CALCIUM COUNTERACTS THE INHIBITORY EFFECT INDUCED BY SALINITY IN *ANABAENA SUBCYLINDRICA* AND *NOSTOC LINCKIA*.

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Abstract

Growth and some metabolic activities of two cyanobacterial species (*Anabaena subcylindrica* and *Nostoc Linckia)* grown under salinity stress with and without exogeneously added calcium chloride were monitored. Salinity treatment (0.3M NaCl) induced pronounced reduction in growth, pigment fractions, carbohydrates, O_2–evolution, respiration, lipids content and increase in the measured elements content (Na^+, K^+, Mg^{++}, Fe^{+++} and Ca^{++}). Presence of Ca^{2+} (0.03 or 0.05 M $CaCl_2$) caused significant recovery of the different measured growth parameters and metabolic activities. The most important changes induced by salinity treatment are:1-Reduction in the polysaccharides content of both organisms accompanied with an increase in the soluble sugars, which proposed that the possible inhibitory effect of salinity associated with osmotic regulation. This effect could be ameliorated by addition of calcium ions. 2- The integrity of the plasma membranes impaired by salinity. Presence of calcium protects the membranes against the injury induced by salinity.

Key words: Salinity, $CaCl_2$, cyanobacteria, growth, photosynthesis.

Introduction

Salinity is an important deterrent to growth and development of plants and microorganisms. Among microorganisms, cyanobacteria which play a fundamental role in supplying the crop plants with nitrogen, growth regulators and increase the yield, and indirectly maintain the fertility status of soils. NaCl stress is well known to suppress the growth of algae (Lehtimaki *et al*., 1997; Masojidek *et al*., 2000; Hagen *et al*., 2001). Salinity induced changes in the morphological characteristics of *Asterocystis Oranta* (Lewin and Robertson 1971), in the physiological characteristics of fresh water Cyanobacterium *Synechococcus* 6311 (Lefort-Tran *et al*., 1988), in membrane surface charge, lipid, fatty acid composition, and carotenoids in *Synechoccus* 6311 (Khomutov *et al*., 1990). Moreover, salinity treatment caused significant reduction in photosynthesis and respiration of some macroalgae (Karsten *et al*., 1991) and enhanced Golgi apparatus and endoplasmic reticulum (Berube *et al*., 1999). Ca^{2+} is repeatedly recorded to have an ameliorative effect on salt stress (Ahmed *et al*.,

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1989; Cramer *et al*., 1988 and Suarez and Grieve, 1988). The protective effect of Ca^{2+} in salt stressed algae has been reviewed by Abdel Basset (1986). Ca^{2+} alleviated the harmful effects of salinity on growth and maintainance respiration of *Chlorella fusca* (Abdel-Bassset *et al*., 1996). Marschner (1995) reported that Ca^{2+} is required in its ionic form extracellulary, for a variety of structural roles, and in the vacuole, as a counter cation for inorganic and organic anions. In addition, it is essential for cell division and expansion (Kiegle *et al*., 2000).

Moreover, photosynthetic electron transport in green plants and algae has an absolute requirement for calcium (Adam and Issa, 2000; Zeng *et al*., 2000). The principle sites of action of this cation have been localized in the water oxidizing photo system (Ono, 2000). Kimura *et al*. (2002) have indicated that Ca^{2+} is necessary for the formation of hydrogen bond network that is involved in the reaction step of water oxidation. It acts as a second messenger in the regulation of a variety of physiological and metabolic processes (Rao, 2001). NaCl causes a rapid increase in cytosolic calcium, although it is still unclear whether this increase mediates salt adaptation or acts as a general stress signal (Niu *et al*., 1995). An increase in external calcium ameliorates the inhibitory effect of salt (Niu *et al*., 1995 and Wu *et al*., 1996).

Since cyanobacteria are subjected to salinity in some natural habitats, the aim of this work was to study the effect of salinity on growth and some metabolic activities of two cyanobacterial species (*Anabaena subcylindrica* and *Nostoc linckia*), and the possible ameliorative effect played by Ca^{2+} on the inhibition induced by salinity.

Materials and Methods

Anabaena subcylindrica and *Nostoc linckia* were isolated from a cultivated fertile soil in Tanta and identified according to Prescott (1978). Axenic cultures of both organisms were obtained by treating the cultures with various antibiotics as described by Venkatarman (1969). The cultures were grown in the nutrient medium recommended by Allen and Stanier (1968). The cultures were maintained at 26 C^5, illuminated with fluorescent light at light intensity of 75 u mol photon m^{-2} s^{-1} PAR on the surface of culture vessels. Dry weights were determined according to Leganes *et al*., (1987). Chlorophyll a was determined spectrophotometrically in 90 % acetone extracts as recommended by Jeffrey and Humphrey (1975). The carotenoids were estimated according to the method described by Jensen and Liaaen-Jensen (1959). Phycobiliproteins were estimated according to Bennett and Bogorad (1973). The concentration of c- phycocyanin, c- phycoerythrin and allophycocyanin in crude extracts were calculated using the measured absorbance at 615, 652 and 526 nm, respectively for distilled water extract of prolonged freezed and sonnicated cells. The different carbohydrate fractions were determined according to the method adopted by Nelson (1944) and

