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Effect of Selenium on Antioxidant Enzymes and Content of Photosynthetic Pigments in algae *Chlorococcum littorale* Chihara,Nakayama &Inouye

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Abstract:

The study provides a new insight into the impact of Se on green algae *Chlorocouyeoccum littorale* and effects of Se on antioxidant system and bioaccumulation. Se positively promoted *C. littorale* growth at lower concentrations (≤50 mg L^{-1}), acting as an antioxidant through the inhibition of lipid peroxidation (LPO) and intracellular reactive oxygen species (ROS). The antioxidative effect was associated with an increase in guaiacol peroxidase (GPX), catalase (CAT), superoxide dismutase (SOD) and photosynthetic pigments. Meanwhile, significant increase in the cell growth rate and organic Se content was also detected in the algae.

Keywords: ***Chlorocooccum***, Selenium, LPO, ROS, GPX, CAT.

Introduction

Selenium (Se) is a natural trace element that acts either as an essential micro-nutrient or as a toxic compound in a dose-dependent manner. At low levels, it shows anti-carcinogenic effects **(Chang *et al*.,2005)** on mammalian development **(Schomburg *et al*., 2004)** and immune function **(Arthur *et al*.,2003)** as well as slowing aging **(Rayman ,2000)**. In contrast high concentrations can cause the generation of Reactive Oxygen Species (ROS), which induce DNA oxidation, DNA double-strand breaks and cell death Se in the aquatic environment comes from both natural and anthropogenic sources, such as irrigation of agricultural lands, coal mining and combustion **(Patrick,2004)**. Typical concentrations in freshwaters are in the range of 0.13–2.50 nmol L^{-1} (equivalent to 0.01–0.5 µg Se L^{-1}) but higher concentrations reaching 5 µmol L^{-1} (equivalent to 400 µg Se L^{-1}) have been observed in contaminated areas **(Conde and Sanz ,1997)**. Se enters freshwaters primarily as selenite (SeO^{32-}) and selenate (SeO^{42-}). Organic selenides

(Se [−II] such as(Se amino acids, Se proteins, methylselenides), produced by biological reduction of selenite, usually occur at lower concentrations in water than inorganic Se species **(Fan *et al* ., 2002)**. In aquatic ecosystems microalgae act as a major vector of Se from water to filter-feeders and other consumers. They accumulate Se from water-column and partially transform it into organic Se before it is transferred by ingestion to higher organisms **(Letavayova *et al* ., 2006)**. most plant species accumulate less than 25 µg Se g^{-1} Dry Weight (DW) both in terrestial and aquatic natural environment and cannot tolerate higher Se concentrations. these are termed nonaccumulators **(White *et al* .,2004)**. In contrast. some microalgae species such as (*Spirulina platensis*) can accumulate Se at as high a concentration as 400 µg Se g^{-1} dry weight in their cells **(Chen *et al*.,2008)**. recently **(Doušková *et al*.2007)** reported about a new selenite resistant strain of *Scenedesmus quadricauda* for Se-enriched microalgal biomass production in photosynthetic mode of cultivation. The selenium effect on the green alga *S. quadricauda* was dose-dependent and the form of the element was also crucial **(Doucha *et al*., 2009)**. the essentiality of Se in algae has been studied mainly in marine species. Selenite bioaccumulation by phytoplankton **(Baines *et al*.,2004)** and Se requirements of many of phytoplankton species from various taxons had been demonstrated. The unicellular marine calcifying alga *Emiliania huxleyi* requires nanomolar levels of Se for growth **(Danbara and Shiraiwa,1999)** Se is essential to many algae and shown to protect them against oxidative damage **(Ekelund and Danilov,2001)**. Sometimes the essentiality is difficult to assess because Se is required at such low levels for most organisms that it is experimentally challenging to generate strong phenotypes of deficiency **(Merchant *et al*.,2006)**. The window between Se requirement and toxicity is the smallest of any element in some freshwater species such as *(S. platensis)* Se concentrations below 20 mg L^{-1} did not inhibit the growth **(Pronina *et al*.,2002)** however, above 500 mg L^{-1} sodium selenite was toxic to this alga. The toxicity of metals to marine algae may be expressed in many ways. among them is alteration in the cellular levels of Reactive Oxygen Species (ROS) which can cause oxidation of proteins and nucleic acids as well as Lipid Peroxidation (LPO) leading further to inactive enzymes, disrupted membranes,

