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GROWTH AND METABOLIC RESPONSE OF THE FRESH WATER UNICELLULAR ALGAE SCENEDESMUS DIMORPHUS, S. QUADRIQCAUDA AND ANKISTRODESMUS FALCATUS TO SALINE ENVIRONMENT.

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ABSTRACT

The effect of salinity on the growth and chemical constituents of the freshwater chlorophytes **Scenedesmus dimorphus** (Trup.) Kuetzing, **Scenedesmus quadricauda** (Trup.) de Brebisson and **Ankistrodesmus falcatus** (Corda) Ralfs are investigated. The results indicate a wide range of responses of the test algae and the wide range of responses of the metabolic dopts within the same species. The growth tolerance of the test algae to salinity increases with increasing exposure time. The 96-h EC_{50} values, however, of salinity for depression of growth in g/l were: 2.25, 2.52 and 2.98 for S. **dimorphus**; 6, 6.5 and 6.64 for S. **quadricauda**; and 1.27, 2.52 and 1.7 for **A. falcatus** after 48, 72 and 96 h exposure, respectively. The dry weight per cell of all the test algae increases with increasing salinity. Furthermore, total chlorophyll a, b, total carotenoids, carbohydrate and protein content per cell of S. **dimorphus** progressively decreases with increasing salinity; the reverse is true with S. **quadricauda** and **A. falcatus** only at low salinity.

INTRODUCTION

The freshwater Lake Wadi El-Rayan is located in the Western Desert of Egypt, about 120 km southwest of Cairo. It has an area of about 12,000 hectares. Lake Wadi El-Rayan (0.65 ‰) will most likely repeat the history of Lake Quarun, i.e. its salinity is progressively increasing with time as a result of high evaporation rate. The increasing salinity of Lake Wadi-El-Rayan will affect the aquatic organisms inhabiting it especially the growth and productivity of phytoplankton representing the base of food-chain.

There are few available data dealing with effect of salinity on growth and metabolic activity of the freshwater algae. Efects of salinity on growth rates and chemical composition of marine algae have been reported by Craigie (1969). Effects of salinity on the rates of photosynthesis of marine algae were discussed by Vosjan and Siezen (1968). Adelman et.al. (1976 a,b) stated that sodium chloride is superior in static bioassay experiments to predict effect of salinity on the freshwater organisms. The correlation

between glycerol synthesis and formation of storage products in salinity adapted as well as NaCl-shocked **Dunaliella parva** cells has been investigated by Cimmler and Moller (1981). The toxic effect of NaCl on **Aerobacter aerogeneis** might be due, at least in part, to its interference with the uptake of magnesium by the growing organisms (Tempest et.al., 1967; Tempest and Meers, 1968). The chlorophyll content of the freshwater algae **Anabaena cylindrica** has been found to be affected by NaCl, decreasing with increase NaCl level in the medium (Brownell and Nicholas, 1967; Wetherell, 1963). Effect of NaCl on growth and chemical constituents of the freshwater chlorophyte **Staurastrum boreale** has been investigated by Ibrahim (1983).

The present experimental study was initiated to predict the effect of increasing salinity in Lake Wadi El-Rayan, by using NaCl, on growth and chemical constituents of the three chlorophytes isolated from the lake, namely **Scenedesmus dimorphus, Scenedesmus quadricauda** and **Ankistrodesmus falcatus.**

MATERIAL and METHODS

Test Algae

Water sample from Lake Wadi El-Rayan (0.65 ‰) was collected by plastic water sampler (Hydro-Bios cap. 1.7 L). This sample was enriched with 0.1 g/l $NaNO_3$, 0.02 g/l K_2HPO_4 and 10 mg/l Na_2SiO_3. 9 H_2O and exposed to light of 4000 lux intensity from "cool white" overhead fluorescent tubes, at 25±1°C for 4 days. The microscopic analysis revealed the dominance of **Scenedesmus dimorphus,** while **Scenedesmus quadricauda** and **Ankistrodesmus falcatus** were abundant. The three algal species were isolated into pure cultures according to the method recommended by Pringsheim (1946).

