

## Alleviation of Salinity Stress on Growth and Some Metabolites of *Anabaena Constricta* and *Nostoc Linckia* Using L- Proline or D L- B Phenylalanine.

Mohamed Goma'a Battah

Department of Botany; Faculty of Science; Benha University; Egypt.

**Abstract:** The objective of this study was to investigate the effects of sodium chloride (0.2M) as salinizing agent on growth, pigment contents and some metabolic activities of the cultures of cyanobacteria *Anabaena constricta* and *Nostoc linckia*. The cell number, dry weight, glucose, protein and nitrogen content of *A. constricta* and *N. linckia* were significantly decrease, while the pigment contents not affected under the stress 0.2M NaCl through 15 days incubation. The addition of any of the two amino acids 5 mM L-proline or 5 mM DL-B-Phenylalanine to salinized culture of both organisms can alleviate or at least modify (mitigatory) the pattern of changes induced by the salinity stress. This means that any of the two amino acids was generally effective in meliorating the adverse effect of salinity on growth, pigment contents and some metabolic activities. Protein electrophoretic pattern of salinized culture of *Anabaena constricta* treated with 5mM of L-proline showed new set of proteins at around 17, 62 and 159 kDa, while treatment with DL-B- phenylalanine showed new proteins at around 21, 62 and 146 KDa. Protein electrophoresis pattern of salinized culture of *Nostoc linckia* when treated with any of the two amino acids showed no difference of protein bands, the difference only in the percentage of intensity.

**Key words:** *Anabaena* sp, *Nostoc* sp, cyanobacterium, Sodium chloride stress, Proline, Phenylalanine.

### INTRODUCTION

Stressors such as heat, cold, salinity and drought play a key role in determining the growth and development of cyanobacterial populations in their particular habitat (Tandeau de Marsac and Houmard, 1993). Salinity has become an ever-increasing problem in irrigated agriculture being cosmopolitan in distribution, cyanobacteria are thought to have been exposed to different levels and types of stressors during their development, thus providing a suitable system for analyzing the adaptive mechanisms developed in response to changing stress conditions (Stainer and Cohen-Bazire, 1977). Certain bacterial strains such as *Rhizobium*, which nodulate a number of economically important crops, are highly sensitive to NaCl (Upchurch and Elkan, 1977). Cyanobacterial N<sub>2</sub>-fixation is supposed to be the most sensitive process in response to enhanced salt concentrations, followed by photosynthesis. Respiration has been reported to be increased in salt adapted cyanobacteria, (Tel or, 1980) and (Vonshak and Guy, 1988). Decreased photosynthesis and chlorophyll a contents have been reported in *Microcystis firma* and *Synechjocystis* sp PCC 6803 following salinity stress, (Erdmann and Hagemann 1992).

In the present investigation an attempt was made to study the effect of NaCl (0.2M) and two amino acids L-proline and DL-B phenylalanine in low concentration 5mM for each one to evaluate the interactive effects of salt stress and the two amino acids, on growth and protein profile of *Anabaena constricta* and *Nostoc linckia*.

### MATERIALS AND METHODS

#### **Organisms:**

Two algal axenic cultures of filamentous heterocystous *Anabaena constricta* and *Nostoc linckia* were isolated from saline alkali soils (pH 9.0), brought from cultivated fields of Sana'a Yemen, using routine microbiological procedures (Kaushik, 1987). The organisms were maintained in BG-11 medium (stainer *et al*; 1971) at an illumination 3500 lux with regime 16/8 hours light / dark at 27 °C.

---

**Corresponding Author:** Biology Department, Faculty of Science, Benha University (Egypt)  
Tel. 0020104493638 – 0020552315971  
E-mail: maamay57@yahoo.com

#### **Growth Estimation:**

Changes in cell number were determined by using homogenizer glass spread the cells of the algal filaments and counted by haemocytometer slide. Dry weight was recorded as mg /100 ml algal suspension (Leganes et al.1987). The chlorophyll "a" contents was determined by spectrophotometric method (Jeffery and Humphrey, 1975). The carotenoids were determined according to (Jensen and liaen, 1959). The phycobiloproteins were determined according to (Bennet and Bogorad, 1973). Glucose contents was estimated the method adapted by Naguib (1963), Nitrogen contents by Jacobs (1958) and total soluble proteins were determined by( Lowery *et al*; 1951).