mutations, and ultimately causing cell death **(Halliwell and Gutteridge,1999)**. The defense of plant cells against the damaging effects of oxidative stress involves both enzymatic and non-enzymatic components **(Candan and Tarhan ,2003)**. A variety of non-enzymatic antioxidants such as carotene and atocopherol may play an important role in the cellular response to oxidative stress by reducing certain ROS **(Mager and Kruijft ,1995)**.

the selected Se resistant strains could be used for bioremediation of Se-contaminated surroundings. The mechanisms of remediation include biosorption and bioconversion. The economic feasibility of algal mass culture for toxic remediation greatly depends on the high biomass productivity **(Liu *et al*.,2008)**. microalgae strains have a great potential to be a resource for toxic remediation due to its faster growth and easier cultivation.

Chlorococcum littorale a unicellular green alga represented common in freshwater. many works have shown that *C. littorale* is a good carrier for Se accumulation. till now less was known about Se-induce oxidative stress and antioxidant response in microalgae. In the present study, we chose *C. littorale* as the model organism to monitor cellular response we grew the alga with Se-amended medium and followed the growth rate biomass the organic and total amount of Se, the activity of antioxidant enzymes (SOD, CAT, GPX), contents of chlorophyll a, carotenoid and ROS and LPO levels. The objective of this study was to investigate the accumulation of Se in *C. littorale* and its effects on the antioxidant systems (including antioxidant enzymes and non-enzyme components) and to show that Se-Chlorococcum could be developed as antioxidative functional foods for aquaculture and human health.

Materials and Methods

Culture conditions and Se treatments

Unialgal stock cultures were propagated and maintained in Erlenmeyer flasks containing BG11 medium (pH 6.8) under incubation conditions of 25°C, a photon flux density of 45 µmol m^{-2} s−1 provided by two white fluorescent tubes, and a light/dark photoperiod of 16:8 h. Flasks were

continuously shaken at 100 rpm. The components of basal culture medium **(Wu *et al*.,1992)**

compound	concentration
KH_2PO_4	0.7 mg L^{-1}
K_2HPO_4	0.3 mg L^{-1}
$MgSO_4 \cdot 7H_2O$	0.3 mg L^{-1}
$FeSO_4 \cdot 7H_2O$	3 mg L^{-1}
glycine	0.1 mg L^{-1}
vitamin B1	0.01 mg L^{-1}
A5 trace mineral solution	1 mg L^{-1}

C. littorale cultures in the late exponential growth phase were decanted into 250 ml flasks containing 100 ml of medium at 25±0.5°C and illuminated with fluorescent lights (45 µmol m^{-2} s^{-1} photon flux intensity) under a 16:8 h light:dark photoperiod. The cultures were initiated at 6×10^7 cells ml^{-1}, shaken periodically and used in triplicate. All solutions and experimental containers were autoclaved at 121°C for 15 min. Sodium selenite (Na_2SeO_3) was added to the medium before inoculation at a concentration of (5, 25, 50, 75,100, 150) mg L−1. cultures grown without sodium selenite served as controls.

The dry weight of *C. littorale* was determined by drying the cells at 70°C in a vacuum oven until constant weight.

The algal growth was monitored spectrophotometrically at optical density at 685 nm. The relative growth rate is Ln $(N_t/N_0)/t$.

N_0 is the value of O.D. at 685 nm measured at time 0

N_t is the value of O.D. at 685 nm measured at time t.

Cell numbers were counted under a microscope with a improved Neubauer haemacytometer.

Determination of total and organic Se content

Total Se concentration was determined by Inductively coupled plasma – mass spectrometer (ICP-MS) **(Lavu *et al*., 2013)**. Cultured after 6 days. the 100 mg dried algal sample was digested with concentrated nitric acid and H_2O_2 (3:1 v/v) in a digestive stove at 180°C for 3 h.