Culture Medium

The medium for both stock and test algal cultures was prepared by dissolving the following major salts in one litre of glass distilled water: 467 mg $NaNO_3$, 18.5 mg $CaCl_2.2H_2O$, 25 mg $MgSO_4$, 31 mg K_2HPO_4, 10.5 mg $NaHCO_3$ and 56 µg Fe^{2+} as $FeSO_4$. To these major salts, 1 ml of the following mixture of trace metals was added: 59 mg $CuSO_4$, 43 mg $ZnSO_4$, 13 mg KI, 15 mg KBr, 18.4 mg Na_2MoO_4, 40 mg $MnSO_4$, 56 mg $CoCl_2$, 91 mg $Na_2B_4O_7$ and 16.7 mg $NaWO_4$. They were all dissolved in 1 litre glass distilled water.

Algal Stocks

Algal stocks were maintained in 200 ml of culture medium in 500 ml Erlenmeyer flasks under the following conditions: light intensity 4000 lux from "cool white" fluorescent tubes; lighting cycle, 14 h light followed by 10 h darkness (14 L: 10 D), temperature 25±1°C.

Sterilization

All glassware and media were sterilized by autoclaving at 121°C and 1.1 kg/cm^2 for 15 minutes. Media were prepared on the day before a test

was to be conducted to allow for pH stabilization at 8.1

Salinity

Test salinities were prepared by adding the appropriate amount of analytical grade NaCl (Fisher Analar). The concentration of NaCl ranges from 0 to that inhibits growth of the respective test alga, namely more than 50 % of the control.

Bioassay Procedure

Algae were grown in 250 ml Erlenmeyer flasks with 100 ml of medium. Treatment and control flasks were inoculated with 5000 algal cells/ml in logarithmic growth phase.

Incubation

Cultures were incubated with vigorous agitation once a day to prevent clumping of the cells and consequent differential exposure to the test medium. The test duration was 96 h, the temperature was $25\pm1^{\circ}C$ and the photoperiod program within the incubator was 14 : 10 h, with a mean light intensity around the flasks of 4000 lux.

Measurements

After 48, 72 and 96 h incubation, four replicate flasks were used for the determination of population growth by counting the cells using a haemocytometer at 300 x magnification. Average values were calculated for all sets and EC_{50} (the calculated salinity which inhibits growth by 50 % of the control) was calculated by straight-line interpolation (APHA, 1975). Four replicate samples were also used for the determination of dry weight, chlorophyll a, b, total carotenoids, carbohydrate and protein contents.

Dry Weight

The dry weight of both the control and treated cultures was measured using Millipore filter paper of 0.45 µm pore size. Before use, filters were washed with distilled water and dried at $60^{\circ}C$ for 24 h, cooled in desiccator over $CaCl_2$ as desiccant and weighed.

The content of four replicate flasks for each treatment were filtered, washed with distilled water, dried cooled, and weighed. Blank samples containing only the NaCl in the medium were also filtered. The dry weight of the cells was calculated by subtracting the value of the blank from the corresponding value of the samples.

Chlorophyll a, b and Carotenoid Contents

Four replicate samples for each salinity gradient were filtered on Whatman glass microfibre filters. Chlorophyll a, b and total carotenoid contents were extracted with 90 % acetone according to Golterman and Clymo (1971). The chlorophyll a content was calculated according to Lorenzen's equation (1967), while chlorophyll b and total carotenoid were calculated according to the method of persons and Strickland (1963).

Total carbohydrate

Total carbohydrate was determined by adding 1 ml of 80% H_2SO_4 to the dry sample (using ice bath). After 20 h at 20°C the mixture was diluted to 7 ml in an ice bath (Myklestad and Haug, 1972). The total amount of carbohydrate in the solution was determined by the phenol sulphuric acid method using glucose as standard (Dubois et.al., 1956.).

Protein-N

Algal nitrogen was determined by the micro-Kjeldahl method (Hiller et.al., 1948). Protein-N content was calculated by multiplying the value of total nitrogen by 6.25.

Using the total cell number at the end of the experiments (96 h) the dry weight, chlorophyll a, b, total carbohydrate and protein contents were calculated on an average per cell basis.

