Iranian Journal of Fisheries Sciences 20(6) 1589-1608 2021
DOI: 10.22092/ijfs.2021.124953

Research Article

Effect of different cobalt ($CoCl_2$) concentrations on cell growth, some biochemical composition, and fatty acids profile of the marine microalga, *Tetraselmis subcordiformis*

Eskandarnia H.[1]; Shamsaie Mehrgan M.[1*]; Rajabi Islami H.[1]; Soltani M.[2]; Kamali A.[1]

Received: October 2019 Accepted: February 2020

Abstract

The aim of this study was to determine the effects of cobalt concentration on cell growth, some biochemical composition, and fatty acids profile of the marine microalga *Tetraselmis subcordiformis*. Cobalt deprivations did not cause a considerable change in growth photosynthesis activity as compared to the control group. Induction of maximum lipid production was achieved using 0.001 mg L^{-1} of cobalt-deprived *T. subcordiformis*. The highest crude protein content (33.75 mg L^{-1}) was observed in 0.001 mg L^{-1} cobalt. The highest and lowest lipid accumulation in *T. subcordiformis* was observed in 0.001 mg L^{-1} and 10 mg L^{-1} cobalt, respectively. Under cobalt deficient conditions, *T. subcordiformis* produced a large quantity of saturated fatty acids and iodine and saponification value. Biodiesel characteristics were enhanced with Co^{+2} reduction. Also, biodiesel quality decreased with increase of cobalt. The results showed that the maximum and minimum carotenoids were observed in 0.1 and 10 mg L^{-1} cobalt, respectively.

Keywords: Cobalt, Proximate composition, Fatty acids profile, Lipid, *Tetraselmis subcordiformis*

1-Department of Fisheries, Science and Research Branch, Islamic Azad University, Tehran, Iran.
2-Department of Aquatic Animal Health, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.
*Corresponding author's Email: m.shamsaie@srbiau.ac.ir

Introduction

Nowadays, microalgae have attracted considerable attention according to their specific features, like rapid growth rate, accumulation of high level of lipid, and high nutritional value (Mata *et al.,* 2010; Hosseini Shekarabi *et al*., 2019).

Microalgae are capable of completing an entire growing cycle every few days, whenever sufficient amounts of sunlight, water, carbon dioxide, and nutrients are available (Liu *et al*., 2008; Brennan and Owende, 2010). Due to the unique biochemistry of algae, they have been probed for several primary (e.g. protein, lipid, and carbohydrates) and secondary (e.g. carotenoids and alkaloids) metabolites (Liu *et al*., 2008; Douskova *et al*., 2009; Hadizadeh *et al*., 2019). Alternative fuel sources such as biofuels have been extensively studied to break the restriction of fossil fuels. Environmental pollution leads to the release of sulfur emission through fossil fuels combustion, as bioremediation microalgae can put forward a promising substitute for petroleum products (Sharma *et al*., 2012; Ghayal and Pandya, 2013; Ullah *et al.,* 2014). Earlier investigation on generating a large quantity of lipid content confirmed that the type of fatty acids and other biochemical compositions of algae have a prominent role in increasing oil for biodiesel production (Palit *et al*., 1994; Sharma *et al.*, 2012; Elsalhin *et al*., 2016; Sabzi *et al.*, 2018).

It has been stated that some factors, including nutrient stress, light intensity, temperature, CO_2, and salinity augment lipid accumulation of microalgae (Layer *et al*., 2010; Rastar *et al*., 2018). In this regards, cobalt (Co^{+2}) as a combination of cyanocobalamin vitamin, is a necessary compound of numerous co-enzymes and enzymes (Elsalhin *et al*., 2016). Also, this chemical element plays a prominent role in the photosynthesis process (El-Sheekh *et al*., 2003). With increasing concentration of cobalt in the medium, the rate of cell volume and cell deviation is reduced linearly (Palit *et al.*, 1994).

Generally, heavy metals are essential at low concentration but they can be toxic at high concentration (Gholamiourimi and Soltani., 2014). Czerpak *et al*. (1994) stated that cobalt in concentration of 5×10^{-6} mol L^{-1} induced growth phase in *Auxenochlorella pyrenoidosa* exponentially. Lustigman *et al.* (1995) demonstrated that growth of *Chlamydomonas reinhardtii* significantly decreased at 20 ppm cobalt. *Tetraselmis sp* is considered to be an appropriate candidate for biofuel because of it's high biomass and starch content. Nevertheless, its biomass product depends not only on primitive nutrients (phosphorus and nitrogen), but also on metals (iron, zinc, cobalt, manganese, copper, molybdenum, zinc), vitamins and other minerals (Dammak *et al*., 2017).

Tetraselmis subcordiformis is suggested as a good candidate green microalgae species for biofuel because of its high biomass and starch production. In this study, it is attempted to investigate the effect of cobalt on *Tetraselmis subcordiformis* growth, lipid accumulation, and biochemical compositions at different dosages. The object of this research was to examine tolerance of the mentioned microalgae to cobalt, and its capacity for production of pigment, and fluctuating the level of polyunsaturated fatty acids (PUFAs) and saturated fatty acids (SFAs). The results of this study can be used for obtaining maximum microalgae biomass production, optimal lipid production, and determining the pollutant-forming chemistry of algae-derived biofuels.

Materials and methods

Culture conditions

The unicellular marine microalga, *T. subcordiformis*, was obtained from Persian Gulf Biotechnology Park (PGBP), Gheshm, Iran. All experiments were fulfilled in Zakariya Razi Laboratory Complex, IAU University, Tehran, Iran. *T. subcordiformis* was cultured in F/2 growth medium correspondent to Guillard *et al.* (1962), with the following ingredients (per liter), 6 mg Na_2HPO_4 $2H_2O$, 75 mg $NaNO_3$, 0.5 µg vitamin B_{12}, 100 µg Thiamine HCl, 0.5 µg biotin, 10 mg Na_2SiO_3 $9H_2O$, 3.16 mg $FeCl_3$ $6H_2O$, 4.4 mg Na_2-EDTA, 21 µg $ZnCO_4$ $7H_2O$, 0.01mg $CoCl_2$ $6H_2O$, 70 µg $CuSO_4$ $5H_2O$, 7 µgNa_2MoO_4 $2H_2O$ and 0.18 mg $MnCl_2$ $4H_2O$. All of the chemicals were from Merck. *T. subcordiformis* strain was grown in 50 mL medium in 100 ml flasks, at an optimum temperature of 25±1°C and under continuous illumination (84 µmol $m^{-2}s^{-1}$) from luminescent with white fluorescent lamps at pH 7. Cultures were continuously aerated to maintain constant CO_2 concentration in the growing medium.

F/2 medium consisted of 0.01 mg L^{-1} $CoCl_2$. To ascertine the effect of various concentrations of $CoCl_2$ on growth and lipid content, the modified solution for experiments was prepared using the concentrations of 0.001, 0.01 (control), 0.1, 1.0 and 10 mg L^{-1} $CoCl_2$. The test flasks were incubated under the same illumination condition and temperature as those used for stock culture. The primary cell density of 5×10^5 cells mL^{-1} was used for starting the experiments. Each experiment consisted of three replicates.

Growth measurement

The biomass (dry weight, DW) was used to measure biomass concentration in microalgae cell suspensions spectrophotometrically at 680 nm (OD_{680}) (UV-2501PC UV-VIS, Shimadzu). Then the OP_{680} ($OD6_{80}$) level was multiplied with 0.38 (the coefficient conversion factor) to convert the OD_{680} value to dry weight (Zhou *et al*., 2013).

