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Preliminary studies on a halotolerant alga: *Dunaliella* sp. from Kuwait salt marshes

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ABSTRACT

Chemical analyses of brine ponds in the Al-Khiran area were carried out. The salinity of the ponds was found to be approximately four times that of sea water. A red form of *Dunaliella* sp. was found to grow in these ponds under extreme conditions of high light intensities ($1700\,\mu E\,m^{-2}\,s^{-1}$), high temperature (50°C) and high ranges of salinities (up to saturation and crystallization of salts). Changes in the pigmentation occurred upon transfer of the organisms from field to laboratory conditions. The effect of various concentrations of $NaNO_3$, NH_4Cl, $Ca(NO_3)_2$, NH_4NO_3, and NaCl on the growth of *Dunaliella* sp. was studied.

INTRODUCTION

Various species of the eukaryotic alga, genus *Dunaliella*, are widespread in saline habitats and a number have been described by Butcher (1959). These species occur, however, in diverse habitats varying from almost fresh water to salt-saturated ground. Great interest in this genus has arisen because of its ability to withstand various environmental stresses, especially those associated with halophilic conditions (Brock 1975).

In Kuwait there are a number of algal mats within various salt marshes (Fig. 1). Such marshes are exposed to extreme environmental conditions, particularly during the summer months when the temperature may reach 50°C with a relative humidity ranging between 1 and 98%. Under such extreme conditions these salt marshes become dry on the surface but saturated with brine underneath and the salt assumes visible red colouration due to large accumulations of the algae.

Here a preliminary work on the genus *Dunaliella* in Al-Khiran area in Kuwait and the effect of sodium chloride and various inorganic nitrogen sources on its growth is described.

MATERIALS AND METHODS

FIELD SAMPLE COLLECTION

Samples were collected in 500 cm^3 clean sterile containers from brine pond water and ground water found in salt marshes in the Al-Khiran area (Fig. 1), and transferred to the laboratory for immediate testing.

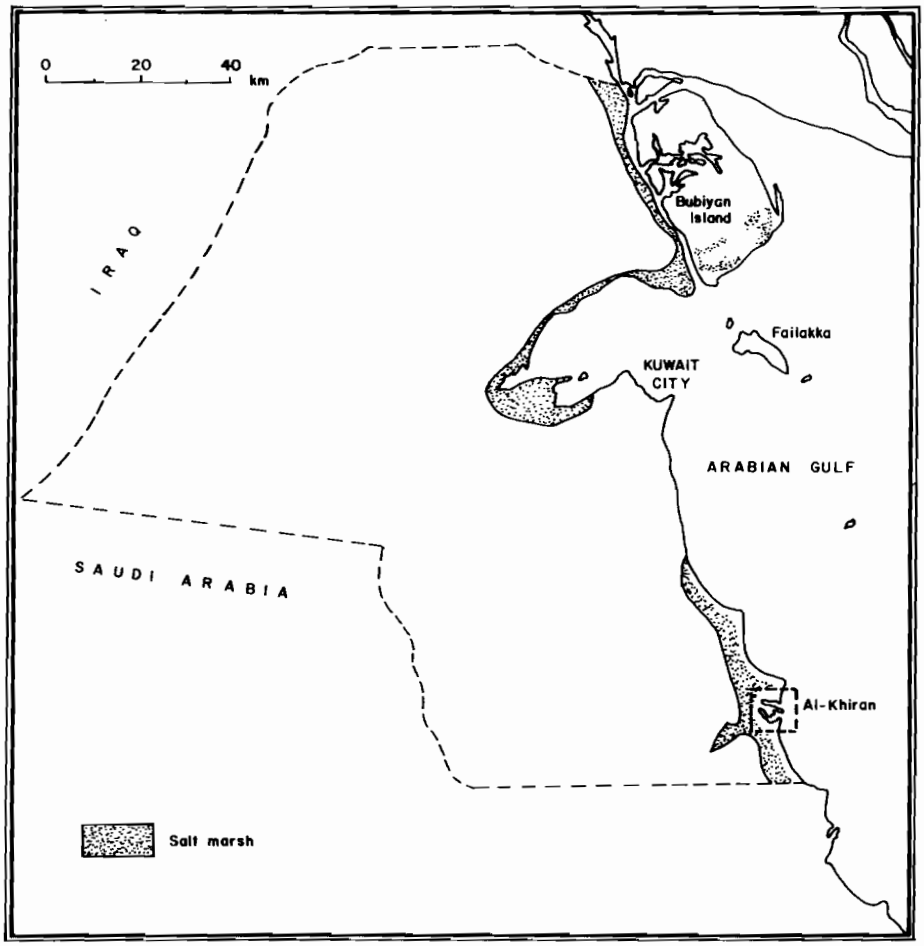

Fig. 1. Map of Kuwait showing Al-Khiran area.

