Bioaccumulation and the Effect of Selenate Concentration on Growth and Photosynthetic Pigment Content of *Spirulina platensis*

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Abstract

Selenium (Se) is a necessary microelement for microalgae growth. Moreover, it might be directly act and cause a decrease in primary production in microalgae. Nowadays, there is a great focus on preparations of Se-enriched products. In the current study, the effects of various selenate concentrations on *Spirulina* evaluated as a primary stage to large-scale production of Se-enriched *S. platensis*. Zarrouk medium supplemented to investigate the stimulatory/inhibitory effects of selenate on the growth at various concentrations. Biomass dry weight and cell mass measured on OD_{550}. Thereafter, inhibitory and algicidal concentrations were determined. Furthermore, its effects on morphology and changes of some important pigments in response to the metal challenge investigated, too. While the results showed that at 5 and 10 ppm concentrations growth was supported, the dry weight of microalgae decreased at selenate levels above 50 ppm. Besides, the inhibitory and lethal effects of selenate were at 100 ppm and 300 ppm, respectively. In addition, morphological changes observed at this concentration. Additionally, chlorophyll, carotenoid and phycobiliproteins, showed a stimulatory effect at 5-50 ppm, 10 ppm, and 10 ppm, respectively.

Moreover, *Spirulina* uses for foods production because some chemicals are unique compounds. Simple cultivation method and high quality of its protein, and no toxic effects, cause its feasibility for large-scale production.

Keywords: *Spirulina platensis*, Selenate, Photosynthetic Pigment Content, Toxicity

Introduction

Disorder in aqueous ecosystems as a result of metal pollution will damage biological diversity and enhance the biological accumulation and concentrated pollution in food chains, as well (Mane et al., 2013). Because many chemical and physical characteristics are effective for determining the metals' impact on aqueous organisms, it is

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difficult to determine the metals' effects on these organisms (Mane et al., 2013).

According to Bertrand's known diagram, there is a certain range of positive effects on humans for each micronutrient, which in lower or higher amount than it becomes harmful for organisms. Therefore, it is obvious that the precious selection of required dosages is the most important work in designing of therapeutical substances (Frontasyeva et al., 2009).

Among these elements, Se is a natural trace element whose essential range can change from natural micronutrients to the toxic compound so that it affects organisms in a dose-dependent manner (Babaei et al., 2017; Gojkovic et al., 2014; Schiavon et al., 2016; Sun et al., 2014; Umysova et al., 2009). This element is essential for many biological functions in human, animals, and microorganisms (Schiavon et al., 2016). Moreover, it plays important role in decrease the harmful effects of free radicals (Frontasyeva et al., 2009). Because of its role in mammalian development, endocrine systems, immune function, slowing down aging, etc., this element has a highly important role in human health (Frontasyeva et al., 2009; Gojkovic et al., 2014; Sun et al., 2014; Umysova et al., 2009). Se function has tight connectivity with vitamin E and beta-carotene (which are present in *Spirulina platensis* biomass); therefore, these elements sometimes utilize together with therapeutical purposes (Frontasyeva et al., 2009).

This opinion generally exists that Se compounds are better and safer than inorganic Se as food supplements, therefore, various biological Se-enriched compounds such as garlic, yeast, lactic acid bacteria (LAB) are commercially available. In this regard, Se supplementation using microorganisms has been attracted attention in the last decades (Chen et al., 2008).

Se toxicity and deficiency are dependent on its availability in the environment. In aqueous environments, Se exists as two main oxidation states (selenite; Se^{IV}, $-SeO_3^{2-}$, Se^{3+} and selenate; Se^{VI}, $-SeO_4^{2-}$, Se^{6+}). Additionally, selenate has high water solubility, therefore, has more bioavailability for aqueous organisms rather than selenite (Gojkovic et al., 2014; Khademi and OraghiArdebili 2017; Schiavon et al., 2012; Schiavon et al., 2016). In aqueous environments, Se exists from natural sources and human activities such as agricultural lands irrigation, mining, and combustion. Common concentration in freshwaters is in the 0.13-2.50 nmol/L range but in polluted areas, higher amounts (up to 5 μmol/L) have been observed (Fournier et al., 2010; Sun et al., 2014).

Phycoremediation is an improvement of environmental pollution decrease/remove using microalgae (Soeprobowati and Hariyati, 2014). In ecosystems, microalgae are the major concern because they accumulate Se from the water column and transform it to organic Se before its transfer to higher organisms (Fournier et al., 2010; Sun et al., 2014).

However, studies showed that both selenate and selenite are toxic for microalgae at higher concentrations, selenate is more lethal

than selenite (Babaei et al., 2017; Khademi and OraghiArdebili, 2017). These types can absorb by t algal cells and affect growth in a dose-dependent manner (Gojkovic et al., 2014; Schiavon et al., 2016). Nevertheless, plant cells have both enzymatic and non-enzymatic strategies against damage effects of oxidative stress (Chen et al., 2008).

Besides Se toxicity effects in microalgae, essential need to Se has been reported in at least 33 species belong to six phyla but its biological importance has been unknown yet (Schiavon et al., 2016). Indeed, Se effects on metabolism and bioaccumulation, studied in various groups of microalgae such as *Spirulina*, *Scenedesmus*, *Chlorella* or *Chlamydomonas* (Babaei et al., 2017; Gojkovic et al., 2014).

Further, at low concentration, Se motivate the growth of some algae (*Thalassiosira pseudonana*) and at high level, it could be toxic for algae and cause growth repression, cell ultrastructure modification, and reactive oxygen species (ROS) transformation which can cause cellular damage, and low storage products (Babaei et al., 2017; Khademi and OraghiArdebili 2017; Schiavon et al., 2012; Schiavon et al., 2016).