RESULTS

Table 1 and Fig. 1 show that the test algae varied greatly in their response to salinity, while **S. quadricuda** was the most tolerant, **A. flacatus** was the most susceptible. The inhibitory effect of salinity on growth of the test algae decreased with increase in the exposure time. The EC_{50} values of salinity for inhibition the growth of the test algae in g/l were: 2.25, 2.52, and 2.98 for **S. dimorphus;** 6, 6.5, and 6.64 for **S. quadricauda** and 1.27, 1.57 and 1.7 for **A. falcatus** after 48, 72 and 96 h exposure, respectively.

The dry weight per cell of the test algae increased with increasing salinity in all the three species investigated; a phenomenon that was reversed with **A. falcatus** at only low salinity of 0.7 g/l (Table 2 and Fig. 1).

Table 3 and Fig. 1 show that the chlorophyll a, b and carotenoid contents per cell of **S. dimorphus** gradually decrease with increase in salinity. On the other hand, low salinities (2 and 4 g/l) stimulated the chlorophyll a content per cell of **S. quadricauda;** a phenomenon that was reversed at high salinities (6, 8 and 10 g/l). Moreover, the chlorophyll b content of **S. quadricauda** cells was still higher than its control value even at salinity of 8 g/l, but at the highest salinity of 10 g/l chlorophyll b decreased below its control value. Furthermore, salinities of 2, 4 and 6 g/l stimulated the total carotenoid of **S. quadricauda** cells, whereas 8 and 10 g/l caused the reverse effect. In case of **A. falcatus,** low salinity of 0.7 g/l increased its chlorophyll a, b and carotenoid contents per cell; the reverse was true at high salinities.

Table 4 and Fig. 1 reveal that low salinities of 1, 2 and 3 g/l caused moderate drop in the carbohydrate content of **S. dimorphus** cells, while the high

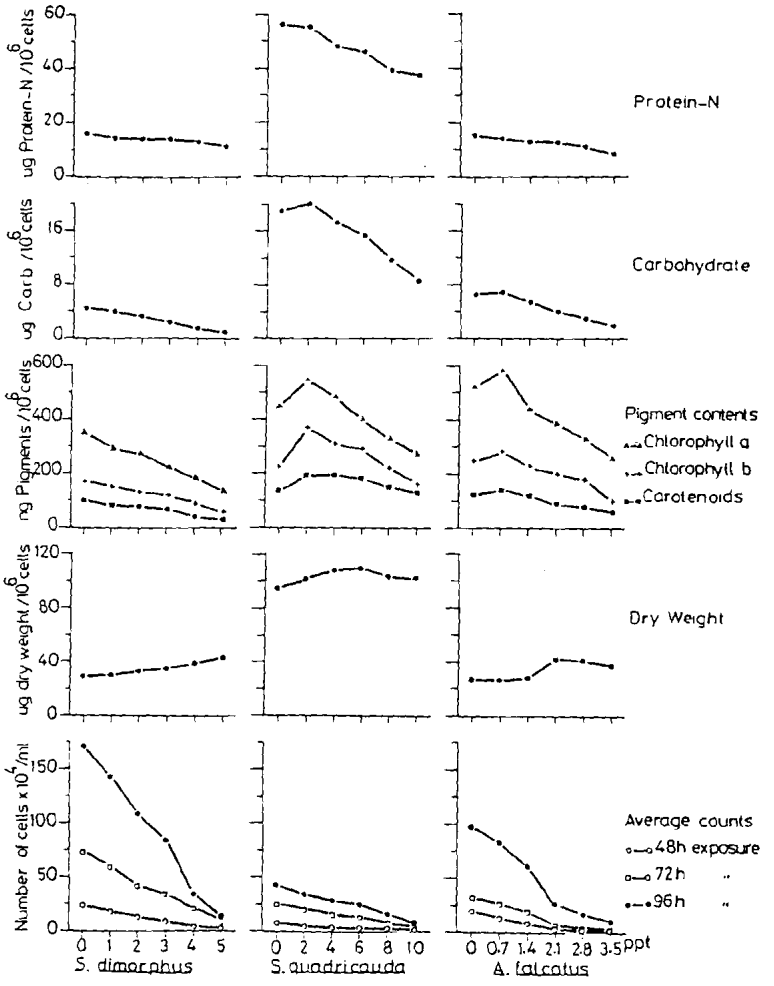

Fig. 1.