Extraction and estimation of pigments procedure

The extraction procedure was performed according to Dammak *et al*. (2017), with some modifications. 2 mL of culture was centrifuged at 5000×g for 10 min. Then, the pellet was suspended with 2 mL methanol (90%) and ethanol and sonicated at 65°C for 30 min. Afterward, the incubation was centrifuged at 5000×g for 5 min, and A_{666}, A_{653}, and A_{470} were measured to determine chlorophyll *a* (Chl *a*), *b* and total carotenoid in days of 4, 6, 8, and 14 as presented by Eqs. 1-3:

$$\text{Chl } a \text{ (mg L}^{-1}) = 15.65 \times A666 - 7.340 \times A653 \quad (1)$$

$$\text{Chl } b \text{ (mg L}^{-1}) = 27{:}05 \times A653 - 11.21 \times A666 \quad (2)$$

$$\text{Total carotenoid content} = [1000\ (A470) - 1.63 \text{ Chl } a - 104.96 \text{ Chl } b]/221 \quad (3)$$

Protein and lipid contents

A small mass of dried *T. subcordiformis* was selected for protein and lipid analysis at the end of the trial (14th day). Protein content was assayed by Zor and Selinger (1996) method. Lipids were extracted from the dried algae by methanol-chloroform extraction (Bligh and Dyer, 1959). Chloroform-methanol (2:1, v/v) was added to the dried cells, then ultrasonication was practiced to destroy cells, and then methanol and water were added to achieve the last solvent ratio of chloroform/methanol/water of 1:1:0.9. After 3-h standing, chloroform and aqueous methanol films were separated. The chloroform film with total lipids was washed with a 5 % NaCl solution and vaporized to dryness. Consequently, the total lipids were assayed gravimetrically (Bligh and Dyer, 1959).

Fatty acids profile

Fatty acid methyl esters (FAMEs) were corrected via acid transesterification. Concisely, lyophilized cells were produced with a solvent mixture of toluene and 1 % sulphuric acid in methanol (1:2, v/v) at 50°C for creating FAMEs, which were then extracted with hexane. The FAMEs were assayed by utilizing an HP 6890 capillary gas chromatography (Hewlett-Packard, Palo Alto, CA) equipped with a flame ionization detector (FID) and capillary column (30m×0.32 mm) (Agilent, Inc., Wilmington, DE). Nitrogen was used as vector gas. The first column heat was set at 170°C, which was progressively increased to 230°C at 1°C min^{-1}. The injector was held at 250°C with an injection volume of 2 µL under splitless mode. The FID heat was set at 270°C. FAMEs were recognized by chromatographic correlation with reliable standards (Sigma).

Iodine value (IV)

IV is a parameter that has been usually used by the vegetable oil industry to evaluate the degree of unsaturation or the number of double bonds in a molecule of oil (Lapuerta *et al*., 2009). One aliquot of the algal lipid extract was used to determine iodine value according to the European standard method EN 14111 and expressed as g I_2 $100g^{-1}$.

Saponification number (SN)

SN is one of the biodiesel quality indicators obtained from the equation 4 (Zhou *et al*., 2013):

SN= ∑(560×N)/M

that N and M are fatty acid percentage, and molecular weight of each fatty acid, respectively.

$$SN = \sum(560 \times Ai)/MWi \quad (4)$$

Statistical analyses

Results are exhibited as mean±standard deviation (SD) from three replicates. A one-way ANOVA followed by Tukey's test was used to recognize differences between treatments. All comparisons were performed by SPSS software version 19.0 (SPSS, Chicago, USA). Also, differences were considered significant at $p<0.05$.

Results

The effect of Co^{+2} on the dry weight of T. subcordiformis

The effect of Co^{+2} concentrations on dry weight of *T. subcordiformis* is presented in Figure 1. As can be seen, there were certain extents of Co^{+2} effect on dry weight of *T. subcordiformis.* It was shown that dry weight of *T. subcordiformis* reached the maximum value (0.0016 mg L^{-1}) when concentration of Co^{+2} was 0.01 mg L^{-1}. The experiment value was followed by the treatment with 0.001 mg L^{-1} Co^{+2} in the 14th day. According to the results, the lowest dry weight (0.0007 mg L^{-1}) was observed in the groups treated with 0.1, 1, and 10 mg L^{-1} Co^{+2} (Fig. 1).

Effect of Co^{+2} on the growth of T. subcordiformis

The data shown in Figure 2 depict the response of growth parameters to Co^{+2} concentrations. There were significant differences in cell density of algal cells between control and treated ones when microalgae cells were exposed to different concentrations of Co^{+2}. It was found that lower and higher Co^{+2} concentrations could not promote the growth of microalgae. As can be seen in Figure 2, *T. subcordiformis* could grow under all experimental conditions, and the cells cultured in 0.01 mg L^{-1} media (control) showed the highest cell density (0.67 cell mL^{-1}) in the 14th day of incubation. The minimum cell density was observed in the group treated with 0.001 mg L^{-1} Co^{+2} (0.42 cell mL^{-1}).

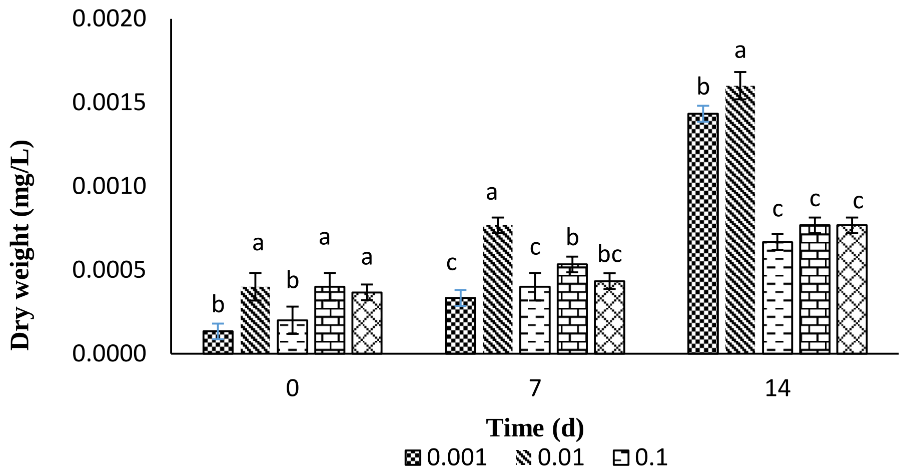

Figure 1: Effect of Co^{+2} concentrations on dry weight of *T. subcordiformis.* All points represent mean of the three individual replicates. Error bars show standard deviation.

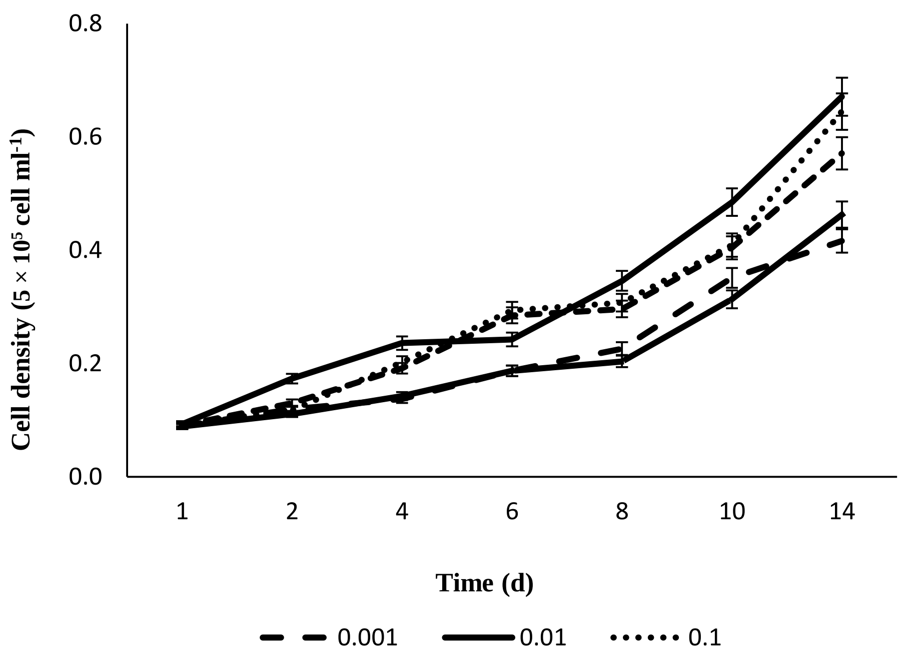

Figure 2: Effect of Co^{+2} concentrations on cell density of *T. subcordiformis*. Error bars show standard deviation.