However, microalgae can metabolize inorganic Se to its organic forms e.g. selenoproteins, volatile compounds, and seleno-aminoacids especially selenomethionine and seleno-cysteine as a part of their detoxification process probably because of their reductive metabolism; Se can stop growth at certain concentrations (Babaei et al., 2017; Khademi and OraghiArdebili 2017). Indded, over absorption of Se can cause metabolic reactions, and raise the probability of photo-oxidative damages (Babaei et al., 2017). Because rapid growth and high amounts of other inorganic substances such as calcium, sodium, potassium, iron micromineral, and nutrients such as protein (58.5%), ash (12%), carbohydrate (7.5%), lipid (7%), and crude fiber (0.95%) in contrast to other organisms, single-cell protein in cyanobacteria are crucial. On the other hand, in some studies on environmental pollution remediation using *S. maxima* Cd^{2+} accumulated in various layers of its cell wall (Costa et al., 2003). In like manner, *Microcyctis aeruginosa* removed Cd^{2+} (90%), Hg^{2+} (90%) and Pb^{2+} (80%) (Chen et al., 2005). In addition, *Spirulina* is an efficient biosorbent so that process equilibrium has reached during 5-10 minutes (Soeprobowati and Hariyati, 2014). *S. platensis* is a filamentous cyanobacteria (Soeprobowati and Hariyati, 2014) enriched by proteins (60-70%). Additionally, *S. platensis* cantains lipids, pigments, antioxidants, and vitamins used commercially as animal feed or human food supplements (Khademi and OraghiArdebili, 2017). Moreover, *Spirulina* becomes the simplest Se-supplementable algae from aqueous environments by changing the culture conditions (Li et al., 2003).

Several studies showed that this alga is a good carrier for Se accumulation. Moreover, Se accumulation could cause an improvement of *S. platensis* quality by enhancement of biomass production, photosynthetic pigments, and protein concentration (Chen et

al., 2008).

Currently, there is a need to investigate the effects of different Se concentrations on *Spirulina* growth to produce Se-enriched *Spirulina* in large-scale amounts (Mane et al., 2013). In the current study, *Spirulina platensis* (from Nogen Company, Ahvaz, Iran) selected as the subject organism because previous studies investigated Se effect on this species. The study aimed to determine the effect of different selenate concentrations on the growth ability of selected strain, selenate tolerance range in *S. platensis* culture, and its effect on photosynthetic pigments (including chlorophyll, phycocyanins, and beta-carotene). Moreover, this study could present some aspects of Se tolerance and increase our perception of Se interaction with *S. platensis*.

Material and methods

Microalgae cultivation

Cyanobacteria strain (*Spirulina platensis* NCC A300) was prepared from the local culture collection of Nogen Company, Ahvaz, Iran. The primary standard inoculated to Erlenmeyer as follows. It was necessary to obtain mid-exponential cells of microalgae. For this, a Zarrouk medium (pH 11.0) was prepared. Medium composition was as follows (g/L): $NaHCO_3$ 16.8, K_2HPO_4 0.5, $NaNO_3$ 2.5, $MgSO_4.7H_2O$ 0.20, EDTA $Na_2.2H_2O$ 0.08, $CaCl_2.2H_2O$ 0.04, $FeSO_4.2H_2O$ 0.01, trace element mixture A 10 ml/L, trace element mixture B 1.0 ml/L. And autoclaved at 121 ºC, 1 atm for 15 min. Then, inoculation was done as 1% of basal medium and incubated at 28±2 ºC with 16/8 hours (light/dark) photo-period for 30 days (Li et al., 2003; Mane et al., 2013).

Growth kinetics study

For the determination of mid-exponential cells, sampling was done at predefined times (every day) during the incubation period and growth rate was determined by Optical Density measurement at 550 nm using a spectrophotometer (analytikjena, spekol, 2000) (Khademi and Oraghi Ardebili, 2017).

It should be noted that growth kinetic studies were done at the presence of 0, 5, 10, 25, 50, 100, 300, 1000, and 2000 ppm selenate concentrations, respectively. Then, the stimulatory or inhibitory effects of selenate on growth profile of *S. platensis* were determind. Moreover, biomass concentration determined by dry weight measurement (Gojkovic et al., 2014). To achieve this goal, 1 ml of cultured media was withdrawn, washed to remove unwanted materials, and centrifuged at 10000 rpm for 10 min. The supernatant discarded and the cell pellet placed at 80 ºC, overnight. All experiments were done in triplicates (Sun et al., 2014). Dry weight expressed as g/L and calculated by the difference between the primary and final weights (Gojkovic et al., 2014; Khademi and OraghiArdebili, 2017). Moreover, pH measured during incubation time to investigate the pH changes during microalgae culture (Sun et al., 2014).

Determination of minimum algistatic and minimum algicidal concentrations

The tolerance of microalgae to selenate toxicity investigated using a broth medium

containing different amounts of selenate oxyanions. In this regard, 30 ml of Zarrouk medium in 100 ml Erlenmeyer was prepared and supplemented with different selenate concentrations (5, 10, 25, 50, 100, 300, 1000 and 2000 ppm), separately (Li et al., 2003). It is noteworthy that, this wide concentration range used to determine the connection between Se dosage and physiological and ultra-structural changes (Schiavon et al., 2016). Then, the Erlenmeyers incubated at predefined conditions. Following, sampling done and growth determination was performed as above to study growth kinetics at the presence of heavy metal stress. Ultimately, those cultures, which did not have any signs of growth, considered as minimum algistatic concentration. While the algicidal effect of selenate was investigated in solid Zarrouk medium by culturing 50 µl of no algae growth cultures (Sun et al., 2014).