modified by Naguib (1964). The polysaccharides content were determined after hydrolysis of a definite weight of the dried residue after determination the direct reducing value. The photosynthetic oxygen evolution in the light and dark respiration was determined polarographically using a Clark-type electrode in 3-ml samples at 25 ^{o}C. The Lipid contents were determined according to the method cited by Varma and Tiwari (1967). Mineral concentrations in the cyanobacterial cells were estimated as described by Allen *et al*. (1974). The samples were analyzed for Na^{+}, K^{+},$Mg^{+}$and Fe^{3+}content in acid digested samples by using Flame Photometer (Clinical Flame Photometer 410 C) and Atomic Absorption (Flame Emission Spectrophotometer, Shimadzu Model A.A-640-12). Residual salt concentrations were determined according to the method adopted by Ting *et al*. (1989).

Results and Discussion

Preliminary investigation had indicated that addition of 0.3 M NaCl in the culture medium of *Anabaena subcylindrica* and *Nostoc linckia* induced 55-60% inhibition in the measured growth parameters of both organisms. Therefore, our experiments were designed to study the ameliorative effect of $CaCl_2$ on 0.3 M NaCl treated cultures.

Supplementation of 0.3 M NaCl to the culture medium of both organisms caused significant reduction in the dry weight throughout the experimental period which amounted to 64 % in *Nostoc linckia* and 65 % in *Anabaena subcylindrica* after 15 days of incubation (Fig. 1 and 2). Presence of $CaCl_2$ (0.03 or 0.05 M) alleviated the inhibitory effect of NaCl on the dry weight production of both organisms. The highest value (about 2.4 fold increase) was observed in the salinized cultures of both organisms treated with 0.05 M $CaCl_2$ after 15 days of incubation.

The toxic action of NaCl on algae was recorded by many workers. An inverse relationship between the algal growth and NaCl concentration was observed in *Chlamydomonas reinhardtii* by Reynose and De- Gamboa (1982); in *Anabaena sp.* PCC7120 by Rai and Tiwari (1999). Sinha and Hader (1996) found that NaCl concentrations >5 mM inhibited growth of *Anabaena* sp. and apparently there was no growth at > 200 mM NaCl. Also, Salinity inhibited the growth of *Chlorococcum* (Masojidek *et al*., 2000) and *Chlorella vulgaris* (El-Sheekh and Omar (2002). However, a number of fresh water algae are able to grow rapidly in saline medium. Chan *et al*. (1979) found *Chlorella salina* grow well in domestic sewage effluent containing relatively high salt concentrations. Furthermore, the cell density of *Aphanothece halophytica* was slightly lowered when transferred from 0.5- 2M NaCl (Incharoensakdi and Wutipraditkul, 1999).

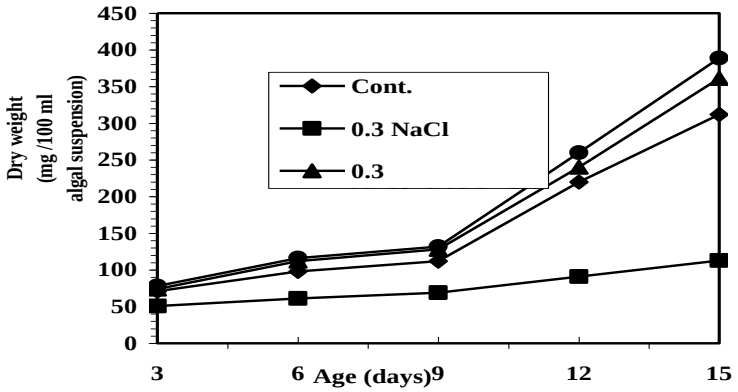

Figure 1: Effect of two different concentrations of $CaCl_2$ on the dry weight of salinized culture of *Nostoc linckia* (mg/ 100 ml algal suspension) grown for 15 days.

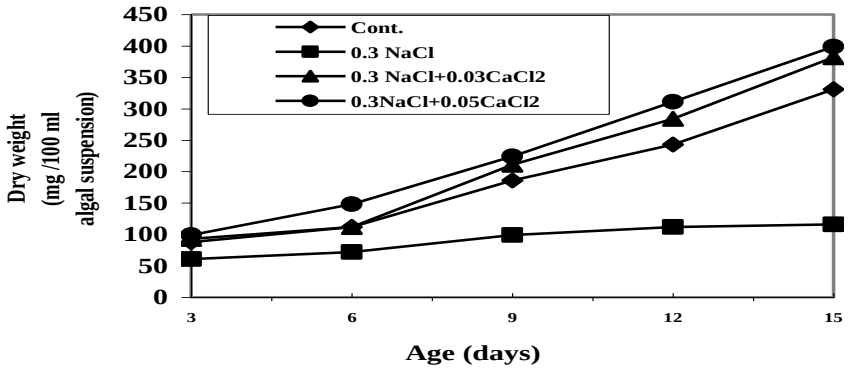

Figure 2: Effect of two different concentrations of $CaCl_2$ on the dry weight of salinized culture of *Anabaena subcylindrica* (mg/ 100 ml algal suspension) grown for 15 days.

