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The Mitigative Effect of Calcium on Two Cyanobacterial Species under Sodium Chloride Stress and its Relation to Some Physiological Activities

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Abstract: The objective of this study was to investigate the effect of two concentrations of $CaCl_2$ (0.04 and 0.06 M) on salinized culture (0.2 M NaCl) of *Anabaena constricta* and *Nostoc linckia* on their growth and metabolic activities. The presence of 0.2M NaCl in culture of *A. constricta and Nostoc linckia* induced a significant decrease in cell number, dry weight, optical density and pigments (chlorophyll "a" and carotenoids,). Also there was a decrease in glucose, protein and nitrogen contents. Addition of two different concentrations of $CaCl_2$ (0.04 or 0.06M) to the salinized cultures of both organisms induced a significant increase in the growth and metabolities activities. The protein electrophoretic patterns of culture of *A. constricta* when treated by 0.2 M NaCl showed disappearance of one protein band at 77 KD_a and appearance of two protein bands at m.w. 171 and70 KD_a . The addition of 0.04M $CaCl_2$ to the salinized culture of *A.Constricta* showed disappearance of one protein at m.w. 77 KDa and appearance of three protein bands at m.w. 17, 61 and 162 KDa, while addition of 0.06 M $CaCl_2$ to salinized culture caused appearance of three protein bands with m.w. 18, 58 and 138 KD_a as compared with control (Salinized culture 0.2 M NaCl alone). Addition of 0.2 M NaCl to culture of *N linckia* and addition of 0.04 M or 0.06 M $CaCl_2$ to salinized culture showed no change of protein patterns as compared with control (culture without NaCl) but differ only in the percentage of intensity of proteins.

Key words: *Anabaena* sp., *Nostoc* sp., *cyanobacteria*., growth, salinity stress, protein profile. mitigative effect of $CaCl_2$.

INTRODUCTION

Salinity is an important deterrent to agriculture in many areas of the world. Salts do not affect only on the growth of plants but inhibit also the proliferation and activity of native or introduced microorganisms. Among these organisms, cyanobacteria which introduced to soil and play a fundamental role in supplying the crop plants with nitrogen, growth regulators , increase the yield, and indirectly maintain the fertility status of soil.

High concentration of NaCl apparently inhibits growth by ionic (Na) stress more than by osmotic stress (Brownell and Nicolas, 1967). Since high intercellular concentrations of Na^+ are toxic to most biological systems and organisms living in sodium rich environments must have developed detoxifying mechanisms. The ability to produce organic osmolytes to cope with ionic and osmotic stresses in the environment is common in nitrogen fixing cyanobacteria (Reed, *et al*., 1986).

Exposure of *Chlorococcum sp.* to 0.2 M NaCl caused an increase in the biomass dry weight due to an increase in the cell size accompanied by massive appearance of secondary carotenoids that reached the maximum after 2-3 days of cultivation (Masojidek, *et al.,* 2000). On the other hand, addition of 40mM NaCl did not increase the carotenoids biosynthesis in the flagellated alga *Haematococcus pluvialis* (Hagen *et al*., 2001).

The influence of salinity (0.03-0.5M NaCl) on the physiological characteristics of fresh water cyanobacterium *Synechococcus* 6311, showed that intercellular granules disappeared, the density of the cytoplasm decreased and the appearance of DNA material was changed (Lefort –Tran *et al*., 1988).

Rai and Abraham (1993), observed that with the increase in NaCl concentration (beyond 200 mM), the filaments of *Anabaena doliolum* were shorter with less heterocysts. Anand *et al*., (1994) studied the effect of

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salinity on the growth of cyanobacteria *Chroococus minor, Gloeocapsa polydermatica, Oscillatoria salina, Lyngbya spiralis, Nostoc piscinate* and *Tolypothrix tenuis*.They observed that *Nostoc piscinate* and *T. fenuis* released phycobilin pigments (phycocyanin and phycoerythrin) in the extracellular medium at salnities of 2.5-3.5%.