Growth and chemical constituents of the test algae grown in medium containing various concentrations of Na Cl (g. L^{-1}, ppt).

Table 1.
Effect of salinity (NaCl) on growth of Scenedesmus dimorphus, Scenedesmus quadricauda, and Ankistrodesmus falcarus after 48, 72, and 96 h exposure.

Test algae.	concentration of NaCl (ppt)	48 h				72 h				96 h			
		Average count (no.x10^4/ml)	Decrease %	Growth rate (K)	EC_{50} (ppt)	Average count (no.x10^4/ml)	Decrease %	Growth rate (K)	EC_{50} (ppt)	Average count (no.x10^4/ml)	Decrease %	Growth rate (K)	EC_{50} (ppt)
S. dimorphus	0	23.75±0.33	-	1.93		73.13±1.66	-	1.66		170 ±2.94	-	1.46	
	1	18.75±0.42	21	1.81		58.25±1.36	20.3	1.59		143 ±2.45	15.9	1.41	
	2	13.0 ±0.50	45.3	1.63	2.25	40.5 ±1.91	44.6	1.46	2.52	109.5 ±1.91	35.6	1.35	2.98
	3	8.75±0.30	63.2	1.43		33.5 ±1.29	54.2	1.40		84.75±1.5	50.1	1.29	
	4	4.59±0.20	81.0	1.10		21.5 ±1.11	70.6	1.25		34.95±0.82	79.4	1.06	
	5	2.10±0.20	91.2	0.72		9.0 ±0.64	87.7	0.96		12.8 ±0.65	92.5	0.84	
S. quadricauda	0	8.0 ±0.18	-	1.39		24.18±0.21	-	1.29		44.4 ±1.30	-	1.12	
	2	5.85±0.14	26.9	1.23		20.78±0.26	14.1	1.24		35.93±1.22	19.1	1.07	
	4	4.98±0.05	37.7	1.15	6	15.63±0.20	35.4	1.15	6.5	29.33±0.53	33.9	1.02	6.64
	6	4.0 ±0.12	50.0	1.04		13.88±0.17	42.6	1.11		25.95±0.46	41.5	0.99	
	8	2.6 ±0.10	67.5	0.82		7.05±0.13	70.8	0.88		17.05±0.27	61.6	0.88	
	10	1.4 ±0.03	82.5	0.51		3.4 ±0.12	85.9	0.64		7.43±0.18	83.2	0.67	
A. falcatus	0	19.85±0.65	-	1.84		32.33±0.62	-	1.39		98.2 ±1.27	-	1.32	
	0.7	13.75±0.82	30.7	1.66		26.18±0.66	19.0	1.32		85.0 ±1.2	13.4	1.28	
	1.4	8.72±0.5	55.8	1.43	1.27	19.0 ±0.16	41.2	1.21	1.57	62.7 ±1.3	36.1	1.21	1.7
	2.1	6.55±0.29	67.0	1.29		6.1 ±0.08	81.1	0.83		26.0 ±0.81	73.5	0.99	
	2.8	3.98±0.10	79.9	1.04		3.33±0.15	89.7	0.63		17.4 ±0.84	82.3	0.89	
	3.5	2.38±0.06	88.0	0.78		2.43±0.10	92.5	0.52		10.6 ±0.52	89.2	0.76	

Table 2.
Effect of salinity (NaCl) on dry weight of the experimental algae after 96 h exposure

Test alage	Concentration of NaCl (g/l)	µg dry weight/10^6 cells	Decrease (%)	Increase (%)
S. dimorphus	0	29.7	---	---
	1	30.2	---	1.7
	2	33.6	---	13.1
	3	35.3	---	18.9
	4	39.7	---	33.7
	5	43.8	---	47.5
S. quadricauda	0	96.6	---	---
	2	111.9	---	15.8
	4	116.6	---	20.7
	6	117.3	---	21.4
	8	113.2	---	17.2
	10	113.1	---	17.1
A. falcatus	0	27.5	---	---
	0.7	27.1	1.5	---
	1.4	28.7	---	4.4
	2.1	42.3	---	53.8
	3.5	37.7	---	37.1

salinities of 4 and 5 g/l caused a pronounced decrease in the carbohydrate content. Conversily, at the salinity concentration of 2 g/l, the carbohydrate content of **S. quadricauda** cells was stimulated; a phenomenon that was reversed at high salinity. Similarly, low salinity of 0.7 g/l stimulated the carbohydrate content of **S. quadricauda** cells, while the reverse was true at high salinities.