Samples were allowed to cool dissolved in 0.6% HNO_3 and filtered through Whattman fulter paper. Further before drying the samples were observed under microscope for removing any contamination of non-algal particulates. The volume of each sample was maintained up to 10 ml with 0.6% HNO_3 and analyzed the total Se content by ICP-MS. For inorganic Se determination, the 100 mg dried algal sample was extracted with 15% HCl and the extract was analyzed by ICP-MS directly. Organic Se concentration was calculated from the difference between the total Se concentration and the inorganic Se concentration.

An Inductively coupled plasma – mass spectrometer Agilent 7500ce was used for analysis of sample solutions. for quantification of Se, a standard addition method was used to eliminate matrix effects of residual carbon and other matrix elements. all data are presented as means ± S.D. of three experiments.and measurement the antioxidant enzymes by the cell algae suspensions were centrifuged and then the pellets were resuspended in pre-chilled phosphate buffer (pH 7.0) and disrupted by ultrasonication. The homogenate was centrifuged at 10,000 g for 30 min and the supernatant was collected. The total protein content in the supernatant of homogenate extracts was determined according to the **(Bradford,1976)** method using bovine serum albumin as a standard. The activities of antioxidant enzymes and level of lipid peroxidation in the protein extract were examined using spectrophotometric diagnostic kit. Basically superoxide dismutase (SOD) activity was determined using the xanthine oxidase method based on its ability to inhibit the oxidation of hydroxylamine by the xanthine–xanthine oxidase system **(Beauchamp and Fridovich,1971)**. Catalase (CAT) activity was measured according to the ammonium molybdate spectrophotometric method, based on the fact that ammonium molybdate could rapidly terminate the H_2O_2 degradation reaction catalysed by CAT and react with the residual H_2O_2 to generate a yellow complex which could be monitored by the absorbance at 405 nm **(Aebi,1984)** Guaiacol-dependent peroxidase (GPX) activity was measured by quantifying the rate of H_2O_2-induced oxidation of GSH to oxidised glutathione (GSSG), catalysed by GPX. The GPX content in the supernatant was measured by reaction with dithionitrobenzoic acid (DTNB) and monitored by absorbance at 412 nm

(Hemeda and Klein,1990). The level of lipid peroxidation (LPO) was estimated by malondialdehyde (MDA) levels, which were measured using the spectrophotometric diagnostic kits as described by**(Buege ,1978).** .determination of reactive oxygen species (ROS) in the algae when (OD685) were incubated for 0.5 h at 37°C in 100 mL of 5 mM ^{2} 7-dichlorofluoresceine diacetate dissolved in double distilled water. After incubation the cells was rinsed in double distilled water. then suspended in 5 mL of 40 mM Tris HCl buffer(pH 7.0) and centrifuged at 15,000 g for 10 min. Fluorescence was determined using spectrofluorometer at an excitation wavelength of 485 nm and an emission wavelength of 535 nm. Fluorescence values were obtained using a standard curve of DCF. using the method of **(Inskeep and Bloom ,1985)** for Photosynthetic pigment extraction and analysis where take 40 ml of each culture were collected at 10,000 g for 10 min to analyze chlorophyll a content. Carotenoids were extracted with 5 ml 100% methanol in the dark at 4°C for 1 day **(Welburn,1994)**. The extracts were centrifuged at 5,000 rpm for 10 min, and the supernatants were used to determine the contents of pigments by spectrophotometer.The contents of phycoerythrin and phycoery-throcyanin were estimated according to Beer's method **(Beer,1985)**.

Statistics

All treatments and controls were performed in triplicates. The significance of the difference between mean values obtained from 3 independent experiments was determined by one-way analysis of variance (ANOVA) at 95% confidence interval by using SPSS software.