CHEMICAL ANALYSIS OF FIELD SAMPLES

Samples from both brine ponds and ground water were analysed for Ca^{2+}, Mg^{2+}, Na^+, K^+, Cl^-, SO_4^{2-}, NO_3^-, CO_3^{2-} and HCO_3^-. All analyses were made in duplicate according to the methods of the American Public Health Association (1975).

IDENTIFICATION

Various species of algae present in the samples collected were identified after Desikachary (1959) and Hendey (1964). The isolated *Dunaliella* sp. matches the description of *Dunaliella bardawil* given by Ben-Amotz *et al.* (1982).

GROWTH CONDITIONS AND MEASUREMENTS

Dunaliella sp. was grown non-axenically in the laboratory using Allen and Arnon medium (Allen & Arnon 1955) containing 5% (w/v) NaCl. The effects of certain

nitrogenous compounds such as $NaNO_3$, NH_4Cl_2, $Ca(NO_3)_2$ and NH_4NO_3 on the growth of *Dunaliella* were determined in duplicate using the following concentrations of nitrogen: 0, 10, 20, 30, 40, 50 mg $N\,l^{-1}$ in 250 cm^3 conical flasks containing 100 cm^3 nitrogen-free medium of Allen and Arnon.

All cultures were shaken at 100 rpm in an illuminated orbital shaker under constant illumination of 61 $\mu E\,m^{-2}\,s^{-1}$ at 30°C.

The growth of *Dunaliella* sp. was estimated spectrophotometrically as chlorophyll *a* concentration according to the method of Kirk (1967).

RESULTS AND DISCUSSION

Two types of brine ponds were observed in the salt marshes of Al-Khiran: red coloured ponds which were dominated by *Dunaliella* sp. and green coloured ponds which showed growth of the blue-green algae *Spirulina* sp., *Lyngbya* sp., *Calothrix* sp. and *Synechocystis* sp. Pennate diatoms were also present as well as the actively grazing *Artemia salina* which most probably affected the abundance of *Dunaliella* sp. in the green ponds.

Chemical analyses of water samples from brine ponds, where *Dunaliella* sp. was growing, showed a high concentration of Ca, Mg, Na and Cl compared with that of sea water (Table 1). Similar analyses of sabkha brine were reported by Bush (1970). Differences in salt concentrations between ground water and brine pond water are mainly attributed to the climatic conditions, especially during summer where the maximum temperature reaches 50°C. However, mineralogical analysis of these ponds indicated that gypsum ($CaSO_4$) is the major salt present. The salinity of these ponds showed some variation and was approximately four times that of sea water (Table 1).

The tolerance of *Dunaliella* sp. to high salt concentrations, which may reach saturation or salt crystallization due to evaporation of water, has been noticed in samples taken from a sun-dried salt pond which showed actively growing cells. This is in agreement with the findings of other workers (Hof & Fremy 1933; Johnson *et al.* 1968; Brock 1975).

Table 1. Chemical analysis of water samples from the Al-Khiran area (in ppm)

Components	Al-Khiran area brine pond	Al-Khiran area ground water	Sea water
Ca^{2+}	4,000	2,800	600
Mg^{2+}	6,240	660	1,500
Cl^-	132,238	29,288	22,000
CO_3^{2-}	240	N.T.*	N.T.*
HCO_3^-	305	427	N.T.*
NO_3^-	29·9	N.T.*	N.T.*
K^+	2,847	585	399
Na^+	45,500	12,650	11,000
SO_4^{2-}	3,500	2,700	2,712
% Salts	23·04	5·76	N.T.*
pH	7·2	7·6	7·5

* N.T. = not tested.

