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RESEARCH PAPER

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Evaluation of Effects of Mg^{2+} and Cu^{2+} on Pigment-Metabolite Production and Photosystem II Activity of *Arthrospira platensis* Gomont 1892

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Abstract

The aim of this study was to investigate the effects of Mg^{2+} (0 - 4 mM), and Cu^{2+} (0 - 5 µM) concentrations on biomass and photosynthetic pigment-metabolites, and photosystem II activity in *Arthrospira platensis* Gomont 1892. The highest biomass was determined on control condition including both Mg^{2+} (0.8 mM) and Cu^{2+} (0.5 µM) as 49.1±1.6 mg mL^{-1}. Although the levels of phycocyanin, allophycocyanin and chlorophyll a were varied due to Mg^{2+} concentration, phycocyanin and allophycocyanin were the major fractions. The highest chlorophyll *a* level was detected at 0.5 µM Cu^{2+}. The major carotenoid was β-carotene, and its highest value was determined in the absence of Cu^{2+} as 2400±60 µg mL^{-1}. In addition, excessive and insufficient amounts of Mg^{2+} and Cu^{2+} caused a decrease in photosystem II activities of *A. platensis*. This work provides a good example for the production of carotenoids for use in various industries, such as food, cosmetic and pharmaceutical products.

Introduction

Microalgae are microscopic unicellular organisms capable to convert solar energy to chemical energy via photosynthesis. The productivity of photosynthetic process which is driven on photosystems (PSII and PSI) depends heavily on absorbing light energy in an efficient way (Hoober, 2012). Capturing light energy effectively is possible by absorbing light in extended spectrum that is achieved by chlorophylls (Chl *a* and Chl *b*) and carotenoids (β-cryptoxanthin, β-carotene, zeaxanthin, lutein) in plants, while cyanobacterial species like *Arthrospira* sp. have additive pigments called phycobiliproteins i.e. phyocyanin (CPC), allophycocyanin (APC), and phycoerythrin (PE) (Stadnichuk & Tropin, 2017).In photosynthesis, contribution of these pigments gives a paramount property to *Arthrospira* species. However, the pigments have chromophore characteristics, and use of carotenoids as well as phycobiliproteins for natural coloring agents in many industries such as food and cosmetics is increasing considerably. Their nutraceutical and pharmaceutical values are also deemed to be raising the *Spirulina* cell production (Prasanna, Sood, Suresh, Nayak, & Kaushik, 2007). Furthermore, some recent studies focus on their potential as therapeutic agents in oxidative stress-induced diseases as well as the great demand on biological sources of natural colors as food and cosmetics colorant and emphasize the need to evaluate production conditions to increase levels of these pigments (Minkova *et al.*, 2003).

The composition of growth medium is particularly prominent to produce target metabolites at high levels.

Production of pigments such as chlorophylls, carotenoids and phycobiliproteins requires many enzymatic procedures which are affected by some essential metal ions such as Mg^{2+}, Cu^{2+} and Zarrouk's medium constituents (Zarrouk, 1966). These metals are also present in reaction centers of photosystems and play a key role in photosynthesis (Mandalam & Palsson, 1998; Tanaka & Tanaka, 2006). In addition, these are structural metal ions found in some pigments (Mg^{2+} in Chl *a*) and some electron-carrier metabolites (Cu^{2+} in plastocyanin). Non-essential metals can interact and change these metals' redox states, or displace them and impact photosynthesis. Some of the most common effects of these have been investigated in algae (Dao & Beardall, 2016; Nowicka, Plucinski, Kuczynska, & Kruk, 2016; Xu & Juneau, 2016). Although the influence of growth conditions on the biochemical composition of *A. platensis* has been investigated by many researchers, research on the effects of Mg^{2+} and Cu^{2+} on pigment-metabolite production and photosystem II activity of *A. platensis* is limited. The aim of this study was to investigate effects of control and stress concentrations of Mg^{2+} and Cu^{2+} on production of some metabolites such as Chl *a*, carotenoids, proline, CPC, APC and malondialdehyde (MDA) as well as PS II activity in *A. platensis* during the incubation period. In this study, increasing microalgae biomass and pigments will cause microalgae use to expand in different areas such as food and aquaculture.

Materials and Methods

Microorganism and Culture Conditions

The cyanobacterium *A. platensis* was provided by the Faculty of Aquaculture of Çanakkale Onsekiz Mart University, Turkey. *A. platensis* was cultivated in batch cultures containing 750 mL Zarrouk's medium at 30°C at pH 9.0±0.2 (Zarrouk, 1966). The culture was inoculated to an initial optical density (OD) (600 nm) of approximately 0.2. The cultures were mixed and bubbled using filtered air continuously. Standard Zarrouk's medium composition containing 0.8 mM Mg^{2+} and 0.5 µM Cu^{2+} was used as control. In this study; when the Mg^{2+} concentration is constant (0.8 mM), the changing concentrations of Cu^{2+} (0-5) and when the Cu^{2+}concentration is constant (0.5 µM), the changing concentrations of Mg^{2+} (0-1.6 mM) were used.

Stock solutions of $MgSO_4$ and $CuSO_4$ were prepared in distilled water and the solutions were further sterilized by passing through membrane filter (0.22 µm). For the growth conditions of *A. platensis*, 0 - 4 mM Mg^{2+} and 0 - 5 µM Cu^{2+} concentrations were used. The reason for examining different Mg^{2+} and Cu^{2+} concentrations in the growth media is to achieve optimum growth of *A. platensis* and also production of metabolites, pigments, and PS II enzyme activity.

Illumination at 2500 lx (30 µmol photon $m^{-2}s^{-1}$) light intensity was provided by white fluorescent lamps continuously. The light intensity was measured by a digital light meter (Luxtron LX-101). The purity of all the reagents used was higher than 99.99%. Sampling vessels are cleaned with 10% (v/v) HNO_3 and subsequent ultra-pure water (18 MΩcm) in order to minimize trace metal contamination.

Analytical Methods

The growth of *A.platensis* was detected by determining dry cell weight (mg mL^{-1}) and optical density (OD) at 600 nm. Harvesting cells were centrifuged at 11180 g at 24°C for 20 min. The supernatant decanted and cell pellets were washed 2 times with distilled water and afterwards dried in the vacuum oven at 105°C until a constant weight was obtained. In this process, dry cell weight was determined gravimetrically.