Table 5 and Fig. 1 show that the protein content of **S. dimorphus** cells decreased with increasing salinity. The protein content of **S. quadricauda** and **A. falcatus** cells, on the other hand, slightly decreased at low salinities of 2 and 0.7 g/l, respectively. Further increase in salinity resulted in pronounced decrease in the protein content.

Table 3
Effect of salinity (NaCl) on chlorophyll a, b and total carotenoid contents of the test algae after 96 h incubation.

Test algae	Concentration NaCl (g/l)	Chlorophyll a ng/10^6 cells	Chlorophyll a Decrease %	Chlorophyll a Increase %	Chlorophyll b ng/10^6	Chlorophyll b Decrease %	Chlorophyll b Increase %	Total carotenoids ng/10^6 cells	Total carotenoids Decrease %	Total carotenoids Increase %
S. dimorphus	0	350	---	---	100	---	---	170	---	---
	1	290	17.1	---	80	20	---	150	11.8	---
	2	270	22.9	---	80	20	---	130	23.5	---
	3	220	37.1	---	70	30	---	120	29.4	---
	4	180	48.6	---	40	60	---	90	47.1	---
	5	130	62.9	---	30	70	---	60	64.7	---
S. quadricuda	0	450	---	---	140	---	---	230	---	---
	2	550	---	22.2	190	---	35.7	370	---	60.9
	4	480	---	6.7	190	---	35.7	310	---	34.8
	6	400	11.1	---	180	---	28.6	290	---	26.1
	8	330	26.7	---	150	---	6.1	220	4.3	---
	10	270	40.0	---	130	7.1	---	160	30.4	---
A. falcatus	0	520	---	---	120	---	---	250	---	---
	0.7	580	---	11.5	140	---	16.7	280	---	12.0
	1.4	440	15.4	---	120	---	---	230	8.0	---
	2.1	390	25.0	---	90	25.0	---	200	20.0	---
	2.8	330	36.5	---	80	33.3	---	180	28.0	---
	3.5	260	50.0	---	60	50.0	---	100	60.0	---

Table 4.
Effect of salinity (NaCl) on total cellular carbohydrate of the test algae after 96 h incubation.

Test algae	Concentration of NaCl (g/l)	µg carbohydrate/10^6 cells	Decrease (%)	Increase (%)
S. dimorphus	0	4.4	---	---
	1	4.0	9.1	---
	2	3.5	20.5	---
	3	2.4	45.5	---
	4	1.6	63.6	---
	5	1.1	75.0	---
S. quadricauda	0	19.0	---	---
	2	20.2	---	6.3
	4	17.5	7.9	---
	6	15.5	18.4	---
	8	11.7	38.4	---
	10	8.8	53.7	---
A. falcatus	0	6.5	---	---
	0.7	6.9	---	6.2
	1.4	5.3	18.5	---
	2.1	4.0	38.5	---
	2.8	3.0	53.9	---
	3.5	2.0	69.2	---

Table 5.
Effect of salinity (NaCl) on total protein
of the test algae after 96 h exposure

Test algae	Concetration of NaCl (g/l)	µg protein/10^6 cells	Decrease (%)
S. dimorphus	0	16.0	---
	1	14.4	10.0
	2	14.1	11.9
	3	14.1	11.6
	4	13.5	15.6
	5	10.9	31.9
S. quadricauda	0	56.3	---
	2	55.1	2.1
	4	48.4	14.0
	6	45.9	18.5
	8	39.3	30.2
	10	37.6	33.2
A. falcatus	0	15.3	---
	0.7	14.0	8.5
	1.4	13.2	13.7
	2.1	12.3	19.6
	2.8	10.9	28.8
	3.5	8.5	44.4

DISCUSSION

This study concerned the examination of the effect of salinity on the growth and chemical constituents of three freshwater green algae. Although the test algae are phylogenetically close, they varied greatly in their responses to salinity.

