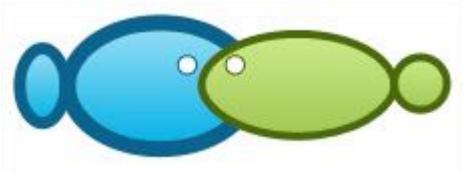

Bioaccumulation of Fe^{+2} and its effects on growth and pigment content of spirulina (*Arthrospira platensis)*

[1]Maryam Akbarnezhad, [1]Mehdi Shamsaie Mehrgan, [1]Abolghasem Kamali, [2]Mehran Javaheri Baboli

[1] Department of Fisheries, Science and Research Branch, Islamic Azad University, Tehran, Iran; [2] Department of Fisheries Science, Ahvaz Branch, Islamic Azad University, Ahvaz, Iran. Corresponding author: M. Shamsaie Mehrgan, drshamsaie@gmail.com

Abstract. A laboratory experiment was conducted to assess the bioaccumulation of Fe^{2+} and its effects on growth and pigment contents of spirulina (*Arthrospira platensis*). The specimens were cultured in Zarrouk medium. The mentioned metal concentration enhanced separately to 10 and 20 fold of the Zarrouk (control) content. Although the values of dry weight in iron treatment were higher than values in control treatment however, the results indicated no significant differences in the dry weights for the different mediums. A gradual increase in the optical density (OD) was seen at all concentration of treatments but with different rates of increase however, there were no significant difference in values of OD for the different mediums. The maximum bioaccumulation value was observed 4465±39.68 mg kg^{-1} in Fe - 10 fold concentration and the extent of iron accumulation and enrichment factor (EF) of spirulina was significantly increased when applying sample with 10 fold fortified iron concentration. Also, the results indicated no significant differences in the Chl-a content after 14 day of inoculation. The maximum production of Chl-a occurred in 7^{th} days of incubation at all concentration of treatments. The maximum Chl-a values being 1.34±0.784 mgL^{-1} in experiment with 20 fold iron fortified concentration, after 14 day of inoculation. Also, the results indicated no significant differences in the carotenoid contents after 14 day of inoculation. It was observed that an increase of Fe concentration caused reduction of phycobiliproteins of *A. platensis*. Consequently, it can be suggested for involvement in further functional food developments.

Key Words: metal concentration, Fe^{+2}, uptake, pigments, growth.

Introduction. Spirulina, *Arthrospira platensis* is a photosynthetic filamentous, helical shaped, multicellular and green-blue microalga (Sanchez et al 2003) that grows vigorously in strong sunshine under high temperatures and highly alkaline conditions (Habib et al 2008). The two most important species of which are *Arthrospira maxima* and *A. platensis* (Sanchez et al 2003). It has gained considerable popularity in the human health food industry as an excellent food, lacking toxicity and having corrective properties against viral attacks, anemia, tumor growth and malnutrition and as an animal food supplement (terrestrial, fresh water and marine) (Belay et al 1996). In aquaculture, spirulina is used as a feed additive to improve growth, feed efficiency, carcass quality, and physiological response to disease in several species of fish (Mustafa et al 1994). Also, it has been used as bio-fertilizers (Vaishampayan et al 2001), as a natural color in food industry (Goh et al 2009), in cosmetic products (Henrikson 1994) and has a wide application in biotechnology (Eriksen 2008) etc because of its valuable constituents such as high content of protein (up to 70%), along with high amounts of essential fatty acids, essential amino acids, minerals, vitamins, antioxidant pigments (phycobiliproteins, carotenoids), chlorophyll and polysaccharides (Vonshak 1997; Belay et al 1993).

Also, *A. platensis* as cyanobacteria is excellent model for bioaccumulation studies (Perales-Vela et al 2006). The majority of nutrition supplements comprise trace elements as inorganic salts but the bioavailability of inorganic forms is not so pronounced. The bioavailability of organic form is larger and more effective than that of inorganic forms (Worms et al 2006; House 1999). Iron in some nutritional complements is not

appropriately absorbed. Iron in spirulina is 60% better absorbed than ferrous sulfate and other complements (Henrikson 1994). Studies on dietary application of *A. platensis* (Varga et al 1999) related the bioaccumulation of iodine, zinc and selenium. It was observed that the uptaken metals are in organic form in the spirulina.

The growth of spirulina and the composition of the biomass produced depend on many factors, the most important of which are nutrient availability, temperature and light (Cornet et al 1992). Trace metals are metals present in algal cells in extremely small quantities (<4 ppm) but that are an essential component of phycophysiology. Iron (Fe), manganese (Mn), cobalt (Co), zinc (Zn), copper (Cu) and nickel (Ni) are the six most important trace metals required by algae for various metabolic functions (Bruland et al 1991). Iron is an important trace metal for normal growth and functioning of photosynthesis and respiration in algae (Terry & Abadia 1986).

A number of studies have been performed to study the effects of metals on growth and pigment contents of cyanobacteria for example Molnár et al (2013) investigated bioaccumulation of 4 important microelements Fe (III), Cu (II), Zn (II) and Mo (VI) by *A. platensis* and *Chlorella vulgaris* and proved to enhanced bioaccumulation ability and biomass in cases of above mentioned alga species. Some experiments demonstrated that Fe limitation also reduces cellular chlorophyll concentration and carotenoid composition (Kudo et al 2000; Van Leeuwe & Stefels 1998; Kobayashi et al 1993; Greene et al 1992). In another study, Kudo et al (2000) observed the effect of Fe stress on growth rate and cellular composition of the marine diatom *Phaeodactylum tricornutum*, over the temperature range of 5–30°C. The optimum temperature for growth of *P. tricornutum* was 20°C. The growth rate of Fe-stressed cells was 50% of Fe-replete cell growth rate at the optimum growth temperature. Also, Rueter & Petersen (1987) reported that Fe promotes the growth of cyanobacteria in natural waters.