Effects of Co^{+2} on pigments content of T. subcordiformis

Figure 3 shows that the treatment with 10 mg L^{-1} Co^{+2} led to a sharp decrease in Chl *a* content compared to the control. Co^{+2} concentration of 1 mg L^{-1} slightly stimulated the Chl *a* content by 4.93 mg L^{-1}. However, other manipulated cultures of Co^{2+} concentrations led to reductions in Chl *a* biosynthesis. The highest (5.13 mg L^{-1}) and lowest (1.99 mg L^{-1}) Chl *a* were observed in 0.01 and 10 mg L^{-1} Co^{+2}, respectively. A similar impact of Co^{2+} on Chl *b* biosynthesis was also noted. The data presented in Figure 4 indicated that application of Co^{+2} in different concentrations led to a significant reduction Chl *b* content. The maximum (2.24 mg L^{-1}) and minimum (1.23 mg L^{-1}) Chl *b* contents were obtained in the groups treated with 0.01 (control), and 10 mg L^{-1} Co^{+2}, respectively.

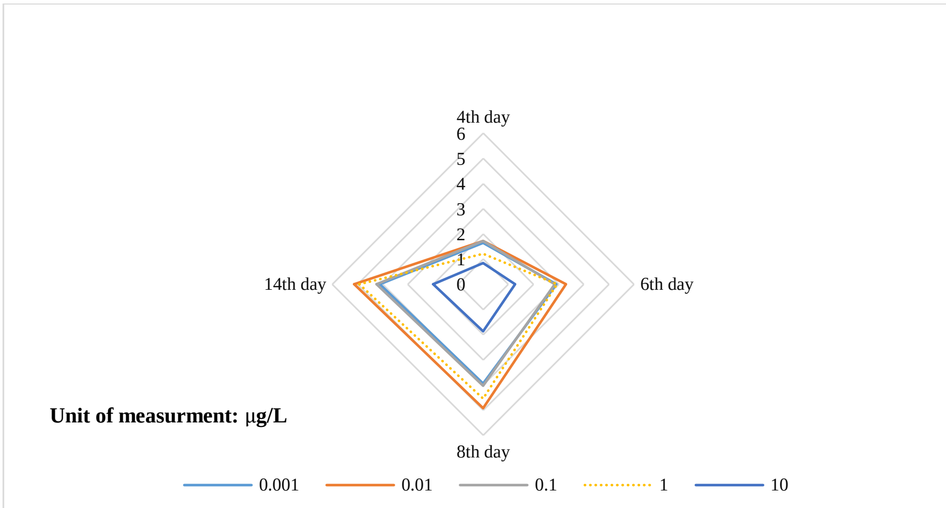

Figure 3: Effect of Co^{+2} concentrations on *Chl a* of *T. subcordiformis*.

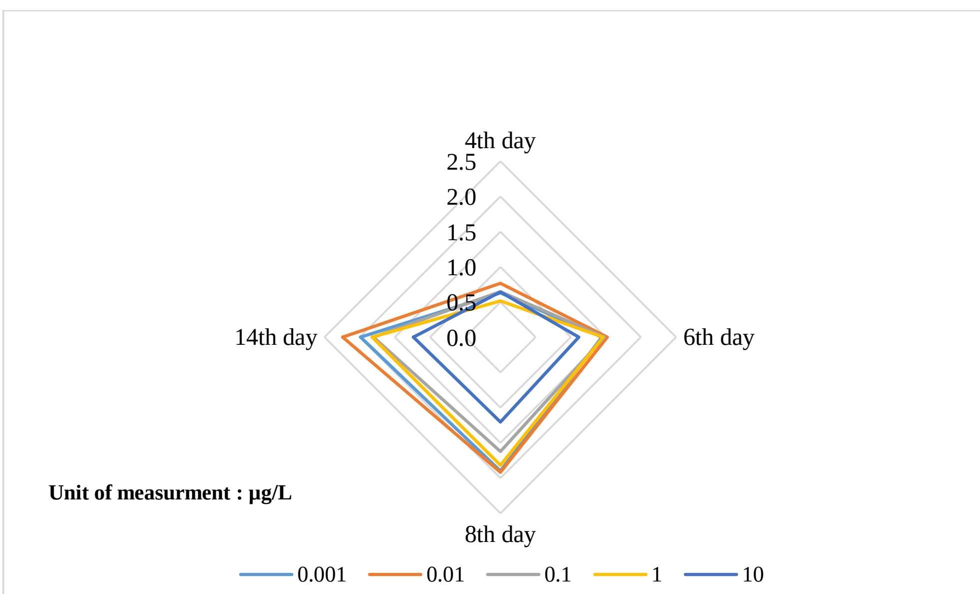

Figure 4: Effect of Co^{+2} concentrations on *Chl b* of *T. subcordiformis*.

In carotenoids study adding 0.01 mg L^{-1} Co^{+2} led to the maximum value (0.64 mg L^{-1}) at the end of the incubation period. Also, the lowest Co^{+2} concentration (0.001 mg L^{-1}) resulted in the lowest carotenoid accumulation (0.32 mg L^{-1}) during 14 days (Fig. 5).

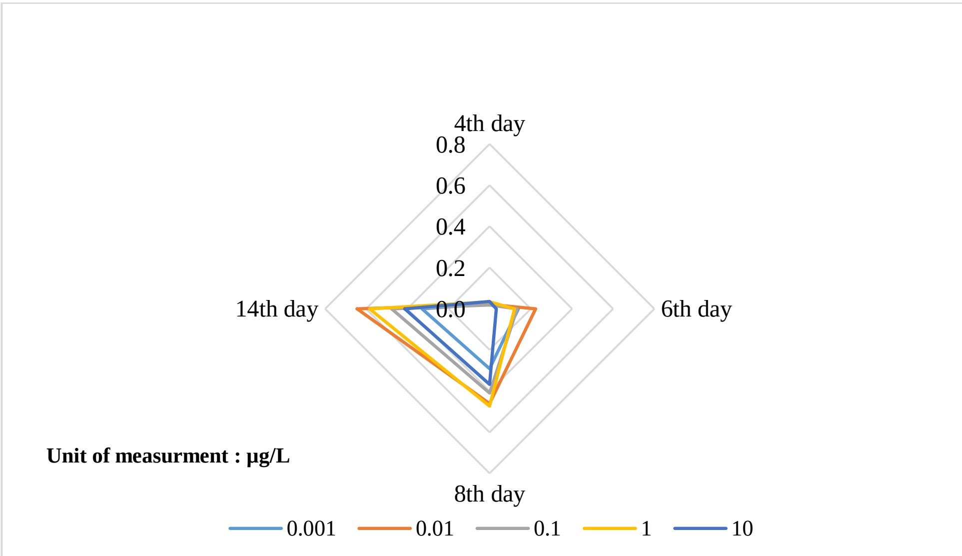

Figure 5: Effect of Co+2 concentrations on carotenoids of *T. subcordiformis*.

