Interaction of nitrogen and silicate fluctuations with salt stress on growth, and lipid production in *Navicula* sp.

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Abstract:

Diatoms have been recognized as source of important and applicable ingredients such as pigments and essential lipids. In this study, the effects of nitrogen and silicate changes accomplished by salt stress were investigated to analysis the chl *a* and chl c_1+c_2 contents, specific growth rate, total lipid percentage and fatty acid composition of *Navicula* sp. Samples were collected using by plankton net (30-micron pores) in spring 2019, from 6 stations in the Persian Gulf, Iran. In this study, samples were cultured under salt stress (control, 20ppt and 45ppt) and nutrients fluctuation (control: N: 0.5 mg, Si:0.3 mg, high nutrient culture: N:0.9 mg, Si:4.5 mg and without nutrient culture). There was a considerable decrease in dry weight and maximum growth rate under 45ppt salt treatment without silicate. The amount of chl *a* was not significantly changed under various treatments. The highest level of chl c_1+c_2 was related to 20ppt with nitrate (3.05 mg L^{-1}) and the lowest value was 0.38 mg L^{-1} for 20ppt with silicate treatment. The highest level of total lipid content was obtained at 45ppt without silicate. Lipid composition analysis under 45ppt without silicate showed a significant increase (2.07 times compared to control) of palmitoleic acid (C16:1n-7) and trace amounts of polyunsaturated fatty acids were detected. Based on these results, it could be suggested that salt stress without silicate has an important effect to accumulate valuable lipids in *Navicula* sp. so that, they could be useful in medical and bioenergy applications.

Keywords: Marine diatoms, Salt stress, Nutrients fluctuations, Palmitoleic acid, *Navicula* sp.

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Introduction

Diatoms are major group of unicellular and photosynthetic organisms that are found in the ocean (Harwood *et al*., 2017). It has been estimated that approximately 2% of total carbon fixation on the planet produce by diatoms (Ebenezer *et al*., 2012; Tréguer and De La Rocha, 2013). This group of microalgae has crucial role in the accumulation of CO_2 evolving, nitrogen and silicate cycles (Reinfelder, 2011).

Rising global demand for energy, population growth, and industrialization, is largely based on using fossil fuels. Today, fossil fuels are classified as unreliable energy sources due to greenhouse gas emissions and global climate change; so there is an urgent need to find alternative fuels (Wang *et al*., 2017). Diatoms can accumulate lipids (i.e. triacylglycerols, TAGs) under stress conditions. The amount and kind of lipids produced by microalgae species are different (Rosenwasser *et al*., 2014). The process of producing lipids in large volume begins with selecting the proper microalgae species, examining the growth rate, lipid content, lipid compounds and their growth medium. Cultivation, drying, and purification of lipids are included about 50% of the total cost of biofuel production, so their evaluation is essential before starting large-scale production processes (Gordon, 2016).

Diatoms are contained variety of nutrients, so that, they could be important sources for pharmaceutical and medical applications. Many researchers have been focused on finding the best species and culture conditions for increasing the nutritional value of microalgae (Mathieu‑Rivet E, *et al*., 2014).

Stress conditions directly affect the pathways of carbon metabolism which deviated them to commercially valuable products (such as lipids and medical compounds). TAG storage in diatoms occurs when photosynthetic assimilation is reduced because of the restriction of cell division by nutrition and salt stress (Shemi *et al*., 2015).

In this study, we have investigated the effect of nitrogen and silicate deficiencies at the same time with salt stress, on the growth rate, chlorophyll *a*, chlorophyll c_1+c_2 for determination of quality and quantity of lipids in the *Navicula* sp.

Materials and methods

Sampling and culturing method

To find the species, six different stations were considered: Station 1 (S1): Imam Khomeini seaport (49.09* 30.45'0 ″ N), Station 2 (S2): Boushehr seaport (50.8* 28.91'0 "N), Station 3 (S3): Mahshahr seaport (49.13* 30.51'9 ‹N), Station 4 (S4): Kangan seaport (52.05* 27.83'0 ″ N), Station 5 (S5): Deilam seaport (50.14* 30'0 N) and Station 6 (S6): Ganaveh seaport (50.5* 29.55'0 ″ N) (Fig. 1).

Figure 1: The map shows the study area (www.nationsonline.org). Scale: 1:600,000 – 1:2,000,000.