Most of the previous studies were focused on the biosorption of heavy metals by algae species in order to eliminate toxic elements from the environment (Romera et al 2007) or reveal amino acid, fatty acid and protein profiles of various alga species although little information is available to support enrichment of natural biomass of edible microalgae with microelement metal ions with the prospects of functional food development. Our objective in this study was to examinate accumulation of iron microelements by *A. platensis* and its effects on growth and biopigment accumulation.

Material and Method

Microorganism and applied chemicals. *A. platensis* used in this study was obtained from Islamic Azad University, Ahvaz, Iran. All the applied reagents and chemicals (analytical purity) was obtained from either Merk or Sigma-Aldrich.

Culture medium. *A. platensis* is cultured in modified Zarrouk medium (Venkataraman 1997) supplemented with 10 fold and 20 fold concentrations of iron ion separately. These ion solutions were prepared from iron sulfate. The organism grown in Zarrouk medium alone was served as control.

Culture conditions and growth. The alga growing apparatus consists of a horizontal glass surface where on the Erlenmeyer flasks were placed. Erlenmeyer flasks of 1000 mL capacity were prepared containing 100 mL *A. platensis* (10%) with initial optical density (OD) 0.019 (biomass concentration of 0.002 g/L dry weight) and 200 mL Zarrouk medium (Zarrouk 1966) at temperature 32°C, pH 8.7, salinity 20 ppt with an illumination of 2500 lux light intensity, with a light/dark cycle of 12/12 h, fresh air was pumped into the solution through plastic tubes in order to avoid the generation of alga film layer on the wall of the flasks. The culture was grown for a period of 14 days.

Growth of microalgae. Biomass concentration was determined every day by measuring the OD at λ 560 nm to produce a standard curve. This standard curve was subsequently used to calculate the biomass of individual samples based on their OD (Gupta et al 2006).

Determination of dry weight. The dry weight of biomass was determined by filtration of sample (15 mL) through dried pre-weighed whatman filter (pore size 0.42 µm) carefully up to 0.0001 g level. The biomass obtained was washed twice with distilled water, dried at 80°C for 4h, cooled in desiccator and the resulting dry weight was determined (Olguin et al 2001).

Bioaccumulation analysis. To estimate of iron metals in the samples of algae, the samples were separated from the solution by centrifugation MICRO 22R model manufactured by Hettich of Germany. One gram of the wet sample more accurately weighed with scale Sartryvs 124S model and was transferred to crucible and then in electric furnaces BATEC PC 21 model ashing process was performed in crucible at 550°C. After cooling, their contents were dissolved in 3 mL 1:1 nitric acid solution and then volumed with 10 mL of distilled water in 10 mL volumetric flask. Decomposed standard solution of iron was prepared from standard stock solutions (1000 mg/L) in the concentration range 0.1 to 10 mg of Fe/L the number three solution with 3 mL solution of 1:1 nitric acid solution and twice distilled water and then samples and standards were analyzed by flame atomic absorption spectrometer PG-990 model manufactured by PG Instrument England. The obtained results were calculated from the values of three parallel measurements and were expressed in mg kg^{-1} dried alga.

Enrichment factor (EF) calculation. Data for the calculation EF gained from the results of measured metal content of 9 samples prepared as follows. Three samples were prepared in triplicates with two different compositions of the metals (with a control sample).
EF was calculated according to the following ratios:

$E = C_E/C_C$
C_E = Microelement concentration of dry alga grown in the media with enhanced metal content
C_C = Microelement concentration of dry alga grown in the control media

Biopigments estimation
Chlorophyll estimation. Chlorophyll content was determined by centrifugation for 10 minute at 4000 rpm. Chlorophyll a (Ch-a) was extracted by using 5 mL 90% acetone. Place the tube in dark for 24 hour. After extraction period centrifuge the sample for 15 minute at 5000 rpm and collect the supernatant. Read the absorbance at 630nm (A630), 645nm (A645), and 665nm (A665) against 90% acetone as blank by using UV/VIS spectrophotometer and concentration of Chl-a was calculated using the formula of Parson & Strickland (1965):

$C = 11.6\ A665 - 1.31\ A645 - 0.14\ A630$
The concentration of Chl-a in a given volume of culture can be determined by formula:

$$\text{Chl-a (mg/L)} = \frac{C \times V_e}{V_c}$$

C = Value obtained from above equation
Ve = Volume of extract (mL)
Vc = Volume of culture (litres)

Phycobiliproteins estimation. 5 mL of cyanobacterial cell suspension was taken and subjected to centrifugation for 10 minute at 4000 rpm then wash the pellet with distilled water. Phycobiliproteins were extracted in 5 mL of phosphate buffer pH 6.7, 0.05M by three times repeated freezing and thawing. Freeze thawed samples subjected to Centrifugation at 1000 rpm for 15 minutes. The final extract was measured at 56 nm (A562), 615 nm (A615), and 652 nm (A652) against phosphate buffer as blank.