Effect of selenate on cell morphology

An approach to investigate the effect of heavy metals on microalgae is the study of ultrastructure and morphology changes. Accordingly, samples were withdrawn from culture media supplemented with various concentrations of selenate and observed using a light microscope (Olympus) (Belokobylsky et al., 2004; Pelah and Cohen, 2005; Schiavon et al., 2012; Schiavon et al., 2016; Umysova et al., 2009).

Pigments extraction and analysis

To determine the effect of selenate toxicity on pigments content, the number of essential pigments (chlorophyll a, carotenoid, phycobilliproteins) was measured using acetone. For this, the culture of *S. platensis* withdrawn on the 30th day of cultivation, then their cells were breakdown using vigorous vortex for 2 min. After centrifugation, supernatant was collected and subjected to further study as follows (Sharma et al., 2014).

Pigment analysis

According to Sharma et al. (2014), the amount of photosynthetic pigments was measured in both types of media: supplemented with various concentrations of selenate; without any metal. Using a vortex mixer breaking microalgae cells done and chlorophyll and total carotenoid contents were determined in 80% acetone. After centrifugation, the supernatant collected (because it contains pigments). The absorbance of the supernatants of all extraction steps was measured using spectrophotometer in the most suitable wavelength for each pigment, as it has been mentioned in the following sections (Nanodrop 1000, ThermoScientific, USA) and the concentrations of pigments were calculated (Babaei et al., 2017).

Chlorophyll estimation

5 ml of homogenized cyanobacterial suspension centrifuged for 10 min at 4000 rpm and supernatants discarded. 5 ml of 90% acetone was used to extract chlorophyll-a (Chl-a). The tubes wrapped with aluminum foil and placed in the dark for 24 hours. Finally, samples centrifuged for 15 min at 5000 rpm, and the supernatant collected for further analysis. Absorbance measured at 630 nm (A630), 645 nm (A645), and 665 nm (A665) and 90% acetone used as blank. The Chl-a concentration calculated using

the following equation (Mane et al., 2013; Sharma et al., 2014):

$C = 11.6\ A665 - 1.31\ A645 - 0.14\ A630$ (1)

In a given volume of culture, Chl-a concentration determined by the following formula:

$\text{Chl-a}\ ((mg/l)) = c_e / v_c$ (2)

Wherein C, Ve, and Vc are value obtained from the above equation (1), volume of extract (ml), and volume of culture (l), respectively (Khademi and OraghiArdebili, 2017).

Phycobiliproteins estimation

5 ml of cell suspension centrifuged for 10 min at 4000 rpm to obtain the pellet. The cell pellet washed with distilled water. Thereafter, washed pellet used for the total extraction of Phycobiliproteins with 5 ml of sterile phosphate buffer (0.05 M, pH 6.7). Extraction repeated by three times freezing and thawing cycles. The samples centrifuged for 15 min at 10,000 rpm and supernatant collected. The absorbance read at 562 nm (A562), 615 nm (A615), and 652 nm (A652) against phosphate buffer as blank. Using the following formula, concentration of allophycocyanin (APC), phycocyanin (PC), and phycoerythrin (PE) were calculated (Sharma et al., 2014):

$APC = A652 - 0.208\ (A615) / 5.09$ (3)

$PC = A615 - 0.474\ (A652) / 5.34$ (4)

$PE = A562 - 2.41(PC) - 0.849(APC) / 5.09$ (5)

Phycobiliprotein's concentration can be determined by the following formula:

$\text{Phycobiliprotein}\ (mg/ml) = c \times v_e / v_c$ (6)

In these equations: APC, PC, and PE represented Phycocyanin, Allophycocyanin, and Phycoerythrin, respectively. C is the sum of PC, APC, and PE. According to equations 3-5, Ve is the volume of extract (l), and Vc is the volume of culture (l) (Sharma et al., 2014).

Carotenoid estimation

5 ml of homogenized cyanobacterial suspension centrifuged as the previous stage at 4000 rpm for 10 min. The pellet washed 2-3 times with distilled water to remove adhering salts and biomass collected. Pellet was broken down with 5 ml of 90% acetone and the samples were centrifuged 15 min at 5000 rpm for carotenoid extraction. The supernatant was collected and read the absorbance rate at 450 nm (A450). Finally, carotenoids (Cart) concentration calculated using the following formula: (Sharma et al., 2014)

$C = A450 \times V \times f \times 10 / 2500$ (7)

Where, C is the total amount of carotenoid (mg/ml), V is the volume of extract (l) and f is the dilution factor.

Statistical analysis

All statistical analysis performed by SPSS software (version, 19). The significant differences level between the investigated pigment content in different medium compared by Chi-Square.

Results

Change in microalgae's culture in response to various concentrations of selenate

In these set of experiments, after addition of various selenate concentrations from low and stimulant (5 ppm) to high, and inhibitor concentration (2000 ppm), growth and physiology of *S. platensis* studied and compared with control.

Figure 1 represents growth kinetic of mi-

croalgae is impressed at the different concentrations of selenate. The concentrations of selenate in 5, 10 and 25 ppm result more regular changes in biomass production yields (Fig. 1, Fig. 2). It should be noted that when culture media supplemented by 50, 100, and 300 ppm of Se, dry weight was increased. However, by increasing the incubation time, dry weight reduced especially at 50, and 300 ppm of selenate concentration.