The cells were harvested periodically by centrifugation (16099 g, 10 min, 4 °C) and washed with distilled water. The precipitated cells were weighed, and 50 mM phosphate buffer (pH 7) was added at a rate of 12.5 mL g $cells^{-1}$. The cells were disrupted by homogenization. Cell debris was removed by centrifugation at 16099 g, 4 °C for 10 min. After the centrifugation, the obtained supernatant was used for determination of phycobiliprotein levels. CPC and APC levels were assayed by the method of Tarko, Duda-Chodak & Kobus (2012). Chl *a* level was measured as described by Lichtenthaler & Wellburn (1983). Carotenoids contents were determined by HPLC (Agilent Technologies 1100, California, USA) (Kraay, Zapata, & Veldhuis, 1992). All standards (β-carotene, zeaxanthin and lutein concentrations in the range 0 - 400 ppm; β-cryptoxanthin concentration in the range 0 - 200 ppm (Sigma-Aldrich, Darmstadt, Germany)) and samples were prepared in chloroform. The reversed phase column was a C18 column (250 X 4.6 mm) (ACE-221-2546, Aberdeen, Scothland). Flow rate was 0.8 mL min^{-1}. The column was equilibrated prior to use by flushing with 60% mobile phase B (v/v) for 5 min. The solvent gradient consisted of (A) 85% methanol / water (v/v), buffered with 0.5 M ammonium acetate (final concentration), (B) 90% acetonitrile / water (v/v), and (C) ethyl acetate. Amounts of produced carotenoids, such as β-carotene (y = 4.6536 X (R^2 = 0.9794)), β-cryptoxanthin (y = 76.952 X (R^2 = 0.989)), zeaxanthin (y = 15.153 X (R^2 = 0.9969)) and lutein (y= 22.035 X (R^2 = 0.9949)), were determined as ppm from the standard HPLC curve equations. Based on standard chromatograms, retention times of β-carotene, β-cryptoxanthin, zeaxanthin and lutein carotenoids were determined as 22.5±0.2, 18.5±0.2, 12.2±0.1, 11.8±0.1 min, respectively.

Proline content was assayed by the method of Bates, Waldren & Teare (1973). In the process, acid-

ninhydrine reagent was used. The absorbance was measured at 520 nm. Lipid peroxidation (LPO) level was estimated based on thiobarbituric acid (TBA) reactivity (Okhawa, Ohishi, & Yagi, 1979). MDA, an end product of fatty acid peroxidation, can react with TBA to form a colored complex that has a maximum absorbance level at 532 and 600 nm. Absorbance at 600 nm was subtracted from absorbance at 532 nm. MDA value, as nanomoles per gram wet *A. platensis* cell was determined with the extinction coefficient of the MDA-TBA complex ($1.56 \times 10^5 cm^{-1}M^{-1}$).

Determination of Photosystem II Activity

Thylakoid membranes of *A. platensis* were prepared in SPC buffer (0.5 M sucrose, 0.5 M K-phosphate and 0.3 M sodium citrate, pH 7.0). The cells were harvested periodically by centrifugation (16099 g, 10 min, 4°C) and washed with distilled water. The precipitated cells were weighed, and SPC buffer was added by a rate of 5 mL g $cells^{-1}$. The cells were homogenized at 10090 g for 30-s with 10-s intervals. Cell debris was removed by centrifugation at 447 g, 4°C for 5 min. Supernatant was centrifuged again at 5000g, for 10 min and the pellet, in which thylakoids were precipitated, was used (Henriques, 2004). Pellet was diluted by SPC buffer at a rate of 5 mL g $pellet^{-1}$. The obtained supernatant was used to calculate chlorophyll level of cells as described before. The standard assay medium contained 50 mM Hepes (pH 7.5), 0.3 M sucrose, 10 mM NaCl, 2 mM $MgCl_2$, 20 µM 2,6-dichlorophenol indophenol (DCIP). Prepared thylakoids were added to assay medium at a chlorophyll concentration of20 µg mL^{-1}. PS II dependent electron transport activity was measured at room temperature with a recording spectrophotometer by following the bleaching of DCIP at 580 nm using water as electron donor (ε = 19 800 $M^{-1} cm^{-1}$).

Statistical Analysis

All experiments were carried out in triplicates (n=3) and repeated 3 times. Each value is an average of 3 parallel replicates. Data were presented as mean±standard deviation (SD). The data were analyzed by analysis of variance (ANOVA) to identify the significantly different groups at (P<0.05) by one-way ANOVA test using SPSS software statistical program (SPSS for windows ver. 21.00, USA).

Results

Stress is an unwelcome condition in which exogenous and endogenous factors operate together or individually to influence the physiological activities of the cyanobacteria (Öztürk Ürek & Tarhan, 2012). The alteration level in the cyanobacteria growth depends on the concentration and/or duration of stress factor. These different stressors, such as concentration of Mg^{2+} and Cu^{2+}, influence the cyanobacterial growth, pigment-metabolite production and other physiological activities like photosynthesis. In this study, it was aimed to investigate the effects of absence, deficiency and excessive concentration levels of Mg^{2+} and Cu^{2+} on the production of some metabolites, pigments, LPO levels and PSII activity in *A. platensis*.

Effects of Mg^{2+} and Cu^{2+} Concentrations on Optical Density, Dry Biomass, Chlorophyll a in *A. platensis*

In the growth media containing different Mg^{2+} and Cu^{2+} concentrations, the growth rate of *A. platensis* was determined by OD_{600} measurement and dry biomass levels depending on incubation time (Figure 1). Although increases in the OD values of *A. platensis* in presence of Mg^{2+} were detected during incubation period, these got much higher only in presence of 0.5 mM Cu^{2+}.

The highest dry biomass level was determined as 56.7±1.8 mg mL^{-1} on the 10th day in the medium containing 1.6 mM Mg^{2+}. The values were found to be significant when compared to the absence of Mg^{2+} (P< 0.05). In the Cu^{2+} containing media, the highest dry biomass (49.1±1.6 mg mL^{-1}) was detected with 0.5 µM Cu^{2+} on the 12th day. The values were found to be significant when compared to the absence of Cu^{2+} (P< 0.05).