The aim of this research was to study effect of low concentrations of $CaCl_2$ on the growth of salinized cultures (0.2M NaCl) of *Anabaena constricta* and *Nostoc linckia* .Also,its effect on their physiological activities was assessed

MATERIALS AND METHODS

Isolation, Purification and Growth Conditions of the Organisms:

The heterocystous and filamentous N_2- fixing cyanobacterium were isolated from cultivated lands near Sana'a city (Yemen). Isolation and purification was made by dilution technique using petridishes (75 mm) containing different synthetic media as Allen and Arnon's and BG -11 medium. They were incubated under fluorescent white light 5000 lux at 27ºC for 9-13 days, then minute colonies began to appear. Well spread individual colonies were isolated and restreaked on fresh agar plates. In parallel, individual colonies were transferred into 10 ml of the above mentioned liquid media in test tubes and incubated under continuous light condations. After 7-10 days of incubation, cultures growing in liquid media and the filaments were broken by gentle shaking with sterilized glass beads. The homogenous suspension containing mostly individual cells or short filamentous was spread on solid agar Petri plates of the above mentioned media.

Individual cells were marked and after 7-10 days of growth, colonies appearing on agar plates were examined microscopically and transferred onto solid agar Petri plates. Restreaking and sub-culturing were repeated several times to obtain a single colony apparently free from contamination. These colonies were manually isolated and routinely grown in liquid medium. The two organisms were isolated and purified and identified morphologically as *Anabaena constricta* and *Nostoc linckia*. The two organisms ($1x10^6$ cells) were grown in an autoclaved modified chu-10 (1942) liquid (devoid of any nitrogen sources) in Erlenmeyer flasks 500 ml volume containing 150 ml medium. Experiments were performed at least three times.

Sodium Chloride and Calcium Chloride Treatments:

Cultures of *A. constricta* and *N. linckia* (7-9 days old) were inoculated with 0.2 M NaCl and the control without sodium chloride. Other flasks were inoculated with 0.2 M NaCl in addition to one of two different concentrations of $CaCl_2$ (0.04 and 0.06 M). All flasks were incubated at a temperature 27+2 and white light 5000 lux in regime 16/8 hours light/dark.

Growth Estimation:

The changes in cell number were determined by Haemocytometer cell. The optical density was determined at 750 nm by spectreophotometer (lefort Tran *et al*., 1988). The dry weigh was estimated by (leganes *et al*., 1987). The chlorophyll "a" was determined by spectrophotometric method recommended by Jeffery and Humphrey (1975). The carotenoids were determined according to Jensen and liaaen (1959). The phycobiloproteins were determined according to the method described by Bennet and Bogorad (1973). The carbohydrate fractions of algal tissues were calculated as mg glucose/100 gm dry weight by using standard curve (Naguib ,1963). The total nitrogen content of the algal cultures was estimated by micro kgeldahl as described by Jacobs (1958). The total soluble proteins were determined quantitavely by lowery *et al,* (1951).

Gradient Gel Electrophoresis:

Vertical polyacryamide Gel electrophoresis (PAGE) was used as described by Laemmli (1970). Applying electrophoresis analysis was made at Faculty of Science in Al-Azhar University, Egypt. Gel lanes were analyzed using gel documentation and analysis system consisting of dark room, transilluminator, integrating CD Video camera and image software (AAB software).

Statistical Analysis:

Data were subjected to the proper statistical analysis according to Snedecor and Cochran (1982).