All samples were collected by a 30-micron phytoplankton net with 80 liters water inlet volume. Samples were placed in the plastic containers on ice for transport to the laboratory. Then they were pregrown in f/2-enriched seawater media (Guillard and Ryther, 1962) at a temperature of 24±1°C, salinity of 35 ppt, and light/dark-cycle of 16 h light and 8 h dark at the intensity of 37 µ mol/m^2/s (optimums for *Navicula* sp. growth (Cooms *et al*., 1967)). The samples were observed under a light microscope for isolation of *Navicula* sp.

The formula $SV = S_1V_1 + S_2V_2$ (where; SV= final salinity and volume, S_1V_1= salinity and volume of seawater and S_2V_2= salinity and distilled water volume) was used to make a medium with different salinities. At the first, for nutrient fluctuation, nitrogen and silicate were removed from the compounds of f/2 medium. Then 0.9 g

of $NaNO_3$ and 5.4 g Na_2SiO_3 were added to the f/2 medium separately, to prepare the medium containing the high level of nitrogen and silicate (Table 1). The selected species were cultured under 9 different conditions which is described in Table 1. These cultures were maintained for 12 days (Zienkiewicz *et al*., 2016).

Determination of dry weight
Dry weight of the samples was measured by drying the biomass at 60 °C for 24 hours. Growth rates of diatoms estimated using dry weight during stationary growth phase based on Fogg and Thake (1987) study using the formula.1:
formula.1: $\mu = \ln(N_2/N_1)/(t_2-t_1)$
where N_1=optimal density at time t_1 and N_2=optimal density at time t_2, t_1 and t_2 were corresponding culture days (Fogg and Thake, 1987).

Table 1: Growth conditions (normal and treatments).

	Low level	Normal	High level
Salinity	20 ppt	35ppt	45ppt
Nitrat	0	0/5 g 100ml^{-1}	0/9 g 100ml^{-1}
Silicate	0	0/3 g 100ml^{-1}	4/5 g 100ml^{-1}

Determination of pigment content

The pigment concentration determined using the method described by Jeffrey and Humphrey (1975). Five ml of the cultures was filtered through GF/C (Whatman) mesh. One ml of 90% cold acetone was added into the tube, mixed well with the glass rod then poured into a centrifuge tube. Another 1 ml of the cold acetone was poured into the tube. Also, 2 ml of the cold acetone was added into the tube to make 4 ml extract. The samples were then centrifuged for 10 minutes at 4000 rpm. For the second extraction, about 2 ml of 90% of cold acetone was added into each tube, shaked, and then centrifuged again at 4000 rpm for 10 minutes. The supernatants were measured at 630, 664 and 647 nm using the UV spectrophotometer (UV601 visible spectrophotometer, Shimadzu) to evaluate chl *a* and chl c_1+c_2. As general protection, all the processes were performed under dim light as pigments easily turn pale by the lights (Jeffrey and Humphrey, 1975).

Extraction and profile composition of lipids

Lipid extraction was performed according to Bligh and Dyer's method (1959), with some modification. Briefly, 1 ml of toluene was added to the lipid sample before adding 2 ml of 1.5% sulfuric acid in methanol. After incubation, about 5 mL of 5% NaCl in deionized water was added to the sample to dissolve water-soluble compounds. The organic phase and the two hexane washes were combined in the vial, and then washed with 4 ml of 2% $NaHCO_3$ in diminished water. The sample was then transferred into the smaller tubes for analysis using gas chromatography. Fatty acid components were analyzed with a Varian CP3800 Gas Chromatograph. The fatty acid profile was determined by dividing the area of the fatty acid of interest by sum of all fatty acid areas present in the sample. All analyses were carried out in triplicate. FAMEs were analyzed by gas chromatography equipped with a flame ionization detector (260), the split less injector (175) and a capillary column (100 m, 0.25 mm and 0.2 μm). The carrier gas was high-purity hydrogen at 18 cm s^{-1}. FAME identification was performed by comparison with standard sources. Three replicates of each FAME analysis were considered (Lu *et al.*, 2011).

Analysis of variance was assessed using the Tukey's test, to compare the differences in conducting one-way ANOVA that was statistically different at a level of 5%. Drawing graphs were preliminarily performed by Origin lab software.