In accordance with our results, Ahmed *et al*. (1989) reported that growth of *Chlorella vulgaris* was markedly inhibited with the rise of NaCl level. This inhibition was ameliorated by addition of $CaCl_2$. This protective action of calcium in stressed photosynthetic organisms could be achieved through membrane stability as suggested by Munns *et al*. (1983). It has been reported that low

calcium content increases membrane permeability at high external NaCl (Greenway and Munns, 1980). Furthermore, Leopold and Willing (1984) indicated that calcium served partially to protect tissues from NaCl damage and reduces the leakiness of organic metabolites. Lynch and lauchli (1988) reported that preloading corn root protoplasm with supplemental calicum counteracted subsequent NaCl effects on membranes. Therefore, it can be generalized that calcium relief accurs in the following sequence: Stabilization and repair of NaCl damaged membranes, less uptake of Na^+ (less toxicity) and preservation of all metabolites from leakiness.

Application of 0.3 M NaCl to the culture media of both organisms caused significant reduction in chl.a content (57% in *N. linckia* and 68% in *A. subcylindrica*) as compared with control after 15 days of incubation. More or less similar results were observed in the other pigments content (carotenoids, phycocyanins, phycoerythrins and allophycocyanin). However, phycobiliproteins showed more resistance to NaCl stress than chl.a and carotenoids (Table 1).

The inhibitory effect of NaCl on chlorophyll biosynthesis is probably due to that the use of radiant energy by the chlorophyll molecule may be inhibited and the chlorophyll may become oxidized and bleached, leading to a lethal photodynamic reaction as suggested by Loebich (1982). In accordance with our results, Vonshak and Richmond (1981) indicated that chlorophyll content in *Anacystis nidulans* was reduced by increasing NaCl concentration in the culture medium. Moreover, NaCl concentrations above 0.4 M caused a marked decrease in chlorophyll content of the cells, and death followed shortly afterwards. Also, NaCl (beyond 200 mM) caused significant reduction in the pigments content of *Anabaena doliolum* (Rai and Abraham 1993). On the otherhand, Anand *et al*. (1994) found that 3% salinity increased chlorophyll a of *Chrococcus minor* and *Oscillatoria salina* while *Gloeocapsa polydermatica* and *Lyngbya spiralis* showed reduction in pigments content beyond 1.5 % salinity. *Nostoc piscinale* and *Tolypothrix teruis* released phycobilin pigments in the extracellular medium at salinities from 2.5 - 3.5%. Also, Zuther *et al*. (1998) showed reduced chlorophyll a content of the *cyanobacterium Synechocystis* sp., leading to an increase in the ratios of carotenoids/ chlorophyll a in the mutant grown in basal medium after transfer to salt medium (684 mM NaCl) for different times.

Again, addition of different concentrations of $CaCl_2$ in 0.3M NaCL treated cultures of both organisms caused significant rise in the values of the measured pigment fractions of both organisms (Table 1). The percentage of the recovery was pigment and organism dependent. Thus, it could be concluded that calcium has a general protective action against the toxic action of salinity on the reactions leading to pigment biosynthesis.

Table 1: Effect of two different concentrations of $CaCl_2$ (0.03, 0.05 M) on the pigments content of salinized culture of *Anabaena subcylindrica* and *Nostoc linckia* (mg / ml algal suspension)grown for 15 days.