Effect of Co^{+2} on protein accumulation of T. subcordiformis

The results obtained for protein content in *T. subcordiformis* revealed that the value of total protein content increased under the effect of 0.001 mg L^{-1} Co^{+2} at the end of the experiment (14 days). On the contrary, excessive increases in Co^{2+} concentrations led to a reduction of protein content (Table 2). In this experiment, the lowest (16.85%) and highest (33.75%) protein contents were obtained in the treatments with 0.001 and 10 mg L^{-1} Co^{+2}, respectively.

Effect of Co^{+2} on lipid accumulation of T. subcordiformis

The bioassay results shown in Table 1 indicated the distinct differences in lipid contents of *T. subcordiformis* cells between the control and treated samples when the microalgae were exposed to different concentrations of Co^{+2}. The lipid content showed a marginal decrease in the cultures supplemented with higher concentrations of Co^{+2} (0.1, 1 and 10 mg L^{-1}). On the other hand, lower Co^{+2} concentrations significantly increased the lipid content. The results showed that low amounts of Co^{+2} (0.001 mg L^{-1}) had a stimulatory effect on lipid content (22.59 %). The minimum lipid value (11.54 %) was observed in the samples which were incubated with 10 mg L^{-1} Co^{+2} (Table 1).

Table 1: Effects of Co^{+2} concentrations on proximate composition in *T. subcordiformis* harvested on 14-day.

Proximate analysis (%)	Co^{+2} concentration ($mg\ L^{-1}$)				
	0.001	**0.01**	**0.1**	**1**	**10**
Protein	33.75±0.03^{a}	32.83±0.5^{b}	29.23±0.0^{c}	25.67±0.02^{d}	16.85±0.09^{e}
Lipid	22.85±0.03^{a}	21.23±0.16^{b}	19.16±0.01^{c}	15.09±0.03^{d}	11.5±0.37^{e}

Values in the same row with different superscripts represent significant difference ($p<0.05$). All points represent mean ± standard deviation of the three individual replicates.

Effect of Co^{+2} on fatty acids composition of T. subcordiformis

The results of fatty acid profile in *T. subcordiformis* are given in Table 2. The significantly increased fatty acids, including C14:0, C16:0, and C15:1, were detected in 0.001 $mg\ L^{-1}$ Co^{+2} medium. The highest amount of Oleic acid (C18:1 cis) was measured in 10 $mg\ L^{-1}$ Co^{+2} medium. However, the percentage of linolenic acid (C18:3n6) significantly increased in 0.01 and 0.1 Co^{2+} media compared to other treatments. Albeit, the dominant saturated fatty acid (SFA), monounsaturated fatty acid (MUFA) and polyunsaturated fatty acid (PUFA) were observed in 0.001, 1, and 0.01 medium, respectively (Table 2).

Table 2: Effect of Co^{+2} concentrations on fatty acids profile of *T. subcordiformis.*

Fatty acid (%)	Co^{+2} concentration ($mg\ L^{-1}$)				
	0.001	**0.01**	**0.1**	**1**	**10**
C14:0	3.82±0.58^{b}	2.00±0.06^{a}	2.06±0.02^{a}	2.19±0.07^{a}	3.42±0.00^{b}
C15:0	0.2±0.05	ND	0.47±0.5	0.33±0.01	0.54±0.00
C16:0	42.83±0.31^{c}	40.49±0.53^{ab}	39.36±0.37^{a}	42.28±0.76^{bc}	41.6±0.06^{bc}
C17:0	0.08±0.1	0.15±0.0	0.19±0.0	0.17±0.0	0.17±0.02
C18:0	0.8±0.02^{a}	0.94±0.31^{ab}	1.38±0.09^{b}	0.79±0.01^{ab}	1.02±0.0^{ab}
C21:0	0.26±0.07	0.48±0.09	0.93±0.36	0.27±0.02	0.35±0.02
C22:0	0.02±0.0^{a}	0.02±0.0^{a}	0.07±0.02^{b}	0.02±0.0^{a}	0.04±0.0^{ab}
C24:0	0.02±0.02^{a}	0.04±0.01^{a}	0.13±0.0^{b}	0.02±0.02^{a}	0.06±0.01^{a}
C14:1	0.05±0.01^{a}	0.23±0.05^{b}	0.13±0.0^{ab}	0.38±0.01^{c}	0.82±0.0^{d}
C15:1	9.52±0.03^{c}	7.22±0.19^{b}	6.54±0.09^{a}	9.09±0.1^{c}	7.09±0.02^{b}
C16:1	3.03±0.07^{a}	4.21±0.19^{b}	3.66±0.12^{ab}	4.27±0.29^{b}	4.28±0.19^{b}
C17:1	0.72±0.0^{b}	0.67±0.0^{a}	0.72±0.01^{b}	0.69±0.01^{ab}	0.67±0.0^{a}
C18:1 tra	0.65±0.08^{b}	0.44±0.03^{b}	1.24±0.17^{a}	0.64±0.0^{b}	0.52±0.0^{b}
C18:1 cis	14.55±0.03^{ab}	14.58±0.34^{ab}	14.31±0.0^{a}	14.58±0.28^{ab}	15.17±0.02^{b}
C20:1	0.3±0.02	0.07±0.0	0.14±0.06	0.15±0.0	0.1±0.02
C22:1	0.04±0.02	0.05±0.02	0.08±0.04	0.04±0.0	0.04±0.0
C24:1	0.02±0.01	0.14±.12	0.25±0.03	0.03±0.0	0.07±0.01
C18:2 tra	0.36±0.01^{b}	0.39±0.03^{b}	0.59±0.02^{a}	0.37±0.04^{b}	0.43±0.0^{b}
C18:2 cis	11.3±0.07^{a}	12.35±0.21^{b}	11.66±0.16^{a}	11.41±0.2^{a}	11.73±0.02^{ab}
C18:3 tra	0.57±0.0^{ab}	0.45±0.06^{ab}	0.41±0.01^{a}	0.47±0.07^{ab}	0.62±0.0^{b}
C18:3n3	0.33±0.02^{a}	0.53±0.0^{b}	0.49±0.03^{b}	0.29±0.0^{a}	0.47±0.02^{b}
C18:3n6	9.99±0.32^{a}	11.68±0.11^{b}	11.14±0.04^{b}	9.89±0.31^{a}	9.61±0.0^{a}
C20:2	0.07±0.04^{ab}	0.02±0.0^{a}	0.08±0.02^{ab}	0.09±0.0^{ab}	0.15±0.0^{b}
C22:2	0.01±0.0	0.3±0.0	0.07±0.02	ND	0.02±0.0
∑SFA^{a}	48.04±0.65^{b}	44.3±0.6^{a}	44.63±0.09^{a}	46.09±0.86^{ab}	47.23±0.0^{b}
∑MUFA^{b}	28.64±0.24^{ab}	27.63±0.52^{ab}	27.1±0.09^{a}	29.89±0.65^{c}	28.77±0.12^{bc}
∑PUFA^{c}	22.65±0.45^{a}	25.46±0.23^{c}	24.46±0.16^{bc}	22.54±0.63^{a}	23.05±0.04^{ab}

Notes: ND, not detected; ∑SFA, total saturated fatty acids; ∑MUFA, total monounsaturated fatty acids; ∑PUFA, total polyunsaturated fatty acids. All points represent the mean ± standard deviation of three individual replicates. Means in the same row sharing the same superscript letter are not significantly different ($p>0.05$). Absence of letters indicates no significant difference among treatments.