RESULTS AND DISCUSSION

As shown in figs (1,2), the addition of 0.2M NaCl to cultures of *Anabaena constricta* or *Nostoc linckia* caused significant reduction in the cell number amounted to 34% in case of *A.constricta* and 23% in case of *N. linckia* at the end of experiments (15 days old). Addition of 0.04 or 0.06 M of $CaCl_2$ to salinized culture caused increase in the cell number of both organisms reached to 1.42 fold and 1.55 fold with *A.constricta* and *N.linckia* respectively. The dry weight of both organisms in salinized culture (0.2M NaCl only) decreased in *A.constricta* and *N.linckia* and this decreasing amounted to 42.1% and 40% respectively, Addition of 0.04 M of $CaCl_2$ to salinized cultures caused significant increase in the dry weight of both organisms. This increase with *A.constricta* and *N.linckia* wase nearly 1.81 and 1.83 folds respectively while addition of 0.06M $CaCl_2$ to salinized culture of both organisms induced increase reached to 1.6 and 1.5 folds in *A.constricta* and *N.linckia* respectively comparing with salinized culture through 15 days of incubation as shown in fig (3,4). The results in figs (5, 6) indicated that there was an increase in optical density with both organisms in the presence of 0. 2 NaCl comparing with control (without NaCl). Addition of 0.04M $CaCl_2$ to salinized culture caused more increase in optical density in both organisms.

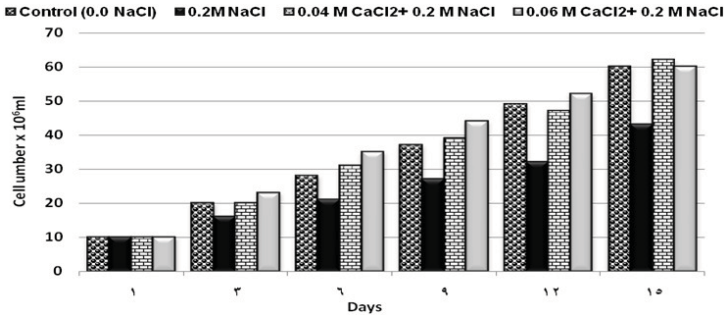

Fig: 1: Effect of two concentrations of $CaCl_2$ on salinized culture of *A. constricta* (Cell number x 10^6/ml).

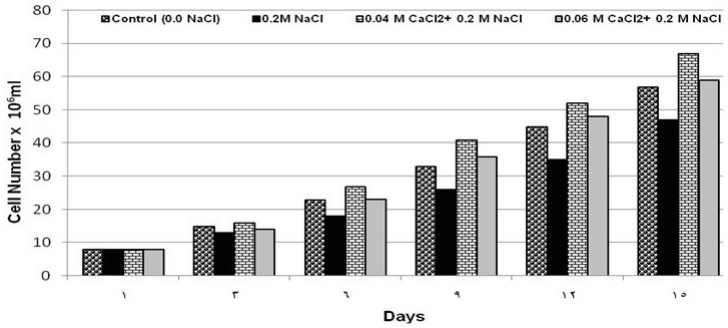

Fig: 2: Effect of two concentrations of $CaCl_2$ salinized culture of *N. linckia* (Cell number x 10^6/ml).

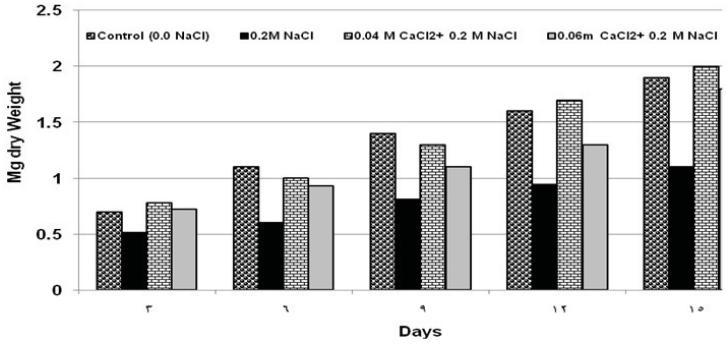

Fig: 3: Effect of two concentrations of $CaCl_2$ salinized culture of *A. constricta* (mg dry weight /ml culture).