Age (days)	Concentrations (M)	*A. subcylindrica*					*N. linckia*				
		Chl.a	Car.	PC x 10^3	PE x 10^3	APC x 10^3	Chl.a	Car.	PC x 10^3	PE x 10^3	APC x 10^3
3	Control	0.21	0.34	2.1	1.9	5.4	0.18	0.41	2.5	3.1	1.9
	0.3NaCl	0.084	0.08	1.7	1.1	3.1	0.099	0.04	0.7	0.8	0.42
	0.3NaCl + 0.03 $CaCl_2$	0.25	0.4	2.5	2.1	5.9	0.2	0.55	2.8	3.9	2.1
	0.3NaCl + 0.05 $CaCl_2$	0.26	0.44	2.6	2.1	5.92	0.22	0.55	2.9	4.02	2.2
6	Control	0.36	0.42	2.5	2.2	5.8	0.2	0.55	3.1	3.5	2.5
	0.3 NaCl	0.103	0.12	2.2	1.5	3.6	0.111	0.06	1.1	1.1	0.59
	0.3 NaCl +0.03 $CaCl_2$	0.39	0.51	2.9	2.5	7.3	0.24	0.64	3.5	4.5	2.8
	0.3 NaCl +0.05 $CaCl_2$	0.42	0.55	2.9	2.7	7.4	0.28	0.68	3.6	5.2	3.1
9	Control	0.41	0.55	3.1	2.5	6.3	0.31	0.68	3.6	4.9	2.8
	0.3 NaCl	0.172	0.16	2.6	2.1	4.2	0.134	0.08	1.6	1.6	0.52
	0.3 NaCl +0.03 $CaCl_2$	0.45	0.62	3.4	3.1	8.2	0.33	0.72	3.9	5.9	3.02
	0.3 NaCl +0.05 $CaCl_2$	0.45	0.62	3.6	3.1	8.1	0.33	0.76	3.9	5.9	3.5
12	Control	0.56	0.69	3.6	3.1	7.1	0.39	0.8	3.9	5.5	3.01
	0.3 NaCl	0.198	0.181	2.6	2.3	5.1	0.179	0.11	1.8	1.8	0.61
	0.3 NaCl +0.03 $CaCl_2$	0.55	0.74	3.9	3.4	9.2	0.42	0.86	4.3	6.2	3.5
	0.3 NaCl +0.05 $CaCl_2$	0.63	0.75	4.1	5.5	9.3	0.46	0.89	4.3	6.1	3.6
15	Control	0.69	0.845	3.9	3.7	7.6	0.45	0.941	4.6	7.9	3.6
	0.3 NaCl	0.218	0.27	3.1	2.6	5.3	0.192	0.125	2.1	2.11	0.9
	0.3 NaCl +0.03 $CaCl_2$	0.73	0.881	4.7	3.8	9.6	0.46	0.963	4.9	8.4	3.8
	0.3 NaCl +0.05 $CaCl_2$	0.79	0.886	4.8	3.9	9.62	0.49	0.992	5.1	8.4	3.95

F- value	Chl.a	Car.	Chl.a	Car.
Day	4185**	4362**	1860**	3982**
Conc.	5740**	11556	2367**	25256
Day x Conc.	208**	160**	73**	276**

Values in row followed by *= significant difference at P ≤ 0.05, **=P≤ 0.01,***=P≤ 0.001 according to F- test. Chl.a = chlorophyll a Car.= carotenoid PC= phycocyanin PE= phycoerythrin APC= allophycocyanin

Addition of 0.3M NaCl in the culture medium of both organisms has resulted in an increase in monosaccharide contents accompanied with a concomitant reduction in polysaccharides and total sugars of both organisms throughout the experimental period (Table 2). Thus, 7 % and 5 % increase in monosaccharide contents was recorded in *N. linckia* and *A. subcylindrica*, respectively at the end of the experimental period. Disaccharides showed non significant reduction in *A. subcylindrica* while increased in *N. linckia*. in response to salinity treatment.

Table 2: Effect of two different concentrations of $CaCl_2$ (0.03, 0.05 M) on the different carbohydrate fractions of salinized culture of *Anabaena subcylindrica* and *Nostoc linckia* (mg glucose/ 100 gm dry weight x 10^{-3}) grown for 15 days.

Age (days)	Concentrations (M)	*A. subcylindrica*				*N. linckia*			
		Monosac.	Disac.	Polysac.	Total sugars	Monosac.	Disac.	Polysac.	Total sugars
6	Control	1.9	4.8	11.1	17.8	2.9	5.8	12.4	21.1
	0.3NaCl	2.3	4	8	14.3	3.3	6	9	18.3
	0.3NaCl + 0.03 $CaCl_2$	2.1	4.9	11.4	18.4	3.1	5.9	12.8	21.8
	0.3NaCl + 0.05 $CaCl_2$	2.2	5	11.6	18.6	3.3	5.9	12.9	22.1
9	Control	3.2	6.6	16	25.8	4.2	6.7	17.6	28.5
	0.3 NaCl	3.3	5.5	12	20.8	4.5	7.5	14	26
	0.3 NaCl +0.03 $CaCl_2$	3.2	6.7	17	26.9	4.2	6.9	18	29.1
	0.3 NaCl +0.05 $CaCl_2$	3.2	6.8	17.6	27.6	4.4	7.1	18.3	29.8
12	Control	4.1	8.9	28	41	4.9	9.3	28	42.2
	0.3 NaCl	4.3	8	22	34.3	5.1	9.9	23	38
	0.3 NaCl +0.03 $CaCl_2$	4.2	8.5	29	41.7	5	9.5	29.2	43.7
	0.3 NaCl +0.05 $CaCl_2$	4.25	8.8	29.2	42.25	5	9.8	29.2	44
15	Control	4.4	11.4	29.2	45	5.5	12.3	30	47.8
	0.3 NaCl	4.6	12	25	41.6	5.9	13	26	44.9
	0.3 NaCl +0.03 $CaCl_2$	4.5	11.6	34	50.1	5.7	12.5	35	53.2
	0.3 NaCl +0.05 $CaCl_2$	4.5	11.8	35.1	51.4	5.75	12.6	37.1	55.45

