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THE EFFECTS OF DIFFERENT CONCENTRATIONS OF CADMIUM ON THE GROWTH RATE AND BETA-CAROTENE SYNTHESIS IN UNICELLULAR GREEN ALGAE *DUNALIELLA SALINA*[*]

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Abstract – The individual effects of cadmium concentrations (0, 0.005, 0.05and 0.5 mg. L^{-1}) on the growth (cell number and chlorophyll content) and beta-carotene synthesis of two strains (Iranian and Australian) of green algae *D.salina* have been studied for a duration of 36 days. The effects of these cadmium concentrations on the amount of cell magnesium and calcium have also been evaluated. An increase in cadmium concentration resulted in a significant reduction in the cell number and chlorophyll content of *D. salina*. In comparison with control, in both strains, the increase of beta-carotene content of cells was observed in the first few days of the experiment, moreover, the amount of magnesium and calcium in cells decreased. The results indicated that the addition of 0.5 mg. L^{-1} of cadmium in the medium results in a 50 percent reduction in cell number after 96h. The decrease in growth rate and the increase in beta-carotene content of cells is probably due to the formation of free radicals and deficiency of essential elements such as magnesium and calcium, which is caused by high concentrations of cadmium in the medium.

Keywords – *Dunaliella salina*, cadmium, heavy metal, beta-carotene, chlorophyll, free radical, antioxidant, magnesium, calcium

1. INTRODUCTION

Dunaliella is an unicellular green algae [1] that belong to the *chlorophyceae* family [2]. The algae that belong to this genus contain no cell wall and thus have been found to change rapidly in the extra cellular osmotic pressure. These algae are able to survive in media containing a wide range of NaCl concentrations ranging from about 0.05M to 5.5mM [3]. Some species of *Dunaliella* such as *Dunaliella salina* can produce large amounts of beta-carotene in response to stresses such as high light intensity, high concentrations of NaCl, nutrient deficiency, etc. [1]. *Dunaliella* is also used as a model system in plant physiology studies [4]. Carotenoids, especially beta-carotene, are the most important and the most abundant pigments in nature, and only plants, algae and microorganisms can produce them naturally [5]. Beta-carotene is very important in photosynthesis, pharmacology and nutrition industries [1, 5]. Heavy metals are important pollutants in water [6], which are toxic for marine organisms including phytoplanktons and algae [7]. Metals not only affect algal cells by disturbing metabolic processes directly, but can also influence algal growth indirectly through the decrease of essential elements [6]. Cadmium is a heavy metal that is widespread in waters and relatively toxic for phytoplanktons, but is not an essential element for algae and plants [7], and can be toxic for organisms [6]. Studies in plants have shown that metal ions such as copper, manganese and iron can produce free radicals that are very toxic for cells [8]. Also, there are some reports about oxidative damage caused by cadmium in animal cells [9]. Moreover, studies have shown that cells produce beta-carotene against the destructive effects of free radicals [5]. It

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has been thought that cadmium ions can also produce free radicals [9], which in turn, as a stressor, can stimulate beta-carotene synthesis in *D. salina*. This study has been carried out for better understanding of beta-carotene synthesis in *Dunaliella*.