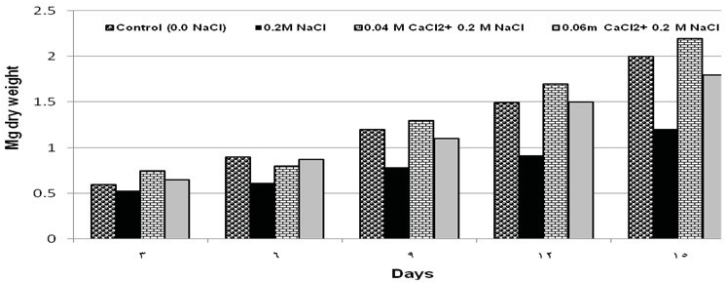

Fig: 4: Effect of two different concentrations of $CaCl_2$ on salinized Culture of *N. linckia* (mg dry weight /ml culture)

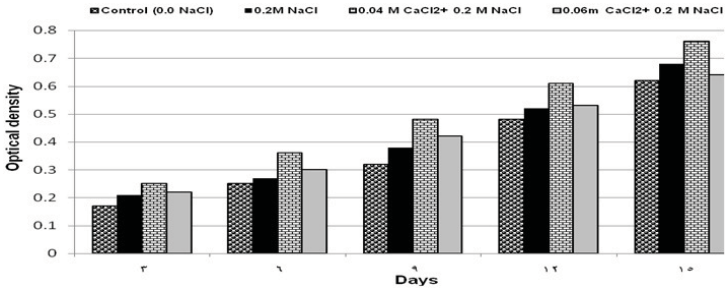

Fig: 5: Effect of two different concentrations of $CaCl_2$ on salinized culture of *A. constricta* (optical density at 750 nm).

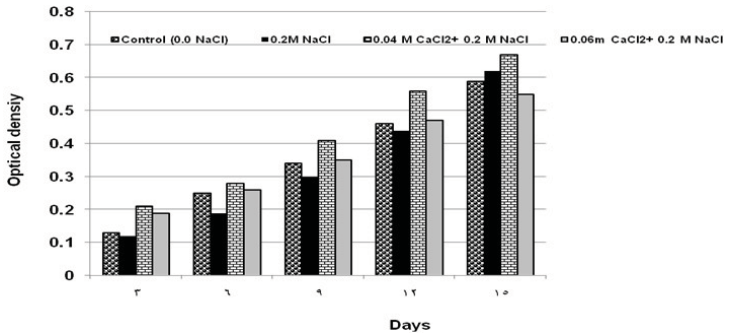

Fig: 6: Effect of two different concentrations of $CaCl_2$ on salinized Culture of *N. linckia* (optical density at 750 nm).

Data in table (1) indicated that the application of 0.2M NaCl only with both organisms leading to sharp decrease in chl"a" content nearly to control, while addition of 0.04 or 0.06M $CaCl_2$ to salinized culture of both organisms caused increase in chl"a" and carotenoid contents as compared to salinized culture. In the same time, the results in tables (2, 3) showed that the presence of 0.2M NaCl in culture medium caused significant decrease in metabolic activities of *A.constricta* and *N.linckia*. Addition of 0.04 or 0.06M $CaCl_2$ to salinized culture of both organisms induced significant increase in metabolic activities as glucose, nitrogen and protein contents. At the same time the effect of 0.04 M $CaCl_2$ was more prominent than the effect of 0.06 M $CaCl_2$.

(a) Protein Electrophoresis Pattern of A.constricta:

As shown in plate (1) and table (4) the structural pattern of 0.2 M NaCl treated culture after 15 days showed disappearance of a protein with an apparent molecular weight of 77 KDa that appeared in control track. In the same time appearance of two protein profiles at m.w. 171 and 70 KDa. Addition of 0.04M $CaCl_2$ to salinized culture (with 0.2 M NaCl) of *A.constricta* showed disappearance of one protein at m.w. 77KDa with appearance of three protein bands at m.w. 17, 61 and 162 KDa, while the treatment with 0.06M $CaCl_2$ appeard three protein bands with an apparent m.w. at 18, 58 and 138 KDa as compared with salinized culture alone after 15 days old.

Table 1: Effect of two concentrations of $CaCl_2$ on the chlorophyll a and carotein contents of solinized culture of *A. constricta* and *N. linckia* (mg/ml).