Concentration of phycocyanin (PC), allophycocyanin (APC), and phycoerythrin (PE) were calculated by using the formula (Bennet & Bogorad 1971):

$$PC = \frac{A615-0.474(A652)}{5.34}$$

$$APC = \frac{A652-0.208(A615)}{5.09}$$

$$PE = \frac{A562-2.41(PC) - 0.849(APC)}{9.62}$$

The concentration of phycobiliprotein in a total volume of culture can be determined as follows:

$$\text{Phycobiliprotein (mg/mL)} = \frac{C \times V_e}{V_c}$$

C = Value of PC, APC and PE obtained from above equations
Ve = Volume of extract (mL)
Vc = Volume of culture (mL)

Carotenoids estimation. 5 mL of cyanobacterial suspension was centrifuged at 4000 rpm for 10 minute. Wash the pellet 2-3 times with distilled water to remove traces of adhering salts. Harvested biomass was homogenized in homogenizer with 5 mL, 90% acetone and centrifuged the sample for 15 min at 5000 rpm. The carotenoids in samples were determined spectrophotometrically at 450 nm is using the fallowing calculation (Jensen 1978):

$$C = \frac{A450 \times V \times f \times 10}{2500}$$

C = Total amount of cart (mg/mL)
V = Volume of extract (mL)
f = Dilution factor

Statistical analysis. Data were analyzed statistically using one way analysis of variance (ANOVA) using SPSS version 18. Duncan multiple range test was used to compare differences among treatment means at ($P \leq 0.05$) level (Duncan 1955).

Results and Discussion

Growth analysis. The effect of different iron concentrations in medium on the growth of *A. platensis* was evaluated daily during 14 days of cultivation and after the incubation period the dry weights of its (gL^{-1}) was determined (Figure 1 and Table 1).

Although the values of dry weight in iron treatment were higher than the control treatment however, the results indicated no significant differences in the dry weights for the different mediums (Table 1). The results are in agreement with views of Molnár et al (2013) who reported that biomass growth of spirulina is not so adversely affected by the increasing iron concentration of the medium, even though slight biomass enhancement is observed in the previous cases. Rueter & Petersen (198) ans Terry & Abadia (1986) reported that iron promotes the growth of cyanobacteria in natural waters. Its additions increase photosynthesis and nitrogen fixation by cyanobacteria. The higher biomass production recorded is most likely due to the high alkalinity, pH 8.7, of such mediums (Pandey et al 2010) or it is most likely due to nutrition. The nutrition condition is one of the key factors that control their growth and productivity (Faintuch et al 1991; Vonshak & Richmond 1988).

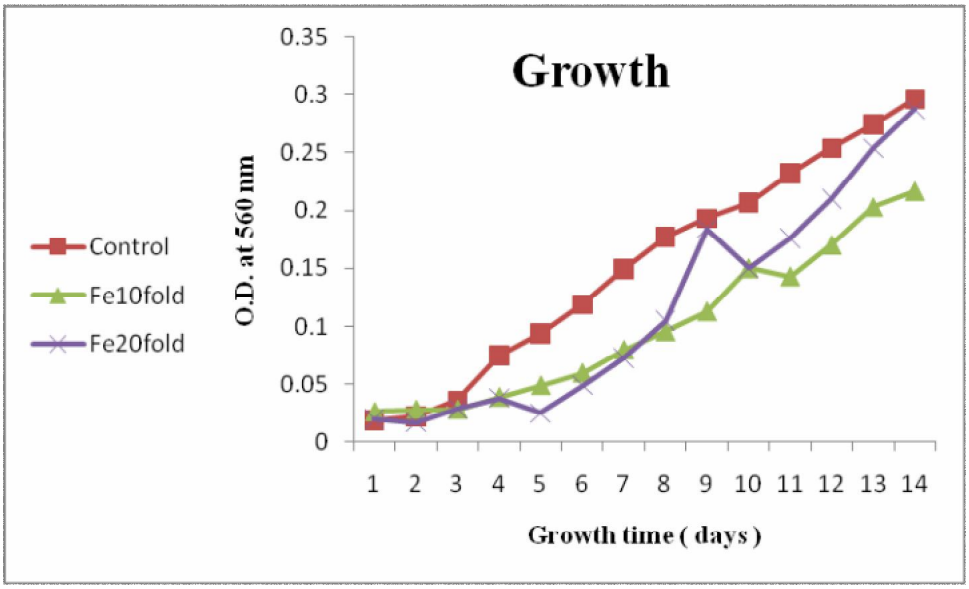

Figure 1. Growth curves of *Arthrospira platensis* cultured in Zarrouk medium (control) and growth mediums with Fe-fortified.

Table 1

Dry weight and optical density of *Arthrospira platensis* cultivated in Zarrouk medium (control) and growth mediums with Fe-fortified

Parameter	*Day*	*Control*	*Fe 10 fold*	*Fe 20 fold*
Dry weight (gL^{-1})	14	1.55 ± 0.02^{a}	1.57 ± 0.03^{a}	1.58 ± 0.05^{a}
OD	1	0.196 ± 0.009^{a}	0.026 ± 0.005^{a}	0.020 ± 0.01^{a}
OD	2	0.022 ± 0.005^{ab}	0.028 ± 0.007^{b}	0.173 ± 0.001^{a}
OD	3	0.036 ± 0.011^{a}	0.028 ± 0.017^{a}	0.030 ± 0.017^{a}
OD	4	0.075 ± 0.010^{b}	0.039 ± 0.014^{a}	0.021 ± 0.012^{a}
OD	5	0.094 ± 0.011^{b}	0.049 ± 0.013^{a}	0.025 ± 0.019^{a}
OD	7	0.149 ± 0.037^{a}	0.080 ± 0.019^{a}	0.073 ± 0.059^{a}
OD	8	0.177 ± 0.044^{a}	0.096 ± 0.026^{a}	0.105 ± 0.090^{a}
OD	9	0.193 ± 0.054^{a}	0.113 ± 0.045^{a}	0.184 ± 0.067^{a}
OD	10	0.207 ± 0.052^{a}	0.150 ± 0.043^{a}	0.150 ± 0.125^{a}
OD	11	0.232 ± 0.067^{a}	0.143 ± 0.083^{a}	0.176 ± 0.130^{a}
OD	13	0.274 ± 0.106^{a}	0.203 ± 0.116^{a}	0.254 ± 0.176^{a}
OD	14	0.296 ± 0.115^{a}	0.217 ± 0.124^{a}	0.287 ± 0.161^{a}

OD – optical density, ($P\geq0.05$).

