanisms activated in algae**

##### *4.1 Se Accumulation*

The level of Se in seawaters ( $0.08 \mu\text{g Se L}^{-1}$ ) and freshwaters ( $0.01\text{--}0.5 \mu\text{g Se L}^{-1}$ ) is generally low (Fournier et al.; 2010). However, in the last years a growing number of aquatic environments where Se concentration is critically increased has been reported (Lemly, 2004; Hartikainen 2005; Chapman et al., 2010). High Se concentrations in waters may cause deleterious effects in the local populations and whole-ecosystem. Selenium-accumulating algae in particular, may represent important vectors for the

transfer of Se from waters to animals that feed on them and other consumers of the food web.

Algae differ in the capacity to take up and accumulate Se in their cells based on algal species, ambient Se concentration and oxidation state, as well as on the array of proteins that mediate Se transport (Wheeler et al. 1982; Dazhi et al. 2003; Abdel-Hamid and Skulberg 2006; Pastierova et al. 2009; Umisová et al. 2009). Many examples exist in this respect.

In *C. reinhardtii*, selenite and selenate uptake rates reflected intracellular Se concentrations, which accounted for 55 mM and 4 mM when the microalga was grown for 24 h with 100  $\mu$ M selenite and selenate, respectively (Vriens et al., 2016). In *S. quadricauda*, total Se and SeMet accumulation was strongly related to the ambient Se concentration (Umisová et al., 2009). Total Se was equal to 4 mg L<sup>-1</sup> and 33 mg L<sup>-1</sup> in cells supplied with 100 mg L<sup>-1</sup> selenite and selenate, respectively (Vítová et al., 2011), and SeMet accounted for 29% and 41% of these values (Umisová et al. 2009). Similar percentage fractions of SeMet over total Se were previously detected in *Chlorella vulgaris* feed with selenite (24%) and selenate (39%) (Neumann et al., 2003).

Despite macroalgae generally accumulate lower levels of Se compared to unicellular algae, they can be used in Se phytoremediation technologies as Se bioindicators or players in Se removal (de Souza et al., 2002). *Chara canescens*, for instance, was tested for its capacity to accumulate Se deriving from the drainage water of a farmland (Lin et al., 2002), whereas *Cladophora hutchinsiae* is recognized as a suitable biosorbent organism for treating Se(IV) ions-containing wastewaters by virtue of its low-cost biomass and high sorption capacity (Tuzen and Sari 2010).

Diverse ranges of Se accumulation have been reported in distinct groups of multicellular algae, with values within 0.014-0.135 mg kg<sup>-1</sup> for Phaeophyceae, 1.153-0.434 mg kg<sup>-1</sup> for Rhodophyceae and 0.053-0.264 mg kg<sup>-1</sup> for Chlorophyceae (Maher et al., 1992). In more recent studies, Se concentration in 14 different species of macroalgae varied between 0.078 to 0.86  $\mu$ g g<sup>-1</sup> (Sánchez-Rodríguez et al., 2001), 0.53 to 0.75  $\mu$ g g<sup>-1</sup> in *Ulva* spp and 1.12 to 1.73  $\mu$ g g<sup>-1</sup> in *Porphyra colombina* (Pérez et al., 2007), while it was equal to 0.549  $\mu$ g g<sup>-1</sup> and 0.289  $\mu$ g g<sup>-1</sup> in *Ulva pertusa* and *Dyctiopters divaricate*, respectively (Hou and Yan, 1998). In *Fucus vesiculosus* and *Fucus ceranoides*, Se concentration accounted for 0.05-0.31  $\mu$ g g<sup>-1</sup> and 0.05-0.51  $\mu$ g g<sup>-1</sup>, respectively (Turner, 2013).

Se accumulation in microalgae is often influenced by the S ambient concentration (Neumann et al., 2003; Fournier et al., 2010; Lo et al., 2015; Vriens et al., 2016). As an example, Se bioaccumulation in *C. reinhardtii* was significantly lower (one tenth) in the presence of 80  $\mu$ mol L<sup>-1</sup> compared to 8  $\mu$ mol L<sup>-1</sup> sulfate given the same selenate concentration (Fournier et al., 2010). On the opposite, in macroalgae like *Ulva* spp., Se accumulation was not reduced by high S in the medium (Schiavon et al., 2012).