F- value	Monosac.	Disac.	Polysac.	Monosac.	Disac.	Polysac.
Day	$8.3+^{04}$***	$1.7+^{06}$***	$2.5+^{07}$***	$8.3+^{04}$***	$1.7+^{06}$***	$2.5+^{07}$***
Conc.	$2.0+^{04}$***	$1.3+^{05}$***	$6.4+^{06}$***	$2.0+^{04}$***	$1.3+^{05}$***	$6.4+^{06}$***
Day x Conc.	$5.0+^{03}$***	$3.3+^{04}$***	$6.4+^{05}$***	$5.0+^{03}$***	$3.3+^{04}$***	$6.4+^{05}$***

Values in row followed by * = significant difference at P ≤ 0.05, ** = P≤ 0.01, *** = P≤ 0.001 according to F-test

On the other hand, polysaccharides contents showed siginficant reduction in both salinized cyanobacterial cultures amounting to 14% and 13 % in *A. subcylindrica* and *N. linckia,* respectively at the end of the experimental period. These results proposed that the possible inhibitory effect of salinity is associated with osmotic regulation, which cause diversion of metabolites from synthesis of cell constituents into synthesis of osmoregulants. In accordance with our results, Erdmann (1984) found that *Microcystis firma* accumulates glycerol glucoside which brings about a high salt resistance. Also, *Anabaena variablis* which is slightly salt resistant accumulate sucrose as osmoregulant. Accumulation of glycine betaine as osmoregulant at high NaCl concentration had been reported in *Aphanothece halophytica* (Incharoensakdi and Wutipra-Ditkul, 1999). The accumulation of soluble sugars was repeatedly recorded under salinity conditions (Erdmann, 1983 and Ahmed *et al*., 1989). However, Hathout (1996) recorded a reduction in carbohydrates in response to salinity treatment.

The results revealed that addition of Ca^{2+} to salinized cultures of both organisms counteracted the salinity effect on the different fractions of carbohydrate. Our results are in a good accordance with those obtained for wheat plants by Abd EL-Samad (1993), who reported that water with $CaCl_2$ or KCl reduced or alleviated the adverse effects of salinity on carbohydrate fractions. This alleviation could be attributed to the enhanced uptake of K^+ in presence of Ca^{2+} resulting in a decreased osmotic potential of the cells which increases the intracellular CO_2 concentration and assimilation (Macrobbie, 1995, Willmer and Fricker, 1996).

The results show that application of 0.3M NaCl to the culture medium of both organisms caused significant reduction in photosynthesis and respiration (Tables 3 and 4). In accordance with our results, Brown (1985) observed a complete inhibition of photosynthesis and respiration of *Nannochloris bacillaris* after transfere from 7% to 200% artificial sea water during the 1-2 days. In addition, Kirst (1990) have been reported that high salinity affect photosynthetic apparatus in at least two sites (the reducing side of PSI and the donor side of PSII). Also, Lu and Zhang (1999) concluded that PSII inactivation by salinity is the major factor of photosynthesis inhibition. Furthermore, Allakhverdiev *et al*. (2000) reported that 0.5 M NaCl induced a rapid loss of PSII activity and the electron transport activity of PSI in *Synechococcus.*

However our results indicate that presence of 0.03 or 0.05 M $CaCl_2$ in the salinized culture medium of both organisms caused significant increase in the photosynthetic activity and respiration (Tables 3 and 4). Similar results were obtained by *Ahmed et al.* (1989) who found that $CaCl_2$ protect the photosynthetic activity and respiration of *Chlorella vulgaris* against the toxic action of NaCl. This protective action could be due to the Ca^{2+} requiment for the proper organization of the hydrogen bond network within the oxygen evolving system

which is involved in the reaction step of water oxidation as suggested by *Kimura et al.* (2002).

Table 3: Effect of two different concentrations of $CaCl_2$ (0.03, 0.05 M) on photosynthetic O_2 evolution (μ mol O_2.mg $Chl^{-1}.h^{-1}$) and respiration (μ mol $O_2.h^{-1}$) of salinized culture of *Nostoc linckia* grown for 15 days.

N. linckia								
Conc. (M)	Control		0.3 NaCl		0.3 NaCl + 0.03 $CaCl_2$		0.3 NaCl + 0.05 $CaCl_2$	
Age (days)	O_2-Evolution	O_2-uptake	O_2-Evolution	O_2-uptake	O_2-Evolution	O_2-uptake	O_2-Evolution	O_2-uptake
3	31.75	7.9	19.25	5.6	49.25	31.1	59.75	16.1
6	37.25	9.1	20.25	5.8	70.75	18.8	77.75	20.7
9	42	9.8	22.9	6.3	72.5	20.8	88.5	25.1
12	48.75	13.1	26.1	8	85.5	23.1	99.5	26.5
15	30.2	7.1	8	4.1	49	13	59.25	16.2

F- value	O_2-evolution	O_2-uptake
Day	$1.2E^{+07}$**	40 **
Conc.	$9.8E^{+07}$**	121**
Day x Conc.	$1.7E^{+06}$**	5**

Values in row followed by *= significant difference at P ≤ 0.05, **=P≤ 0.01, ***=P≤ 0.001 according to F-test

Table 4: Effect of two different concentrations of $CaCl_2$ a(0.03, 0.05 M) on photosynthetic O_2 evolution (μ mol O_2.mg $Chl^{-1}.h^{-1}$). and respiration (μ mol $O_2.h^{-1}$) of salinized culture of *Anabaena subcylindrica* grown for 15 days.