#### **Gradient Gel Electrophoresis:**

Vertical polyacryamide Gel electrophoresis (PAGE) was used as described by Laemmli (1970). Analysis of gel lanes were carried out using gel documentation and analysis system consisting of dark room transilluminator, integrating CD video camera and image software ( AAB software). The analyses were carried out at the end of experiment after 15 days of incubation.

#### **Statistical Analysis:**

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

## **RESULTS AND DISCUSSION**

#### **Effect of 0.2M NaCl and two amino acids on growth of *A. constricta* and *N. linckia*:**

As shown in Figs (1,2) the presence of 0.2 M NaCl in the medium decreased the cell number through the experiment when compared with control. Addition of 5mM of L- proline or DL-B phenylalanine to slainized culture increased the cell number as compared with salinized (0.2 M NaCl only ) culture of *A. constricta* and *N linckia*. The results in figs (3,4) showed that the dry weight in both organisms was decreased when treated by 0.2 M NaCl as compared with control, while addition of 5 mM proline or 5 mM phenylalanine to salinized culture increased the dry weight as compared with salinized culture only.

The data present in tables(1,2) indicated that the application of 0.2 M NaCl caused 46.3% reduction in chlorophyll "a" contents of *A. constricta* and 24.7% of *N. linckia* after 15 days of incubation as compared with control. Addition of 5 mM of proline to salinized culture of the two organisms caused significant increase in chlorophyll "a" contents reached 1.89 fold in *A. constricta* and 1.58 fold in *N. linckia*, while addition of 5 mM of phenylalanine caused increase reached 1.48 fold in *A. constricta* and 1.7 fold in *N. linckia* after 15 days incubatio. The other pigments such as carotenoids, phycocyanin, phycoerythrin and allophycocyanin significantly decreased in culture of both organisms when treated with 0.2 M NaCl, while addition of 5 mM proline and 5mM phenylalanine caused increase in the above pigments.

#### **Effect of 0.2 M Nacl and Two Amino Acids on the Metabolic Activity of *A. Constricta* and *N. Linckia*:**

The results obtained in tables (3&4) showed that addition of 0.2 M NaCl to culture medium of both organisms induced decrease in glucose content when it amounted in 6% and 14% in *A. constricta* and *N. linckia*, receptively at the end of experiment (15 days old). Addition of 5 mM of proline or 5 mM of phenylalanine to salinized culture caused an increase in glucose content reached 1.38 and 1.23 fold in *A. constricta* with proline and phenylalanine, respectively, while the increase amounted to 1.18 fold and 1.0 fold in *N. linckia* with both amino acids, respectively at the end of experiment (15 days old).

Addition of 0.2 M NaCl reduced the nitrogen content by 46% and 29% in *A. constricta* and *N. linckia* respectively at the end of experiment (15 days old ). Presence of 5 mM proline or 5 mM DL-B phenylalanine caused 2.0 and 1.5 fold increase in *A. constricta* while presence of amino acids in salinized culture of *N. linckia* caused increase reached 1.37 and 1.2 fold with proline and phenylalanine respectively as compared with slainized culture.

Data presented in tables (3 &4) showed that inclusion of 0.2 M NaCl in the culture media of both organisms caused remarkable decrease in the protein contents throughout the experimental period. The reduction reached 26.5% in *A. constricta* and 36% in *N. linckia* at the end of experiment (15 days old). Application of 5 mM proline and 5 mM DL-B phenylalanine induced a significant increase in protein contents, reached 1.3 fold in both organisms with proline while phenylalanine caused 1.3 fold and 1.16 fold in *A. constricta* and *N. linckia* respectively as compared with salinized culture at the end of experiment.