2. MATERIALS AND METHODS

The Australian strain of *D. salina* was a gift from the Dr. R. McC. Lilley collection at the University of Wollongong in Australia. The Iranian strain of *D. salina,* isolated from the salt marsh of Gavekhoni [10, 11], was obtained from the biology department of the University of Isfahan. These strains were cultured in a modified [12] Johnson's growth medium [13] containing 5mM KNO_3, 5mM $MgSO_4$, 0.2mM $CaCl_2$, 0.2mM KH_2PO_4, 2+5μM $FeCl_3$ + Na_2EDTA, 7mM $MnCl_2$, 1μM $CuCl_2$, 1μM $ZnCl_2$, 1μM $(NH_4)_6MoO_{24}$, $4H_2O$, 25mM $NaHCO_3$ and 1M NaCl at pH 7.5 and different concentrations of cadmium (0, 0.005, 0.05 and 0.5 mg. L^{-1}). Cadmium was added as $CdCl_2$. The cultures were illuminated at a light intensity of $100\mu E.m^{-2}.s^{-1}$ for a light/dark regime of 16/8 at 27±2˚C. Light intensity was measured by Quantum Sensor QSPAR Hansatech, UK. The test was carried out in sterile conditions in a mid-logarithmic phase. Algal suspensions were inoculated into a new culture medium at concentrations of 24×10^4 cells. mL^{-1} [14]. Ten mL of cell suspension was taken at two day intervals for 36 days of the test duration. The cell number was determined by counting fixed cells in a homocytometer [15]. Total chlorophyll and beta-carotene content were measured by a spectrophotometer (Pharmacia LKB, VASPECII, UK) at 412, 431, 460 and 480nm wavelengths [16]. Data were organized based on four experiments. For measuring magnesium and calcium content of the cells, on day 15, 3mL of algal suspension was centrifuged (SIGMA 3E-1) at 5000 rpm for 5 minutes and the pellet washed with an isotonic medium [14]. The procedure was repeated and finally the pellet dissolved in nitric acid [17]. Then magnesium and calcium contents were measured using an atomic absorption spectrophotometer (Perkin Elmer, 2380, USA). Morphological changes in algal cells also were investigated using a light microscope.

3. RESULTS AND DISCUSSION

Cadmium is widespread in waters and relatively toxic to marine organisms including phytoplanktons [6, 7, 18]. Studies have shown that cadmium affects cells through variation in secondary messengers, the level of uptake and gene expression [19]. The aims of this research were to study physiological indices (cell number and chlorophyll content) and beta-carotene content of *D. salina* cells exposed to cadmium.

Concentrations of cadmium used in this work were not lethal, but they did affect growth rate. Also the light microscope observation indicated that there were no changes in cell sizes during experiments. Figures 1a and 1b show the effect of different concentrations of cadmium (0.005, 0.05 and 0.5 mg. L^{-1}) on the growth rate (cell number) of Australian and Iranian strains of *D. salina.* Results indicate that, at all three concentrations of cadmium, the growth rate is lower than the control. At the highest concentration of cadmium (0.5 mg. L^{-1}) there is a 50% reduction in cell number after 96h. Pistocchi *et al.,* [18] have shown that increasing the concentration of cadmium to 0.5 mg. L^{-1}, is concomitant with the reduction of growth rate of some algae. Table 1 also shows the effect of cadmium on cell content of magnesium and calcium. After 15 days of cadmium treatment, magnesium and calcium content of the cells have decreased significantly (using t-test, p>5%). The reduction was more significant at the highest concentration of cadmium in the medium. It seems that such a decrease in the growth rate is due to a disorder in the uptake of essential elements such as magnesium and calcium, caused by the existing cadmium in the medium, which in turn affects both cell division and the metabolism of the cells. In both Australian and Iranian strains (Figs. 1a and 2b), at all three concentrations of Cd^{+2} in the medium, the total chlorophyll content of the cells decreased after five days, in comparison with the control group. In general, results (Figs. 1 and 2)

show that the presence of cadmium in the medium disturbs chlorophyll biosynthesis and decreases the chlorophyll content of the cells. It seems that such fluctuations in the total chlorophyll content are caused by the deficiency of essential elements such as magnesium, which is necessary for chlorophyll biosynthesis [20]. It has also been suggested that heavy metals ions such as cadmium interact with cell membrane proteins, disturbing their permeability [21]. Also in *Anabaena* cadmium, photosynthetic pigments decrease a 96 hours [22].