A. subcylindrica								
Conc. (M)	Control		0.3 NaCl		0.3 NaCl + 0.03 $CaCl_2$		0.3 NaCl + 0.05 $CaCl_2$	
Age (days)	O_2-Evolution	O_2-uptake	O_2-Evolution	O_2-uptake	O_2 Evolution	O_2-uptake	O_2-evolution	O_2-uptake
3	32.25	8	20.25	5.8	50	13.2	60.25	16.3
6	38	9.5	21.5	6	71.25	18.9	78.25	20.8
9	43	10	23	7	73.25	20.9	89.25	25.3
12	49.25	13.3	26.2	8.3	86.25	23.3	100.25	26.7
15	31.1	7.8	8.1	4.3	50.25	13.4	60.25	16.4

F- value	O_2-evolution	O_2-uptake
Day	$1.9E^{+04}$**	$1.4E^{+06}$**
Conc.	$1.2E^{+03}$**	$8.2E^{+06}$**
Day x Conc.	$1.5E^{+02}$**	$1.1E^{+07}$**

Values in row followed by *= significant difference at P ≤ 0.05, **=P≤ 0.01, ***=P≤ 0.001 according to F-test

Inclusion of 0.3 M NaCl in the culture medium of both organisms showed significant reduction in the lipids content, which amounted to 48% in *N. linckia* and 45% in *A. subcylindrica* after 15 days of incubation (Figs 3 & 4). Presence of $CaCl_2$ in the salinized culture caused significant increase in the lipid content of both organisms, which exceeded the control levels and reached 1.4 fold in *N. linckia* and 1.3 fold in *A. subcylindrica* after 15 days of incubation. lnhibition of lipid biosynthesis in response to NaCl stress was reported in *Porphyridium cruentum* by Lee *et al*. (1989); in *Calothrix* by Senthil *et al*. (1993) and in *Chlorella vulgaris* by El-Sheekh and Omar (2002). Such effect may represent a mechanism or a consequence of osmotic adaptation probably through the alteration in the activity of dehydrase / desaturase enzymes. Furthermore, salinity induced increase in the unsaturation of fatty acids in the membrane lipid which significantly enhanced the tolerance of photosynthetic machinary to salt stress (Allakhverdiev *et al*., 2000 and Nichols *et al*., 2000). The ameliorative effect of Ca Cl_2 on lipid biosynthesis could be attributed to the requirements of Ca^{2+} for the activity of phospholipases as suggested by Qin and Wang (2002).

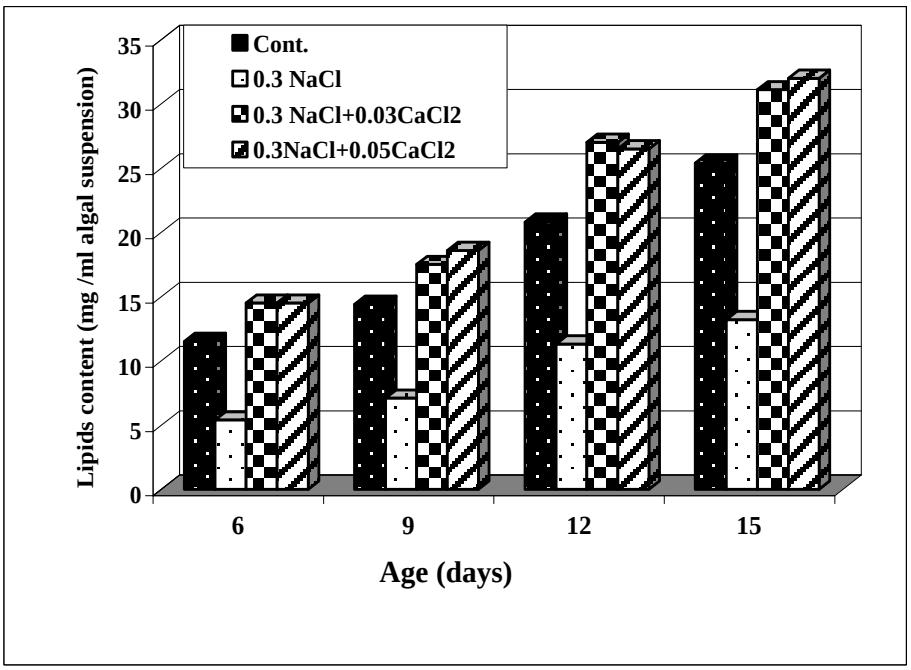

Figure 3: Effect of two different concentrations of $CaCl_2$ on the lipids content of salinized culture of *Nostoc linckia* (mg/ 100 ml algal suspension) grown for 15 days.