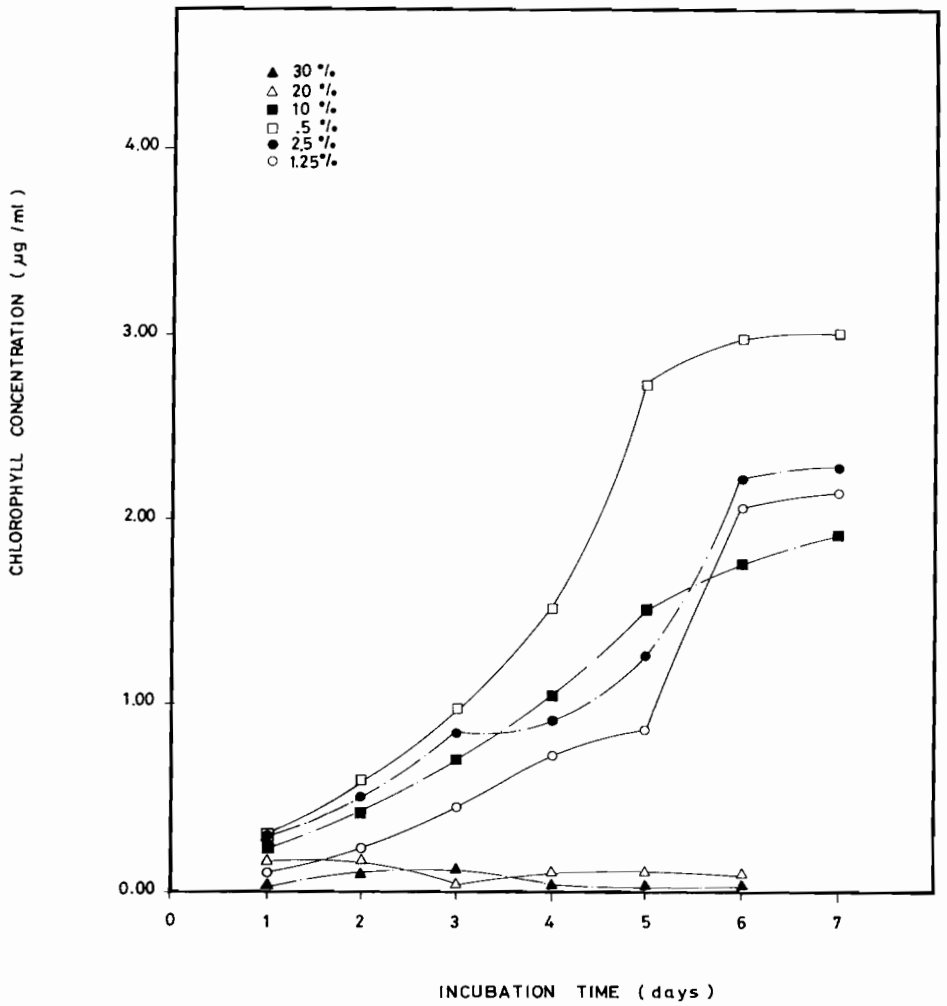

Fig. 2. Effect of various concentrations of sodium chloride on the growth of *Dunaliella* sp. using Allen and Arnon medium under constant illumination of 61 $\mu E\,m^{-2}\,s^{-1}$ at 30°C.

Dunaliella sp. transferred from brine ponds, was cultivated in Allen and Arnon medium and the effect of various concentrations of NaCl (0–30%) under a constant illumination of 61 $\mu E\,m^{-2}\,s^{-1}$ at 30°C was studied. The optimum growth was obtained at 5% NaCl (Fig. 2). Loeblich (1969) found that 0–8% NaCl with an optimum of 4% was the best concentration of NaCl for the growth of *Dunaliella salina*. However, a decrease in the growth of *Dunaliella* sp. under laboratory conditions was noticed using 20 and 30% NaCl (Fig. 2). A slower rate of growth of *Dunaliella salina* at high salinities was attributed by Loeblich (1970) to an increase of carbonic anhydrase activity. The effect of NaCl was also found by Ginzburg & Ginzburg (1981) to vary according to the light intensity, temperature and CO_2 concentration in the medium.