Age (days)	Treatments	*A. constricta*		*N. linckia*	
		Chl"a"	"Car"	Chl"a"	"Car"
3	Control	0.220 ± 0.006	0.280 ± 0.006	0.150 ± 0.006	0.320 ± 0.006
	0.2 M NaCl	0.060 ± 0.006	0.058 ± 0.006	0.013 ± 0.003	0.020± 0.006
	0.2 M NaCl + 0.04 M $CaCl_2$	0.230 ± 0.006	0.230 ± 0.006	0.180 ± 0.006	0.400 ± 0.006
	0.2 M NaCl + 0.06 M $NaCl_2$	0.220 ± 0.012	0.220 ± 0.012	0.160 ± 0.006	0.380± 0.003
6	Control	0.307 ± 0.012	0.390 ± 0.006	0.200 ± 0.006	0.390± 0.006
	0.2 M NaCl	0.120 ± 0.058	0.130 ± 0.006	0.130 ± 0.006	0.070± 0.006
	0.2 M NaCl + 0.04 M $CaCl_2$	0.353 ± 0.088	0.45 ± 0.006	0.250 ± 0.006	0.480± 0.003
	0.2 M NaCl + 0.06 M $NaCl_2$	0.320 ± 0.058	0.400 ± 0.006	0.210 ± 0.006	0.450± 0.003
9	Control	0.303 ± 0.013	0.570 ± 0.006	0.290 ± 0.006	0.480± 0.006
	0.2 M NaCl	0.200 ± 0.058	0.250 ± 0.006	0.190 ± 0.006	0.116 ± 0.009
	0.2 M NaCl + 0.04 M $CaCl_2$	0.486 ± 0.033	0.680 ± 0.006	0.310 ± 0.006	0.540± 0.006
	0.2 M NaCl + 0.06 M $NaCl_2$	0.460 ± 0.058	0.620 ± 0.006	0.260 ± 0.006	0.520± 0.006
12	Control	0.456 ± 0.008	0.690 ± 0.006	0.37 ± 0.006	0.590 ± 0.06
	0.2 M NaCl	0.276 ± 0.008	0.250 ± 0.006	0.313 ± 0.003	0.180± 0.06
	0.2 M NaCl + 0.04 M $CaCl_2$	0.573 ± 0.012	0.683 ± 0.009	0.420 ± 0.006	0.630± 0.03
	0.2 M NaCl + 0.06 M $NaCl_2$	0.510 ± 0.006	0.623 ± 0.007	0.373 ± 0.007	0.570± 0.06
15	Control	0.570 ± 0.006	0.756 ± 0.007	0.490 ± 0.006	0.670± 0.06
	0.2 M NaCl	0.353 ± 0.007	0.320 ± 0.006	0.356 ± 0.003	0.240± 0.06
	0.2 M NaCl + 0.04 M $CaCl_2$	0.0680 ± 0.006	0.74 ± 0.006	0.820 ± 0.006	0.770± 0.03
	0.2 M NaCl + 0.06 M $NaCl_2$	0.0646 ± 0.012	0.680 ± 0.006	0.740 ± 0.006	0.710± 0.06
	Significance	**	**	**	**

*= Significant difference at P £ 0.05 , ** = Significant difference at P £ 0.01, ***= Significant difference at P £ 0.001 ,and N.S.=non significant according to F-test. Chlorophyll "a" = Chl"a" and Carotein = car.

Table 2: Effect of two concentrations of $CaCl_2$ on some metabolites of salinized culture of *A. constoicta*