Chl *a* levels of *A. platensis* with different Mg^{2+} and Cu^{2+}concentrations with respect to incubation time are shown in Figure 2. The highest levels of Chl *a* were determined on the 6th (with 0.2 mM Mg^{2+}) and 12th (with 1.6 mM Mg^{2+}) days as 123±5 µg mL^{-1} and 118.1±5 µg mL^{-1}, respectively. The maximum levels of Chl *a* were observed on the 12th day of incubation period for all tested Cu^{2+} concentrations. The highest Chl *a* level was determined as 94.4±4 µg mL^{-1} in the medium containing 0.5 µMCu^{2+}.

Effects of Mg^{2+} and Cu^{2+} Concentrations on Carotenoids and Phycobiliproteins in *A. platensis*

Evidence based on standard HPLC chromatograms suggested that *A. platensis* contained higher amounts of β-carotene, zeaxanthin, β-cryptoxanthin while lutein was found in lower amounts (Table 1).

The highest CPC level of *A. platensis* was recorded in Mg^{2+} and Cu^{2+} culture conditions on the 10th day (Figure 3(a) and (b)). CPC was one of the major pigments in *A. platensis* which grew in all the media containing Mg^{2+}. However, in the media containing Cu (0.5 µM Cu^{2+} control condition), the highest CPC value was determined as 1967±72 µg mL^{-1}.

As seen in Figure 4(a); APC level reached the highest value as 567±25 µg mL^{-1} on the 10th day of

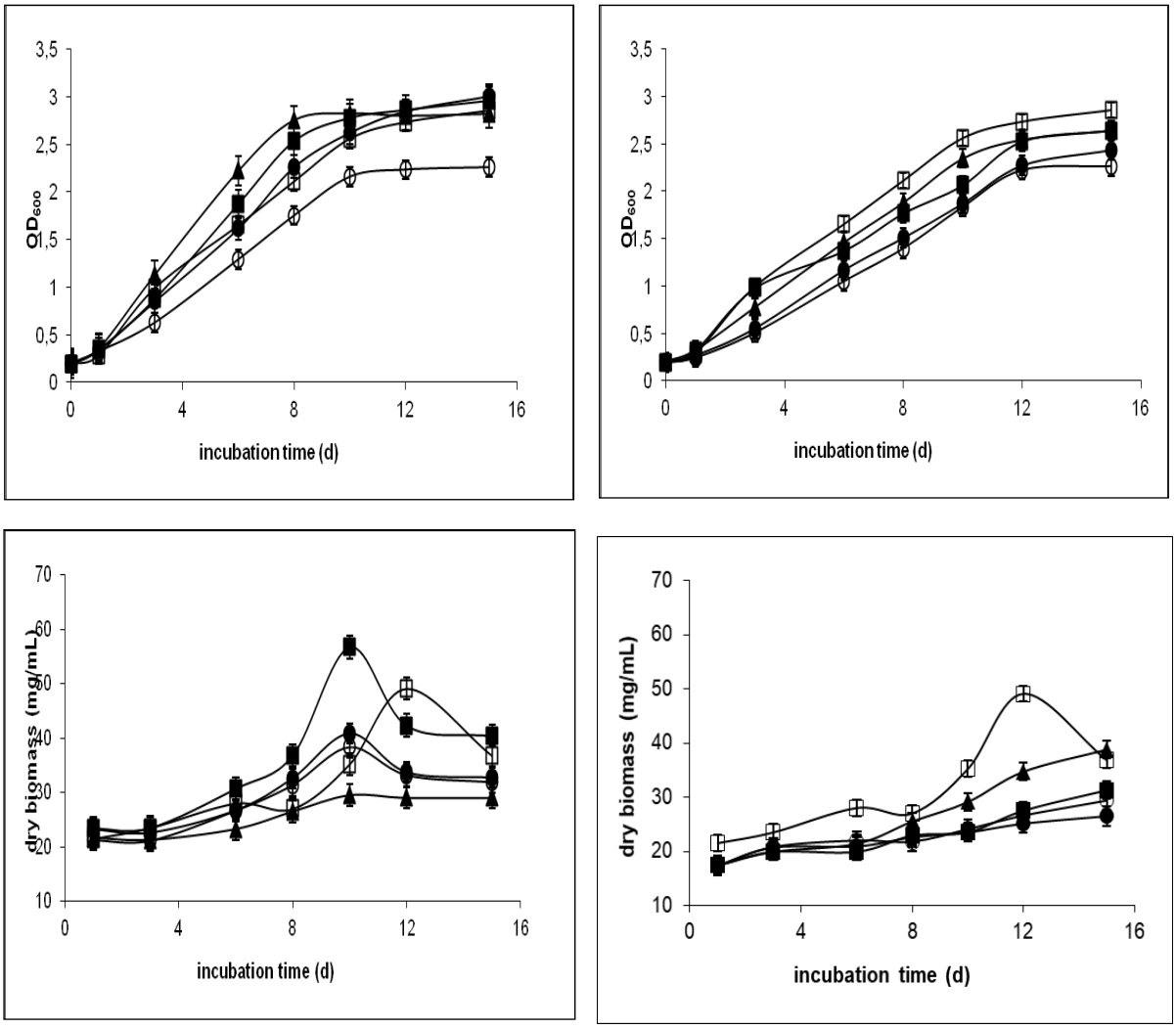

Figure 1. Variations of OD and dry biomass in *A. platensis* depending the incubation period in medium containing Mg^{2+} (A, C) [0 mM (o); 0.2 mM (●); 0.8 mM -control (□); 1.6 mM (■); 4 mM (▲)] and Cu^{2+} (B, D) [0 µM (o); 0.1 µM (●); 0.5 µM-control (□); 1 µM (■); 5 µM (▲)] concentrations. The values are the mean ± SD for 3 independent experiments.