The effect of certain nitrogenous compounds such as $NaNO_3$, NH_4Cl, $Ca(NO_3)_2$ and NH_4NO_3 on the growth of *Dunaliella* sp. was also tested (Figs 3–6). The highest

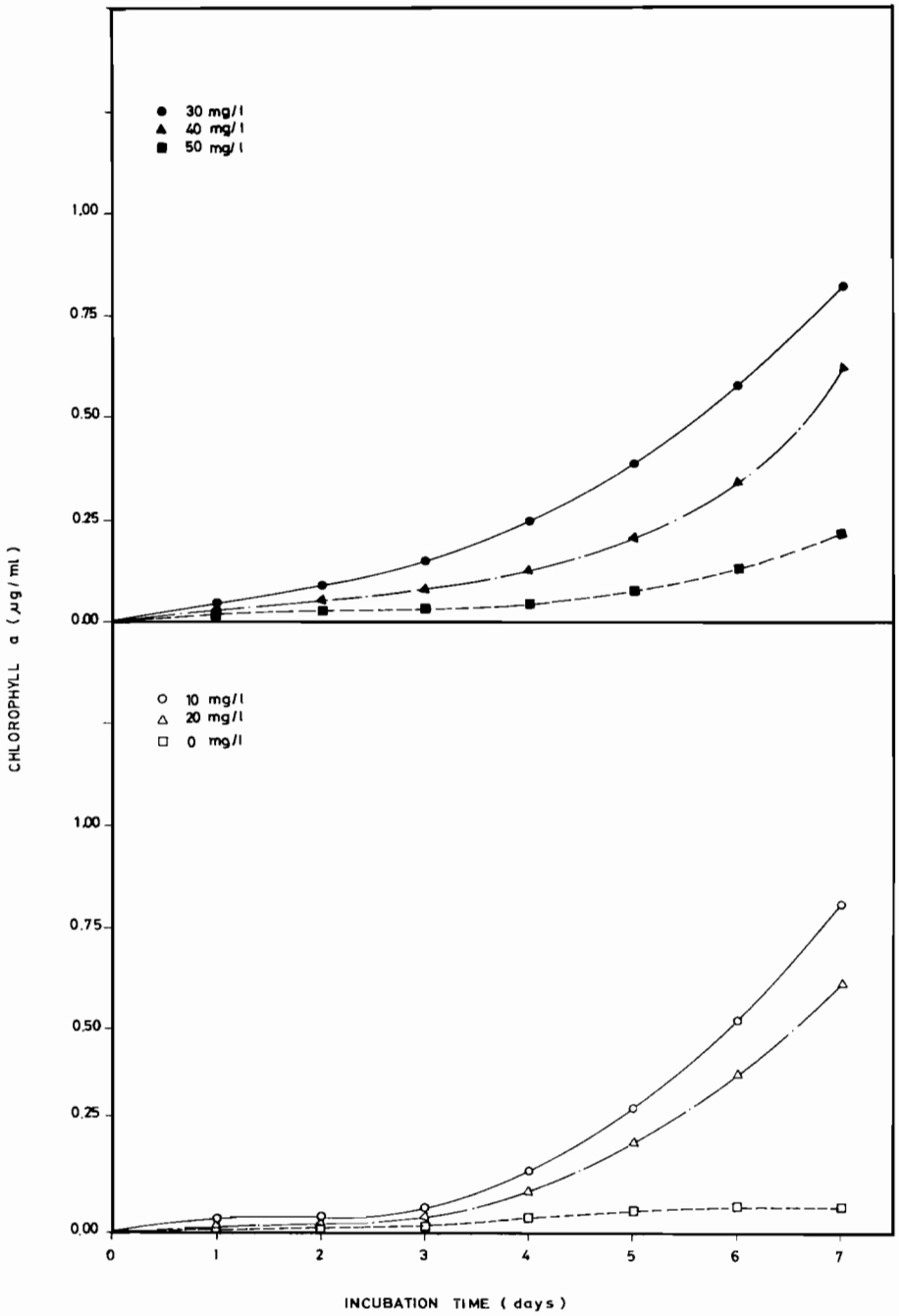

Fig. 3. Effect of various concentrations of $NaNO_3$ on the growth of *Dunaliella* sp. using Allen and Arnon medium containing 5% NaCl under constant illumination of 61 $\mu E\,m^{-2}s^{-1}$ at 30°C.