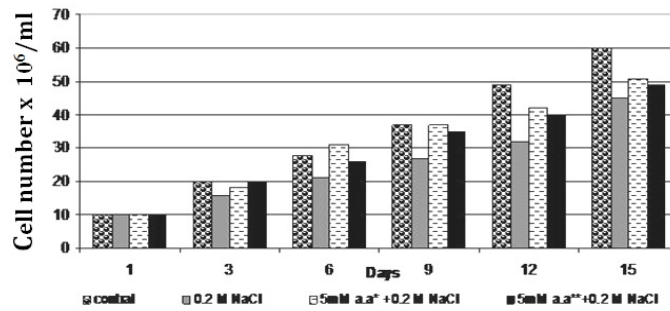


Fig. 1: Effect of L-proline (a.a\*) and DL-B-phenylalanine (a.a\*\*), amino acids on salinized culture of *A. constricta* (Cell number x 10<sup>6</sup>/ml)

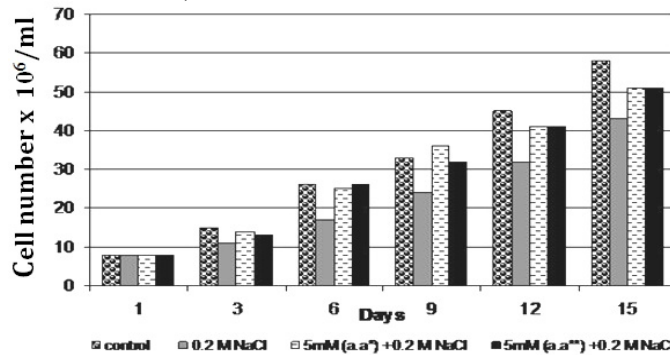


Fig. 2: Effect of L-proline (a.a\*) and DL-B-phenylalanine (a.a\*\*), amino acids on salinized culture of *N. linckia* (Cell number x 10<sup>6</sup>/ml)

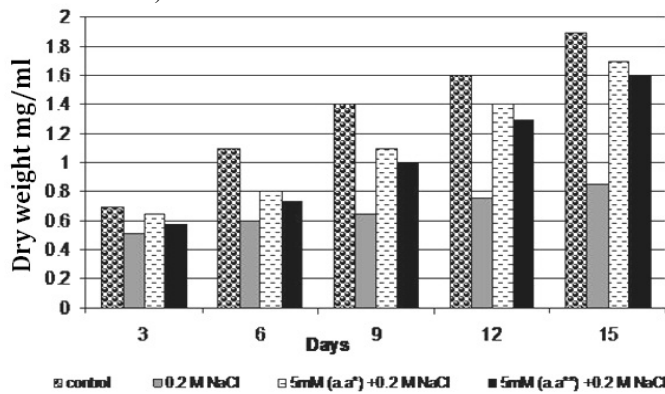


Fig. 3: Effect of L-proline (a.a\*) and DL-B-phenylalanine (a.a\*\*) amino acids on salinized culture of *A. constricta* (Dry weight mg/ml culture)

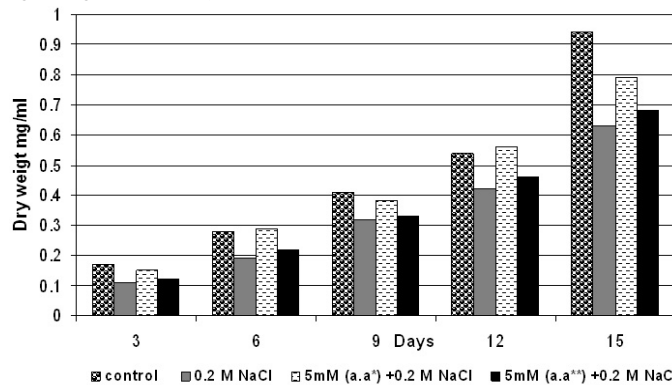


Fig. 4: Effect of L-proline (a.a\*) and DL-B-phenylalanine (a.a\*\*), amino acids on salinized culture of *N. linckia* (Dry weight mg/ml culture)