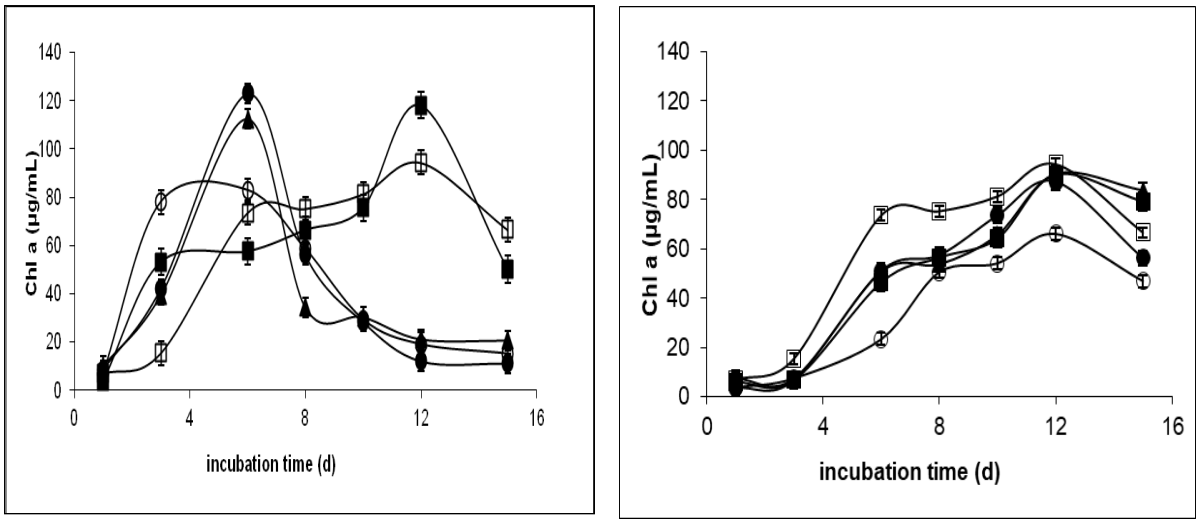

Figure 2. Variations of Chl *a* values in *A. platensis* depending the incubation period in medium containing Mg^{2+} (A) [0 mM (o); 0.2 mM (●); 0.8 mM-control (□); 1.6 mM (■); 4 mM (▲)] and Cu^{2+} (B) [0 µM (o); 0.1 µM (●); 0.5 µM-control (□); 1 µM (■); 5 µM (▲)] concentrations. The values are the mean ± SD for 3 independent experiments.

Table 1. Carotenoids levels of *A. platensis* by varying concentration of Mg^{2+} (a) and Cu^{2+} (b) on the 12th day. The values are the mean ± SD for 3 independent experiments

a	Mg^{2+} concentration (mM)				
Carotenoids (μgmL^{-1})	0	0.2	0.8 –control	1.6	4
β-Carotene	1464.1 ± 51	1822.3 ± 57	2013.3 ± 59	2354.2 ± 61	2299.4 ± 60
β-Cryptoxanthin	455.1 ± 17	669.6 ± 18	681.5 ± 18	713.6 ± 19	719.1 ± 19
Zeaxanthin	448.1 ± 16	674 ± 18	644.2 ± 18	700.1 ± 19	705.9 ± 19
Lutein	156.5 ± 4	194 ± 4.5	201.6 ± 5	215.9 ± 5	208.6 ± 5
b	Cu^{2+} concentration (µM)				
Carotenoids (μgmL^{-1})	0	0.1	0.5 –control	1	5
β-Carotene	2396.7 ± 61	2253.2 ± 60	2013.3 ± 59	1453.7 ± 51	1211.9 ± 43
β-Cryptoxanthin	900.4 ± 22	765.3 ± 19.6	681.5 ± 18	536.9 ± 17.7	412.5 ± 17
Zeaxanthin	821.7 ± 21	704 ± 19	644.2 ± 18	495.8 ± 17.2	445.3 ± 17
Lutein	214.1 ± 5	223.8 ± 5	201.6 ± 5	232.9 ± 5.2	121.1 ± 3.7

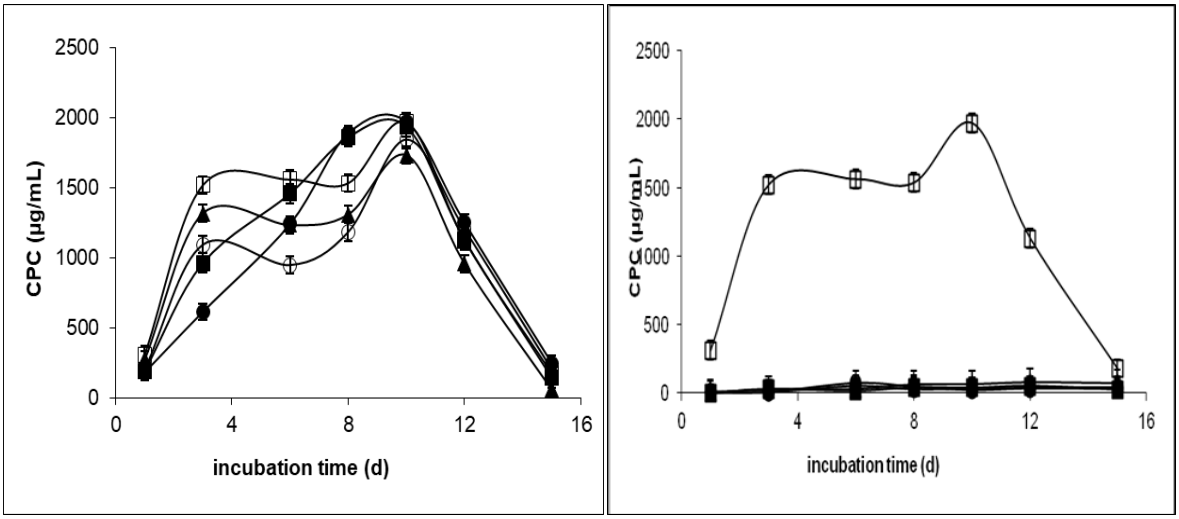

Figure 3. Variations of CPC values in *A. platensis* depending the incubation period in medium containing Mg^{2+} (A) [0 mM (o); 0.2 mM (●); 0.8 mM -control (□); 1.6 mM (■); 4 mM (▲)] and Cu^{2+} (B) [0 µM (o); 0.1 µM (●); 0.5 µM -control (□); 1 µM (■); 5 µM (▲)] concentrations. The values are the mean ± SD for 3 independent experiments.