Furthermore, Se toxicity evaluated by the decrease the exponential growth rate (Khademi and OraghiArdebili, 2017). Moreover, the minimum growth rate considered as a key determinant of Se toxicity. So that with impressing of any metabolic reaction, a corresponding amount of this determinant will be decreased (Gojkovic et al., 2015; Khademi and OraghiArdebili, 2017).

Effect on pH value

While the pH of culture media before selenate addition and *S. platensis* inoculation was 9.0, the overall pH during incubation period tends to increase (Fig. 3).

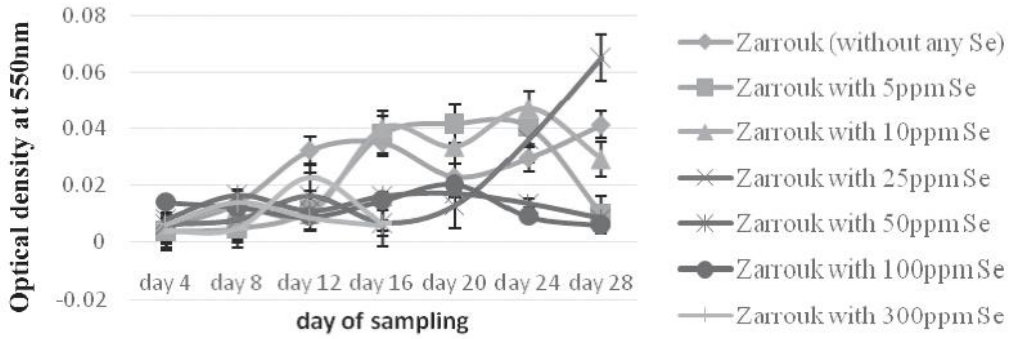


Fig. 1. Growth curve of *S. platensis* in Zarrouk medium supplemented with and without various selenate concentrations during 30 days. The error bars of the mean OD_{550} data and dry weight measurement represents the standard error

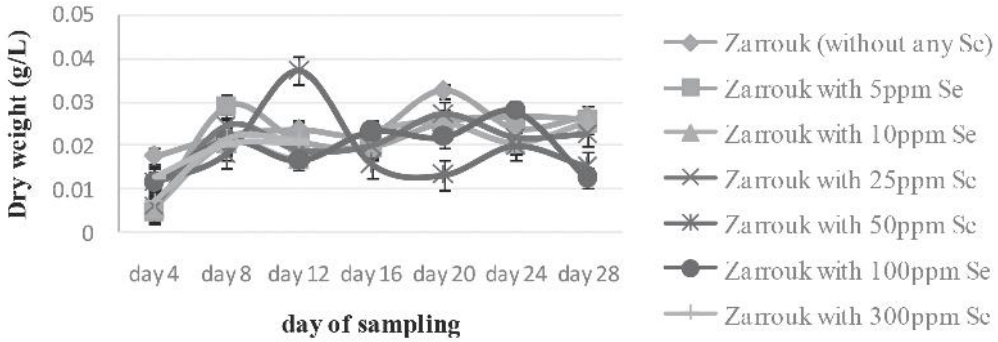


Fig. 2. The dry weight of *S. platensis* in Zarrouk medium during 30 days. The error bars of the mean OD_{550} data and dry weight measurement represents the standard error

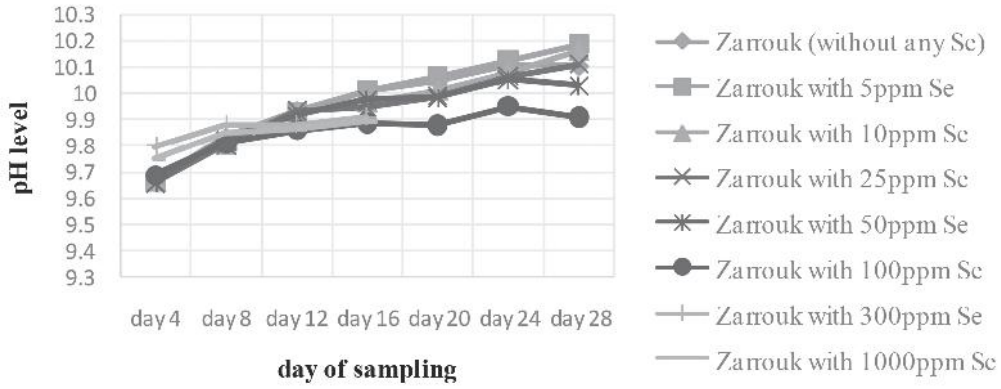


Fig. 3. pH changes of *S. platensis* culture in different selenate concentrations during 30 days. The error bars of the mean gathered data from OD_{550} and dry weight measurement represents the standard error

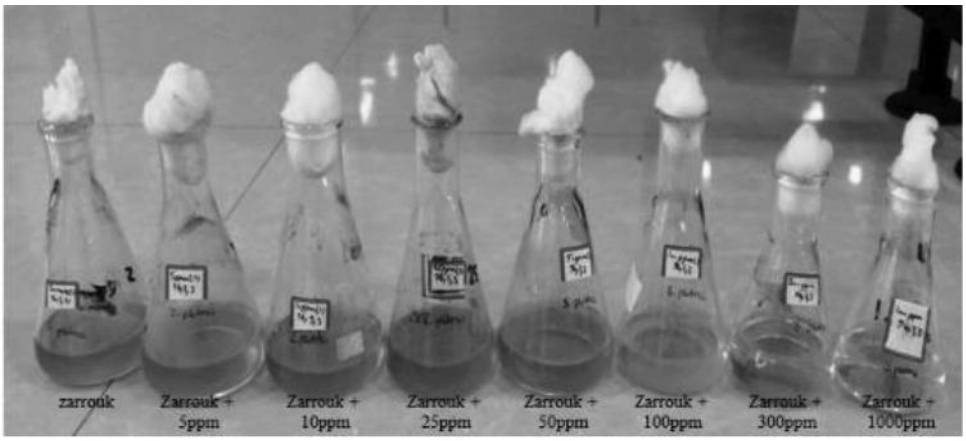


Fig. 4. Growth response of *S. platensis* at the MIC determination stage at various selenate concentrations after 7 days