Results

After microscopic observations and isolation, *Navicula* sp. was found only in two areas (Emam Khomeini (Station 1) and Boushehr see port (Station 2).

Fig. 2 is related to the samples collected from S1. Dry weight in the early days of cultivation shows the highest value for 45 ppt without nitrogen medium (0.17 mg ml^{-1}), while the lowest value assayed under normal medium (0.12 mg ml^{-1}). During lag growth days the lowest dry weight was related to *Navicula* sp. in 45 ppt without silicate medium (0.07 and 2 0.06 mg

ml^{-1}), and the highest dry weight (0.12 mg ml^{-1}) was related to the *Navicula* sp. in normal f/2 medium and normal with 20 ppt salt stress. The lowest dry weight was belonged to 45 ppt without-Si medium in S2 station, as shown in Fig. 3. The highest dry weight was experienced in *Navicula* sp. growing in 45 ppt-with N (0.13 mg ml^{-1}).

In this study, nitrate deficiency decreased the growth rate during the first days of cultivation, but microalgae growth was not completely stopped following this deficiency.
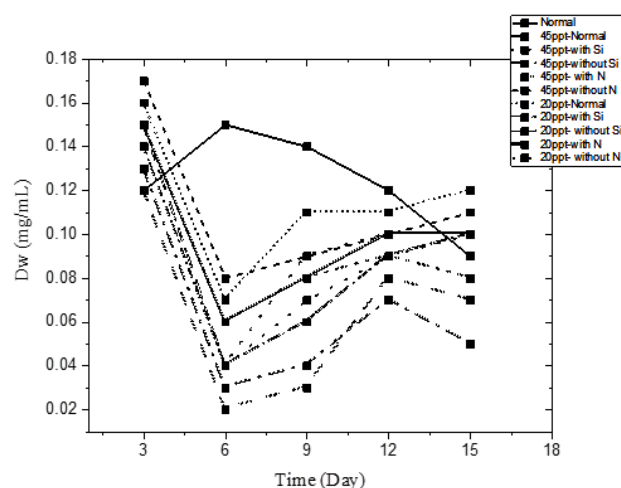

Figure 2: Dry weight trend of *Navicula* sp. collected from S1 in 15 days of preliminary culture.
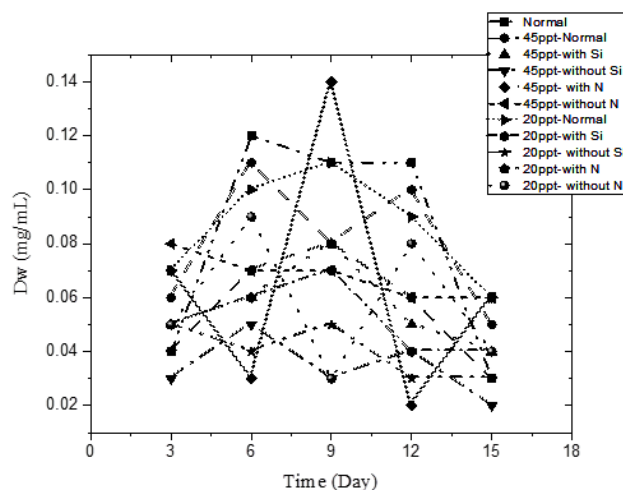

Figure 3: Dry weight trend of *Navicula* sp. collected from S2 in 15 days of preliminary culture.

Specific growth rate

The maximum growth rate was estimated in samples that grown in 45ppt without-Si medium (2.8 and 1.18 d^{-1}, respectively). The lowest specific growth rate in S1 measured for samples grown in normal and 20 ppt-normal mediums (0.84 d^{-1}). In S2 lowest specific growth rate was recorded for microalgae grown in 45 ppt-with N and 20 ppt-normal medium with a 0.45 split in a day (d^{-1}) (Table 2).

Table 2: Average specific growth rates (d^{-1}) of *Navicula* sp. from S1 and S2 in f/2 enriched medium and artificial mediums at stationary growth days. (values in table show significant differences ($p<0.05$)).