Effect of Co^{+2} on biodiesel quality of T. subcordiformis

The high iodine values (IV) of 81.62 and 78.29 gI_2 $100g^{-1}$ were obtained from *T. subcordoformis* grown in the media supplemented with 0.001 and 0.01 mg L^{-1} (control), respectively. The excessive increase of Co^{+2} concentrations resulted in lower IV. The lowest iodine value (34.60 gI_2 $100g^{-1}$) was observed in the samples treated with 10 mg L^{-1} Co^{+2} (Fig. 6).

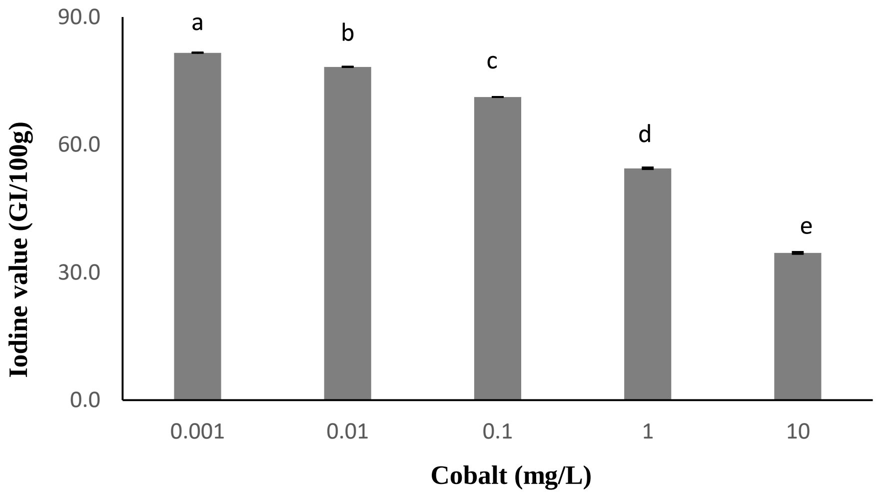

Figure 6: Effect of Co^{+2} concentrations on iodine value of *T. subcordiformis* harvested on day 14. Error bars show standard deviation.

Effect of Co^{+2} on saponification number of T. subcordiformis

As shown in Figure 7, the maximum (233.33 mg KOH g^{-1}) and the minimum (172.67 $mgKoHg^{-1}$) SN numbers were obtained at 0.001 and 10 mg L^{-1} Co^{+2} in the 14^{th} day of culture (Fig. 7).

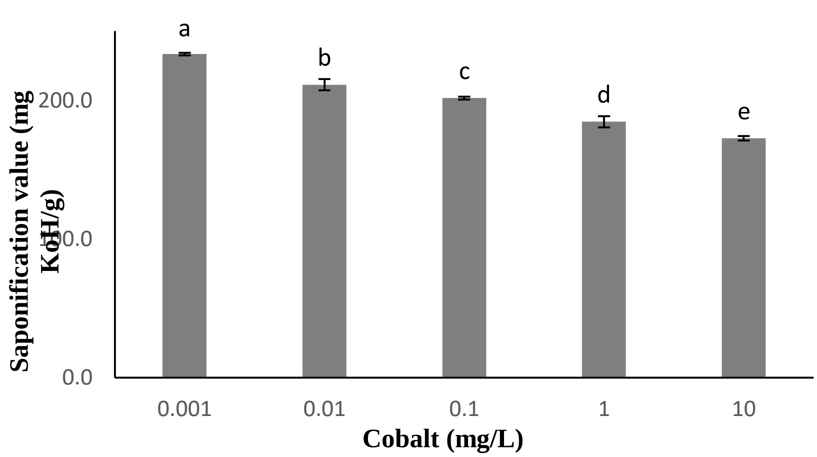

Figure 7: Effect of Co^{+2} concentrations on saponification number of *T. subcordiformis* harvested on day 14. Error bars show standard deviation.

Discussion

The outcome of this study revealed that increasing Co^{+2} concentration to 0.001 mg L^{-1} resulted in a decrease in crude protein and lipid content and an elevate in SFA that has biodiesel property. Co^{+2} is a crucial element for the synthesis of the vitamin cobalamin which catalyzes the reactions of adenosyl cobalamin and methyl cobalamin, two vital cofactors in the activity of methylmalonyl-coenzyme A mutase and methionine synthetase (Layer *et al*., 2010).

Accessibility of nutrients, such as phosphorus, nitrogen, metals, and vitamins, is the most crucial agent adjusting cell growth, photosynthesis, and other processes in microalgae (Yao *et al*., 2013; Rastar *et al.*, 2018). El-Sheekh *et al*. (2003) stated that high levels of heavy metals could diminish the number of Chl and carotenoids. They also suggested that algae exposure to Co^{+2} reduces pigments leading to the chlorophyll-degrading enzyme activity. Earlier reports indicated that growth promotion under low Co^{+2} concentrations could be attributed to replacement with Zn^{2+} in some metalloenzymes in vitro and in vivo (Price and Morel, 1990). As indicated by Lustigman *et al*. (1995), the *Chlamydomonas reinhardtii* culture medium supplemented with 10 ppm Co^{+2} reduced the growth, without changing the morphology of the cells or pH. Conversely, at 20 ppm Co^{+2}, the growth was considerably reduced compared to the control.

The results obtained in the present study illustrated that 0.01 mg L^{-1} Co^{+2} (control) was the optimal range of Co^{+2} for *T. subcordiformis* growth rate. However, Co^{2+} deficiency and excessive Co^{2+} resulted in growth inhibition. As previously reported, the metal deficiency could hinder growth rate in marine phytoplankton (Hokin *et al*., 2004; Jiang *et al*., 2012). The results of the our research showed that both treatment methods (using low and high Co^{+2} doses) were not able to improve *T. subcordiformis* cell densities. Elibol and Çakmak (2016) stated that Co^{+2} deprivation did not make a significant difference in the growth of *Dunaliella* strain during an incubation period of 30 days, which was not in agreement with the presented results. They reported that this noncompliance could be due to different growth solutions.

The efficiency performance of the photosynthetic process in microalgae is predominantly exhibited by the Chl and carotenoid levels. Production of Chl pigments needs a proper and sufficient supply of metal ions (Küpper *et al.*, 2002). A high concentration of Co^{+2} seems to impede the incorporation of iron in protoporphyrin molecule, leading to Chl pigment decrease. Heavy metals in growth media can impair Chl synthesis by blocking the synthesis and activities of the enzyme proteins responsible for Chl biosynthesis (Singh *et al.*, 2012). El-Naggar and Osman (1999) noticed that lower Co^{2+} concentrations (0.01 ppm) excited the growth of *Desmonostoc muscorum* and had an insignificant effect on the growth of *Calothrix fusca*. The results of the present research showed that Co^{+2} treatments (both low and high doses) had a negative effect on *T. subcordiformis* Chl *a*, *b* and total carotenoid. Gholamiourimi and Soltani (2014) reported the highest rates of photosynthetic pigments in *Chara sp* (Chl *a*, *b* and total carotenoids) in control samples (without Co^{+2}). They concluded that the amount of photosynthetic pigments was reduced with increase of Co^{+2} concentration from 10^{-6} to 10^{-3} μM. Their results were in line with the presented data.

As previously reported, algae have a tremendous ability to grow in presence of heavy metals concentrations by a variety of tolerance mechanisms, including binding to the cell wall, sedimentation in vacuole and synthesis of heavy metals binding compounds such as organic acids, proteins, and phenolic compounds (Mehta and Gaur, 2005). Olafson *et al*. (1979) elucidated the importance of proteins and lipoproteins in trapping metals as a means to detoxify them within the cell.