Results

C. littorale Se content increased as a function of the concentration in the medium and biotransformed the inorganic Se to organic Se at a high rate (Fig. 1). Se content increased significantly in algae as Se concentration increased from 0 to 150 mg L−1 ($P<0.05$). The control groups was spiked with 5–150 mg L^{-1} selenite within 5 minutes before harvesting samples and the total Se content was determinated. Then, the total Se content of each spiked control was compared with those in the experiment groups at different Se concentrations cultured for 6 days (Fig. 1A). At low temperature, algae cells are deactivation to absorb Se into the

cells within the five minutes. There is a rapid Se absorption within the first few minutes at the cell surfaces where it is irreversibly fixed and cannot be absorbed by the human body **(De-Alcantara *et al* .,1998)**. The alga cells fixed the highest Se on the surfaces at 50 mg L^{-1} Se concentration. The highest total Se content was 852 ug g^{-1} dry weight (DW). The best growth condition of the microalgae was found at 50 mg L^{-1} Se concentration. Under Se treatment of 5–150 mg L^{-1}, the amount of organic Se accumulated in *C. littorale* increased proportionately with the cultivation time, and no lag time was observed. About 74% of organic Se was accumulated during the first 3 days of exposure. Slower increase was found from day 4 to day 6. after day 6 lower decrease was found. Maximum accumulation of organic Se was recorded at 318 ug g^{-1} under 50 mg L^{-1} Se concentration for 6 day (Fig. 1B, 1C). The Se species accumulated in *C. littorale* cells consisted of organic Se (>65%) and inorganic Se (<35%), showing that algae was an efficient Se accumulator. There was a direct correlation between cell numbers and the optical density at 685 nm wavelength. A regression was obtained from the data analysis.

$$y=2\times10^{7}XR^{2} = 0.992$$

y: cell numbers per milliliter culture; x: optical density of cultures at 685 nm.

Based on the result the algal growth was determined by measuring the values of OD685 with a spectrophotometer every day. Se accumulation increased the growth with increasing Se concentrations (5–50 mg L^{-1}) significantly ($P<0.05$) and was reflected by changes in biomass concentration at the same time (Fig. 2A and 2B). The increase in biomass concentration and cell density was obtained in cells exposed to low Se concentrations (≤50 mg L^{-1}), with the highest (1.4 g L^{-1} and 1.3×10^{8} cells ml^{-1}) found at a Se concentration of 75 mg L^{-1} at day 6. However, higher Se concentrations (>50 mg L^{-1}) decreased the biomass concentration and the growth of *C. littorale* significantly ($P<0.05$), possibly due to the toxic effects of high Se stress. Cultures turned red due to the occurrence of elemental Se when algae was exposed to Se at concentrations over 50 mg L^{-1} after 6 days. by day 6 the control and 75 mg L^{-1} Se treatment groups

reached the stationary phase while cultures supplemented with (5-75) mg L^{-1} Se were still in the exponential growth phase.

Effect of Se on the content of photosynthetic pigments :

and continuous increase of carotenoid and chlorophyll a content was observed in *C. littorale* when it was exposed to Se concentrations up to 50 mg L^{-1} Significant ($P<0.05$). Maximum levels of carotenoid and chlorophyll a reach 24.6 and 29.7 mg L^{-1}, respectively 6 days (Fig. 3). However, at higher Se concentrations (>50 mg L^{-1}), a significant decline ($P<0.05$) in the overall content of photosynthetic pigments (including carotene and chlorophyll a) was evident when compared to the 50 mg L^{-1} Se concentrations groups.

Effects of Se on the activities of antioxidant enzymes :

Selenium is known to act as an antioxidant at low concentrations, but as a pro-oxidant at higher ones. In these studies a concentration-dependent increase in activities of the antioxidant enzymes was evident since a stimulation of activity was observed under Se treatment. In comparison the control. the activities of GPX were increased when the Se concentration increased (Fig. 4A). the activities of CAT increased with increasing levels of Se from 0 to 50 mg L^{-1} (Fig. 4B). However, the control the activities of SOD decreased when the Se concentration increased (Fig. 4C). higher Se concentration (>50 mg L^{-1}) inhibited the activities of CAT compared to the low Se concentration (Fig. 4B). The maximum activities of CAT were found under the Se treatment of 50 mg L^{-1}, and were 1.26 ± $^{0.04}$ IU/ug protein. A sharp elevation in GPX activities was observed when cells were exposed to Se at 150 mg L^{-1}, with maximum activity, 334 ± $^{6.65}$ IU/mg protein, showing a positive correlation with Se concentration and GPX activity. higher Se concentrations (100–150 mg L^{-1}) resulted in significant decrease in CAT activities ($P<0.05$) compared to the lower Se concentrations (5–50 mg L^{-1}) which reached their minimum (0.45±$^{0.03}$ IU/ug protein) under 150 mg L^{-1} Se.