	Normal	45ppt Normal	45ppt with Si	45ppt without Si	45ppt with N	45ppt without N	20ppt Normal	20ppt with Si	20ppt without Si	20ppt with N	20ppt without N
Station 1	1.29± 0.11[d]	1.18± 0.05[d]	1.18± 0.07[d]	2.80± 0.12[a]	1.84± 0.23[b]	1.27± 0.11[d]	0.84± 0.02[d]	0.98± 0.18[d]	1.18± 0.11[d]	1.58± 0.09[c]	0.98±0 .1[d]
Station 2	0.84± 0.13[b]	0.53± 0.11[b]	0.65± 0.09[b]	1.18± 0.04[a]	0.45± 0.1[c]	0.84± 0.09[b]	0.45± 0.12[c]	0.65± 0.14[b]	0.49± 0.08[c]	0.84± 0.11[b]	0.65± 0.14[b]

Evaluation of chl a and chl c_1+c_2:

Comparison analysis of variance for chlorophyll *a* and chlorophyll c_1+c_2 showed that the amounts of chlorophyll *a* were usually lower than chlorophyll c_1+c_2. The highest amount of chlorophyll c_1+c_2 was measured in 20 ppt treatment with nitrogen (3.55 L mg^{-1}) and the lowest amount was measured in 20 ppt with silicate (0.38 L mg^{-1}) (Fig. 4).
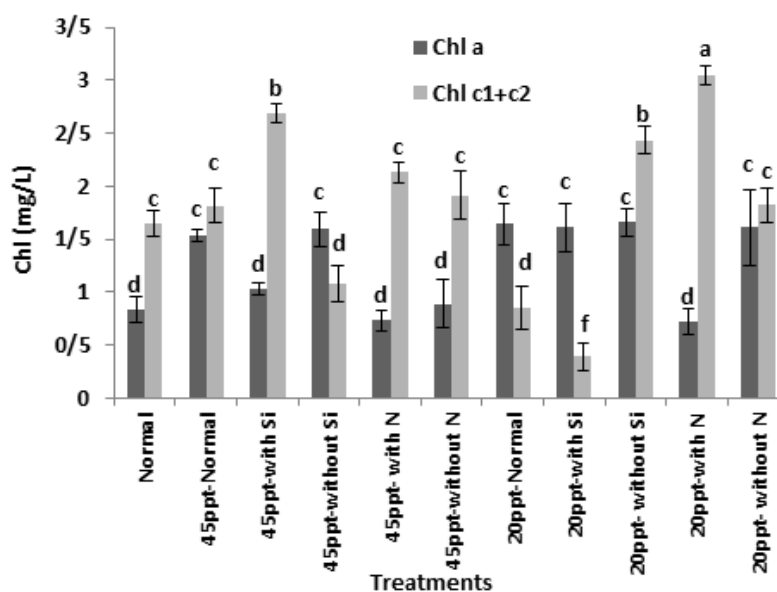

Figure 4: Chl *a* and chl c_1+c_2 content in cells of *Navicula* sp. grown in different artificial mediums collected from S1. (values in table show significant differences ($p<0.05$)).

According to the Figure 5, the amount of chl a for microalgae collected from S2 in different treatments had the highest and lowest values in 45 ppt

without-N medium (2.03 and 0.47 mg ml^{-1}). Also, the mean values of chl c_1+c_2 content in *Navicula* sp. from S2 had a significant increase in 20 ppt-with N medium (3.45 mg ml^{-1}).

Lipid content
The lipid content in microalgae was

measured during stationary phase, and *Navicula* sp. released no or very low amount of lipids into the medium. From day 15, a net increase in 45 ppt without-Si mediums from both stations was observed. The lowest content with no significant difference was measured in another medium (Fig. 6).
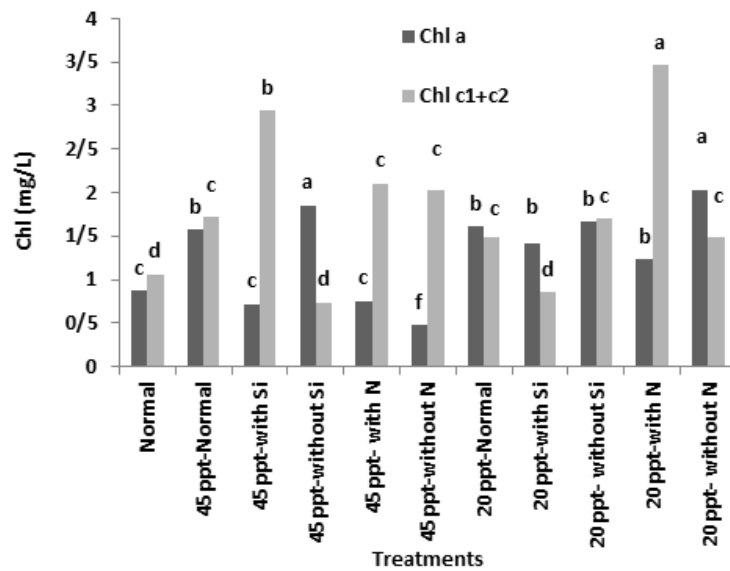