Similar trend of these results was obtained in the OD. The growth curves lacked a lag phase for the Zarrouk medium and the growth mediums with Fe-fortified. A gradual increase in the OD was seen at all concentration of treatments but with different rates of increase however, there were no significant difference in values of OD for the different mediums (Figure 1). As agreed by these results, also, Xing et al (2007) reported that under the iron-replete condition, the OD values of *Microcystis aeruginosa* and *Microcystis wesenbergii* increased during culture period. Some experiments demonstrated that iron limitation might affect phytoplankton in two independent ways: reduced rate processes (photosynthesis) and/or biomass yield (Davey & Geider 2001; Wilhelm & Trick 1994; Wells et al 1994).

Bioaccumulation analysis. The extent of bioaccumulation and EF of Fe by *A. platensis* grown in media with diverse Fe content were measured (Table 2).

Table 2
Bioaccumulation and enrichment factor of Fe by *A. platensis* grown in media with diverse Fe content after two-week-long incubation

Treatment	*Bioaccumulation(mg kg^{-1})*	*EF*
Control	668±25.53^{a}	-
Fe 10 fold	4465±39.68^{c}	6.68
Fe 20 fold	1717±20.66^{b}	2.53

Also, the results indicated significant differences in the bioaccumulation of iron by *A. platensis* for the different mediums ($p \leq 0.05$). The maximum bioaccumulation value was observed 4465±39.68 mg kg^{-1} in Fe-10 fold concentration and the extent of Fe accumulation and EF of spirulina was significantly increased when applying sample with 10 fold fortified Fe concentration. In case of higher concentration of iron (Fe 20 fold) less amounts of Fe accumulation was occurred (Table 2) which is in agreement with findings of Molnár et al (2013). Also, this conclusion is in agreement with Mane & Bhosle (2012) who indicates that both algae are able to remove the metals from aqueous solution at lower concentrations. At higher concentration there might be toxicity of metals which can reduce the biosorption capacity of the microorganisms. It has been described that microalgae can protect themselves against the toxicity caused by heavy metals using several mechanisms such as: exclusion mechanisms, adsorption to cell surface or intracellular accumulation.

Each of the metals showed different affinity toward algae. This could be due to the difference in cell wall composition and the intra group differences which cause significant differences in the type and amount of metal ion binding to them. The cell wall consists of variety of polysaccharides and proteins which offers a number of active sites capable of binding metal ions (Mane & Bhosle 2012).

Literature reports that the accumulation of metal ions depends on external concentration of metal ions in the solution until their concentration leads to toxic effects and which leads to decreased performance of bioaccumulation (Yan & Pan 2002).

***Pigment content*:**

Chlorophyll-a and carotenoids. Figure 2 shows the concentration of chlorophyll accumulation in *A. platensis* grown in different media.

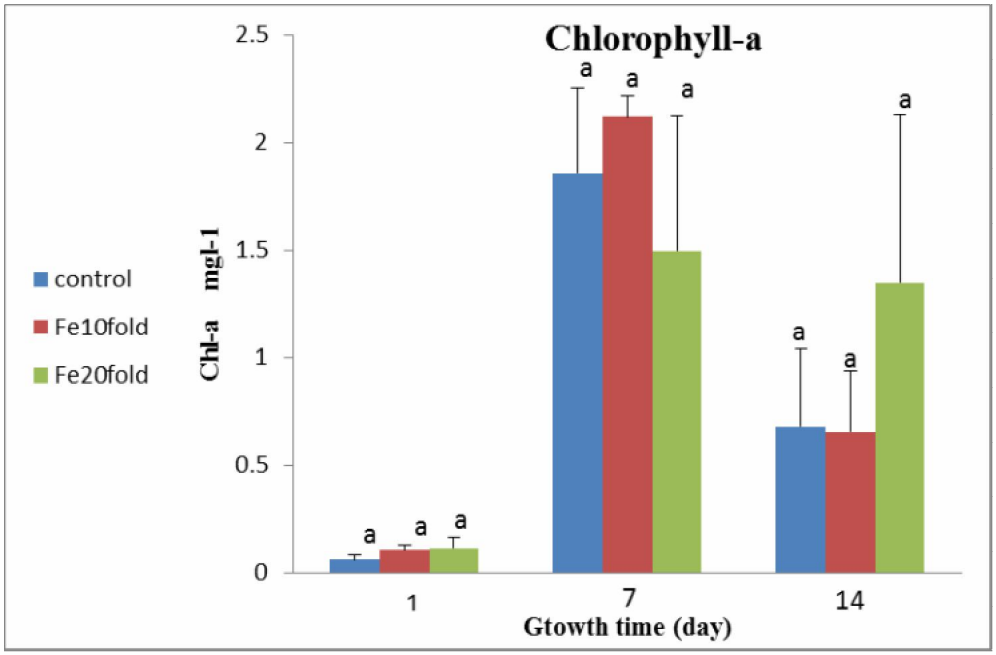

Figure 2. Changes in the chlorophyll-a content of *Arthrospira platensis* on Zarrouk medium (control) and growth mediums with Fe-fortified ($p \geq 0.05$).