The data presented in this study revealed the sensitivity of *T. subcordiformis* to high concentrations of Co^{+2}. The results indicated that the protein content increased at a low concentration of Co^{+2} (0.001 mg L^{-1} Co^{+2}). However, the microalgae protein content was clearly decreased with the increase of Co^{+2} during the trial. Moreover, the algae treated with 10 mg L^{-1} Co^{+2} led to the least protein yield (16.85%) at the end of the experiment. Osman *et al*. (2004) clarified that low Co^{2+} concentrations of 0.1 and 1 ppm for *Scenedesmus obliquus*, and 0.5 and 1.5 ppm for *Navicula perminuta* significantly increased the protein content. They also reported that further increases in Co^{+2} concentration (2-4 ppm for *S. obliquus* and 2.5, 3.5 and 5 ppm for *N. perminuta*) were correlated with a decline in protein content of the two algae tested.

Elsalhin *et al*. (2016) stated that low concentrations of Co^{+2} (1.0 and 1.5 mg L^{-1}) induced a significant increase in protein content of *Arthrospira platensis* until the 4th day of culture. They found that more increase in Co^{+2} levels was responsible for a continuous reduction in protein content of the algae. The same outcomes were obtained by Larsen and Nilsson (1983). The results obtained in our study were in agreement with all the mentioned reports regarding the positive effects of low concentrations of Co^{+2} on protein accumulation. It is assumed that they employed protein accumulation by microalgae at a low heavy metals level as a method to eliminate the toxic effects. It should be noted that increased respiration can also contribute to protein accumulation in response to carbohydrate consumption (Mohammady and Fathy, 2007; Coesel *et al*., 2008).

In line with our study, Jiang *et al*. (2012) stated that nutrient deprivation is one of the lipid induction techniques widely used in microalgae TAG production for many species.

However, nutrient deprivation often alters the cellular macromolecular composition (Mittelbach, 1996; Yodsuwan *et al*., 2017). The results obtained in our investigation indicated that different concentrations of Co^{+2} affected the lipid content of *T. subcordiformis*. Increasing in Co^{+2} concentration in the experiments were associated with low lipid accumulation in *T. subcordiformis*. The chemical composition of the microalgae which are exposed to a range of environmental stresses is extremely affected by nutrient availability and light intensity. Therefore, it is conceivable that different cultivation growth conditions lead to different results (Mittelbach, 1996; Kyriakidis and Katsiloulis, 2000).

The results showed that 0.001 mg L^{-1} Co^{+2} roughly boosted the microalgae lipid accumulation compared to the control treatment (0.01 mg L^{-1} Co^{+2}). The results achieved by Elibol and Çakmak (2016) revealed that Co^{+2} deprivation prompted neutral lipid and β-carotene production in *Dunaliella tertiolecta*. Chia *et al.* (2013) noted that with the increase of total lipid concentration, the growth rate of *Chlorella vulgaris* decreased under cadmium stress. Lipid accumulation under high concentrations of Co^{2+} attributed to interruption of algal metabolism by inactivation of the photosynthetic machinery. This phenomenon leads to the formation of lipids as storage compounds in favor of carbohydrates (Bellou and Aggelis, 2013).

The present study showed that different Co^{+2} concentrations slightly affected the fatty acids profile in *T. subcordiformis*. Application of 0.001 and 10 mg L^{-1} Co^{+2} increased the SFA by 48.04% and 47.23%, respectively, while 1, 10 and 0.001 mg L^{-1} Co^{+2} slightly enhanced the MUFAS in *T. subcordiformis*. Heavy metals can alter the fatty acid structure of algae. Pinto *et al*. (2011) showed that SFA and MUFA contents of *Agarophyton tenuistipitatum* increased with the increase of cadmium (Cd) concentration. Also, Chia *et al*. (2013) reported the same results and concluded that SAFA and MUFA productions in *Chlorella vulgaris* increased in the presence of Cd in the culture media. Mohammady and Fathy (2007) revealed that proportions of fatty acids composition of *Dunaliella salina* remarkably changed under $NiCl_2$ treatment. In the presence of Ni^{+2}, the saturated fatty acids (including C14:0, C16:0, C20:0, and C22:0) strongly increased and the synchronous unsaturated fatty acids compositions, including C16:1, C16:4, C18:3, C22:4, and C24:1, reduced.

In our study, the accumulation of PUFAs under Co^{+2} concentrations was significantly differnent in 0.01 mg L^{-1} treatment, and the process of increasing cobalt did not match PUFA enhancement. The mechanisms include regularly oxidative stress and generation of reactive oxygen/nitrogen species that lead to oxidation of lipids (Pinto *et al*., 2011; Battah *et al*., 2015). Battah *et al*. (2015) showed that the treatments with 12 μM manganese chloride, 2.5 μM Co^{+2} nitrate and 4 mM hydrogen peroxide significantly decreased the total SFAs to 15% and 19% (lower than the values obtained in the control treatment), with a noticeable increment in unsaturated fatty acids.

Biodiesel quality considerably depends on feedstock such as chain length, degree of unsaturation and fatty acids compositions in TAG molecules which determine physical characteristics of these molecules (Hempel *et al*., 2012). In the present study, biodiesel characterization was determined using IV and SN. Calculated SN increased in algal cultures with supplementation of 0.001 and 0.01 mg L^{-1} (control) Co^{+2}. According to Yodsuwan *et al*. (2017), SN of *Phaeodactylum tricornutum* increased with addition of $NaNO_3$ (16.45, 32.09, and 64.29 mg L^{-1}). IV is regularly applied to verify the quality of biodiesel, and its permissible level in Europe is 120 gI_2 $100g^{-1}$ (Mittelbach, 1996). IV is a measure of the relative value of unsaturation in oil ingredients. The lower the IV is, the better the oil quality (Kyriakidis and Katsiloulis, 2000; Knothe, 2005; Sabzi *et al*., 2018). In the present study, IV decreased with increase of Co^{2+} concentration in the media, and the mean IV was calculated as 34.60 gI_2 $100g^{-1}$ in the samples treated with 10 mg L^{-1} Co^{+2}. On the other hand, the maximum IV (81.62 gI_2 $100g^{-1}$) was observed in the samples which were treated with 0.001 mg L^{-1} Co^{2+}. The obtained results implied that *T. subcordiformis* has SFA-rich lipids (48.04%) and low PUFA (22.65%), which may exhibit better IV levels according to the European Standard parameters for biodiesel synthesis. The degree of oxidation in unsaturated fatty acids compounds progresses with different rates depending on the abundance and location of double bonds. In the current study, *T. subcordiformis* lipids showed low levels of linoleic acid (C18:2n6) and linolenic acid (C18:3n3), which can provide methyl ester fuels with high oxidative stability (Ramos *et al*., 2009). Because USFAs heating results in polymerization and better oil quality, a low IV for biodiesel FAME would be necessary (Mittelbach, 1996; Kyriakidis and Katsiloulis, 2000; Knothe, 2002).

Results of the present study suggested that Co^{+2} deficiency was effective in enhancing lipid production by *T. subcordiformis*. The dry weight of *T. subcordiformis* reached the highest level at the Co^{+2} concentration of 0.01 mg L^{-1}. In spite of growth inhibition by Co^{+2} deprivation, protein content, biodiesel characteristics, and proportion of saturated fatty acids (SFA) were enhanced in the culture treated with 0.001 mg L^{-1} Co^{+2}. The highest and lowest Chl *a* and *b* were detected in 0.01 and 10 mg L^{-1} Co^{+2}, respectively. In the case of lipid, Co^{+2} deprivation (0.001 mg L^{-1}) led to an increase in lipid accumulation. The maximum IV and SN were achieved in the group treated with 0.001 mg L^{-1} Co^{+2}, and the lowest were obtained in 10 mg L^{-1} Co^{+2} treatment. Therefore, it was concluded that 0.001 mg L^{-1} Co^{+2} was suitable for lipid and protein accumulations. Measurement of some properties such as density, viscosity, flash point, and cold filter plugging point is essential to determine the suitability of *T. subcordiformis* lipids as a feedstock for biodiesel production.