Age (days)	Treatments	Glucose ug/ml	Nitrogen mg N/100 ml	Protein mg/100 ml
3	Control	23.0 ±0.250	0.57 ± 0.006	7.09 ± 0.012
	0.2 M NaCl	28.0 ± 0.180	0.35 ± 0.006	5.68 ± 0.046
	0.2 M NaCl + 0.04 M $CaCl_2$	35.8 ± 0.320	0.78 ± 0.006	7.68 ± 0.063
	0.2 M NaCl + 0.06 M $NaCl_2$	35.2 ± 0.610	0.60 ± 0.006	7.08 ± 0.004
6	Control	44.8 ± 0.810	1.20 ± 0.060	8.29 ± 0.006
	0.2 M NaCl	36.0 ± 0.580	0.75 ± 0.06	6.74 ± 0.063
	0.2 M NaCl + 0.04 M $CaCl_2$	55.0 ± 0.580	1.52 ± 0.060	10.34 ± 0.063
	0.2 M NaCl + 0.06 M $NaCl_2$	47.0 ± 0.580	1.30 ± 0.003	12.20 ± 0.115
9	Control	62.4 ± 0.660	1.91 ± 0.006	8.50 ± 0.057
	0.2 M NaCl	57.0 ± 0.580	1.20 ± 0.110	13.20 ± 0.058
	0.2 M NaCl + 0.04 M $CaCl_2$	88.6 ± 0.330	2.11 ± 0.009	11.610 ± 0.063
	0.2 M NaCl + 0.06 M $NaCl_2$	84.0 ± 0.580	1.97 ± 0.006	15.40 ± 0.067
12	Control	94.6 ± 0.580	2.42 ± 0.006	10.00 ± 0.577
	0.2 M NaCl	84.0 ± 0.580	1.51 ± 0.020	16.40 ± 0.067
	0.2 M NaCl + 0.04 M $CaCl_2$	128.0 ± 0.580	2.80 ± 0.060	13.167 ± 0.091
	0.2 M NaCl + 0.06 M $NaCl_2$	119.0 ± 0.580	2.50 ± 0.115	17.21 ± 0.063
15	Control	120.4 ± 0.580	3.10 ± 0.115	14.40 ± 0.067
	0.2 M NaCl	130.0 ± 0.580	1.70 ± 0.060	18.20 ± 0.067
	0.2 M NaCl + 0.04 M $CaCl_2$	160.0 ± 0.580	3.68 ± 0.060	16.60 ± 6.057
	0.2 M NaCl + 0.06 M $NaCl_2$	151.0 ± 0.580	3.22 ± 0.060	11.44 ± 0.122
	Significance	**	**	**

*= Significant difference at P £ 0.05 , ** = Significant difference at P £ 0.01, ***= Significant difference at P £ 0.001 , and N.S.=non significant according to F-test

Table 3: Effect of two concentrations of $CaCl_2$ on some *metabolites activities of salinized* culture of *N. linckia*.

Age (days)	Treatments	Total Glucose ug/ml	Total Nitrogen mg N/100 ml	Total Protein mg/100 ml
3	Control	32.63 ± 0.66	0.560 ± 0.06	3.80 ± 0.17
	0.2 M NaCl	29.0 ± 0.58	0.340 ± 0.06	4.20 ± 0.12
	0.2 M NaCl + 0.04 M $CaCl_2$	52.6 ± 1.20	0.600 ± 0.06	4.0 ± 0.06
	0.2 M NaCl + 0.06 M $NaCl_2$	43.0 ± 0.58	0.54 ± 0.06	4.0 ± 0.12
6	Control	40.53 ± 0.57	0.72 ± 0.006	4.00 ± 0.058
	0.2 M NaCl	43.00 ± 0.58	0.63 ± 0.006	4.20 ± 0.115
	0.2 M NaCl + 0.04 M $CaCl_2$	61.00 ± 0.58	0.96 ± 0.006	3.21 ± 0.121
	0.2 M NaCl + 0.06 M $NaCl_2$	50.00 ± 0.58	0.84 ± 0.006	5.40 ± 0.230
9	Control	65.30 ± 0.124	1.120 ± 0.023	6.45 ± 0.030
	0.2 M NaCl	51.67 ± 0.667	0.60 ± 0.251	4.93 ± 0.38
	0.2 M NaCl + 0.04 M $CaCl_2$	95.00 ± 0.577	1.52 ± 0.058	9.60 ± 0.036
	0.2 M NaCl + 0.06 M $NaCl_2$	86.00 ± 0.577	4.31 ± 3.24	7.80 ± 0.058