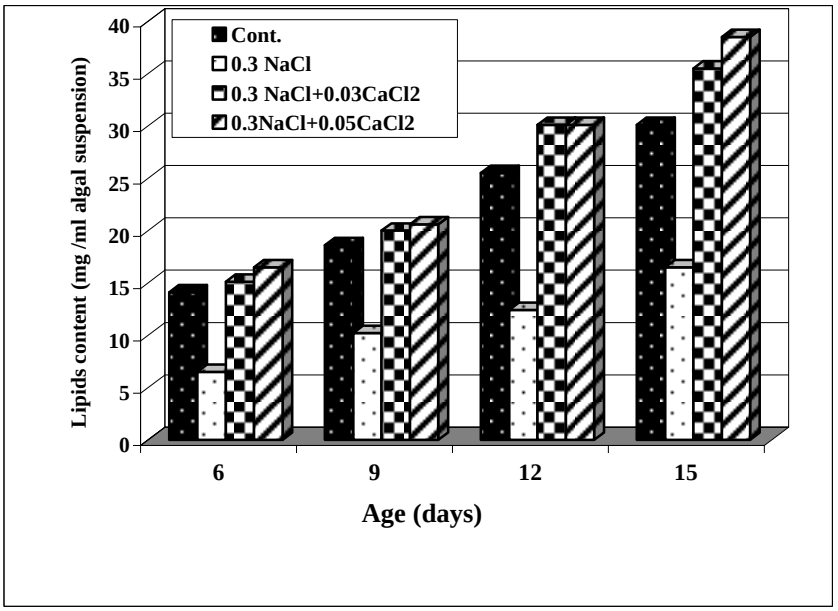

Figure 4: Effect of two different concentrations of $CaCl_2$ on the lipids content of salinized culture of *Anabaena subcylindrica* (mg/ 100 ml algal suspension) grown for 15 days.

The results show significant accumulation of the different cations content (Na^+, Ca^{2+}, K^+, $Mg2^+$and Fe^{2+}) in response to salinity treatment throughout the experimental period of both organisms (Fig. 5 A, B). The percentage of accumulation is cation and organism dependent. In accordance with our results Ahmed *et al*. (1984) found that all minerals content were increased in *Ankistrodesmus falcatus* in response to salinity treatment. This may be explained on the basis that the integrity of plasma membranes may be partially injured by salinity and as a result, the minerals moved freely into the cell without any clear selection. However, Wang (1998) reported that increasing salinity caused significant rise in Ca^{2+} and Na^+ concentrations in *Phalaenopsis orchidis* without affecting the concentration of other elements in the leaves. This result indicates that the mechanism of cation accumulation in response to salinity is species dependent. The results show that Ca^{2+} addition to the salinized cultures of both organisms caused significant reduction in the concentration of the accumulated cations. More or less similar results were obtained by Fernandz-pinas *et al*. (1997) for Cd^{2+} treated culture of *Nostoc* UAM208. This may indicate that Ca^{2+} is able to protect the cells against the toxic action of some cations. The ameliorative behavior of calcium could be attributed to its homologous chemistry rather than