Also, the results indicated no significant differences after 14 day of inoculation. The maximum production of Chl-a occurred in the 7th days of incubation at all concentration of treatments. The maximum Chl-a values being 1.34±0.784 mgL^{-1} in experiment with 20 fold Fe fortified concentration, after 14 day of inoculation.

Figure 3 shows the concentration of carotenoid accumulation in *A. platensis* grown in different media. Also, the results indicated no significant differences in the carotenoid contents after 14 day of inoculation.

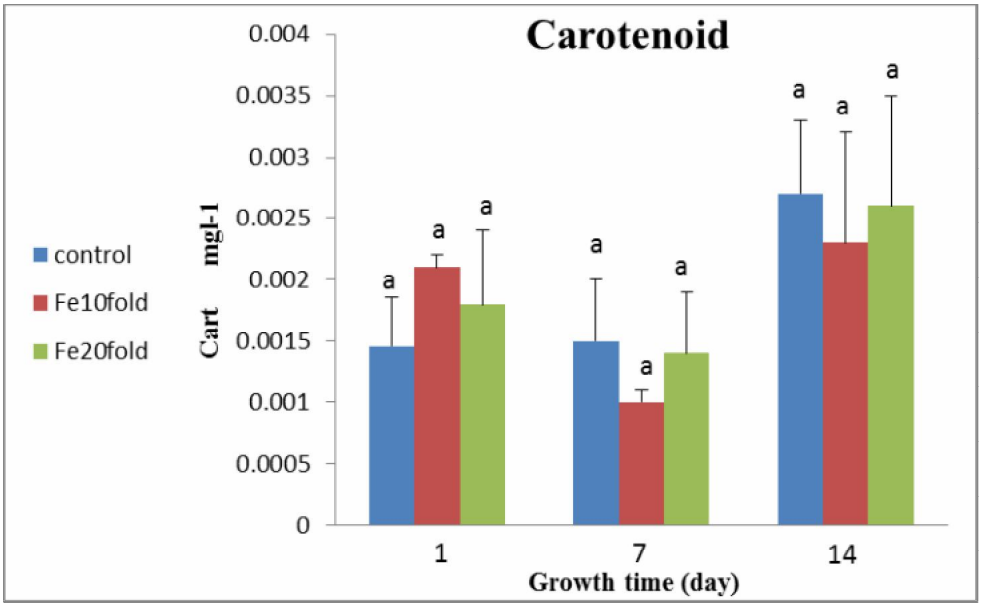

Figure 3. Changes in the carotenoid content of *Arthrospira platensis* on Zarrouk medium (control) and growth mediums with Fe-fortified ($p \geq 0.05$).

Unlike the effect on the growth, the impacts on the photosynthesis were more complex. Dinesh et al (2014) also reported that, growth and photosynthesis are independent processes unrelated to each other.

Iron is an important trace metal for normal growth and functioning of photosynthesis and respiration in algae. It acts as redox catalyst in photosynthesis and nitrogen assimilation and mediates electron transport reactions in photosynthetic organisms (Terry & Abadia 1986). Iron limitation significantly depresses photosynthetic electron transfer, resulting in a reduction in NADPH formation. Reduction in Fe decreases the cellular abundance of ferredoxin, which contains Fe, and forces the substitution of flavodoxin, a non iron functional equivalent, in the cell (Straus 2004; McKay et al 1999; Roche et al 1993; Sandmann & Malkin 1983). Since the catalytic capacity of ferredoxin is much higher than flavodoxin, this can be problematic (Raven 1984). Iron limitation also reduces cellular Chl concentration (Greene et al 1992). Decrease in Fe content reduces carotenoid composition (Van Leeuwe & Stefels 1998; Kobayashi et al 1993).

Xing et al (2007) found that under the iron-replete condition, pigments and photochemical efficiency of *M. aeruginosa* and *M. wesenbergii* were promoted. Chl-a was influenced by iron limitation and iron enrichment. Although Chl-a itself does not contain iron, there are both direct and indirect requirements for iron by enzymes involved in the Chl-a biosynthetic pathway.

Concerning the influence of medium type on carotenoids concentrations, it was found that similar trend of total Chl content profile.

This may indicate a strong relation between both chlorophyll and carotenoids contents. Such correlation could be attributed to that the carotenoids protect chlorophyll molecules against photo destruction and oxidation by molecular oxygen (Krinsky 1979). Similarly, Vonshak (1997) reported that there was a positive correlation between chlorophyll and carotenoids content of *A. platensis*.