Acknowledgments

The authors thankfully acknowledge the staff of the Zakaria-e-Razi Laboratory Complex of Science and Research Branch for their valuable participations during the project.

References

Battah, M., El-Ayoty, Y., Abomohra, A.E.F., El-Ghany, S.A. and Esmael, A., 2015. Effect of Mn^{2+}, Co^{2+} and H_2O_2 on biomass and lipids of the green microalga *Chlorella vulgaris* as a potential candidate for biodiesel production. *Annals of Microbiology*, 65(**1**), 155-162. DOI:10.1007/s13213-014-0846-7.

Bellou, S. and Aggelis, G., 2013. Biochemical activities in *Chlorella sp*. and *Nannochloropsis salina* during lipid and sugar synthesis in a lab-scale open pond simulating reactor. *Journal of Biotechnology*, 164(**2**), 318-329. DOI:10.1016/j.jbiotec.2013.01.010.

Bligh, E.G. and Dyer, W.J., 1959. A rapid method of total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology*, 37(**8**), 911-917. DOI:10.1139/o59-099.

Brennan, L. and Owende, P., 2010. Biofuels from microalgae-A review of technologies for production, processing, and extractions of biofuels and co-products. *Renewable and Sustainable Energy Reviews*, 14(**2**), 557-577. DOI:10.1016/j.rser.2009.10.009.

Çakmak, Z.E. and Çakmak, T. 2016. Evaluation of an indigenous *Dunaliella* strain for β-caroten and neutral lipid production as a response to Cobalt deprivation. *Advances in Renewable Energy*, 3, 1-6.

Chia, M.A., Lombardi, A.T., Melão, M.G.G. and Parrish, C.C., 2013. Effects of cadmium and nitrogen on lipid composition of *Chlorella vulgaris* (Trebouxiophyceae, Chlorophyta). *European Journal of Phycology*, 48(**1**), 1-11. DOI:10.1080/09670262.2012.750687.

Coesel, S.N., Baumgartner, A.C., Teles, L.M., Ramos, A.A., Henriques, N.M., Cancela, L. and Varela, J.C.S., 2008. Nutrient limitation is the main regulatory factor for carotenoid accumulation and for Psy and Pds steady state transcript levels in *Dunaliella salina* exposed to high light and salt stress.

Marine Biotechnology, 10(**5**), 602-611. DOI:10.1007/s10126-008-9100-2.

Czerpak, R., Bajguz, A., Chodkowski, K. and Popow, H., 1994. Influence of nickel and cobalt on the growth and biochemical changes of *Chlorella pyrenoidosa* (Chlorophyceae). *Polskei Archiwum Hydrobiologii*, 41(**2**), 161-169.

Dammak, M., Hadrich, B., Miladi, R., Barkallah, M., Hentati, F., Hachicha, R., Laroche, C., Michaud, P., Fendri, I. and Abdelkafi, S., 2017. Effects of nutritional conditions on growth and biochemical composition of *Tetraselmis sp*. *Lipids in Health and Disease*, 16(**1**), 41. DOI:10.1186/s12944-016-0378-1.

Douskova, I., Doucha, J., Livansky, K., Machat, J., Novak, P., Umysova, D. and Zachleder, V., 2009. Simultaneous flue gas bioremediation and reduction of microalgal biomass production costs. *Applied Microbiology and Biotechnology*, 82(**1**), 179-185. DOI:10.1007/s00253-008-1811-9.

El-Naggar, A. and Osman, M.E., 1999. Cobalt and lead toxicities on *Calothria fusa* and *Nostoc muscorum*. *Egyptian Journal of Botany*, 7, 421-441.

Elsalhin, H.E., Abobaker, H.M. and Ali, M.S., 2016. Toxicity effect of Cobalt on total protein and carbohydrate of cyanobacteria *Spirulina platensis*. *IOSR Journal of Environmental Science, Toxicology and Food Technology*, 10(**9**), 114-120. DOI:10.9790/2402-100902114120.

El-Sheekh, M.M., El-Naggar, A.H., Osman, M.E.H. and El-Mazaly, E., 2003. Effect of cobalt on growth, pigments and the photosynthetic electron transport in *Monoraphidium minutum* and *Nitzchia perminuta*. *Brazilian Journal of Plant Physiology*, 15(**3**), 159-166. DOI:10.1590/s1677-04202003000300005.

Ghayal, M.S. and Pandya, M.T., 2013. Microalgae biomass: A renewable source of energy. *Energy Procedia*, 32, 242-250. DOI:10.1016/j.egypro.2013.05.031.

Gholamiourimi, A. and Soltani, S., 2014. Effects of cobalt on biological activities of green algae *Chara Sp*. *International Journal of Current Life Sciences*, 4, 13004-13008.

Guillard, R., Ryther, J., Hustedt, I.C.N. and Cleve, D.C., 1962. Studies of marine planktonic diatoms. I. *Cyclotella nana* Hustedt, and *Detonula confrevacea* (cleve) Gran. *Canadian Journal of Microbiology*, 8, 229-239. DOI:10.1139/m62-029.

Hadizadeh, Z., Shamsaie Mehrgan, M. and Hosseini Shekarabi, S.P., 2019. The potential use of stickwater from a kilka fishmeal plant in *Dunaliella salina* cultivation. *Environmental Science and Pollution Research*, 27, 2144-2154.

DOI:10.1007/s11356-019-06926-w.

Hempel, N., Petrick, I. and Behrendt, F., 2012. Biomass productivity and productivity of fatty acids and amino acids of microalgae strains as key characteristics of suitability for biodiesel production. *Journal of Applied Phycology*, 24(**6**), 1407-1418. DOI:10.1007/s10811-012-9795-3.

Hokin, B., Adams, M., Ashton, J. and Louie, H., 2004. Comparison of the dietary cobalt intake in three different Australian diets. *Asia Pacific Journal of Clinical Nutrition*, 13(**3**), 289-291.

Hosseini Shekarabi, S.P., Shamsaie Mehrgan, M., Razi, N. and Sabzi, S., 2019. Biochemical composition and fatty acid profile of the marine microalga *Isochrysis galbana* dried with different methods. *Journal of Microbiology, Biotechnology and Food Sciences*, 9(**3**), 521-524. DOI:10.15414/jmbfs.2019/20.9.3.521-524.

Jiang, Y., Yoshida, T. and Quigg, A., 2012. Photosynthetic performance, lipid production and biomass composition in response to nitrogen limitation in marine microalgae. *Plant Physiology and Biochemistry*, 54, 70-77. DOI:10.1016/j.plaphy.2012.02.012.

Knothe, G., 2002. Structure indices in FA chemistry. How relevant is the iodine value? *Journal of the American Oil Chemists' Society*, 79(**9**), 847-854. DOI:10.1007/s11746-002-0569-4.

Knothe, G., 2005. Dependence of biodiesel fuel properties on the structure of fatty acid alkyl esters. *Fuel Processing Technology*, 86(**10**), 1059-1070. DOI:10.1016/j.fuproc.2004.11.002.

Küpper, H., Šetlík, I., Spiller, M., Küpper, F.C. and Prášil, O., 2002. Heavy metal-induced inhibition of photosynthesis: Targets of in vivo heavy metal chlorophyll formation. *Journal of Phycology*, 38(**3**), 429-441. DOI:10.1046/j.1529-8817.2002.01148.x.