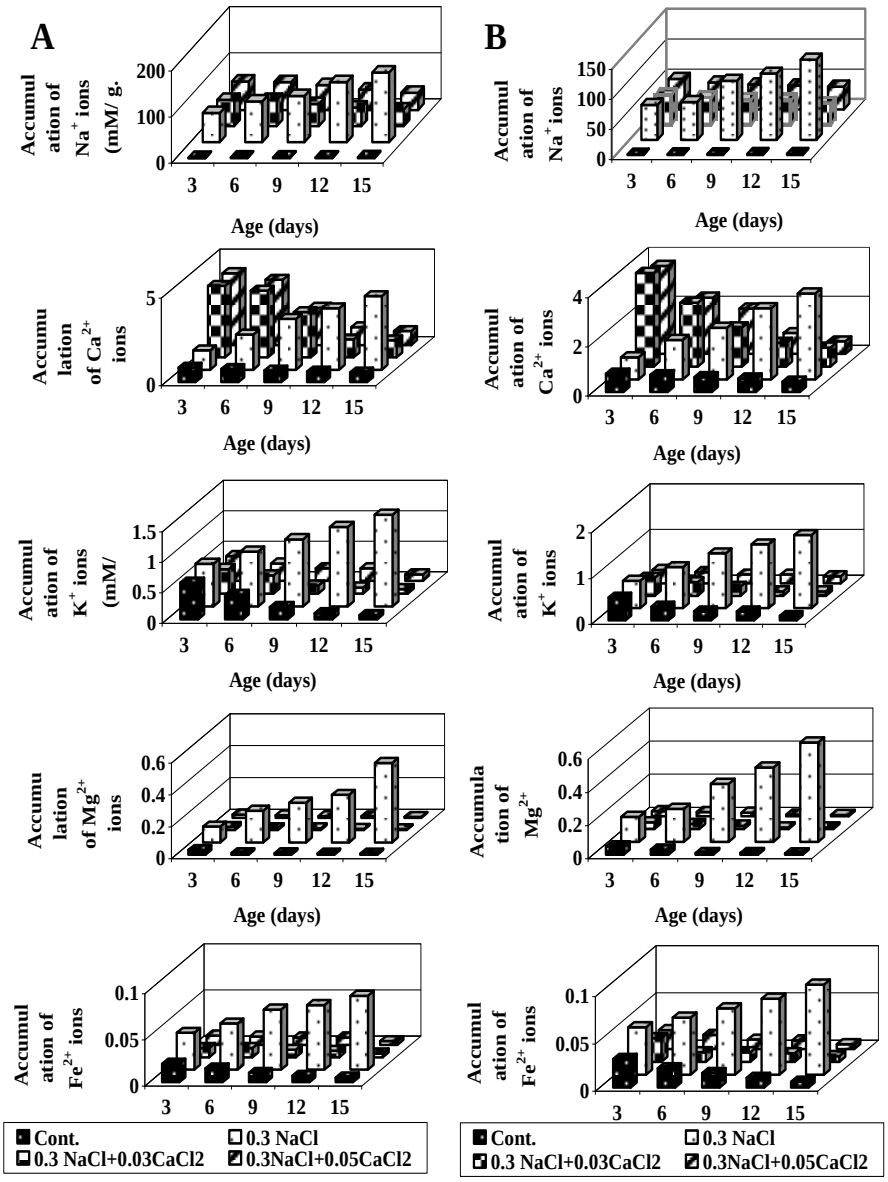

Figure 5: Effect of two different concentrations of $CaCl_2$ on the accumulation of Na^+, Ca^{2+}, K^+, $Mg^{2+}$and Fe^{2+} of salinized culture of A- *Nostoc linckia* B- *Anabaena subcylindrica* (mM / gm. Dry weight) grown for 15 days.

the cation size. On the other hand, Kinraide (1998) found that Ca^{2+} appears to alleviate the effects of rhizotoxic cations (Al^{3+}, H^{+}, Na^{+}, or other cationic toxicant) by multiple mechanisms. First, the electrostatic displacement of toxicant from plasma membrane surfaces may be the most important mechanism in most cases, although it is less important for Na^{+} toxicity than Al^{3+} and H^{+} toxicities. Second, the restoration of toxicant-displaced Ca^{2+} at plasma membrane surfaces is unlikely to be an important mechanism in the most cases, although it is more important for Na^{+} toxicity than for Al^{3+} and H^{+} toxicities. Third, a class of interactions between Ca^{2+} and toxicants is highly specific and may reflect in part Ca^{2+} blockade of plasma membrane channels that admit toxicant.

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الكالسيوم يضاد التأثير المثبط للملوحة فى طحلبى أنابينا صبسيليندريكا والنوستوك لينكيا.

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**قسم النبات – كلية العلوم – جامعة طنطا.*

***مركز تكنولوجيا و تطوير التعليم – طنطا.*

تم فى هذه الدراسة تتبع النمو وبعض الأنشطة الأيضية لنوعين من السيانوبكتيريا (أنابينا صبسيليندريكا والنوستوك لينكيا) النامية تحت تأثير الملوحة فى وجود وعدم وجود كلوريد الكالسيوم الذي تم إضافته خارجياً .

أدت المعاملة بالملوحة (0.3 جزيئ من كلوريد الصوديوم) إلى انخفاض ملحوظ فى النمو ومكونات الأصباغ والمواد الكربوهيدراتية وتصاعد الأكسجين والتنفس ومحتوى الدهون ومن ناحية أخرى قد أدت الملوحة إلى زيادة فى محتوى بعض العناصر التي تم قياسها (الصوديوم ، البوتاسيوم ، الماغنسيوم ، الحديد والكالسيوم) .

وقد أ ظهرت النتائج انه بإضافة الكالسيوم (03 , أو 05 , جزيئ من كلوريد الكالسيوم) قد نتج عنه شفاء معنوي فى معايير النمو المختلفة التي تم قياسها وكذلك الأنشطة الأيضية ومن أهم التغيرات التي أحدثتها الملوحة هي :-

1- الانخفاض فى محتوى السكريات العديدة لكلا الكائنين والمصحوبة بزيادة فى السكريات الذائبة والتي ترجح أن التأثير المثبط للملوحة يرتبط بالتوازن الأسموزى الذي يمكن معادلته عند إضافة أيونات الكالسيوم .

2- أثرت الملوحة على كفاءة الأغشية البلازمية مسببة ضرراً بها إلا أن وجود الكالسيوم أدى إلى حماية الأغشية البلازمية من هذا الضرر الواقع عليها بسبب الملوحة .