Figure 5: Chl *a* and chl c_1+c_2 content in cells of *Navicula* sp. grown in different artificial mediums collected from S2. (values in table show significant differences (*p*<0.05)).
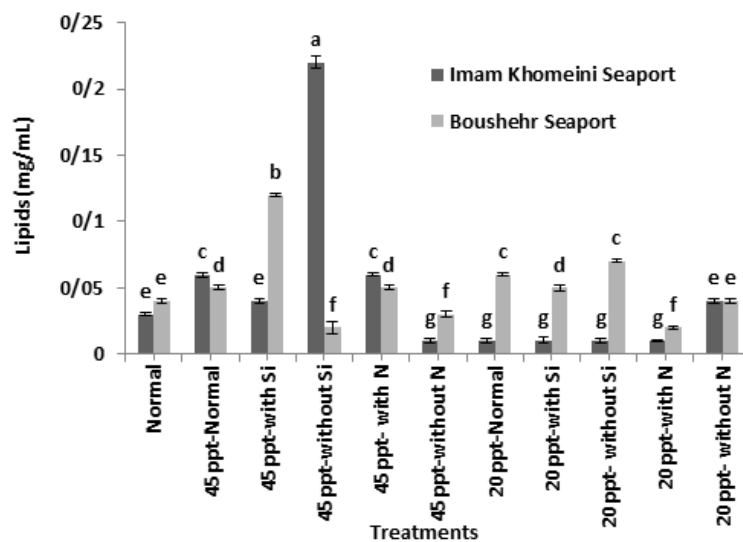

Figure 6: Lipids base on the percentage of dry weight of *Navicula* sp. from Station1 and Station2 (mg ml^{-1}) regardless of growth medium. (Lipid values for S1 and S2 were (*p*>0.5) analyzed separately).

Fatty acid composition

Lipid profile in *Navicula* sp. showed different kinds of saturated, monounsaturated and polyunsaturated fatty acids.

As shown in Table 3, the highest amount of fatty acids in *Navicula* sp. cultured in the normal medium was measured in palmetholic acid (7-n1:16) as a monounsaturated fatty acid (60.67%). Palmitic acid (16:00) and echosapentaenoic acid (3-n5: 20) had higher values (11.45 and 7.38%, respectively) in the fatty acid profile. These microalgae showed a trace amount of polyunsaturated fatty acids (Table 3).

Table 3: Fatty acids composition of late-stationary-phase in 45 ppt without-Si medium of *Navicula* sp.

Saturated	Myristic acid 14:00	Pentade cylic acid 15:00	Palmitic acid 16:00	Heptadeca noic acid 17:00	Stearic acid 18:00	Arachidic acid 20:00	Behenic acid 22:00	Lignoceric acid 24:00:00	
	2.11 ± 0.12	2.45± 0.12	13.59 ± 0.83	0.52± 0.06	0.34 ± 0.05	0.78± 0.03	0.06 ± 0.06	0.05 ± 0.009	
Monounsa turated	Cis^{-1}0-Pentadeca noic acid 15:01	Palmitol eic acid 16:1n-7	Cis^{-1}0-Heptadeca noic acid 17:1n-7	Oleic acid 18:1n-9c	Erucic Acid 22:1n-9	Nervonic acid 24:1n-9			
	0.43 ± 0.05	60.67 ± 1.7	2.67 ± 0.2	3.67 ± 0.34	0.032 ± 0.005	0.334 ± 0.01			
Polyunsat urated	Linoleic acid 18:2n-6c	Linolen ic acid 18:3n-6	Cis^{-1}1,14-Ecosadie noic acid 20:2n-6	Dihomo-gamma-linolenic *acid* 20:3n-6	Eicosatri enoic acid 20:3n-3	Docosadi enoic acid 22:2n-6	Eicosapent aenoic acid 20:5n-3	Docosap entaenoi c acid 22:5n-3	Docosa hexaen oic acid 22:6n-3
	3.03 ± 0.23	0.21 ± 0.002	0.15± 0.043	0.025 ± 0.001	1.12± 0.007	1.34± 0.05	5.2 ± 0.23	0.45 ± 0.67	0.78 ± 0.21