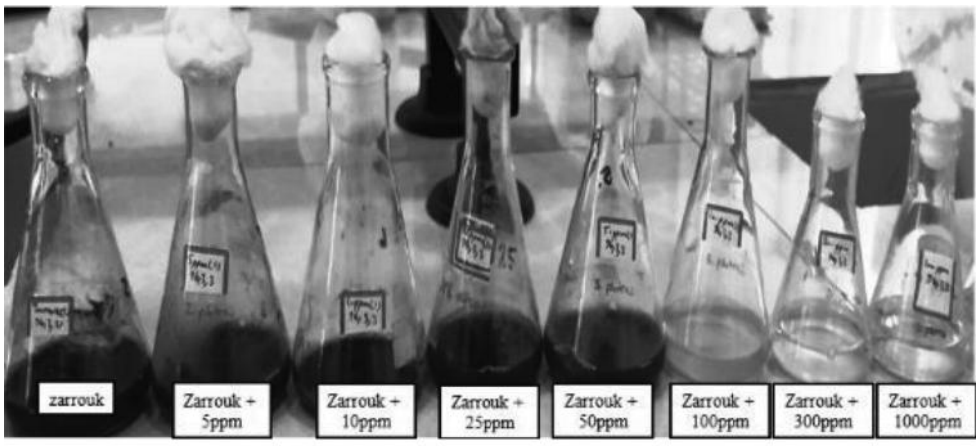


Fig. 5. Growth response of *S. platensis* in the MIC determination stage to different selenate concentrations after 30 days

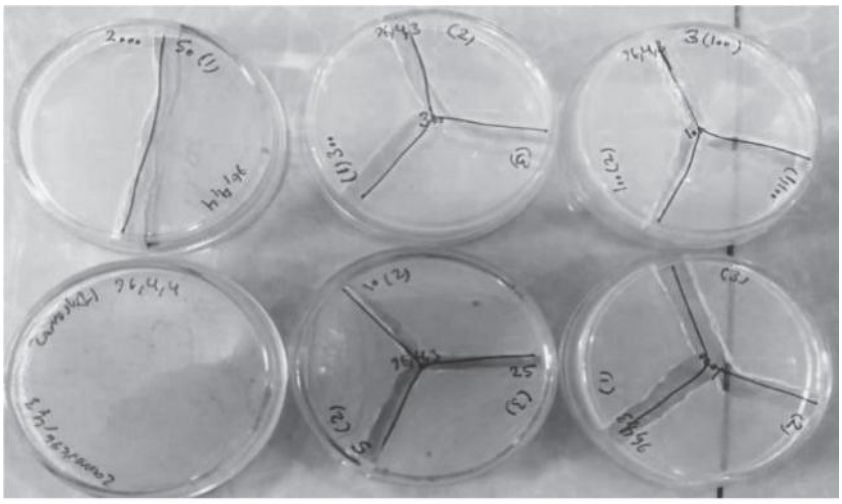
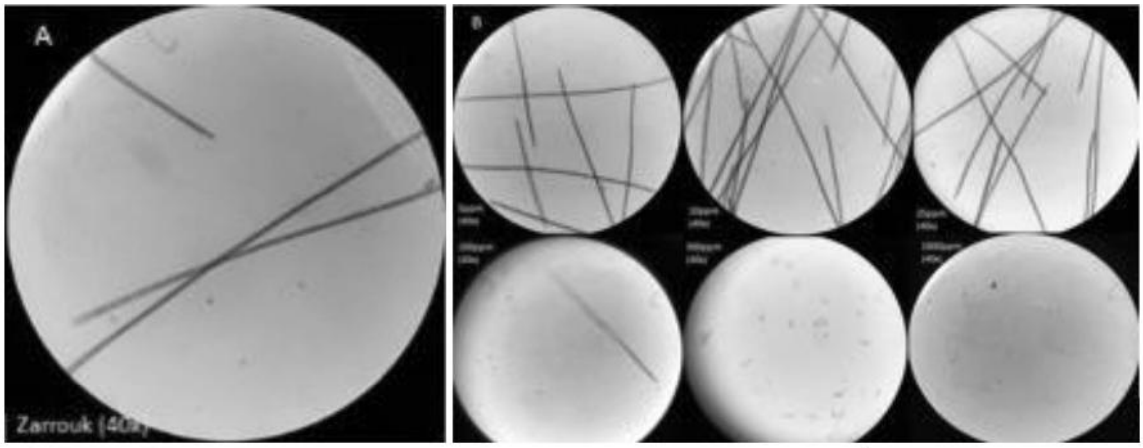


Fig. 6. The algicidal effect of *S. platensis* at various selenate concentrations in Zarrouk medium



a b

Fig. 7. Microscopic shape of *S. platensis* in (a) Standard Zarrouk medium, and (b) Zarrouk medium supplemented by various selenate concentrations

Minimum Algistatic and Minimum Algicidal determination

To investigate inhibitory or lethal effects of selenate on microalgae growth pattern, Zarrouk media which was supplemented with various dosages (5, 10, 25, 50, 100, 300, 1000, and 2000 ppm) of selenate was applied. Growth changes monitored after 7 and 30 days of the incubation (Figs 4 and 5, respectively). At the end of the incubation period, the growth rate recorded visually and the first culture, which did not have any sign of growth, was considered as minimum algicidal concentration (Fig. 4).