Phycobiliproteins

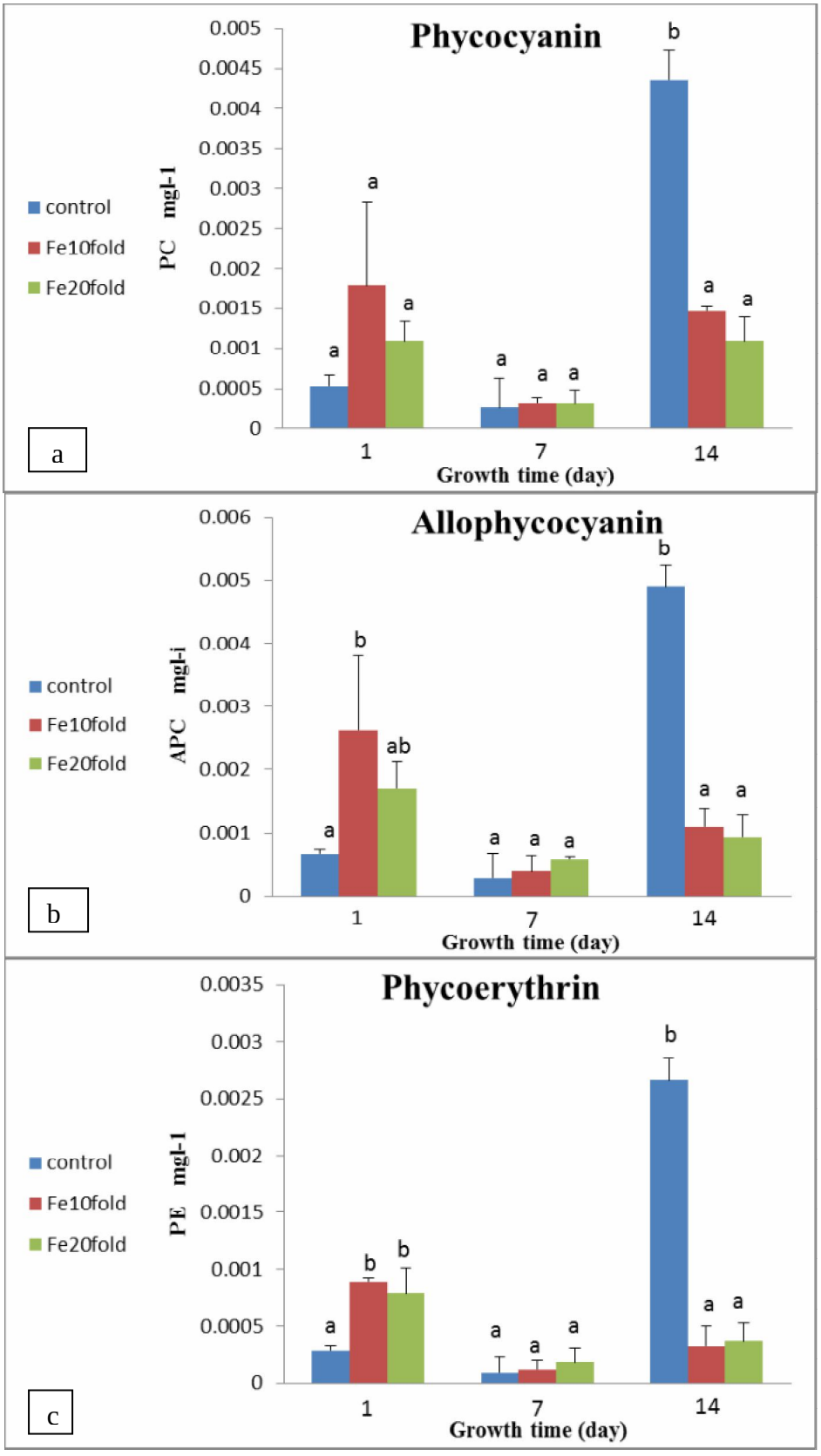

Figure 4. Changes in phycocyanin, allophycocyanin and phycoerythrin content of *Arthrospira platensis* on Zarrouk medium (control) and growth mediums with Fe-fortified ($p \leq 0.05$).

Figure 4 (a,b and c) shows the concentration of phycobiliproteins accumulation in Zarrouk medium (control) and growth mediums with enhanced Fe^{+2} element level. The results showed significant difference in content of phycobiliproteins for the different mediums as compared to control medium. It was observed that an increase of Fe concentration caused reduction of phycobiliproteins of *A. platensis*.

Iron is an essential trace element for biological requirements of photoplankton. It can be involved in chlorophyll and phycobilin pigment biosynthesis, in many components of photosynthetic (PS I and PS II) and electron transport systems, and in nitrate assimilation as an enzyme cofactor (nitrate reductase and nitrite reductase) (Geider & La Roche 1994).

The phycobiliproteins are located in the phycobilisomes outside the chloroplast thylakoids. In red algae, PE is used during the acclimation process; therefore, it is located more externally in the phycobilisomes (Talarico 1996). Our results demonstrated that phycobiliprotein levels, including APC, PC, and PE, decreased in *S. platensis* after Iron treatments. These molecules absorb solar energy, transferring it to the reaction center of photosystem II, where Chl a is excited by the flow of electrons (Gantt 1981). Also, it could be due to its peripheral position in phycobilisomes on the thylakoid membrane (Gantt 1981) and attributable to its sensitivity to metals (Kiran & Thanasekaran 2011).

A large decrease in the amount of phycocyanin and chlorophyll-a is accompanied by structural alterations of the thylakoid membranes and phycobilisomes, and the number of iron-containing proteins within the photosynthetic apparatus is reduced (Kudo et al 2000).

Conclusions. This study used blue green algae to test for trace metal accumulation because they have high growth rates, rich source, cheap and are easy to separate from solution by simple filtration. *Arthrospira sp*. have the ability to uptake and accumulate metal in their cells and known as one of the most efficient microalgae in this process. Most of utilized mineral feed additives nowadays are used from non renewable sources. Utilization of microalgae as a base to produce such a feed additives cause less mineral mining, as a result of better bioavailability of minerals supplied in biological form. The concept of using biological pathways to produce a wide variety of valuable compounds offers many opportunities for innovative products in the areas of nutrition in agriculture. Algal strains like spirulina have bioaccumulation trace elements although little information is available to support this subject.. Although the values of dry weight in iron treatment were higher than the control treatment however, the results indicated no significant differences in the dry weights for the different mediums. The maximum production of Chl-a and carotenoid occurred in the 7^{th} and 14^{th} days of incubation at all concentration of treatment, respectively. The maximum bioaccumulation value was observed 4465±39.68 mg kg^{-1} in Fe-10 fold concentration. *A. platensis* has the ability to uptake and accumulate iron metal in its cells, as a result of better bioavailability of minerals supplied in biological form.