The results indicate that the growth tolerance of the test algae to salinity increased with increasing exposure time. The growth inhibition of the test algae by NaCl may be due to improper monovalent-divalent ion balances or Ca/Mg ratios as suggested by Provasoli et.at., (1954). On the other hand, the increasing growth tolerance of the test algae to salinity with increasing exposure time reflects their capability to resist high osmotic pressure in the external medium. This leads to the conclusion that, the osmotic resistance of the test algae increased with increasing exposure time. In this connection, Threader and Houston (1982) found that the LC_{50} values of the gold fish **Carassius auratus** for NaCl vary discontinuously with time, which may reflect time dependent variations in cause of death.

The data reveal a significant increase in dry weight of **S. dimorphus** cells, while pigment, carbohydrate and protein contents were inhibited at all salinities. In Case of **S. quadricauda** and **A. falcatus**, the dry weight per cell increased with increase in salinity, while their chemical constituents slightly increased at only low salinities. Conversely, at high salinities the chemical constituents of **S. quadricauda** and **A. falcatus** cells were depressed. This is because under NaCl stress, the dividing cells lost the chance to enter into the resting phase or continue cell enlargment. Also, the ability of cells to absorb salts from the medium containing different concentrations of NaCl, caused increase in the dry weight per cell without proportional increase in the metabolic dopts. This is substantiated by the finding of Wetherell (1963) whose conductivity measurements of the extract of saline-grown cells of **Scenedesmus obliques**, indicated the presence of approximately one third as much total salt in cell-extract as in the culture medium.

The metabolic dopts of **S. dimorphus** were more susceptible to salinity than those of both **S. quadricauda** and **A. falcatus**. The chemical consitutents (pigment, carbohydrate and protein) of **S. dimorphus**, however, decreased progressively with increasing salinity. On the other hand, at low salinities the chemical constituents of **S. quadricauda** and **A. falcatus** cells increased, whereas at high salinities showed the reverese effect. In this connection, one may attribute this stimulatory effect of low salinity to increase in volume of **S. quadricauda** and **A. falcatus** cells due to increase in the uptake of Mg which consequently increased contents. The increase in NaCl concentration probably interferes with the absorption of divalent cations, particularly Mg, impeding its role in chlorophyll formation and other magnesium-dependent pathways particularly enzyme activity. This probably corroborated by the finding that the pigment content of cabbage plant

depends on the salt resistance and the degree of salinization (Dostanove, 1966) and that low osmotic stress of 9-14 atmospheres increases chlorophyll content of **Atriplex halimus** via swelling the chloroplastt (Blumenthal and Poljakoffmayber, 1968).

On the other hand, low salinities inhibited the carbohydrate content of **S. dimorphus** cells, whereas they stimulated the carbohydrate content of **S. quadricauda** and **A. falcatus** cells. High salinities inhibited carbohydrate content of the three algae investigated. The decrease in the carbohydrate content under the influence of NaCl is probably due to the increase in the endogenous inorganic phosphate level which plays an important role in controlling the carbon distribution between starch and glycerol in salinity adapted and NaCl-shocked cells (Gimmler and Moller, 1981). The increased level of inorganic phosphate in the cytoplasm of NaCl-shocked cells resulting from the hydrolysis of either glycerol-3-phosphate or dihydroxy acetone phosphate (DHAP) induce an increased export of DHAP from the chloroplast via translocation in exchange for inorganic phosphate. The increased chloroplast inorganic phosphate level reduces the formation of starch by inhibiting adenosine-diphosphoglucose pyrophosphorylase. Consequently, photosynthetically-fixed carbon is diverted from storage dopts to the synthesis of glycerol (Giersh,et.al., 1980).

On the other hand, the inhibition of protein synthesis of the test algae with high doses of NaCl agrees with the finding of Hellebust (1976), who attributed this phenomenon to the accumulation of proline from glutamate or arginine. Also, the actively growing algal cells posses sufficient amounts of storage dopts which can be degradated under NaCl stress and converted into osmotically active material (Gimmler and Moller, 1981).

The results of the present investigation indicate the obvious inhibitory effect of increasing salinity on growth and chemical constituents, at least in the three algae investigated. Therefore, the behavior of the freshwater algae inhabiting Lake Wadi-El-Rayn in correlation with the rate of increase of Lake water salinity should be investigated.

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