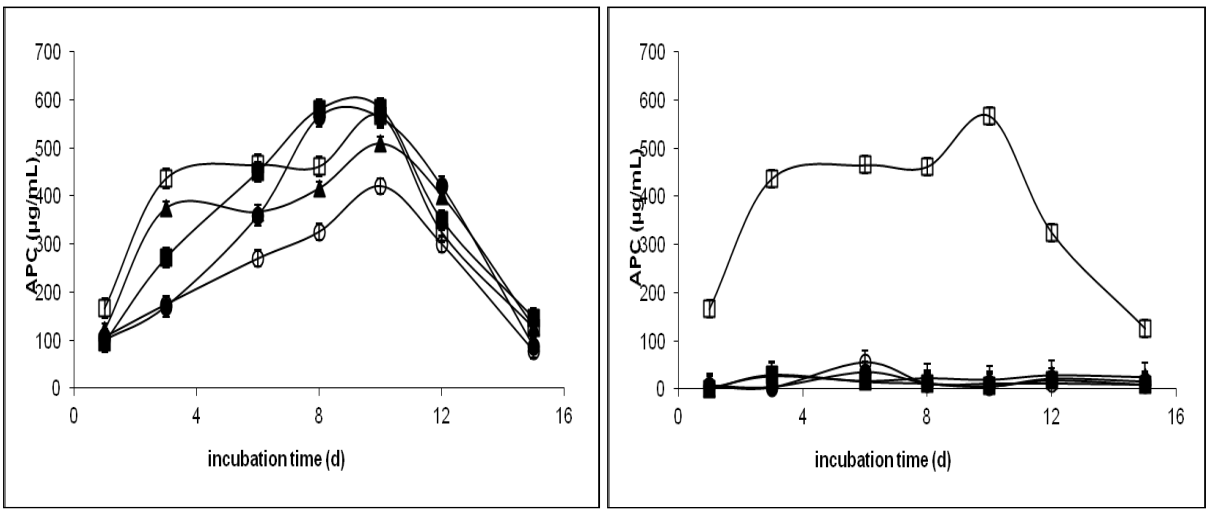

Figure 4. Variations of APC values depending the incubation period in medium *A. platensis* containing Mg^{2+} (A) [0 mM (o); 0.2 mM (●); 0.8 mM -control (□); 1.6 mM (■); 4 mM (▲)] and Cu^{2+} (B) [0 µM (o); 0.1 µM (●); 0.5 µM-control (□); 1 µM (■); 5 µM (▲)] concentrations. The values are the mean ± SD for 3 independent experiments.

incubation. Even so, CPC level was generally higher than APC level, although they were both major pigments. In the Cu^{2+} conditions, maximum APC level was observed on the control condition (Figure 4(b)). In absence of Cu^{2+}, the levels of CPC and APC were detected to be decreasing due to the decline of Chl *a*. The levels of APC also followed a similar trend with CPC levels during the incubation period in both metal media. While CPC and APC pigments were produced in high levels in *A. platensis* cultures, PE was insignificantly produced so it was not depicted here ($P > 0.05$).

Effects of Mg^{2+} and Cu^{2+} Concentrations on the Proline and Lipid Peroxidation Levels in *A. platensis*

The proline levels raised depending on increasing concentration of Mg^{2+} (from 0.8 (control) to 4 mM) on the 3rd day (Figure 5(a)). The highest proline level was determined as 263.2±12 µmol g^{-1} in presence of 4 mMMg^{2+}. The maximum proline values were positively correlated with the Mg concentration ($P<0.05$; r=0.839). This level was found to be approximately 27% higher than the value obtained in the presence of 5 µM Cu^{2+} ($P<0.05$). As it can be seen on Figure 5(b), at

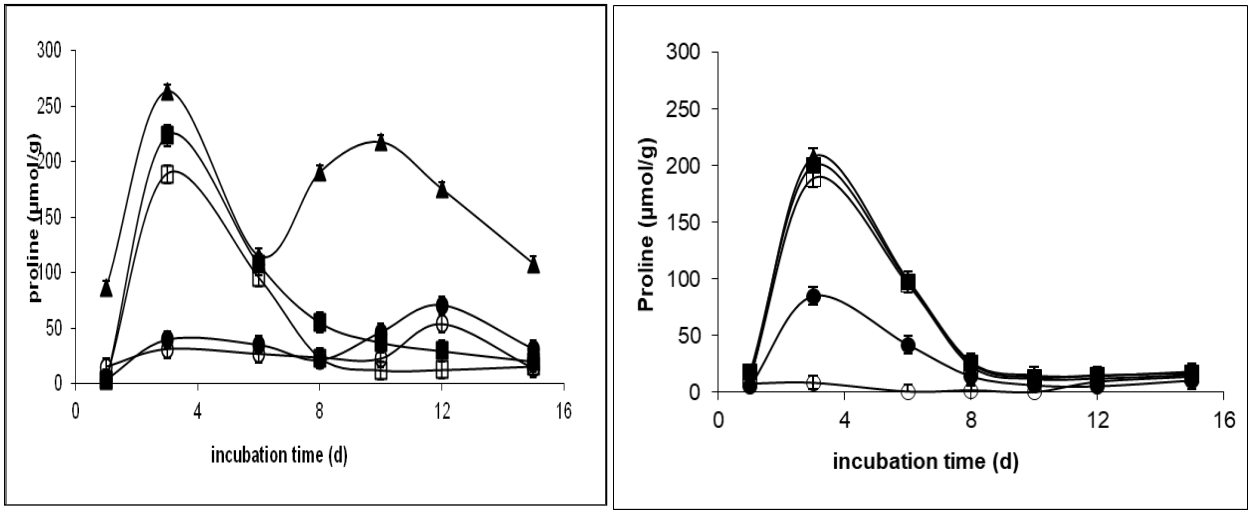

Figure 5. Variations of proline level in *A. platensis* depending the incubation period in medium containing Mg^{2+} (A) [0 mM (o); 0.2 mM (●); 0.8 mM -control (□); 1.6 mM (■); 4 mM (▲)] and Cu^{2+} (B) [0 µM (o); 0.1 µM (●); 0.5 µM -control (□); 1 µM (■); 5 µM (▲)] concentrations. The values are the mean ± SD for 3 independent experiments.