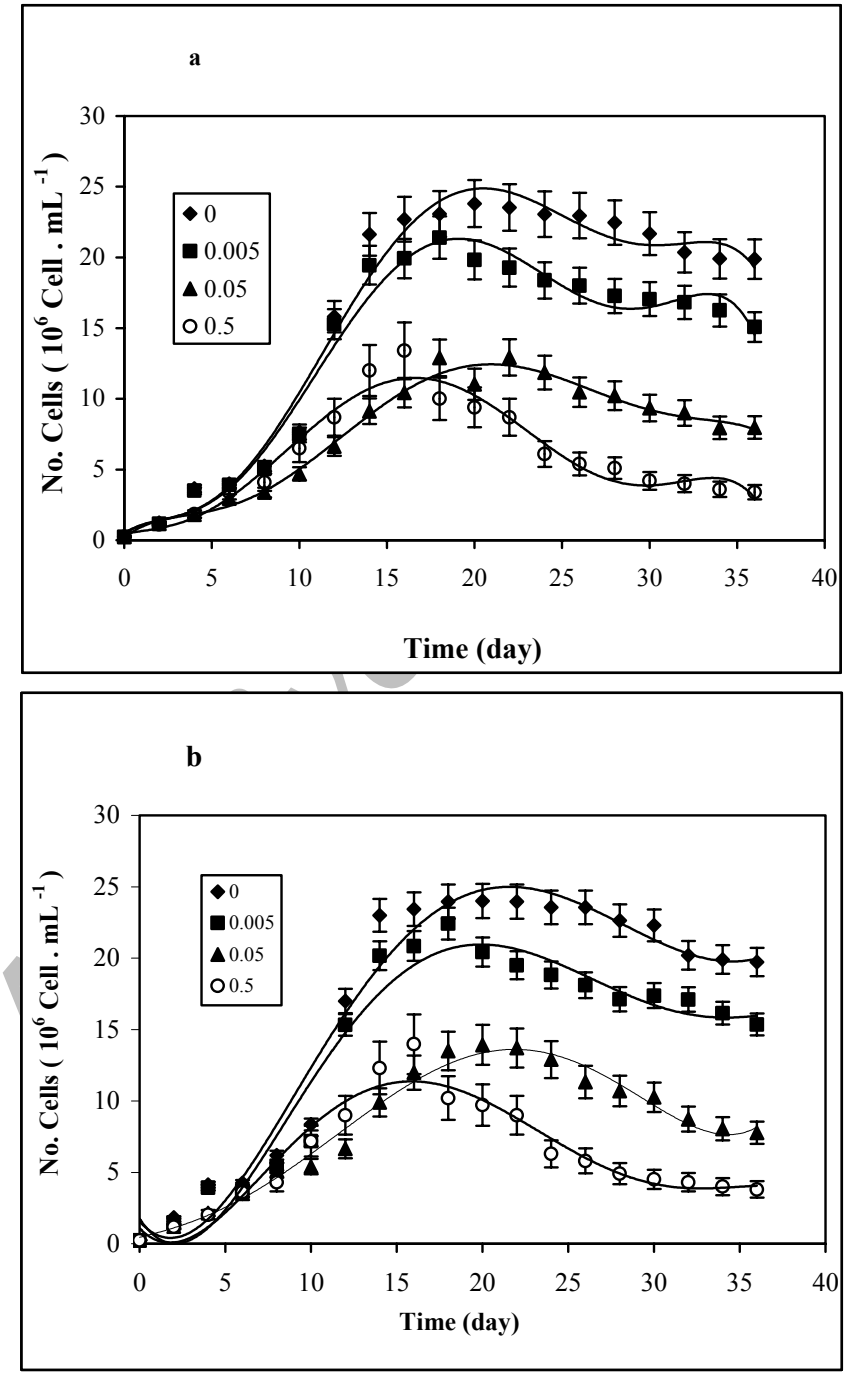

Fig. 1. Growth curve of two strains (a, Australian and b, Iranian) of *D.salina* at different concentrations of cadmium in the medium containing 1M NaCl. Each value is mean±std of four replicates

Table 1. Ca^{+2} and Mg^{+2} content of the cells in two strains (A, Australian and I, Iranian) of *D.salina* after 15 days exposure of the cells to different concentrations of cadmium in the medium containing 1M NaCl. Each value is mean±std of four replicates

Cd^{+2} concenration in the medium (mg . L^{-1})	mg . gr dw $^{-1}$			
	Ca^{+2}		**Mg^{+2}**	
	A	I	A	I
0	594±2	590±3	235±2	240±2
0.005	570±5	580±2	220±5	230±5
0.05	560±4	560±5	202±4	210±3
0.5	529±7	530±6	189±1	189±22