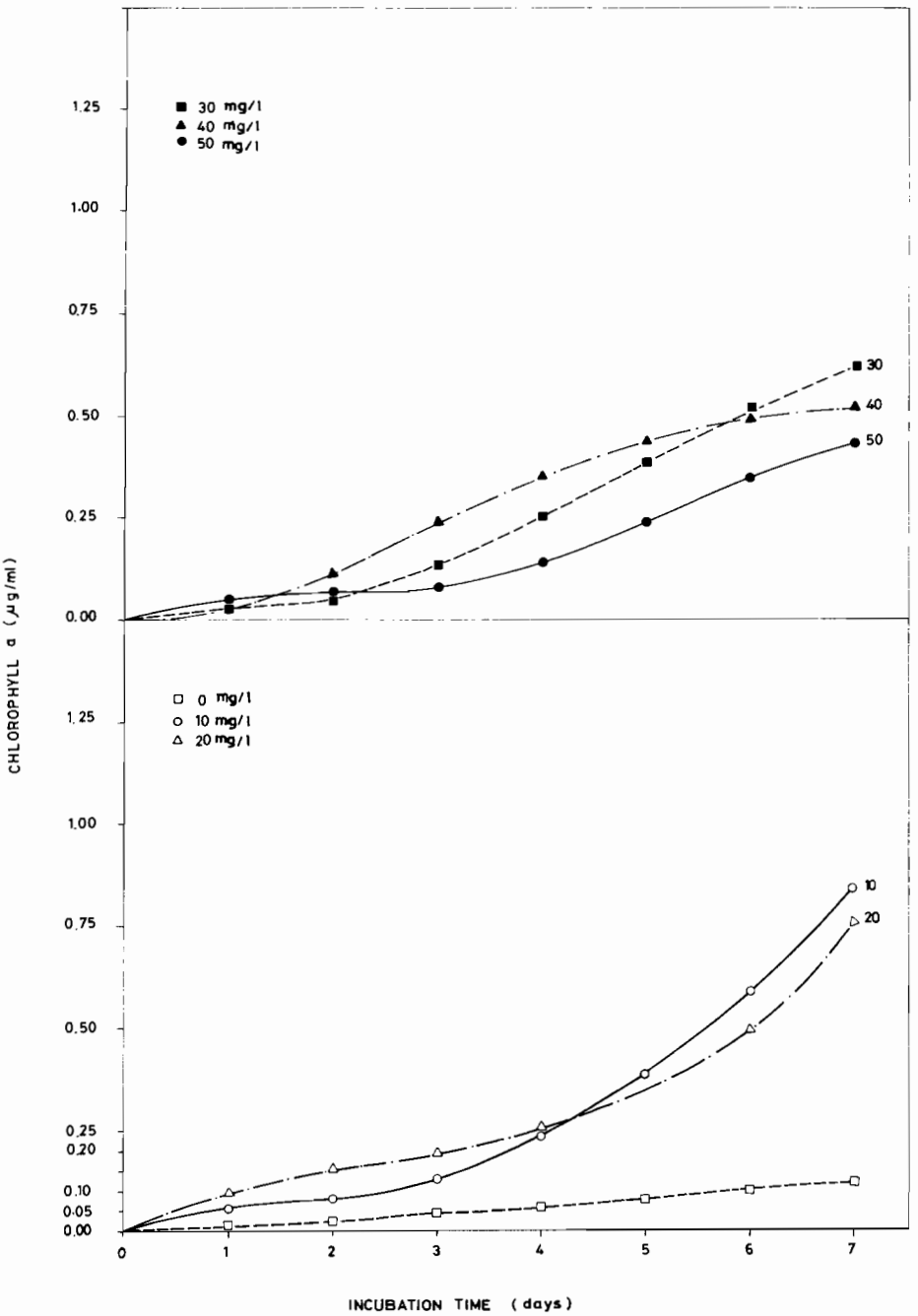

Fig. 4. Effect of various concentrations of NH_4Cl on the growth of *Dunaliella* sp. using Allen and Arnon medium containing 5% NaCl under constant illumination of 61 $\mu E\,m^{-2}\,s^{-1}$ at 30°C.