Those cultures, which did not have any sign of growth were cultivated to investigate the lethal activity of selenate oxyanion. For this mean, 50 µl inoculum was withdrawn and cultivated on solidified Zarrouk medium without any metal presence. After the incu-

bation, plates investigated and the first plate that did not have any growth considered as minimum algicidal concentration (MIC). Moreover, Selenate in this concentration has a lethal effect on the growth of this microalga (Fig. 6).

Furthermore, selenate has inhibitory and lethal effects on the studied strain at 100 ppm and 300 ppm concentrations, respectively (Fig. 6).

Effect of selenate on the microscopic shape of S. platensis

Investigation the heavy metal effect on the ultrastructure of microalgae and cyanobacteria is an issue to show the ability of these microorganisms to cope with heavy metal stresses. In this regard, selenate effect on filament and septa shape of *S. platensis* was investigated (Fig. 7). The results show that filamentous shape would start to change at 100 ppm and disrupted at 300 and 1000 ppm of selenate concentration.

Effect of selenate on pigment content of microalgae

The results of the selenate effect on studied pigments of *S. platensis* revealed different chl-a content at various selenate concentrations (Table 1). This order is 5 ppm> 25 ppm> 50 ppm> 10 ppm> zarrouk medium> 1000 ppm> 300 ppm> 100 ppm= 2000 ppm. Furthermore, Se-Toxicity affects several physiological features of *S. Platensis*.

While, carotenoid content changes in response to various selenate concentrations was in this order: 10 ppm> 25 ppm> 50 ppm> 5 ppm> zarrouk medium> 100 ppm> 300 ppm> 1000 ppm> 2000 ppm.

As the results show, this trend is somewhat rational. An increase in the carotenoid amount in response to the high level of Se accumulation, may be present a mechanism for resistance against selenate toxicity because of their protective effects on membrane integrity. Carotenoids may also protect chloroplast membranes from generated damages of produced ROS. However, some studies report different results, which show Se concentrations do not have negative effects on pigment contents.

The results show that the highest amount of each phycobilliprotein is 300 ppm, allophycocyanin in 1000 ppm and phycoerythrin in 300 ppm.

Discussion

Several studies reported therapeutical and preventive substances based on blue-green algae (*S. platensis*) (Frontasyeva et al., 2009). On the other hand, some studies investigated the stimulatory/inhibitory effects of selenate on this valuable cyanobacterium. In this regard, our study provides new insight into Se effects on microalgae, especially concerning to its toxicity.

Se is an important microelement because its narrow concentration range placed between its essential and toxic effect on human and animal health (Schiavon et al., 2012; Zheng, 2017). Furthermore, low Se uptake can cause cancer stimulation, immune system dysfunction, cardiovascular diseases, fertility reduction, and hypothyroidism, while high Se level in food regime, affects on cardiometabolic and induce some acute

Table 1. Changes in pigment content of *S. platensis* mg/l in response to various selenate concentrations

	Chl a (mg/l)	Cartenoid	Phycobiliprote in (mg/l)	phycobiliprotein			
				PC	APC	PE	Phycobiliprotein (mg/l)
Zarrouk	0.034180467	2.2667E-06	3.77783E-07	0.00012806	9.01768E-05	0.000237195	7.59053E-05
5ppm	0.102760065	1.6667E-06	2.778E-07	0.000131303	0.000207414	0.000260651	9.98947E-05
10ppm	0.041732198	0.000062	1.03333E-05	below the detection limit	0.000144527	0.000329409	5.88221E-05
25ppm	0.066564525	2.7533E-06	4.589E-07	below the detection limit	below the detection limit	0.000180256	below the detection limit
50ppm	0.043502142	2.3467E-06	3.91117E-07	below the detection limit	9.28016E-05	0.000238547	0.000029387
100ppm	0.014163735	1.2533E-06	2.08883E-07	below the detection limit	0.000354884	7.17971E-05	2.55406E-05
300ppm	0.015770555	0.00000088	1.4666E-06	0.001192539	below the detection limit	0.000812214	0.000317884
1000ppm	0.016610001	8.267E-07	1.37783E-07	below the detection limit	0.000641206	0.00017519	9.71508E-05
2000ppm	0.012948333	0.0000003	0.00000005	0.000187266	below the detection limit	0.000268543	6.91574E-05

$P<0.05$ is considered statistically significant (0.95 confidence level). Chl a: chlorophylle A; Cart: cartenoid; PC: phycocyanin; APC: allophycocyanin; PE: Phycoerythrin.

toxicity signs which is naturally occurred and are known as selenosis (Frontasyeva et al., 2009; Schiavon et al., 2016). Indeed, these high concentrations are toxic because producing reactive oxygen species (ROS) which induce DNA oxidation, DNA double strands breakage, and cell death (Schiavon et al., 2016). This assumed that Se substitution with sulfur in S-containing proteins, and its prooxidant ability for catalysis thioloxidation and concomitant production of superoxide, might be its toxicity agent (Chen et al., 2008; Fournier et al., 2010). Additionally, during protein synthesis, this substitution, will change protein structure and function and thereby will cause a teratogenic effect on animals such as fish (Fournier et al., 2010).