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References

Bennett A., Bogorad L., 1971 Properties of subunits and aggregates of blue green algal biliproteins. Biochemistry 10:3625-3634.

Belay A., Ota Y., Miyakawa K., Shimamatsu H., 1993 Current knowledge on potential health benefits of *Spirulina*. Journal of Applied Phycology 5:235–241.

Belay A., Kato T., Ota Y., 1996 *Spirulina* (*Arthrospira*): potential application as an animal feed supplement. Journal of Applied Phycology 8:303-311.

Bruland K. W., Donat J. R., Hutchins D. A., 1991 Interactive influences of bioactive trace metals on biological production in oceanic waters. Limnology and Oceanography 36:1555–1577.

Cornet J. F., Dussap C. G., Dubertret G., 1992 A structured model for simulation of cultures of the cyanobacterium *Spirulina platensis* in photobioreactors. I. Coupling between light transfer and growth kinetics. Biotechnology and Bioengineering 40:817–825.

Davey M., Geider R. J., 2001 Impact of iron limitation on the photosynthetic apparatus of the diatom *Chaetoceros muelleri* (Bacillariophyceae). Journal of Phycology 37(6):987-1000.

Dinesh S., Santhanam P., Ananth S., Shenbaga A., Nandakumar R., Balaji B., Jeyanthi S., Jayalakshmi T., Ananthi P., 2014 Effect of different dosages of zinc on the growth and biomass in five marine microalgae. International Journal of Fisheries and Aquaculture 6(1):1-8.

Duncan D. B., 1955 Multiple-range and multiple F tests. Biometrics 11:1–42.

Eriksen N. T., 2008 Production of phycocyanin-a pigment with applications in biology, biotechnology, foods and medicine. Applied Microbiology and Biotechnology 80:1-14.

Faintuch B. L., Sato S., Aquarone E., 1991 Influence of the nutritional sources on the growth rate of cyanobacteria. Arquivos de Biologia e Tecnologia 34:13–30.

Gantt E., 1981 Phycobilisomes. Annual Review of Plant Physiology 32:327–347.

Geider R. J., La Roche J., 1994 The role of iron in phytoplankton photosynthesis, and the potential for iron-limitation of primary productivity in the sea. Photosynthesis Research 39:275-301.

Goh L. P. Jr, Loh S. P., Fatimah M. Y., Perumal K., 2009 Bioaccessibility of carotenoids and tocopherols in marine microalgae, *Nannochloropsis sp*. and *Chaetoceros sp*. Malaysian Journal of Nutrition 15(1):77-86.

Gupta V. K., Rastoqi A., Saini V. K., Jain N., 2006 Biosorption of copper(II) from aqueous solutions by *Spirogyra* species. Journal of Colloid and Interface Science 296(1):59-63.

Greene R. M., Geider R. J., Kolber Z., Falkowski P. G., 1992 Iron-induced changes in light harvesting and photochemical energy conversion processes in eukaryotic marine algae. Plant Physiology 100:565–575.

Habib M. A. B., Parvin M., Huntington T. C., Hasan M. R., 2008 A review on culture, production and use of *Spirulina* as food for humans and feeds for domestic animals and fish. FAO Fisheries and Aquaculture Circular, No. 1034, FAO, Rome, pp. 33.

Henrikson R., 1994 Microalga *Spirulina*. Superalimento del futuro. Ediciones S. A. Urano, Barcelona, Spain.

House W. A., 1999 Trace element bioavailability as exemplified by iron and zinc. Field Crops Research 60:115-141.

Jensen A., 1978 Chlorophylls and carotenoids. In: Handbook of phycological methods, physiological and biochemical methods. Hellebust J. A., Craigie J. S. (eds), pp. 59-70, Cambridge University Press, Cambridge.

Kiran B., Thanasekaran K., 2011 Metal tolerance of an indigenous cyanobacterial strain, *Lyngbya putealis*. International Biodeterioration and Biodegradation 65:1128-1132.

Kobayashi M., Kakizono T., Nagai S., 1993 Enhanced carotenoid biosynthesis by oxidative stress in acetate-induced cyst cells of a green unicellular alga, *Haematococcus pluvialis*. Applied and Environmental Microbiology 59:867–873.

Krinsky I., 1979 Carotenoid protection against oxidation. Pure and Applied Chemistry 51:649-660.

Kudo I., Miyamoto M., Noiri Y., Maita Y., 2000 Combined effects of temperature and iron on the growth and physiology of the marine diatom *Phaeodactylum tricornutum* (Bacillariophyceae). Journal of Phycology 36:1096–1102.

Mane P. C., Bhosle A. B., 2012 Bioremoval of some metals by living algae *Spirogyra* sp. and *Spirulina sp*. from aqueous solution. International Journal of Environmental Research 6(2):571-576.

McKay R. M. L., La Roche J., Yakunin A. F., Durnford D. G., Geider R. J., 1999 Accumulation of ferredoxin and flavodoxin in a marine diatom in response to Fe. Journal of Phycology 35:510–519.