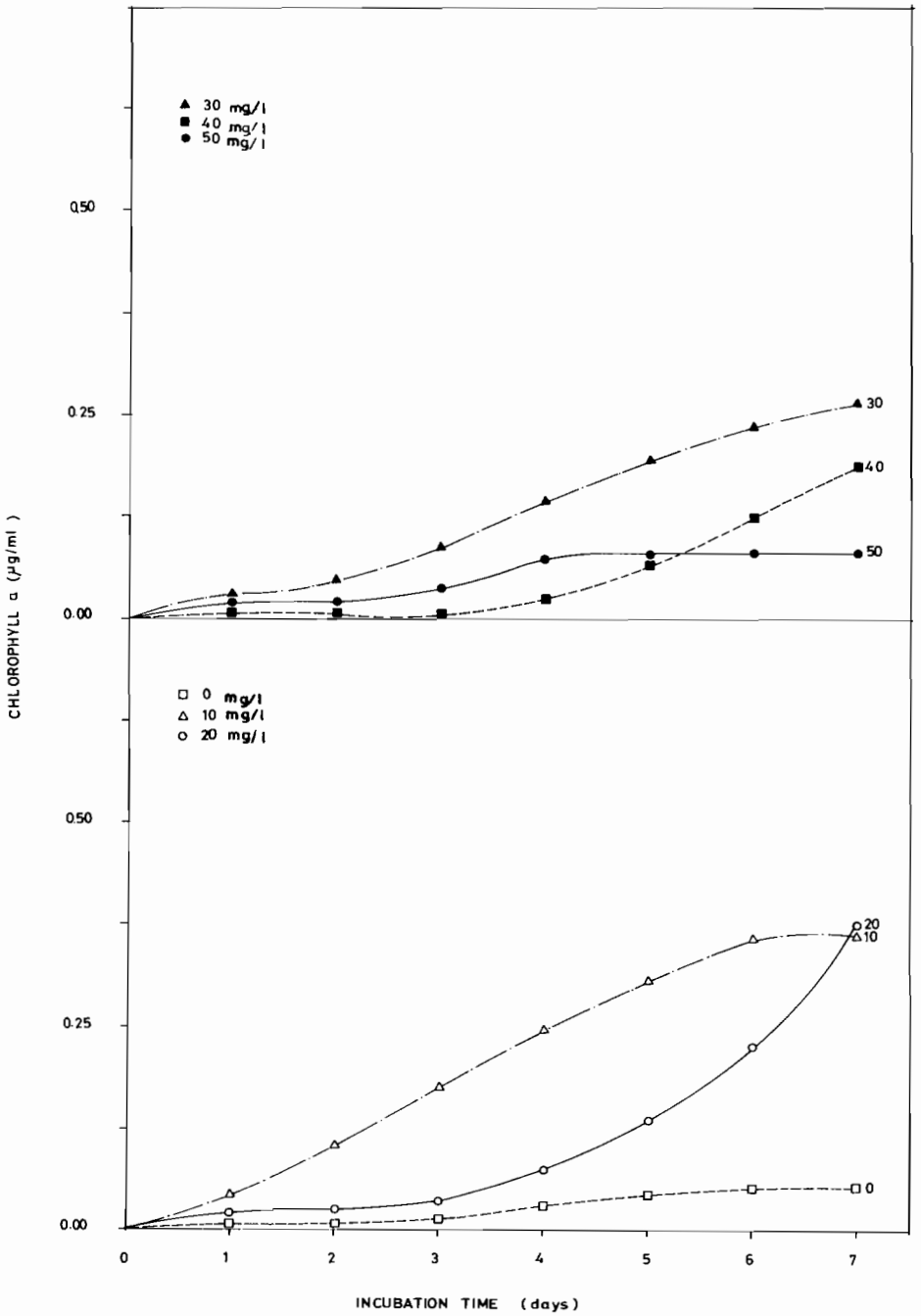

Fig. 5. Effect of various concentrations of NH_4NO_3 on the growth of *Dunaliella* sp. using Allen and Arnon medium containing 5% NaCl under constant illumination of 61 $\mu E\,m^{-2}s^{-1}$ at 30°C.