According to Table 4, the fatty acids profile in different treatments was consisted of 19.9% saturated fatty acid, 67.93% monounsaturated fatty acid and 12.30% polyunsaturated fatty acid. Comparison of fatty acids profile in the control and treated samples is presented in Fig. 7. Estimation of the ratio of the samples exposed to 45 ppt without silicate treatment showed a 2.07 times increase compared to the control for predominant fatty acid C16:1n-7 (Table 5).

Table 4: Percentage amounts of lipids from 45 ppt without-Si medium (% of total fatty acids).

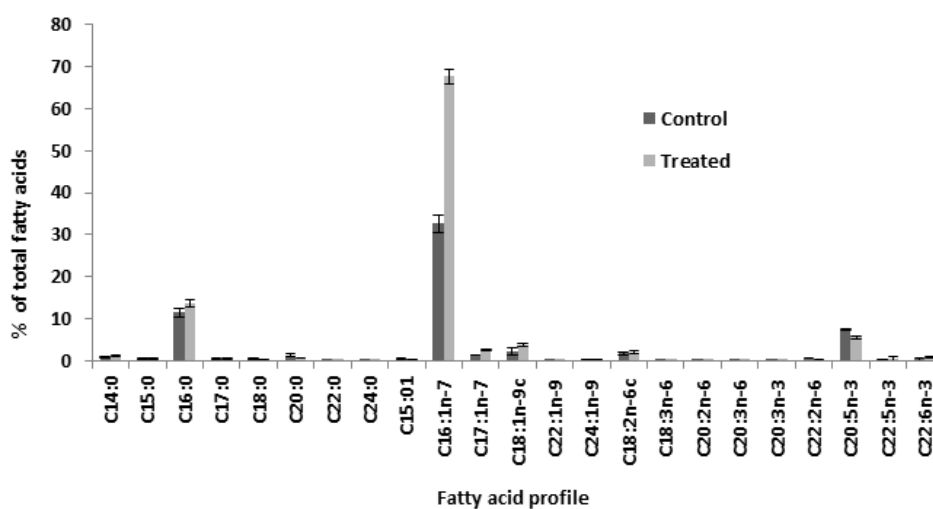| % Composition	Saturated	Monounsaturated	Polyunsaturated
Normal f/2 medium	45.3%	43.5%	11.2%
45 ppt without Si medium	19.9%	67.80%	12.30%

Figure 7: Fatty acid comparison of *Navicula* sp. under normal and 45 ppt without Si treatment.

Table 5: The ratio of the sample under treatment compared to the control.

Saturated					
C16:0	C17:0	C18:0	C20:0	C22:0	C24:0
1.18±0.69[a]	1.20±0.08[a]	0.74±0.61[b]	0.58±0.08[b]	1.28±0.73[a]	1.53±0.79[a]

Monounsaturated					
C15:01	C16:1n-7	C17:1n-7	C18:1n-9c	C22:1n-9	C24:1n-9
0.95±0.43[c]	2.07±0.83[a]	1.84±2.06[b]	1.56±0.38[b]	0.47±0.28[c]	1.25±0.191[b]

Polyunsaturated								
C18:2n-6c	C18:3n-6	C20:2n-6	C20:3n-6	C20:3n-3	C22:2n-6	C20:5n-3	C22:5n-3	C22:6n-3
1.14±0.64[a]	1.47±0.18[a]	0.64±0.2[b]	1.77±0.38[a]	1.37±1.9[a]	0.52±1.7[b]	0.75±1.86[b]	1.95±0.58[a]	2.19±0.85[a]

Discussion

The most suitable stations for microalgae collection would be tropical climates, where the temperature is almost above 15°C (Lundquist *et al.*, 2007). The estimates of biomass and lipid production are largely dependent on the conditions and potential sites.

Like advanced plants, light is an energy source for microalgae. In this study, the light intensity at 37 μmoles m^{-2} s^{-1} adjusted to promote photosynthesis rates. According to Takeshita *et al.* (2014), aeration in the culture medium has a significant performance on the microalgae growth

rate and production, so aeration is supplied twice in a day to the liquid mediums.