Kyriakidis, N.B. and Katsiloulis, T., 2000. Calculation of iodine value from measurements of fatty acid methyl esters of some oils: Comparison with the relevant American Oil Chemits Society method. *Journal of the American Oil Chemists' Society*, 77(**12**), 1235-1238. DOI:10.1007/s11746-000-0193-3.

Lapuerta, M., Rodríguez-Fernández, J. and de Mora, E.F., 2009. Correlation for the estimation of the cetane number of biodiesel fuels and implications on the iodine number. *Energy Policy*, 37(**11**), 4337-4344. DOI:10.1016/j.enpol.2009.05.049.

Larsen, J. and Nilsson, J.R., 1983. Effects of nickel on the rates of endocytosis, motility, and proliferation in *Tetrahymena* and determinations on the cell content of the metal. *Protoplasma*, 118(**2**), 140-147. https://doi.org/10.1007/bf01293071.

Layer, G., Jahn, D., Deery, E., Lawrence, A.D. and Warren, M.J., 2010. Biosynthesis of heme and vitamin B12. Comprehensive Natural Products II. Elsevier, Oxford. DOI:10.1016/b978-008045382-8.00144-1.

Liu, Z.Y., Wang, G.C. and Zhou, B.C., 2008. Effect of iron on growth and lipid accumulation in *Chlorella vulgaris*. *Bioresource Technology*, 99(**11**), 4717-4722. DOI:10.1016/j.biortech.2007.09.073.

Lustigman, B., Lee, L.H. and Weiss-Magasic, C., 1995. Effects of cobalt and pH on the growth of *Chlamydomonas reinhardtii*. *Bulletin of Environmental Contamination and Toxicology*, 55(**1**), 65-72. DOI:10.1007/bf00212390.

Mata, T.M., Martins, A.A. and Caetano, N.S., 2010. Microalgae for biodiesel production and other applications: A review. *Renewable and Sustainable Energy Reviews*, 14(**1**), 217-232. DOI:10.1016/j.rser.2009.07.020.

Mehta, S.K. and Gaur, J.P., 2005. Use of algae for removing heavy metal ions from wastewater: Progress and prospects. *Critical Reviews in Biotechnology*, 25(**3**), 113-152. DOI:10.1080/07388550500248571.

Mittelbach, M., 1996. Diesel fuel derived from vegetable oils, VI : Specifications and quality control of biodiesel. *Bioresource Technology*, 56(**1**), 7-11. DOI:10.1016/0960-8524(95)00172-7.

Mohammady, N.G.E. and Fathy, A.A., 2007. Humic acid mitigates viability reduction, lipids and fatty acid of *Dunaliella salina* and *Nannochloropsis salina* growth under nickel stress. *International Journal of Botany*, 3(**1**), 64-70. DOI:10.3923/ijb.2007.64.70.

Olafson, R.W., Abel, K. and Sim, R.G., 1979. Prokaryotic metallothionein: Preliminary characterization of a blue-green alga heavy metal-binding protein. *Biochemical and Biophysical Research Communications*, 89(**1**), 36-43. DOI:10.1016/0006-291x(79)90939-2.

Osman, M.E.H., El-Naggar, A.H., El-Sheekh, M.M. and El-Mazally, E.E., 2004. Differential effects of Co(2+) and Ni(2+ on protein metabolism in *Scenedesmus obliquus* and *Nitzschia perminuta. Environmental Toxicology and Pharmacology*, 16(**3**), 169-178. DOI:10.1016/j.etap.2003.12.004.

Palit, S., Sharma, A. and Talukder, G., 1994. Effects of cobalt on plants. *Botanical Review*, 60, 149-181. DOI:10.1007/bf02856575.

Pinto, E., Carvalho, A.P., Cardozo, K.H.M., Malcata, F.X., dos Anjos, F.M. and Colepicolo, P., 2011. Effects of heavy metals and light levels on the biosynthesis of carotenoids and fatty acids in the macroalgae *Gracilaria tenuistipitata* (var. liui Zhang and Xia). *Brazilian Journal of Pharmacognosy*, 21(**2**), 349-354. DOI:10.1590/s0102-695x2011005000060.

Price, N.M. and Morel, F.M.M., 1990. Cadmium and cobalt substitution for zinc in a marine diatom. *Nature*, 344, 658-660. DOI:10.1038/344658a0.

Ramos, M.J., Fernández-Marchante, C.M., Casas, A., Rodríguez, L. and Pérez, Á., 2009. Influence of fatty acid composition of raw materials on biodiesel properties. *Bioresource Technology*, 100(**1**), 261-268. DOI:10.1016/j.biortech.2008.06.039.

Rastar, M., Hosseini Shekarabi, S.P., Shamsaie Mehrgan, M. and Sabzi, S., 2018. Effects of iron and zinc concentrations on growth performance and biochemical composition of *Haematococcus pluvialis*: a comparison between nanoparticles and their corresponding metals bulks. *Journal of Algal Biomass Utilization*, 9(**2**), 59-67.

Sabzi, S., Shamsaie Mehrgan, M., Rajabi Islami, H. and Hosseini Shekarabi, S.P., 2018. Changes in biochemical composition and fatty acid accumulation of *Nannochloropsis oculata* in response to different Iron concentrations. *Biofuels*, 12(**1**), 1-7. DOI:10.1080/17597269.2018.1489672.

Sharma, K.K., Schuhmann, H. and Schenk, P.M., 2012. High lipid induction in microalgae for biodiesel production. *Energies*, 5(**5**), 1532-1553. DOI:10.3390/en5051532.

Singh, G., Agnihotri, R.K., Reshma, R.S. and Ahmad, M., 2012. Effect of lead and nickel toxicity on chlorophyll and proline content of Urd (*Vigna mungo* L.) seedlings. *International Journal of Plant Physiology and Biochemistry*, 4(**6**), 136-141. DOI:10.5897/ijppb12.005.

Ullah, K., Ahmad, M., Sofia, Sharma, V.K., Lu, P., Harvey, A., Zafar, M.,Sultana, S. and Anyanwu, C.N., 2014. Algal biomass as a global source of transport fuels: Overview and development perspectives. *Progress in Natural Science: Materials International*, 24(**4**), 329-339.
DOI:10.1016/j.pnsc.2014.06.008.

Yao, C.H., Ai, J.N., Cao, X.P. and Xue, S., 2013. Characterization of cell growth and starch production in the marine green microalga *Tetraselmis subcordiformis* under extracellular phosphorus-deprived and sequentially phosphorus-replete conditions. *Applied Microbiology and Biotechnology*, 97(**13**), 6099-6110. DOI:10.1007/s00253-013-4983-x.

Yodsuwan, N., Sawayama, S. and Sirisansaneeyakul, S., 2017. Effect of nitrogen concentration on growth, lipid production and fatty acid profiles of the marine diatom *Phaeodactylum tricornutum*. *Agriculture and Natural Resources*, 51(**3**), 190-197. DOI:10.1016/j.anres.2017.02.004.

Zhou, X.P., Xia, L., Ge, H.M., Zhang, D.L. and Hu, C.X., 2013. Feasibility of biodiesel production by microalgae *Chlorella sp.* (FACHB-1748) under outdoor conditions. *Bioresource Technology*, 138, 131-135. DOI:10.1016/j.biortech.2013.03.169.

Zor, T. and Selinger, Z. 1996. Linearization of the Bradford protein assay increases its sensitivity. *Analytical Biochemistry*, 236(**2**), 302-308. DOI:10.1006/abio.1996.0171.