Molnár S., Kiss A., Virág D., Forgó P., 2013 Comparative studies on accumulation of selected microelements by *Spirulina platensis* and *Chlorella vulgaris* with the prospects of functional food development. Journal of Chemical Engineering and Process Technology 4(7):1-6.
Mustafa M. G., Umino T., Nakagawa H., 1994 The effect of *Spirulina* feeding on muscle protein deposition in red sea bream, *Pagrus major*. Journal of Applied Ichthyology 10:141–145.
Olguin E. J., Galicia S., Angulo-Guerrero O., Hernandez E., 2001 The effect of low light flux and nitrogen deficiency on the chemical composition of *Spirulina sp*. (*Arthrospira*) grown on digested pig waste. Bioresource Technology 77:19–24.
Pandey P., Pathak N., Tiwari A., 2010 Standardization of pH and light intensity for the biomass production of *Spirulina platensis*. Journal of Algal Biomass Utilization 1(2):93-102.
Parson T. R., Strickland J. D. H., 1965 Particulate organic matter III. I. pigment analysis III, I.I. Determination of phytoplankton pigments. Journal of Fisheries Research Board of Canada 18:117-127.
Perales-Vela H. V., Pena-Castro J., Canizares-Villanueva R. O., 2006 Heavy metal detoxification in eukaryotic microalgae. Chemosphere 64:1-10.
Raven J. A., 1984 Energetics and transport in aquatic plants. MLB Lectures in Biology, vol 4, Alan R. Liss, New York, NY, USA.
Roche J., Geider R. J., Graziano L. M., Murray H., Lewis K., 1993 Induction of specific proteins in eukaryotic algae grown under iron- phosphorus- or nitrogen-deficient conditions. Journal of Phycology 29:767–777.
Romera E., Gonzales F., Ballester A., Blazquez M. L., Munoz J. A., 2007 Comparative study of biosorption of heavy metals using different types of algae. Bioresource Technology 98:3344-3353.
Rueter J. G., Petersen R. R., 1987 Micronutrient effects on cyanobacterial growth and physiology. New Zealand Journal of Marine and Freshwater Research 21:435-445.
Sandmann G., Malkin R., 1983 Iron-sulfur centers and activities of the photosynthetic electron transport chain in iron-deficient cultures of the blue-green alga *Aphanocapsa*. Plant Physiology 73(3):724–728.
Sanchez M., Bernal-Castillo J., Rozo C., Rodriguez I., 2003 *Spirulina* (*Arthrospira*): an edible microorganism: a review. Universitas Scientiarum Revista de la Facuitad de Ciencias Pontificia Universidad Javeriana 8(1):7-24.
Straus N. A., 2004 Iron deprivation: physiology and gene regulation. In: The molecular biology of cyanobacteria. Bryant D. A. (ed), 1:731–750, Springer: Berlin, Germany.
Talarico L., 1996 Phycobiliproteins and phycobilisomes in red algae: adaptative responses to light. Scientia Marina 60:205–222.
Terry N., Abadia J.,1986 Function of iron in chloroplasts. Journal of Plant Nutrition 9(3-7):609–646.
Vaishampayan A., Sinha R. P., Häder D.-P., Dey T., Gupta A. K., Bhan U., Rao A. L., 2001 Cyanobacterial biofertilizers in rice agriculture. The Botanical Review 67(4):453-516.
Van Leeuwe M. A., Stefels J., 1998 Effects of iron and light stress on the biochemical composition of *Antarctic Phaeocystis sp*. (Prymnesiophyceae). II. Pigment composition. Journal of Phycology 34(3):496–503.
Varga L., Szigeti J., Ordog V., 1999 Effects of a *Spirulina platensis* biomass enriched with trace elements on combinations of starter culture strains employed in the dairy industry. Milchwissenschaft 54:247-248.
Venkataraman L. V., 1967 *Spirulina platensis* (Arthrospira): physiology, cell Biology and biotechnology. Edited by Avigad Vonshak. Journal of Applied Phycology 9(3):295-296.
Vonshak A., 1997 Spirulina: growth, physiology and biochemistry. In: *Spirulina platensis (Arthrospira):* physiology, cell biology and biotechnology. Vonshak A. (ed), pp. 43-66, London: Taylor and Francis.
Vonshak A., Richmond A., 1988 Mass production of blue-green alga *Spirulina* – an overview. Biomass 15:233–247.

Wells M. L., Price N. M., Bruland K. W., 1994 Iron limitation and the cyanobacterium *Synechococcus* in equatorial Pacific waters. Liminology and Oceeanography 39(6):1481-1486.
Wilhelm S. W., Trick C. G., 1994 Iron-limited growth of cyanobacteria: multiple siderophore production is a common response. Limnology and Oceanography 39(8):1979-1984.
Worms I., Simon D. F., Hassler C. S., Wilkinson K. J., 2006 Bioavailability of trace metals to aquatic microorganisms: importance of chemical, biological and physical processes on biouptake. Biochimie 88:1720-1731.
Xing W., Huang W., Li D., Liu Y., 2007 Effects of iron on growth, pigment content, photosystem II efficiency, and siderophores production of *Microcystis aeruginosa* and *Microsystis wesenbergii*. International journal Current Microbiology 55:94-98.
Yan H., Pan G., 2002 Toxicity and bioaccumulation of copper in three green microalgal species. Chemosphere 49:471-479.

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Authors:
Maryam Akbarnezhad, Islamic Azad University, Science and Research Branch, Department of Fisheries, Iran, Tehran, P.O. Box 14515-775, e-mail: maryam.a0707@yahoo.com
Mehdi Shamsaie Mehrgan, Islamic Azad University, Science and Research Branch, Department of Fisheries, Iran, Tehran, P.O. Box 14515-775, e-mail: drshamsaie@gmail.com
Abolghasem Kamali, Islamic Azad University, Science and Research Branch, Department of Fisheries, Iran, Tehran, P.O. Box 14515-775, e-mail: kamali.abolghasem@gmail.com
Mehran Javaheri Baboli, Islamic Azad University, Branch of Ahvaz, Department of Fisheries Science, Iran, Golestan Highway, Farhangshahr Square, P.O. Box 1915, e-mail: mehranjavaheri@gmail.com

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