Mean while time-dependent variations in the activity of antioxidant enzymes were shown in Figure 4. compared with the control GPX and CAT activities in cells under Se treatments both increased for up to day 3 and then significantly decreased after wards ($P<0.05$). The maximum CAT activities were $1.22 \pm {}^{0.02}$ IU/ug protein under Se treatments at day 3 (Fig. 4B) compared with the control SOD activity in cells under both Se treatments decreased for up to day 4 and then increased significantly afterwards ($P<0.05$) (Fig. 4C).

Effect of Se on the intracellular ROS generation and LPO :

Additions of Se to culture medium ranging from 5–50 mg L^{-1}, decreased the accumulation of ROS and MDA contents cultured after 2 days (Fig. 5)

The contents of ROS under the concentrations of 50 mg L^{-1} at day 4 were lower significantly ($P<0.05$) than the control (Fig. 5A). under Se concentration of (75-150) mg L^{-1}, the content of ROS was significantly higher than the control ($P<0.05$). The LPO level decreased with the increase of Se concentrations (5–50 mg L^{-1}) and reached the minimum ($37.4\pm {}^{4.43}$ nmol MDA/mg protein) at 50 mg L^{-1} Se, but increased significantly ($P<0.05$) with a positive correlation to Se concentration from 50 to 150 mg L^{-1} (Fig. 5B). The maximum LPO level ($154.8\pm{}^{1.1}$ nmol MDA/mg protein) was obtained at 150 mg L^{-1} Se. Significant ($P<0.05$) and continuous increase in the LPO level was observed in controls and cells under Se treatment from day 0 to day 6. However the increase trend under Se treatment of 50 mg L^{-1} was the minimum.Cultured at day 2 under Se treatment of (5-50) mg L^{-1}. the LPO levels and ROS generation were higher than control indicating that *C. littorale* was sensitive to Se initially even under low Se concentration. However, significant ($P<0.05$) decrease in the LPO level and ROS content at day 4 under Se treatment ranging from 5 to 50 mg L^{-1}, showing that 50 mg L^{-1} Na_2SeO_3 concentration is optimum for the growth of *C. littorale*.

Discussion

Se can be transported into the cell as anionic macronutrients via a transport system but with low affinity In green algae **(Neumann *et al***

.,2003). Some algae showed growth stimulation at lower selenium levels within a defined range but almost all were inhibited at higher concentrations **(Douskova *et al* .,2007)**. In this study *C. littorale* showed a tolerance at high Se levels indicating that it is a good Se bioaccumulator. However Se has either stimulating or toxic effects on algae depending on the Se concentration. according to our results the algae cells can accumulate Se efficiently during cultivation at concentration ≤50 mg L^{-1}.higher Se concentrations led to much lower biomass (Fig. 2) and decreased in content of photosynthetic pigments (Fig. 3). most of the total Se was transformed to organic Se (>50%) at the concentration of Se (≤50 mg L^{-1}) (Fig. 1). The function of Se is mediated mostly by selenoproteins to which the Se is inserted as a selenocysteine during translation **(Kryukov *et al*.,2003)**. Selenoproteins include enzymes such as GPX and proteins with unknown functions (**Kiffney and Knight,1990)**. some studies have already revealed Se is needed as a nutrient of algae in the synthesis of proteins and lipids to enhance cellular division **(Furness and Rainbow,2009)**. The growth-stimulating effect of Se may be related to its antioxidative function as demonstrated by high contents of chlorophyll a (Fig. 3), and diminished LPO and ROS (Fig. 5). The increased chlorophyll content in low Se treated cells (5–50 mg L^{-1}) over control might be attributed to efficient scavenging of ROS by CAT, SOD and GPX or otherwise they would have destroyed the chlorophyll pigments**(Thomas *et al*.,2001)**. Carotenoids as one of the non-enzymatic antioxidants play an important role in the cellular response to oxidative stress by reducing ROS **(Sager,2006)** .under stress conditions singlet oxygen in the pigment bed might accumulate **(Wada *et al*.,2010)** and could seriously disrupt metabolism through oxidative damage to cellular components **(Foyer *et al*.,2014)**. Carotenoids were found to be able to protect the photosynthetic membrane from photo-oxidation by effectively scavenging singlet oxygen and quenching the triplet state of chlorophyll **(Demmig ,1990)**. The increase in carotenoid content in *C. littorale* with cultivation time was observed in both control and Se treatment cells which may be due to these antioxidant mechanisms. However, the contents of carotenoids and chlorophyll decreased under high Se concentration (100–150 mg L^{-1}). which may be due to lipid peroxidation