Table 3: Continue

12	Control	95.40 ± 0.230	1.50 ± 0.058	9.40 ± 0.00
	0.2 M NaCl	81.00 ± 0.577	1.140 ± 0.058	6.40 ± 0.230
	0.2 M NaCl + 0.04 M $CaCl_2$	125.00 ± 0.577	1.80 ± 0.058	12.13 ± 0.075
	0.2 M NaCl + 0.06 M $NaCl_2$	101.00 ± 0.577	1.43 ± 0.044	10.16 ± 0.08
15	Control	126.33 ± 0.190	2.00 ± 0.077	13.60 ± 0.346
	0.2 M NaCl	116.55 ± 0.293	1.400 ± 0.058	9.110 ± 0.063
	0.2 M NaCl + 0.04 M $CaCl_2$	146.00 ± 0.577	2.320 ± 0.058	16.13 ± 0.075
	0.2 M NaCl + 0.06 M $NaCl_2$	129.33 ± 1.201	2.100 ± 0.058	15.60 ± 0.057
	Significance	**	N.S	**

*= Significant difference at P £ 0.05 , ** = Significant difference at P £ 0.01, ***= Significant difference at P £ 0.001 and N.S.=non significant according to F-test

Table 4: The percentage of intensity of molecular weights (W.M.) of protein bands of *A. constricta* after 15 days incubation.

Bands	control		0.2 M NaCl		0.2 M NaCl + 0.04 M $CaCl_2$		0.2 M NaCl + 0.06 M $CaCl_2$		M.W Standard (Kda)
	AMT%	M.W	AMT%	M.W	AMT%	M.W	AMT%	M.W	
1	46.98	77	4.03	171	4.74	162	14.06	138	211
2	4.22	37	65.84	70	4.1	70	1.95	70	122
3	3.19	33	5.79	36	16.01	61	17.51	58	80
4	45.61	19	3.2	32	1.77	36	1.1	36	51
5			21.11	19	1.56	33	1.42	33	35
6					71.82	17	63.97	18	28
7									21
8									7

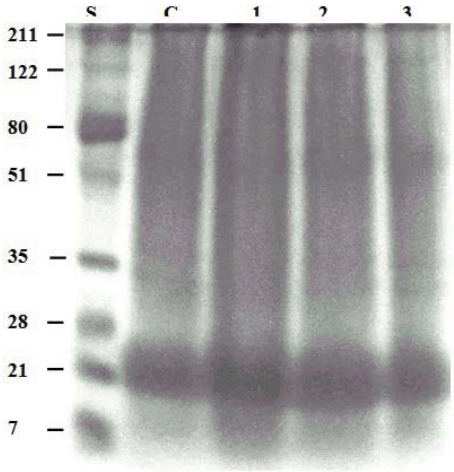

Plate 1: Photographic picture of the gel electrophoresis of protein in *A. constricta*.[Lane S=Standard, C=Control , Lane 1=0.2M.NaCl , Lane 2= 0.2M NaCL+0.04M $CaCL_2$ and Lane 3= 0.2M NaCL+0.06M.

(b) Protein Electrophoresis Pattern of N.linckia:

The results in plate (2) and table (5) showed changes in protein electrophoreses pattern of *N.linckia* after 15 days of incubation as affected by different salts. The structure pattern of 0.2M NaCl treated culture showed no major changes of protein patterns as compared with control. Treated culture by 0.2M NaCl appeared two proteins with apparent protein profiles with m.w. 44 and 6 KDa that appeared in control track but the difference between them was in the percentage of intensity. Addition of 0.04 or 0.06 M $CaCl_2$ to salinized culture of *N.linckia* showed appearance the same proteins with an apparent m.w. 43 and 6 KDa that differed only in the percentage of intensity (AMT %).

Discussion:

In the course of our experiment, we found that salinity induced a significant decrease in the value of parameters tested (growth, pigment content and metabolic activities). The addition of 0.2 M NaCl in culture media of *Anabaena constricta* and *Nostoc linckia* caused significant decrease in cell number, dry weight, optical density and different pigments. The results obtained agreed with Blumwald and Tel-or (1984),who

observed that, the chlorophyll contents of *Synechococcus* 6311 was essentially stable through the process of salt adaption, with an observed enhancement in the synthesis of biloprotein pigments (phycocyanin and phycoerythrin). There was an enhancement in the synthesis of salt adapted cells of *synechococcus* 6311, while they indicated that the synthesis of both pigments in the heterocystous *Nostoc muscorum* was slightly lower at high salt concentration of NaCl. In this connection the growth rate of *Porphyridium cruentum* was influenced by the connection of NaCl, optimum growth was found with salinities ranging between 0.45 M and 0.8M NaCl. A further increase in salinity to 1.5 M NaCl resulted in a drastic drop in algal growth Lee, *et al.,* (1989).