#### 4.2 Se toxicity and defense mechanisms

Depending on its concentration, Se can exert either beneficial or adverse effects on algae. In trace amounts, it can promote algal growth and photosynthesis, and activate antioxidant defense systems (Sun et al., 2014). For instance, selenite at low



dosages ( $\leq 75 \text{ mg L}^{-1}$ ) may act as an antioxidant in *Chlorella vulgaris* by inhibiting lipid peroxidation and intracellular reactive oxygen species (ROS) formation via increased activity of the antioxidant enzymes guaiacol peroxidase (GPX), catalase (CAT) and superoxide dismutase (SOD) (Sun et al., 2014). Similarly, selenite concentrations lower than  $750 \text{ mg L}^{-1}$  enhanced the activity of antioxidant enzymes in the macroalga *Ulva fasciata* (Zhong et al. 2015).

However, elevate Se concentration can induce toxicity in algae, thus impacting on primary production (Morlon et al., 2005). The major effects of Se toxicity include the formation of malformed proteins (Vallentine et al., 2014), inhibition of cell division, and reduction of algal growth (Geoffroy et al. 2007, Umisová et al. 2009; Zheng et al., 2017). Decrease in growth is partly ascribed to damages to the photosynthetic apparatus, as the chloroplast ultrastructure and photosynthetic electron transport may be impaired by Se-dependent ROS production (Morlon et al., 2005; Geoffroy et al. 2007; Vítová et al., 2011).

The chemical form of Se in aquatic environments is also important in predicting toxicity to algae. In the forms of Se(IV), for instance, Se was reported to be less toxic than Se(VI) towards microalgae (Wheeler et al. 1982). The opposite was predicted in the macroalga *U. fasciata* based on changes in glutathione levels (Schiavon et al., 2016).

Algae can activate specific defense mechanisms in response to Se toxicity, primarily the stimulation of antioxidant enzyme activity and/or synthesis of non-enzymatic molecules, as well as the activation of pathways that promote Se conversion to the less toxic volatile compounds DMDS<sub>e</sub> and/or DMSe (Neumann et al., 2003; Schiavon et al., 2012, 2016; Vriens et al., 2016; Zheng et al., 2017). Se toxicity was reported to increase the activity of thioredoxin reductase and glutathione peroxidase in *S. quadricauda* (Umisová et al. 2009; Vítová et al., 2011), and the activity of SOD and CAT in *Ulva* spp. (Schiavon et al. 2012). Se can also enhance the content in non-enzymatic antioxidants like carotenoids (e.g. astaxanthin) (Schiavon et al. 2012; 2016; Zheng et al., 2017), phenolic compounds and oxidized glutathione (GSSG) in algae (Schiavon et al. 2012; 2016).

The ubiquitin–proteasome pathway (UPP) has been recently identified as an additional important mechanism for algae protection against moderate Se stress via increased proteasome activity, protein ubiquitination and proteasomal removal of malformed selenoproteins in *C. reinhardtii* (Vallentine et al., 2014). However, this mechanism seems inefficient under severe Se stress because of excessive ROS accumulation.

Se-induced toxicity might be also mitigated by the ambient S concentration as observed in *S. quadricauda* (Umisová et al. 2009), *C. reinhardtii* (Fournier et al., 2010) and *Pseudokirchneriella subcapitata* (Lo et al., 2015). High S depletes the amount of Se that can access the S pathway to form Se-amino acids. As a result, algae tolerance to Se increases as the rate of misincorporation of Se into proteins declines.

## **5. Toxicological and nutritional aspects of Se accumulation in algae**

### *5.1. Toxicology of Se accumulation in algae for aquatic organisms and other consumers*

Organisms can be exposed to Se in different ways, but the most prevalent is via dietary intake (Hamilton, 2004). In this respect, micro- and macro-algae represent one of the primary routes of Se movement along the food web, especially in freshwaters, as the S relative abundance in marine systems may limit their Se uptake capacity.