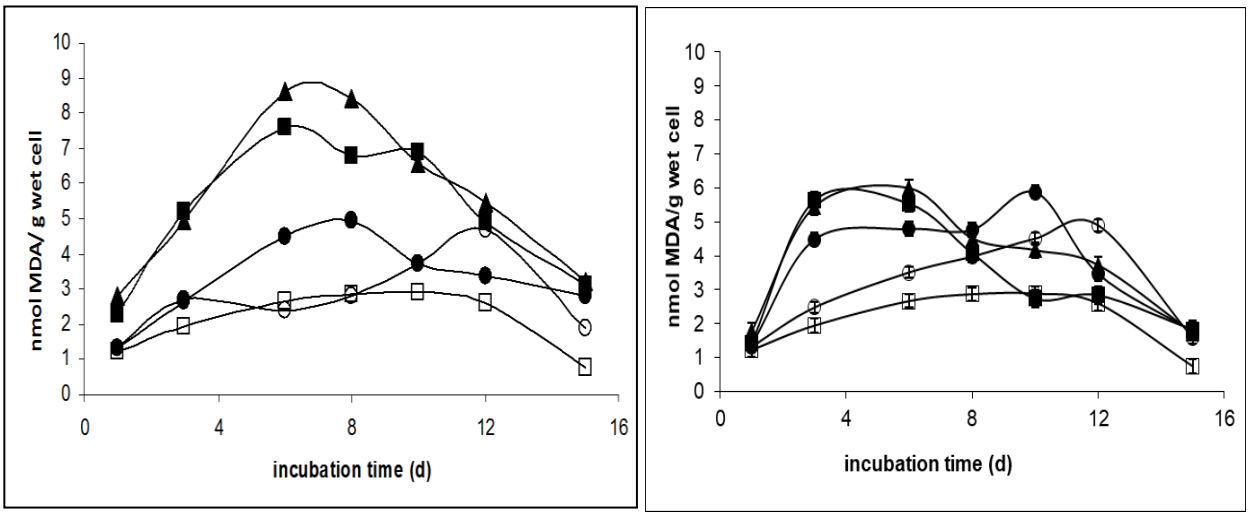

Figure 6. Variations of LPO levels in *A. platensis* depending the incubation period in medium containing Mg^{2+} (A) [0 mM (o); 0.2 mM (●); 0.8 mM -control (□); 1.6 mM (■); 4 mM (▲)] and Cu^{2+} (B) [0 µM (o); 0.1 µM (●); 0.5 µM -control (□); 1 µM (■); 5 µM (▲)] concentrations. The values are the mean ± SD for 3 independent experiments.

higher Cu^{2+} concentrations proline levels decreased after the 3rd day of incubation.

MDA is a cytotoxic product of LPO and an indicator of free radical production and consequent tissue damage (Okhawa *et al.*, 1979). Variations of LPO levels in the media containing Mg^{2+} and Cu^{2+} depending on incubation period are shown in Figure 6. In the growth media containing Mg^{2+}, maximum LPO values were observed on the 10th day for 0.8 mM (control) and 0.2 mM Mg^{2+}. However, in presence of 1.6 and 4 mM Mg^{2+} maximum LPO values were detected on the 6th day. It can be stated that, in comparison to control, LPO levels were found higher at different concentrations of Mg^{2+} during the incubation period.

Effects of Mg^{2+} and Cu^{2+} Concentrations on the Photosystem II Activities of *A. platensis*

Metal ions of a biological system are normally combined with proteins of enzymes for effective biological catalysis. As shown in Table 2, the highest PSII activities were determined as 55.8 µmol DCIP mg^{-1} chl h^{-1} (10th day) under control condition.

Discussion

In the growth medium of *A. platensis* effects of Mg^{2+} (0-4 mmol L^{-1}) and Cu^{2+} (0-5 µmol L^{-1}) concentrations on the biomass and photosynthetic pigment- metabolites such as chlorophyll *a,* phycobiliproteins (phycocyanin, allophycocyanin), proline, lipid peroxidation, carotenoids levels and photosystem II activity were investigated. During the first eight days of incubation, biomass levels indicated same trends, yet it was higher at control level compared to that of assayed concentrations of Cu^{2+}. The evidence suggested that excessive concentration of Cu^{2+} was quite likely to suppress growth. Perales-Vela, Gonzales-Moreno, Mentes-Horcasitas & Canizares-Villanueva (2007) reported that growth rates of *Tetradesmus incrassatulus* (Bohlin) M.J. Wynne 2016 were the most sensitive to high levels of Cu^{2+} ions, followed by photosynthesis and respiration. Due to the need for Cu at that certain concentration level to grow cells, at 0.1 µM and absence of Cu^{2+} concentrations the dry biomass values were well below the half of control condition ($P<0.05$). Consequently, it can be concluded that the lag and exponential phases of growth were prolonged when compared with the control Cu^{2+} concentration.

Chl *a* levels decreased in the media containing 0.2 mM and absence of Mg^{2+} after the 6th day due to lower dry biomass level at these concentrations. This might be attributed to Mg^{2+}, an essential nutrient, and to the fact that deficiency or absence of Mg^{2+} did not contribute to an increase on dry biomass. An increment in dry biomass may result in a rising trend in Chl *a*. A positive correlation was found between maximum dry biomass and Chl *a* values ($P<0.05$; $r=0.605$). For this reason, Chl *a* values are used to evaluate microalgae growth. After the 6day, Chl *a* levels decreased in the deficiency and absence of Mg^{2+} concentration. Accordingly, it can be stated that Chl molecules may be lost Mg ions in the active center. Chl *a* can normally be degraded into pheophytin a (derivative of Chl *a* without Mg ion), but in the absence of Mg^{2+}, this conversion is possible to increase. At higher Cu^{2+} concentrations, Mg^{2+} may be replaced by Cu^{2+} so that Chl *a* may be degraded. Similarly, in algae exposed to higher Cu^{2+} concentrations decrease in chlorophyll pigment levels have been reported (Schiariti, Juarez & Rodrigues, 2004).