of chloroplast membranes, resulting from cell damage or death by Se toxicity**(Health and Packer ,2015)**. One of the Se-induced selenoproteins can protect cells from oxidative stress. A previous study showed that GPX catalyses of peroxides reduction would damage cells (**Jeong *et al*.,2002)** .In this study compared to the control the activities of GPX increased when the Se concentration increased (Fig. 4A). SOD acts as an opposite way to GPX, indicating that lower amounts of superoxide anion radicals were produced in cells due to high GPX activity**(Hartikainen *et al*., 2000)**.It is hypothesized that the increase in GPX which is a scavenger of H_2O_2 and lipid hydroperoxides may resulted in reduced formation of superoxide anion radicals (O^{2-}) through the dynamic inter-transformation among oxygen species. superoxide radicals can be used in spontaneous disproportion reactions producing H_2O_2 and singlet oxygen **(Thompson *et al*.,2013)**. This supports that Se promotes through increased GPX the scavenge of produced H_2O_2 while it enhances the spontaneous disproportion of superoxide radicals and consequently reduces the need for their scavenger SOD (Fig. 4). During oxidative stress, excess ROS production causes membrane damage eventually leading to cell death. For protection against ROS. plants contain antioxidant enzymes such as SOD, CAT and GPX, as well as a wide array of non-enzymatic antioxidants **(Mittler,2002)**. ROS and LPO are the well known indices for determining the degree of oxidative stress and considered main contributors for growth retardation **(Blokhina *et al*.,2003).** se acts as an antioxidant at low concentrations. In this study it was observed that GPX and CAT activity increased significantly and LPO level decreased significantly in cells treated with increasing levels of Se from 0 to 50 mg L^{-1} (Figure 4 and and5). However, high Se concentration may cause oxidative stress and membrane damage through production of ROS in *C. littorale* (Fig. 4). compared to the low Se concentration group (5–50 mg L^{-1}), the activities of CAT and GPX decreased. while the LPO and ROS levels increased under the high Se concentration (>50 mg L^{-1}). from the first to the third exposure day in spite of the decrease in SOD activity the GPX activitiy and carotenoid concentrations increased significantly ($P<0.05$).

Refernces

Arthur, J.R; McKenzie, R.C and Beckett, G,J (2003) .Selenium in the immune system. J Nutr 133: 1457–1459.

Aebi, H. (1984). Catalase in vitro. Method Enzymol 105: 121–126.

Baines, S.B.; Fisher, N.S.; Doblin, M.A.; Cutter, G.A. and Cutter, L.S. (2004). Light dependence of selenium uptake by phytoplankton and implications for predicting selenium incorporation into food webs. Limnol Oceanogr 49: 566–578.

Beauchamp, C. and Fridovich ,I. (1971). Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. Anal Biochem 44: 276–87.

Beer,S.E.(1985). Determining phycoerythrin and phycocyanin in concentrations in aqueous crude extracts of red algae. Aust J Mar Freshwater Res 36: 785–792.

Blokhina, O.; Virolainen, E. and Fagerstedt, K.V. (2003). Antioxidants, oxidative damage and oxygen deprivation stress: a review. Ann Bot 91: 179–194. [PMC free article]

Bradford ,M.M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principles of protein-dye binding. Anal Biochem 72: 248–254.