Further, Se toxicity impressed by various factors such as oxidation state, Se concentration, microalgae species and characteristic, and environmental condition. So that, it is difficult to realize Se toxicity in aqueous environment (Babaei et al., 2017; Fournier et al., 2010; Schiavon et al., 2016). Therefore, analysis of specific toxicity is required for selected species before Se-enriched biomass production (Babaei et al., 2017). On the other hand, algal sensitivity to elements is strongly depends on species. Umysova et al., (2009) showed that selenate concentrations depend on studied species and may be inhibited 3-fold growth (Umysova et al., 2009). Different organisms react differently to the same metal and may be affect more or less by various metals (Mane et al., 2013).

Li et al. (2003) investigated sodium selenite effect on various parameters of *Spirulina* and showed that sodium selenite causes growth stimulation at concentrations lower than 400 mg/L (especially at 4-50 mg/L). However, they emphasized that the enrichment of *Spirulina* cultures by ranges of 0.5-40 mg/L selenite concentrartion is the most appropriate concentration; this salt has a toxic effect at concentrations above 500 mg/L on algae.

Se toxicity mainly attributed to stronger bioaccumulation of microalgae in comparison to macroalgae, inactivation of Cys-contained enzymes, inhibitory effects on photosynthetic electron transfer chain, which overall result of these mechanisms causes reduction of photosynthetic yield, metabolism disorder, and finally constrained of growth rate (Khademi and OraghiArdebili, 2017).

S. platensis growth is supported at 5 and 10 ppm concentrations (Fig. 1). On the other hand, the dry weight of microalgae decreased at selenate levels above 50 ppm. Khademi and OraghiArdebili (2017) reported that an increase of selenate concentration up to 10 mg/L reduced significantly the growth rate of *Spirulina* sp. This trend is observed in the current study at above 50, 100, and 300 ppm concentrations (Fig 1.). Sun et al. (2014) showed that Se is required as a nutrient to biosynthesis of proteins and lipids. They represented that growth stimulatory effect of Se is attributed to its antioxidant activity which increases chlorophyll-a and reduces LPO and ROS.

According to Babaei et al. (2017), 2.5 mg

Se/g DW and 8.5 mg Se/g DW of biomass, caused a slight stimulatory effect on growth rate during the first three days of experiment. Growth and photosynthetic inhibition observed up to 85 mg/g DW Se concentration. Besides, our results are in consistent with this study.

In the current study, the dry weight of microalgae decreased at selenate levels above 50 ppm which is in accordance with Khademi and OraghiArdebili (2017). This is probably due to the selenate effect on the cell division in microalgae (Geoffroy et al., 2007; Khademi and OraghiArdebili, 2017). In accordance with Sun et al. (2014), reduction in biomass at 50, 100, and 300 ppm of selenate concentration, is result of toxic effects of high concentration of selenate. The effect of 0, 5, and 10 mg/L of selenate concentration on the growth and antioxidant activity of *Spirulina platensis* investigated by Khademi and OraghiArdebili (2017) during 7 days cultivation period. The results showed that 5 mg/L of selenate concentration caused growth improvement but it was toxic in 10 mg/L concentration. Moreover, chlorophyll and carotenoid content in selanate-treated microalgae did not have any significant change in comparison to control culture. They stated that Se affects on *Spirulina* physiology at low concentrations.

The results of Chen et al. (2008) determined that using various Se concentrations (0-250 mg/L) caused Se accumulation in a dose-dependent pathway. Results indicated that at concentrations lower than 150 mg/L, Se causes increase in biomass concentration, photosynthetic pigment content including Lutein, beta-carotene and chlorophyll a, and the activities of glutathione peroxidase (GPX), superoxide dismutase (SOD), catalase (CAT), and Guaiacol-dependent peroxidases (POD). These results confirm that antioxidant enzymes play a crucial role in cell protecting against Se stress. Higher Se concentrations above 175 mg/L cause more accumulation and activities of GPX, SOD, CAT, POD and reduction in biomass concentration and photosynthetic pigments contents. Moreover, continuous reduction of chlorophyll-a content has been reported in control cultures and treated cells with 200 mg/L of Se after 0-11 days of incubation (Chen et al., 2008).

Umysova et al. (2009) investigated the effects of Se compounds at 0-100 mg/L ranges on *Scenedesmus quadricauda* and the level and chemical form of Se are crucial factors in cellular response and revealed that Se toxicity increased by sulfate-deficiency conditions and this is a result of Se interference by sulfur metabolism. It has also been reported that at the above concentration of 50 mg/L, most cells died after 1 or 2 days of incubation. Moreover, Se toxicity cause chloroplasts bleaching, cell malformation, and finally death.

Fournier et al. (2010) also studied the toxic effect of selenate on unicellular green algae *Chlamydomonas reinhardtii* as a function of sulfate ion concentration. It showed that maximum cell density and growth rate decreased by elevation of selenate in culture medium especially in the presence of

low-level sulfate ion. The same results obtained in the current study. According to these results, selenate toxicity correlates with its bioaccumulation in algae and shows that toxicity depends on intracellular effects of selenate rather than surface effects. Mane et al. (2013) reported that Se has stimulatory effects on *Anabaena ambigua* (at 3.0 mg/L), *Anabaena subcylindrica* (at 0.5 mg/L), *Nostoc commune* (at 0.5 mg/L), *Nostoc muscorum* (0.5 mg/L), *Spirogyra* sp. (0.1 mg/L) and *Spirulina* sp. (0.1 mg/L).