Because of its propensity to bioaccumulate at the base of the food chain, Se is of primary concern compared to other elements (Hamilton, 2004). According to the model depicted in Figure 2, which illustrates selenium species associated with major processes in water systems, aquatic primary producers (algae but also bacteria, fungi, and submerged macrophytes) can efficiently convert inorganic Se forms into organic species, mainly amino acids. These compounds are further transferred through primary and secondary consumers (invertebrates and vertebrates) to higher-order consumers or predators (e.g. birds, mammals, humans).

Depending on the dose of Se ingested by these organisms and whether they require or not this element, Se can exert healthy or deleterious metabolic effects. For animals that need Se, this element in traces ensures normal growth and development. However, high Se exposure can generate oxidative stress via increased ROS production (Spallholz and Hoffman, 2002). Excess Se in the form of the amino acid SeCys can also cause the inhibition of selenium methylation metabolism, which results in accumulation of the hepatotoxic hydrogen selenide (Sayato et al. 1997), and may disrupt the function of S-containing enzymes and structural proteins via replacement of the S analog amino acid cysteine (Spallholz and Hoffman, 2002).

In literature a number of examples related to Se toxicity in birds and fish have been reported in natural environments, as well as the toxicological impact of Se bioaccumulation on change in aquatic communities (for more details, see review from Hamilton 2004). The prevalent effects induced by toxic Se concentration in these organisms are growth impairment, failure in reproduction, embryo deformities and mortality.

The most famous example of Se toxicity to aquatic wildlife occurred in the mid 1980s at Kesterson Reservoir located near Kesterson National Wildlife Refuge (NWR) in the San Joaquin Valley of California. Irrigation of seleniferous soils in this valley caused the increase of Se in drainage water that further moved to the Kesterson Reservoir. Dramatic effects of this Se contamination were observed on water bird embryos, which exhibit significant deformations (Ohlendorf et al., 1988a, 1990; Hoffman et al., 1988). Fish disappearances were also reported at Kesterson NWR (Harris, 1986; Vencil, 1986) and further studies were conducted on other animals, such as reptiles and amphibians (Ohlendorf et al., 1988b), and mammals (Clark, 1987; Paveglia and Clifton, 1988; Clark et al., 1989).

The species of Se ingested by fish and birds is also important to determine the magnitude of Se-induced toxicity. Various investigations have reported a major role of SeMet in inducing Se toxicity in these animals (Bell and Cowey, 1989; Hamilton et al., 1990; Hoffman et al., 1996; Heinz et al., 1996; Hamilton, 2005).

In addition to Se-rich drainage waters, other potential sources of environmental contaminations with Se are represented by coal fly ash emissions derived from coal-fired electric power industries (Gutenmann et al., 1976; Kashiwakura et al., 2011). Only United States have been estimated to produce over 120 million tons of this material annually (Lemly, 2002). High Se concentration associated with this fly ash from coal combustion poses environmental concern because of the issues related to

the disposal of this contaminated landfill leachate. Episodes of Se pollutions due to this coal industry waste are reported and mainly affected aquatic life (Lemly, 2002)

Based on these studies, it is clear that depuration systems and treatments of drainage waters and coal industry waste rich in Se are crucial practices to prevent or reduce Se-pollution in natural sites in order to avoid detrimental effects of high Se concentrations in wildlife.

### *5.2. Se-enriched microalgae as functional food*

Despite their role as vectors of Se transfer in aquatic ecosystems, algae enriched in Se attract interest in the field of human nutrition. According to USDA (2012), the daily reference Se intake for human diet ranges between 50 and 70  $\mu\text{g}$  Se, based on fully expression of plasma glutathione peroxidase.