Buege, J.A. (1978). Microsomal lipid peroxidation. Method Enzymol 52: 302–306.

Candan, N. and Tarhan, L. (2003). Relationship among chlorophyll-carotenoid concentration, antioxidant enzyme activities and lipid peroxidation levels by Mg^{2+} deficiency in the Mentha pulegium leaves. Plant Physiol Biochem 41: 35–40.

Chang, W.P.; Combs, G.F.J.; Scanes ,C.G. and Marsh, J.A. (2005). The effects of dietary vitamin E and selenium deficiencies on plasma thyroid and thymic hormone concentrations in the chicken. Dev Comp Immunol 29: 265–273.

Chen, T.F.; Zheng, W.J.; Wong, Y.S. and Yang, F. (2008). Selenium-induced changes in activities of antioxidant enzymes and content of photosynthetic pigments in *Spirulina platensis*. J Integr Plant Biol 50: 40–48.

Conde, J.E. and Sanz, A. M. (1997). Selenium concentrations in natural and environmental waters. Chem Rev 97: 1979–2003.

De- Alcantara ,S.; Lopes, C.C. and Wagener, K. (1998). Controlled introduction of selenium into *Chlorella* cells. Indian J Exp Biol 36: 1286–1288.

Danbara, A. and Shiraiwa, Y. (1999). The requirement of selenium for the growth of marine coccolithophorids, *Emiliania huxleyi*, *Gephyrocapsa oceanica* and *Helladosphaera sp.* (Prymnesiophyceae). Plant Cell Physiol 40: 762–766.

Demmig, A. B. (1990). Carotenoids and photoprotection in plants: a role for the xanthophyll zeaxanthin. Biochim Biophys Acta 1020: 1–24.

Doucha, J.; Lívanský, K.; Kotrbáček, V. and Zachleder,V.(2009). Production of *Chlorella* biomass enriched by selenium and its use in animal nutrition: a review. Appl Microbiol Biotechnol 83: 1001–1008.

Doušková, I.; Machát, J.; Umysová, D.; Vítová, M. and Doucha, J. (2007). *Scenedesmus quadricauda* - a promising microorganism for selenium-enriched algal biomass production. SEFS-5 Symposium for European Freshwater Sciences. Palermo, Italy. Book of Abstracts, p. 197.

Ekelund, N.A. and Danilov, R.A. (2001). The influence of selenium on photosynthesis and "light-enhanced dark respiration" (LEDR) in the flagellate *Euglena gracilis* after exposure to ultraviolet radiation. Aquat Sci 63: 457–465.

Fan,T.M.;The,S.J.;Hinton,D.E. and Higashi, R.M. (2002). Selenium biotransformation into proteinaceous forms by foodweb organisms of selenium-laden drainage waters in California. Aquat Toxicol 57: 65–84.

Foyer, C.H.; Lelandais, M.L. and Kunert, K.J. (2014). Photooxidative stress in plants. Physiol Plant 92: 696–717.

Furness, R.W.and Rainbow, S. (2009). Heavy Metals in the Marine Environment. CRC Press Inc., Boca Raton, Florida.

Halliwell, B. and Gutteridge ,J.C. (1999). Free Radicals in Biology and Medicine. Oxford Science Publications, Oxford.

Hartikainen, H.; Xue, T. and Piironen, V. (2000). Selenium as an anti-oxidant and pro-oxidant in ryegrass. Plant Soil 225: 193–200.

Heath, R.L.and Packer, L. (2015). Photoperoxidation in isolated chloroplasts: kinetics and stoichiometry of fatty acid peroxidation. Arch Biochem Biophys 125: 189–198.

Hemeda, H.M.and Klein, B.P. (1990). Effects of naturally occurring antioxidants on peroxidase activity of vegetable extracts. J Food Sci 55: 184–185.

Inskeep, W.P. and Bloom, P.R. (1985). Extinction coefficients of chlorophyll a and b in N,N-dimethylformamide and 80% acetone. Plant Physiol 77: 483–485. [PMC free article] .