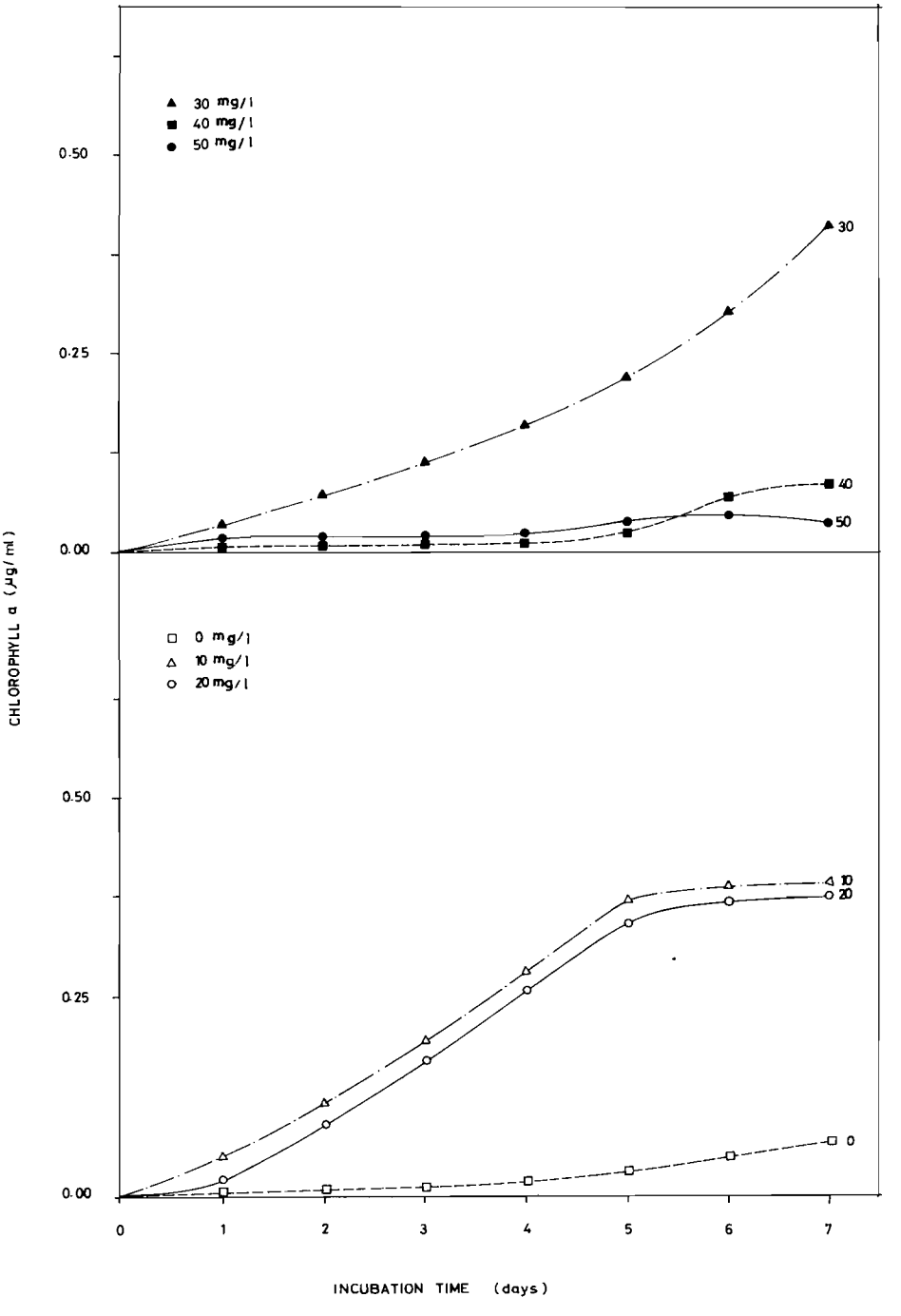

Fig. 6. Effect of various concentrations of $Ca(NO_3)_2$ on the growth of *Dunaliella* sp. using Allen and Arnon medium containing 5% NaCl under constant illumination of 61 $\mu E\,m^{-2}\,s^{-1}$ at 30°C.