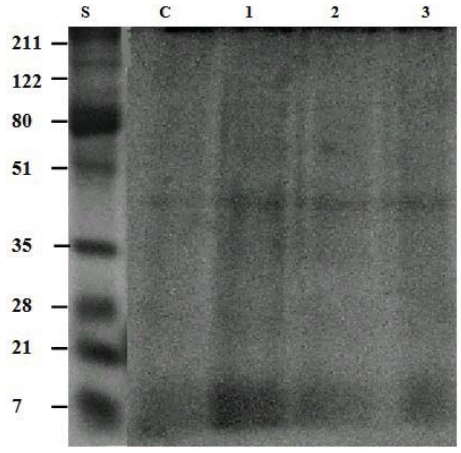

Plate 2: Photographic picture of the gel electrophoresis of protein in *N. linckia*.[Lane S=Standard, C=Control, Lane 1=0.2M.NaCl, Lane 2= 0.2M NaCL+0.04M $CaCL_2$ and Lane 3= 0.2M NaCL+0.06M.

Table 5: The percentage of intensity of molecular weights (W.M.)of protein bands of *N. linckia* after 15 days old incubation.

Bands	control		0.2 M NaCl		0.2 M NaCl + 0.04 M $CaCl_2$		0.2 M NaCl + 0.06 M $CaCl_2$		M.W Standard (Kda)
	AMT%	M.W	AMT%	M.W	AMT%	M.W	AMT%	M.W	
1	32.44	44	16.34	44	68.61	43	39.82	43	211
2	67.56	6	83.66	6	31.39	6	60.18	6	122
3									80
4									51
5									35
6									28
7									21
8									7

The combination between low concentration of $CaCl_2$ (0.04 or 0.06 M) with salinized culture (0.2 M NaCl) of *A constricta* and *N. linckia*, caused significant increase in the growth parameters and metabolic activity as compared with control (0.2M NaCl only). In accordance with the present results, Ahmed et.al., (1989) found that the growth of *Chlorella vulgaris* was markedly inhibited with the rise of NaCl level, however, a marked growth stimulation was observed under certain combination of NaCl and $CaCl_2$.

The mechanism of calcium in stressed plants could be activated through membrane stability Munns, *et al.,* (1983). Also low calcium increased membrane permeability at high external NaCl (Greenway, and Munns, (1980). Leopold, and Wilting, (1984) found that calcium served partially to protect tissues from NaCl damage and lessens the leakiness of organic metabolites. Therefore, it could would be generalized that calcium relief occurs in the following sequence: Stabilization and repair of NaCl damaged membrane including thylakoids, less uptake of Na^+ (less toxicity) and preservation of cell metabolites from leakiness.

It has been documented that most organisms investigated respond to shock treatment by synthesizing a new set of proteins. Bhagwat and Apte (1989); Schubert, *et al.*(1993); Thomas *et al;* (1990) and Rajesh war and Donat (1996). Our results indicate that the *cyanobacteria A.constricta* and *N. Linckia* respond to shock treatments. The protein electrophoresis pattern of both organisms under shock of 0.2 M NaCl alone or with addition of 0.04 or 0.06 M $CaCl_2$ provided major changes (appearance disappearance) of protein patterns.

In conclusion, our results indicate that any substantial increase in the above mentioned stress in nature might be related to the ecological and economically important cyanobacterial communities, which in turn, may affect the productivity of higher plants. With increasing presence of stressors, there might be a setback in the agriculture economy of all countries where cyanobacteria are being considered as an alternate natural source of nitrogenous fertilizers for rice paddies and other crops.

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