Occurring pH increases during the day as HCO_3^-/CO_2 uptake and consumption cause an alkalization in the mediums, while the pH reduces during the night due to CO_2 production via respiration (Asma *et al.*, 2015). Therefore, to keep up the growth phase, adding CO_2 to mediums performed for stabilizing the buffer system (pH= 8) (Rai *et al.*, 2015).

The growth rate and lipid content are the only two important characteristics of biodiesel production that have been

estimated for a variety of microalgae species (Gammanpila *et al*., 2015). As shown in Figure 2, microalgae cultivated in the normal medium began to grow exponentially from about the third day and they reached the maximum growth on the sixth day. A comparison of dry weight and growth rate indicated that variation in salt caused the delayed onset of growth. The inhibitory effect of salt on microalgae growth could be attributed to an imbalance ion homeostasis, osmotic change, and accumulation of reactive oxygen species (ROS), which finally caused programmed cell death. The decrease in microalgae growth with variety levels of salt and nutrition often has reported in some literature (Ravelonandro *et al*., 2011). It could be because of the effect of salt and nutrient stresses on light utilization and metabolism (particularly carbohydrates involved in osmoregulation) (Roleda *et al*., 2013, Yang *et al*., 2018). In salt stress, higher levels of silicate and nitrogen were increased dry weight, and we could suppose that the amount of nitrogen and silicate required by *Navicula* sp. in salt stress is greater than the normal f/2 medium. These results were consistent with the previous study on marine diatoms (Wu *et al*., 2015).

The type and amount of biodiesel produced by an algae species, e.g. chain length, degree of saturation and triglycerides in total lipids are important for biodiesel production (Griffiths and Harrison 2009, Dickinson, 2017).

Pigments values are indicated the activity of the photosynthetic apparatus as well as physiological state and growth, under a variety of conditions (Suggett *et al*., 2011). Diatoms have two types of pigments: chls and carotenoids. Chls trap light energy which is used in photosynthesis. Chls are a magnesium coordination complex. Jeffrey and Vesk (1975) published data for 47 of the most important chls and carotenoids found in marine algae (Lepetit *et al*., 2010). Only two forms of chls are found in diatoms: chl *a* and *c*. Chl *a* plays a central role in the photochemical energy conversion of the photosynthesizing organisms, while chl *c* plays a role as an accessory pigment in photosynthesis like the functional activity of chl *b* of advanced plants. The most abundant of chl *c* in diatoms is chl c_1 and chl c_2 (Peloquin *et al*., 2013). The special structure of chl *c* causes changes in the absorption spectrum at~ 630 nm (blue region) and~ 580 mm (red region) (Zapata *et al.*, 2006). In the aquatic environment, pigment response to biotic and abiotic stresses. It is suggested that evaluation of pigment values in marine microalgae being one of the important indexes to survey stresses in the environment (Rontani *et al*., 1995, Zigmantas *et al*., 2004). Changes in culture conditions like salt and nutrient oscillations modify pigment amounts in the algae (Ahmad *et al*., 2010). Chl is a compound rich in nitrogen and accessible, so it is used as an intracellular nitrogen pool to support further cell growth and biomass

production when the nitrogen in the medium decreased (Li *et al*., 2008). As nitrogen was added to the nitrogen-free medium of *Crematogaster minutissima*, an increase in chls levels was reported (Ördög *et al*., 2012). Restore of chls would cause for the faster growth rates evaluated in the higher nitrogen treatments. However, as growth, deprived nitrogen mediums, chl would degrade to reuse the nitrogen for growth with decreasing chl levels. In this study, significant increase in chl c_1+c_2 rather than the chl *a* was observed after salt treatments in cultures with nitrogen and silicate treatments.

Silicate metabolism in diatoms is extensively linked to the regulation of cell growth and division. Also, because the regenerating of Si is a slow process in comparison with the rapid cycling of N, growth rate and chls amounts of *Navicula* sp. would be late in the effect of silicate deficiency (Pandit *et al*., 2017).