Algae generally contain high levels of bioactive compounds or phytochemicals, which may improve health and offer a variety of physiological benefits (Stengel et al. 2011; Cornish et al. 2015; Hafting et al. 2015). In Eastern countries, fresh marine macroalgae are widely consumed by local populations because of their content in proteins, polysaccharides, fiber, vitamins and trace elements (Chapman and Chapman 1980; Dawczynsky et al. 2007; Cornish and Garbary 2010).

In a recent work, Gerrad et al. (2017) predicted that changes in climate and soil organic carbon content will reduce soil Se concentrations, especially in agricultural regions. As a result, the incidence of Se deficiency-related health issues will increase. In this context, Se supplementation via Se-enriched algae may be envisioned as a tool to correct or prevent Se deficiency-related diseases that may affect populations living in low Se-areas (Yan et al., 2004; Garcia-Vaquero and Hayes, 2106). Algae enriched in Se can also contain organic Se-metabolites that might function as anticarcinogens and promoting agents of immune system and thyroid metabolism (Yan et al. 2004).

## **6. Conclusion**

The element Se may act as pro-oxidant or antioxidant in algae, depending on several factors. The knowledge of the mechanisms involved in its uptake and assimilation in algae are only partly understood and need more investigations, especially because algae play a pivotal role in Se cycling in the environment. Macroalgae in particular, are mainly studied for their bioaccumulation capacity in natural environments, but detailed studies focusing on the molecular and physiological responses to Se in these organisms are limited. Being both micro- and macro-algae crucial in Se transfer throughout the food chain, toxicological and nutritional aspects deriving from Se bioaccumulation and transformation into organic forms should be carefully evaluated.

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## Figure legends.