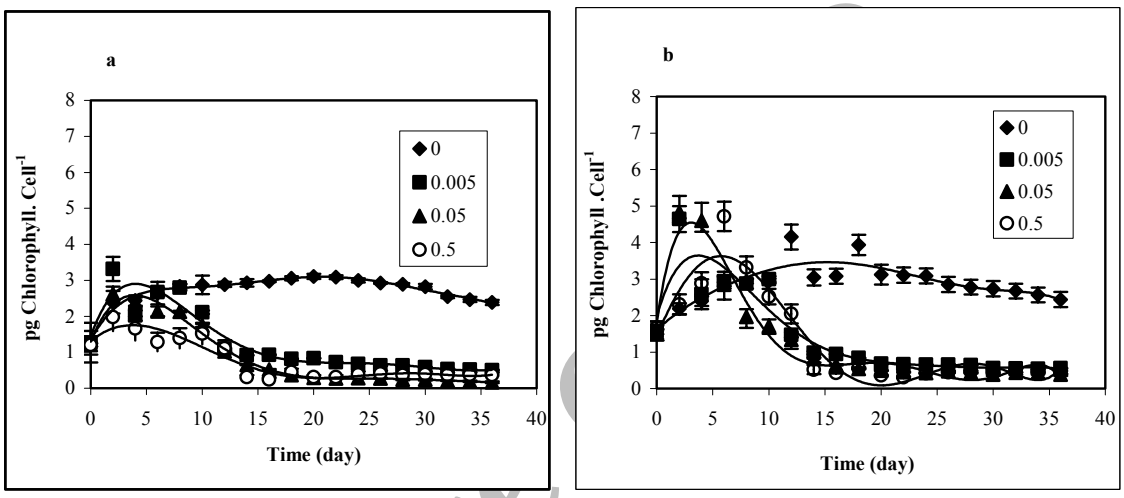

Fig. 2. The effect of different concentrations of cadmium on total chlorophyll content of the cells in two strains (a, Australian and b, Iranian) of *D.salina* in the medium containing 1M NaCl. Each value is mean±std of four replicates

Figure 3, shows the effect of different concentrations of cadmium on beta-carotene synthesis. In the Australian strain (Fig. 3a) beta-carotene content of the cells is higher in 0.5 mg. L^{-1} of Cd^{+2} in the medium is compared with control and remains high for 5 days. This increase in beta-carotene content is correlated with the increase in cadmium concentrations in the medium, and the order is 0.5 > 0.05, 0.005 > 0. In the Iranian strain (Fig. 3b), there is also a correlation between the amount of beta-carotene and concentration of cadmium. In the presence of higher concentrations of cadmium (in 0. 5 mg. L^{-1}) the level of beta-carotene of cells increases until day 5, and then decreases again. However, as it is shown in Fig. 3b, the rate of beta-carotene synthesis at 0.05 and 0.005 mg. L^{-1} of Cd^{+2} is not the same as the Australian strain (Fig. 3a) and the order for the Iranian strain is 0.5 > 0.005 > 0.05 > 0. It seems that these differences between two strains are related to genetic differences.

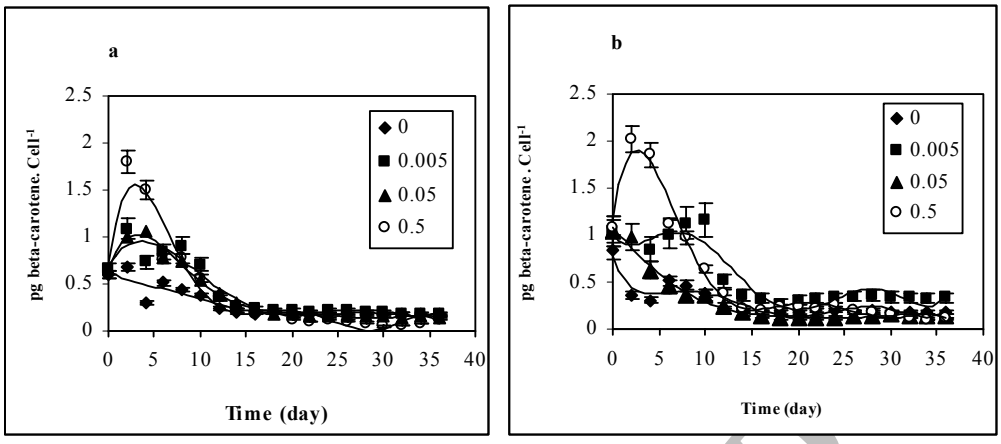

Fig. 3. The effect of different concentrations of cadmium on beta-carotene content of the cells in two strains (a, Australian and b, Iranian) of *D.salina* in the medium containing 1M NaCl.
Each value is mean±std of four replicates