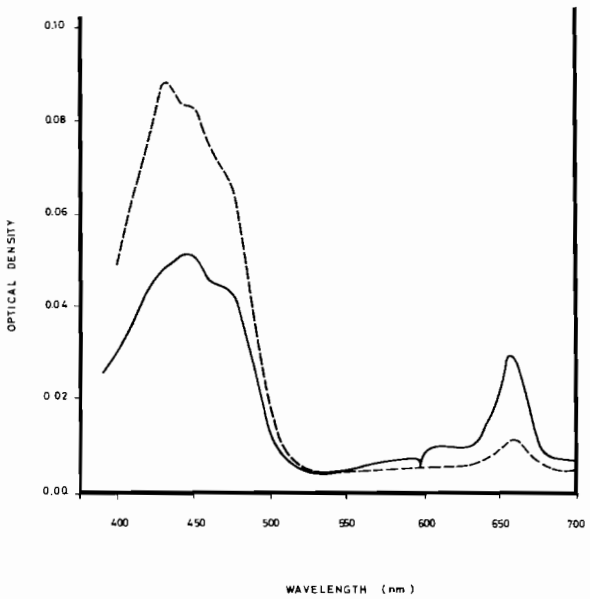

Fig. 7. Absorption spectra of 80% acetone extract of a red form (---) and a green form (——) of *Dunaliella* sp.

growth of *Dunaliella* sp. in all the nitrogenous compounds tested was found to be between 10 and 20 mg Nl^{-1}. However, NH_4Cl and $NaNO_3$ enhanced a better growth of *Dunaliella* sp. compared with $Ca(NO_3)_2$ and NH_4NO_3.

Upon the transfer of the red-coloured *Dunaliella* sp. from the brine pond to the laboratory growth conditions, a remarkable change in the colour of the alga from red to green was observed. This is most probably due to changes in light intensity, temperature and salinity which were lower under laboratory conditions (see growth conditions) than in field conditions. Ginzburg & Ginzburg (1981) found that certain halophilic varieties of *Dunaliella* (red *Dunaliella*) remained green at 4000 Lux, turned bright yellow and dark brown outside at 16,000 Lux due to the production of more carotenes. A sample of red *Dunaliella* which was transferred to the dark at 10°C, maintained its colour for several weeks but did not grow. Ben-Amotz & Avron (1983) found that under various stresses of growth conditions, such as increasing light intensities, nutrient deficiency or high salt concentrations, chlorophyll content of the algal cells decreases while β-carotene increases. As a result, the green coloured culture turned to deep orange. Clear differences were observed in the pigmentations of the red and the green forms of *Dunaliella*. The influence of chlorophyll *a* and *b* on the absorption spectrum of an 80% acetone extract of the red *Dunaliella* was significantly lower than that of the green *Dunaliella* (Fig. 7). However, the ratio of carotenoid to chlorophyll concentration was much higher in the red form than in the green form of *Dunaliella* sp. Ben-Amotz *et al.* (1982) found that the absorption spectrum of an 80% acetone extract of purified globules from *Dunaliella bardawil* produced maxima at 452 and 478 nm which indicates the presence of large amounts of β-carotene. Studies on the ultrastructure of the red and green forms of *Dunaliella* sp. revealed that in the red form the lamellae of the chloroplast were degenerated and masked with numerous

osmiophilic bodies, while the green *Dunaliella* had well-developed lamellae and very few osmiophilic bodies could be observed in the cell (Jafri & Al-Hasan 1982).

The great diversity and adaptability of this organism is of scientific and economic importance. *Dunaliella* is being considered as a source of glycerol and carotenoid production on a commercial level. Therefore, further ecological, physiological and biochemical studies are required.

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دراسات اولية على طحلب يتحمل الملوحة من نوع دونااليلا من مستنقعات الكويت المالحة

رضا الحسن وعبدالكريم السلال
قسم علم النبات والميكروبيولوجيا بجامعة الكويت

خلاصة

أجريت دراسات اولية على طحلب من نوع دونااليلا (أحمر اللون) والذي ينمو في برك مالحة بمنطقة الخيران تحت ظروف بيئية قاسية متميزة بشدة الضوء والحرارة وكذلك الملوحة العالية التي قد تصل إلى درجة التشبع .

وقد أظهرت التحاليل الكيميائية لمياه البرك أن ملوحتها تعادل أربعة اضعاف ملوحة مياه البحر . وقد أدى نقل هذا الطحلب من بيئته الطبيعية وانمائه تحت ظروف مخبرية إلى حدوث تغيرات في المواد الصبغية الموجودة فيه . وقد تطرق البحث إلى دراسة تأثير بعض المركبات النيتروجينية غير العضوية مثل نترات الصوديوم ، وكلوريد الامونيوم ، ونترات الكالسيوم ، ونترات الامونيوم وكلوريد الصوديوم على نمو الطحلب .