Jeong, D.W.; Kim ,T.S.; Chung, Y.W.; Lee, B.J.and Kim, I.Y. (2002). Selenoprotein W is a glutathione-dependent antioxidant in vivo. FEBS Lett 517: 225–228.

Kiffney, P.and Knight, A. (1990). The toxicity and bioaccumulation of selenate, selenite and seleno-L-methionine in the cyanobacterium *Anabaena flosaquae*. Arch Environ Contam Toxicol 19: 488–94.

Kryukov, G.V.; Castellano, S.; Novoselov, S.V.; Lobanov, A.V. and Zehtab, O. (2003). Characterization of mammalian selenoproteomes. Science 300: 1439–1443.

Lavu, R.S.; Schepper, V.D.;Steppe, K.; Majeti, P.V. and Tack, F. (2013). Use of selenium fertilizers for production of Se-enriched Kenaf (*Hibiscus cannabinus*): Effect on Se concentration and plant productivity. J Plant Nutr Soil Sci 176: 634–639.

Letavayova, L.; Vlckova, V. and Brozmanova, J. (2006). Selenium: From cancer prevention to DNA damage. Toxicol 227: 1–14**.**

Liu, Z.Y.; Wang, G.C. and Zhou, B.C. (2008). Effect of iron on growth and lipid accumulation in *Chlorella vulgaris*. Bioresource Technol 99(11): 4717–4722.

Mager, W.H. and Kruijft, A.I. (1995). Stress-induced transcriptional activation. Microbiol Rev 59: 506–531. [PMC free article] .

Merchant, S.S.; Allen, M.D.; Kropat, J.; Moseley, J.L. and Long ,J.C. (2006). Between a rock and a hard place: Trace element nutrition in *Chlamydomonas*. Biochim Biophys Acta 1763: 578–594.

Mittler, R. (2002). Oxidative stress, antioxidants and stress tolerance. Trends Plant Sci 7: 405–410.

Neumann. P.M.; De- Souza, M.P.;Pickering, I.J. and Terry, N. (2003). Rapid microalgal metabolism of selenate to volatile dimethylselenide. Plant Cell Environ 26: 897–905.

Patrick, L. (2004). Selenium biochemistry and cancer: A review of the literature. Altern Med Rev 9: 239–258.

Pronina, N.A.; Kovshova, Y.I.; Popova, V.V.; Lapin, A.B.and Alekseeva, S.G. (2002). The effect of selenite ions on growth and selenium accumulation in *Spirulina platensis*. Russian J Plant Physiol 49: 235–241.

Rayman, M.P. (2000). The importance of selenium to human health. Lancet 356: 233–241.

Sager ,M. (2006). Selenium in agriculture, food, and nutrition. Pure Appl Chem 78: 111–133.

Schomburg. L.;Schweizer,U.and Kohrle,J.(2004). Selenium and selenoproteins in mammals: extraordinary, essential, enigmatic. Cell Mol Life Sci 61: 1988–1995.

Thomas, H.; Ougham, H. and Hortensteiner, S. (2001). Recent advances in the cell biology of chlorophyll catabolism. Adv Bot Res 35: 1–52.

Thompson, J.E.; Legge, R.I. and arber, R. (2013). The role of free radicals in senescence and wounding. New Physiol 105: 317–344.

Wada, O.; Kurihara, N.and Yamazaki, N. (2010). Essentiality and toxicity of trace elements. Jap J Nutr Assess 10: 199–210.

Wellburn, A.R. (1994). The Spectral Determination of Chlorophylls a and b, as well as Total Carotenoids, Using Various Solvents with Spectrophotometers of Different Resolution. J Plant Physiol 144: 307–313.

White, P.J.; Bowen, H.C.; Parmaguru, P.; Fritz, M. and Spracklen ,W.P. (2004). Interactions between selenium and sulphur nutrition in Arabidopsis thaliana. J Exp Bot 55: 1927–1937.

Wu, Q.Y.; Yin, S.; Sheng, G.Y. and Fu, J.M. (1992). A comparative study of gases generated from simulant thermal degradation of autotrophic and heterotrophic *Chlorella*. Prog Nat Sci (in Chinese) 3: 435–440.