Diatoms in response to salt stress can change their lipid and fatty acid compositions to modify their membrane permeability. For example, examination of various salt concentrations (from 10 to 30mg L^{-1}) in *Nitzschia loevis* have indicated that the highest amounts of eicosapentaenoic acid was obtained with a salt concentration of 20 mg L^{-1} that was more than 70% of the total fatty acids in these polar fractions (Chen *et al*., 2012). These changes in the unsaturated membrane lipids seemed to be related to changes in membrane permeability and fluidity

under salt concentrations as acclimation to salt stress. Another study demonstrated the important role of nitrogen levels in lipid biosynthesis (Shakouri and Balouch, 2016). In nitrogen, deficiency indicated protein biosynthesis reduction and cell division suppression, therefore reduced microalgae growth and shunted about 40% more carbon towards TAG accumulation (Kalita *et al*., 2017).

In the present study the level of lipid production showed no direct relation with nitrogen deficiency in the cultures from station 1, therefore samples from Station 2 indicated increasing lipid production in nitrogen depletes cultures. In this context, Breuer *et al.* (2013) reported that under nitrogen stress the *Scenedesmus obliquus* and *Navicula saprophila* increased their total lipid content. Also in our study for all samples, combining salt and silicate stress has a significant effect on total lipids.

Study of fatty acid profiles in diatoms showed that there were medium-chain fatty acids as well as very long-chain polyunsaturated fatty acids (VLC-PUfAs), which are rare in both chlorophytes and flowering plants (Merz and Main, 2014). The main fatty acids in diatoms are 14:0, 16:0, 16:1 and 20:5 (Wacker *et al*., 2015) concerning biodiesel production; medium-chain fatty acids are preferred because they result in less viscous biodiesel (Merz and Main, 2014). In most studies, it is reported that diatoms have high contents of arachidonic acid

(ARA 20:4n6) and eicosapentaenoic acid (EPA, 20:5n-3) (Yi *et al.*, 2017).

In our study, lipid composition analysis in 45 ppt without silicate culture showed a significant increase (2.07 times) compared to the control culture in palmitoleic acid (C16:1n-7). Palmitoleic acid is essential ω7 fatty acid that is applicable in the pharma and food industries. Based on the other studies, depending on the growth conditions, medium stresses, culture age, etc. the amount of ω7 fatty acid could be modified. These modifications are different from one species to another one (Yi *et al.*, 2017).

Earlier researches indicated that diatoms in the mediums with silicate deficiency have higher proportions of saturated and monounsaturated fatty acids (Demimbras and Demimbras, 2010). In our study, *Navicula* sp. cultured in 45 ppt without-Si medium had more saturated and monounsaturated fatty acid than polyunsaturated fatty acid. Moreover, according to biofuel standards, the concentration of linolenic acid (polyunsaturated fatty acid) should not exceed the limit of 12%. In this research, *Navicula* sp. cultured in salt and silicate stresses indicated only 0.2% of linolenic acid and a low amount of other polyunsaturated fatty acids that may lead to oxidation. As a result, mediums with salt and silicate stress would be good conditions for lipid production in *Navicula* sp. Wacker *et al.*, (2015) recognized that the main fatty acids in diatoms are palmitoleic

acid, palmitic acid, EPA and oleic acid. Palmitoleic acid is effective against different life-threatening disorders, such as cancers and metabolic syndrome.

Diatoms are unicellular algae responsible for approximately 40% of aquatic carbon fixation. The use of microalgae as microorganisms capable of converting energy from light and carbon dioxide into the valuable and sustainable products has become a new and growing area of research in recent years. In this study, significant differences in specific growth rates were found in *Navicula* sp. during exposure to different salt stress and nutrients fluctuations. Significant increase in chlorophyll *a*, c_1, and c_2 was observed in 20 ppt salt stress and increase in nitrogen for *Navicula* sp. cultures. These results suggest the specific intracellular acclimation processes which give possible explanations for the species involved in osmotic regulation, to withstand ionic and osmotic stresses towards environmental short-time variations. These factors can also cause bioenergetics and biochemical changes in the pigments of photosynthetic organisms. Lipid composition analysis in culture with salt and silicate stress indicated a significant increase (2.07 times) in palmitoleic acid (C16:1n-7). Based on these results, it could be suggested that salt stress without silicate has an important effect to accumulate valuable lipids in diatoms. Also, the relatively low levels of linolenic acid in the adjusted stress

treatments for cultures enhance prospects for future use of *Navicula* sp. in the biofuel production.

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