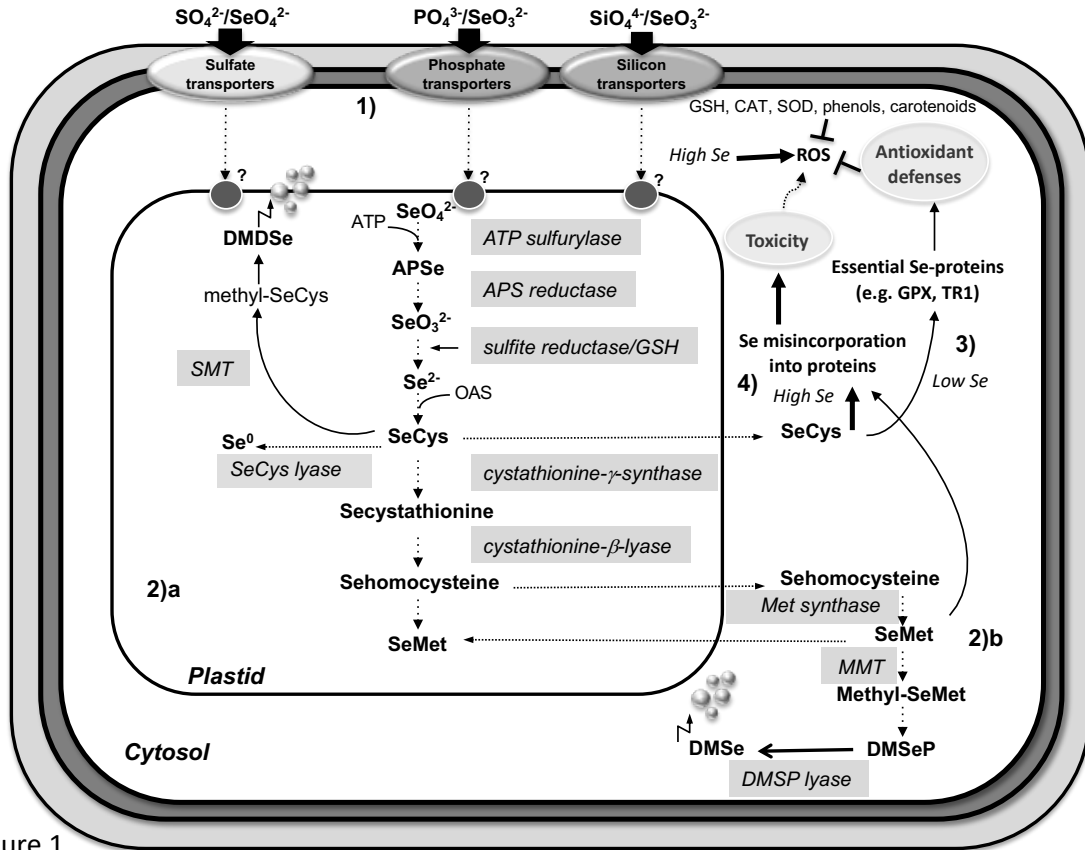
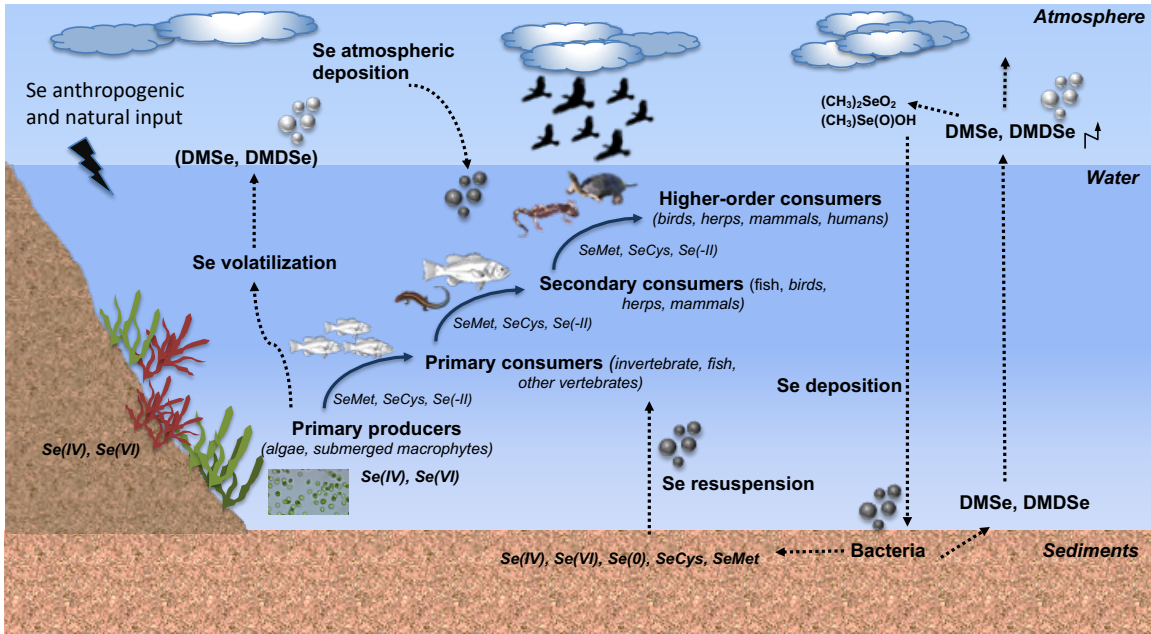


Figure 1

### Figure 1.

Schematic model of Se metabolism in algal cells. 1) Selenium uptake via sulfate, phosphate and silicon transporters. 2) Selenium/sulfur assimilation pathway: APSe: adenosine phosphoselenate; GSH: glutathione; OAS: O-acetylserine; (Se)Cys: (seleno)cysteine; (Se)Met: (seleno)methionine; MMT: methylmethionine methyltransferase; DMSeP: dimethylselenopropionate; DM(D)Se: dimethyl(di)selenide (volatile); SMT: selenocysteine methyltransferase. 3) SeCys is incorporated in essential Se-proteins that play a key role in ROS scavenging and defense systems. 4) However, if intracellular Se concentration is high, Se amino acids can be also incorporated into other proteins non-specifically, thus producing malformed structures. This event causes toxicity via increased production of ROS, which algae try to scavenge by enhancing the synthesis of enzymatic and non-enzymatic antioxidant compounds.



**Figure 2.**

Selenium species associated with major processes occurring in aquatic environments and partitioning of Se among environmental compartments and in the food web.