Based on Schiavon et al. (2012) after cultivation of *Ulva* sp. at the 6 first days of incubation, the pH of culture media was identical between all treatments but a considerable increase of pH from 8.46 for 0 concentration to 9.71 for 100 µM concentration of selenate observed at the end of the experiment (incubation period was 10 day). Nonetheless, there was no significant difference between control culture and supplemented medium with 2.5, 10, and 50 µM of selenate. Based on the results, the plastids are major aim for Se toxicity, ultrastructure studies in various microalgae, e.g. *Chlamydomonas reinhardtii* showed that both selenate and selenite could damage thylakoid membranes and cause overproduction of starch granules (Schiavon et al., 2016; Umysova et al., 2009).

According to Mane et al. (2013), at lower Se levels, Se has stimulatory effects on total chlorophyll content, protein, carbohydrate, starch, and free amino acids of studied algae. Moreover, all biochemical parameters of algal strains decreased gradually as in a dose-dependent manner. Furthermore, Babaei et al. (2017), indicated that a low Se level (about 19-65 µM) partially stimulates photosynthetic activity during 24 hours after cultivation. Sun et al. (2014) emphasized a similar trend and the inhibitory effect of Se salts on photosynthetic yield depends on ultrastructural changes. Probably, chloroplasts are the first goal of Se toxicity, so that their stroma, thylakoid, and pyrenoids are impressed and extra accumulation of starch and formation of condensed Se granules in their structures is reported using electron microscope. Finally, increasing in Se concentration (190 µM) causes a significant decrease in photosynthetic activity and at the growth rate. This matter is consistent with the data other studies on various algae such as *Spirulina*, *Chlamydomonas* or *Chlorella sorokiniana* (Geoffroy et al., 2007; Morlon et al., 2006; Schiavon et al., 2016).

Schiavon et al. (2016), reported that thallus morphology did not impress by selenite/selenate. They concluded that resistance to high selenate concentrations (200 and 400 mg/L) had been probably due to the exclusion mechanism for Se which may be include down regulation of at least one sulfate transporter. Although there was no effect on total morphology but chloroplast ultrastructure had affected, our results showed that in concentrations above 100 ppm, microscopic morphology of microalgae starts to change and the overall structure disrupted finally. However, in another study conducted by Belokobylsky et al. (2004), an increase of selenate level did not affect the morphol-

ogy of *Arthrospia platensis* Gomont, Gojkovicet al. (2014) considered this result. Likewise, they observed typical changes on chloroplast structure due to challenge with selenate oxyanions using electronic microscopi. Indeed, granulated stroma and reduction of its density and variations in thylakoids observed in contact with 40 and 100 mg/L of selenate (Gojkovic et al., 2014).

Effect on the pigment amount:

Carotenoids play crucial role in cell response to oxidative stress as one non-enzymatic antioxidant, which arises from ROS. Indeed, singlet oxygen in stress situations causes severe metabolic disorders using oxidative damage to cell components (Sun et al., 2014). Further, it found that carotenoids protect the photosynthetic membrane from photo-oxidation using effective removal of singlet oxygen and returning chlorophyll to the primary state. Sun et al. (2014), reported an increase in carotenoid content during the cultivation. However, in our study, the chlorophyll and carotenoid contents decreased at Se concentrations above 50 and 25 ppm, respectively. Subsequently, it can be a result of lipid peroxidation in chloroplast membranes. These effects caused by cellular damage or death from Se toxicity (Sun et al., 2014).

Mane et al. (2013) reported the inhibitory effects of some metals (such as iron, copper, silver, zinc, etc.) on chlorophyll contents in algae, which are in accordance with this study.

As it was demonstrated in the present study, when the selenate concentrations increase to upper levels (100-2000 ppm) chlorophyll amount will increase in 5, 10, 25, and 50 ppm of selenate concentration and were showed reduction of this the pigments is reduced. On this basis, an increase of chlorophyll amount in treated cells with low levels of Se (5-50 mg/L) may be due to efficient removal of ROS using CAT, SOD, and GPX. Also, these results stated by Sun et al. (2014).

Gojkovic et al. (2014), investigated the effect of 40 mg/L (212 μM) selenate on *Chlorella sorokiniana*. The results indicated that chlorophyll and carotenoid amounts did not change but the growth rate decreased up to 25%. Schiavon et al. (2012) reported that chlorophyll amount did not have any significant change in cultured thallus at the presence of selenate in comparison to control culture. However, an increase of these pigments observed after three days of cultivation in the presence of high selenate levels. In contrast, the carotenoid amount significantly rose during 3 to 10 days of experiment especially in a 50-100 mg/L range. On the other hand, at low levels of selenate (2.5-50 mg/L) any effect on the microscopic structure of thallus did not observe and in the higher concentrations (100 mg/L) thallus thickness decreased.

Gojkovic et al. (2015), reported that the total amount of chlorophyll and carotenoid increased during the first 48 hours of cultivation at control culture and nearly constant up to 96 hours of cultivation. Thereafter, the levels of pigments in the control culture de-

creased as the self-shading effect.

For Se-enriched biomass production and development of biotechnological processes, a better perception of total Se effect on microalgae is required (Babaei et al., 2013). Due to the increase in bioavailability, Se is essential both in biomedicine and as a complementary diet for domestics. It seems that further studies need to conduct usage of Se-*Spirulina* as antioxidant for aquaculture feed and human food (Sun et al., 2014). Further, more studies need to find key variables, which cause growth inhibition or even growth failed on large scales at different Se Concentration.

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