As shown in Table 1, β-carotene was the major carotenoid pigment and the carotenoid levels of *A. platensis* were influenced by concentrations of Mg^{2+} and Cu^{2+}. The carotenoids were accumulated depending on conditions such as metal stress, nutrient levels, and salt concentrations which cause limitation on growth of cells (Abd El- Baky, El Baz & El-Baroty, 2007). Results demonstrated that carotenoid levels increased gradually depending on Mg^{2+} concentrations, whereas increasing of Cu^{2+} levels caused a significant decrease ($P<0.05$). One positive effect of divalent cations has been accounted for a stimulatory effect on carotenoids-synthesizing enzymes. The evidence suggested that carotenoids composition was affected by varying concentrations of Mg (El-Banna, El-Razek & El-Mahdy, 2012). Likewise, Fang, Ku, Lee & Su (2010)

Table 2. Variations in PSII activities (µmol DCIP mg^{-1} chl h^{-1}) of *A. platensis* depending on stress conditions by incubation time

	Cu^{2+}concentration (µM)			
Incubation time (day)	0	0.1	0.5 – control	5
8	24	30.6	43.2	13.2
10	28.2	39.6	55.8	16.8
	Mg^{2+}concentration (mM)			
Incubation time (day)	0	0.2	0.8 – control	1.6
8	8.4	15.6	43.2	28.2
10	7.2	22.2	55.8	31.8

found that carotenoids production increased if a certain level of magnesium sulfate was added into the medium. Magnesium in the medium is used to modulate the environmental osmosis, reduce the cell lysis and increase the yield of carotenoids. However, when the magnesium sulfate exceeded 8%, the yield of pigments did not increase, and even showed a slight decline. In this study, the highest levels of carotenoids were detected in absence of Cu^{2+}. This level was 1.64 times higher than the value in the absence of Mg^{2+}. The values obtained in the absence of Cu^{2+} were found to be significant when compared to the absence of Mg^{2+} ($P< 0.05$). The results indicated that increasing concentration of Cu^{2+} gradually decreased carotenoid levels and these results showed consistency with results of Deniz, Saygıdeger & Karaman (2011). An implication of this is the possibility that Cu^{2+} has a more toxic effect at higher concentrations. At high concentration levels of Cu^{2+}, pigment synthesis could be inhibited and pigment degradation could be increased. The carotenoids accumulation is often regarded as one of the mechanisms to counteract stress in organisms. Carotenoids are also sensitive to metal oxidative stress and the β-carotene is the most resistant. Wisniewski & Dickinson (2003) proposed that the xanthophyll cycle is a mechanism that protects the photosynthetic apparatus and showed a clear decline in metal stress studies. Carotenoid analysis of their study pointed out that xanthophylls cycle pool size was lower in copper-treated plant which was in parallel with results obtained by our study. According to Lombardi & Maldonado (2011), NPQ (non-photochemical quenching) which mostly reflects heat dissipation, had a tendency to decrease at the highest Cu^{2+} concentrations. The general NPQ, a process requiring the presence of the xanthophyll cycle, decreased under high Cu^{2+} concentrations and suggested that the photoprotection mechanisms could have been impaired. One of the most significant findings to emerge from this study was that photosynthetic light reactions especially PSII was affected by stress level of Cu^{2+}.

Phycobiliproteins, which consist of CPC, APC and PE, are important accessory pigments of *A. platensis*. Vonshak (1997) proved that 20% of dry weight of *A. platensis* fraction is constituted by phycobiliproteins. The synthesis of phycobiliproteins was affected by Cu^{2+} concentration as a cofactor (Vonshak,1997; Jaouen, Lepine, Rossignol, Royer & Quemeneur, 1999). The levels of phycobiliproteins were low in presence of 1 and 5 µM Cu^{2+} due to its toxic effects. This result was consistent with Hemlata Tasneem (2009), who explained that the decline in phycobiliprotein content depended on varying metal concentrations. Phycobiliproteins production in absence and presence of 0.1 µM Cu^{2+} was very low compared to control values due to its necessity as a trace metal ($P< 0.05$). Thus, absence of Cu^{2+} led to negative effects on the production of CPC and APC ($P> 0.05$). Tredici, Papuzzo, and Tomaselli (1986) emphasized in their study that stress induced by cultures of *Arthrospira maxima* Setchell & N.L.Gardner in N.L.Gardner 1917resulted in strong reduction of the phycobiliprotein content. However, cell concentrations of these conditions were not very different from values obtained under control cultures.

On the 3rd day of incubation period, maximum proline level was determined in presence of 5 µM Cu^{2+}. This bears a resemblance to the results achieved by Choudhary, Jetley, Khan, Zutsi & Fatma (2007). In their study, they indicated that proline accumulation increased with increasing concentration of metals. On initial days of cultivations, in higher concentrations of $Cu^{2+}$and Mg^{2+}, the proline levels were extremely high. Thus, it could be deduced that the *A. platensis* cells were on the adaptive period at metal stress conditions. Especially higher concentrations of both metals (Mg^{2+} and Cu^{2+}) caused higher proline accumulation as led by stress. Stabilization of proteins and protein complexes in the chloroplast and cytosol could be provided by proline accumulation during stress. Therefore, proline accumulation played a vital role as a functioning agent on protection mechanism of the photosynthetic apparatus and enzymes involved in detoxification of ROS (Szabados & Savoure, 2009). Additionally, amino acids like proline could have involved metal chelating which was the possible enhancement of metal solubility (Viehweger, 2014).

In the growth medium containing Cu^{2+}, LPO levels of control condition (0.5 µM Cu^{2+}) were observed to change insignificantly depending on the incubation period. LPO level increased owing to a rise in heavy metal concentration in the culture medium. This situation might have caused increasing concentration-dependent free radical generation. The LPO levels showed similar trends in presence of 1 and 5 µM Cu^{2+} during incubation period. The highest LPO level was determined in presence of 5 µM Cu^{2+} on the 6th day due to its toxic effect. The Cu^{2+} concentration had major impacts on pigments and photosynthetic activities due to imbalanced metabolism and ROS. Additionally, Cu^{2+} could have destroyed the structure and function of membrane due to membrane protein inactivation and degeneration (Viehwegwe, 2014). These results have contributed enormously to the fact that the increase in both proline and LPO levels with increasing metal ion concentration could be an indicative of a correlation between free radical generation and proline accumulation (Choudhary *et al.*, 2007). According to Çelekli, Kapı, Soysal, Arslanargun & Bozkurt (2017) this peroxidation can induce cell damage by changing membrane permeability which promotes the synthesis of proline. In their study, Wu, Hseieh & Know (1998) observed proline accumulation in response to Cu^{2+} stress in *Chlorella*. These data obviously show parallelism to our results.