In both strains, after day 5, the cell division is very high, thus beta-carotene content is diluted after this time for each cell division and its content is decreased per cell. After day 15, cells reach the stationary phase. After this time, in spite of our expectation, there is no increase in the beta-carotene content of cells. As mentioned earlier, by increasing concentrations of cadmium in the medium, the amount of cell magnesium and calcium decreased. Magnesium is an essential element for chlorophyll biosynthesis and calcium is necessary for cell growth and metabolism [8]. It has been reported that uptake of calcium and photosynthesis are inhibited by cadmium [7]. It seems that a slight increase in beta-carotene content of cells in the first few days of the experiment is due to the disordering in plasmallema functions, changing in absorption of other essential elements [21] and probably existing free radicals caused by cadmium ions. Both strains have a similar behavior. Figure 4 shows the effect of different concentrations of cadmium on the ratio of beta-carotene to chlorophyll in both strains. In both strains, in the first few days of the experiment, this ratio at 0.5 mg. L^{-1} of Cd^{+2} was higher than the others, but during the rest of the experiment there were no significant differences. Although the ratio of beta-carotene to chlorophyll content of the cells is a good indicator of some stress responses in *D. salina*, it seems that there are no clear responses in our experimental condition.

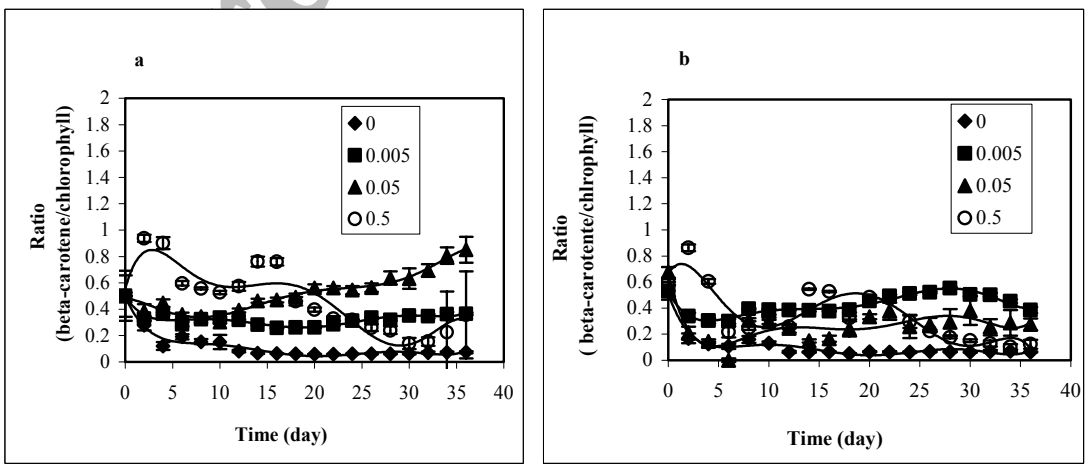

Fig. 4. The effect of different concentrations of cadmium on beta-carotene / chlorophyll ration of the cells in two strains (a, Australian and b, Iranian) of *D.salina* in the medium containing
1M NaCl. Each value is mean±std of four replicates

In conclusion, cadmium is a metal inhibitor of cell growth (cell division) and chlorophyll biosynthesis in *D. salina*. Also, we might suggest that, *D. salina* cells produce the beta-carotene to prevent cell damage, probably from free radicals and deficiency of essential elements resulting from high concentrations of cadmium in the medium. Also the decrease in beta-carotene content of cells after a few days and the decline again after cessation of the cell division is probably due to other unknown mechanisms in response to cadmium stress by *D. salina* cells such as synthesis of phytochelatins, or an increase in the activity of antioxidant enzymes. It is reported that *chlorella sp*. can produce phytochelatins in response to cadmium [23]. It has also been reported that unicellular algae *Gonyaulax polyedra* exposed to the acute stress of Cd^{+2} in the medium exhibit beta-carotene synthesis [24]. In contrast, cells chronically subjected to Cd^{+2} display a high activity of the antioxidant enzymes with no significant changes in the beta-carotene content of the cells.

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