In presence of 5 µM Cu^{2+}, the PS II activity was obtained as 16.8 µmol DCIP mg^{-1} chl h^{-1} on the 10^{th} day while it was 13.2 µmol DCIP mg^{-1} chl h^{-1} on the 8^{th} day. In absence of Cu^{2+}, PSII activities decreased in comparison to control values. These results could be associated with metal interactions which are required at lumen side of PSII and may change regarding the lack of Cu^{2+} (Sersen, Kralova, Bumbalova & Svajlenova, 1997; Miqyass, van Gorkom & Yocuum, 2007). In the green alga *Chlamydomonas*, Cu^{2+} deficiency has been reported to change the composition of the thylakoid membrane lipids, thus affecting the integrity and function of the photosystems (Castruita *et al.*, 2011). Furthermore, Peers and Price (2006) suggested that electron flow downstream of PSII was impeded by a lack of Cu^{2+} and proposed that Cu^{2+}-limited *Thalassiosira oceanica* Hasle 1983was unable to assemble adequate amounts of functional Cu^{2+}-containing plastocyanin, thus limiting electron flow. Results revealed that excessive amounts of Cu^{2+} concentration had toxic effects on the PSII activity. Therefore, it could be argued that excessive amounts of Cu^{2+} may damage photosynthetic apparatus and cause reduction in pigment content (Ciscato, Valcke, Van Loven, Clijsters & Navari-Izzo, 1997). Besides, the disorganization of the chloroplast structure may lead to inactivation of oxygen evolving centers and it can impair electron transport. Mg^{2+}, central atom of Chl molecules, may be replaced by Cu^{2+} atom as it loses its function at higher Cu^{2+} concentrations (Küpper, Setlik, Spiller, Küpper & Prasil, 2002). It was demonstrated that Cu^{2+} toxicity is closely related to the damage of PSII reaction centers or their antenna pigments (Lombardi & Maldonado, 2011). Nonetheless, presence of Cu^{2+} as micronutrient may be essential for the whole photosynthetic process. Excessive Cu^{2+} concentrations may induce an increase in plasmalemma permeability which might lead to ionic imbalance, loss of turgor, and subsequent breakdown of cell metabolism (Sicko-Goad, 1982). Pätsikkä, Kairavuo, Sersen, Aro & Tyystijarvi (2002) indicated that when Cu^{2+} is given in excessive amounts in a growth medium, it causes Fe deficiency as both metal ions have partially common pathways. What's more, Cu^{2+} treatment showed the potential to result in a larger light harvesting antenna. Decreasing of Mg^{2+}concentration gradually caused a decline on PSII activities. It might be due to the need for Mg ions in biosynthesis of the Chl molecules. It was demonstrated that Mg^{2+} deficiency impairs the whole photosynthetic electron transport chain (Hermans, Johnson, Strasser & Verbruggen, 2004; Tang, Li & Chen, 2012). Numerous researchers indicate a substantial decrease of PSII photochemistry in Mg^{2+}-deficient plants (Baszynski *et al.*, 1980). The lowest PSII activity was determined in the absence of Mg^{2+} on the 10^{th} day. This result might be accounted for Chl *a* molecules which were already present in the cells of *A. platensis*. The highest PSII activity, which was found at 0.8 mM Mg^{2+}, proved that this concentration of Mg^{2+} might improve PSII conformation and promote energy transfer and water splitting (Liang *et al.*, 2009). In the presence of 1.6 mM Mg^{2+}, the PSII activities were detected as 28.2 U and 31.8 µmol DCIP mg^{-1} chl h^{-1} on the 8^{th} and 10^{th} day, respectively. It was observed that the PSII activities in this condition were higher than that detected in absence and deficiency of Mg^{2+}. Evidence showed that excessive concentration of Mg^{2+} could have a lower effect on PSII activity if compared to decreasing concentration of Mg^{2+}. In addition, Mn deficiency resulted in a decrease in the number of chloroplasts in the leaves of pecan, but no significant effect on PSII activity was detected (Henriques, 2004). Compared to this study, higher PSII activities were found due to differences in organism and stress factor.

In conclusions; this study demonstrated that different concentrations of Mg^{2+} and Cu^{2+} had a profound influence on production of biomass, Chl *a*, CPC, APC, carotenoids, proline, LPO levels and PSII activity of *A. platensis*. The highest levels of CPC and APC were obtained at control condition of Cu^{2+} (0.5 µM) while the highest β-carotene level (2400±60 µg mL^{-1}) was detected in the absence of Cu^{2+} leading to stress on the *A. platensis*. In addition, increasing Mg^{2+} concentration supported carotenoids production while the lack of Mg^{2+} resulted in lower value of carotenoids as 1464.1±51 µg mL^{-1}. As far as we are concerned, only a few studies have been carried out on effects of Mg^{2+} concentrations on levels of carotenoids produced by *A. platensis*. In this study, the biomass and carotenoids produced can be used in a variety of industrial fields such as food, fisheries, cosmetics and pharmaceuticals. As a result, it could be concluded that increase in both proline and LPO levels with rising metal ion concentration points to a correlation between free radical generation and proline accumulation. Even though PSII activities were affected by Mg^{2+} and Cu^{2+} concentrations, they were affected seriously by the absence of Mg^{2+} compared to lack of Cu^{2+} condition. On the contrary, PS II activities caused a decrease in excessive amounts of Cu^{2+} concentration compared to excessive amounts of Mg^{2+} concentration. This present study indicates that different concentrations of Mg^{2+} and Cu^{2+} had important roles on biomass and photosynthetic pigment-metabolites as well as photosystem II activity in *A. platensis*.

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