**Effect of 0.2 M NaCl and Two Amino Acids on Protein Profile of *A. Constricta* and *N. Linkia*:**

The SDS-PAGE protein profile of *A. constricta* and *N. Linkia* of control and 0.2M NaCl treated culture and after addition of 5 mM proline and 5 mM phenylalanine is illustrated in plates( 1& 2 ) and in tables (5& 6). It is evident that the electrophoretic pattern of culture that exposed to salinity stress resulted in alteration in cyanobacterial protein content. Cultures of *A. constricta* treated with 0.2M NaCl showed a number of new proteins bands at 70 and 171 KDa, in the same time disappearance of a protein around 77KDa as compared with control (without salt) table 5. Addition of 5 mM proline to salinized culture of *A. constricta* showed new proteins bands at 17, 62 and 159 KDa, while addition of 5 mM of DL-B phenylalanine showed a number of new proteins at around 21.62 and 146 KDa as compared to salinized culture. Application of proline or phenylalanine in table (6) leads to appearance of three proteins but not disappear any type of proteins comparing with control. Treatment of *N. Linkia* with 0.2 M NaCl showed no difference of protein bands as compared with control, but there was difference in the intensity of all protein bands appeared. Addition of proline or phenylalanine to salinized culture of *N. Linkia* showed no changes in protein bands but only in the percentage of intensity.

**Table 1:** Effect of L-proline (a.a\*) and DL-B-phenylalanine (a.a\*\*), amino acids on the pigment contents of salinized culture of *Anabaena constricta* (mg/ml).

Age (days)	Treatments	Chl"a"	Car.	PC.(X10 <sup>-3</sup> )	PE.(X10 <sup>-3</sup> )	APC.(X10 <sup>3</sup> )
3	Control	0.213	0.002	2.9	1.8	5.6
	0.2 M NaCl	0.351	0.002	2.6	1.4	3.2
	0.2 M NaCl + 5mM (a.a*)	0.560	0.003	1.6	2.3	4.5
	0.2 M NaCl + 5mM (a.a**)	0.440	0.002	1.5	2.0	3.8
6	Control	1.212	0.006	4.3	2.1	5.9
	0.2 M NaCl	1.100	0.003	3.5	1.6	3.7
	0.2 M NaCl + 5mM (a.a*)	1.201	0.011	4.0	2.6	4.8
	0.2 M NaCl + 5mM (a.a**)	0.962	0.006	3.5	2.2	4.0
9	Control	1.401	0.011	6.1	2.8	6.3
	0.2 M NaCl	1.321	0.010	4.1	2.0	4.8
	0.2 M NaCl + 5mM (a.a*)	1.661	0.015	6.2	2.9	5.7
	0.2 M NaCl + 5mM (a.a**)	1.230	0.013	4.3	2.5	4.4
12	Control	2.612	0.015	11.2	3.0	6.6
	0.2 M NaCl	2.190	0.013	7.3	2.6	5.2
	0.2 M NaCl + 5mM (a.a*)	2.853	0.025	4.4	3.4	6.7
	0.2 M NaCl + 5mM (a.a**)	2.253	0.024	7.2	2.9	5.6
15	Control	3.135	0.028	13.3	3.5	7.2
	0.2 M NaCl	2.690	0.019	10.2	2.9	6.3
	0.2 M NaCl + 5mM (a.a*)	3.664	0.031	11.6	3.9	7.4
	0.2 M NaCl + 5mM (a.a**)	2.736	0.026	14.8	2.6	6.8

Chlorophyll "a" = Chl"a" , Caroten = car. Phycocyanin = PC. Phycoerythrin = PE and Allophycocyanin = APC.

**Table 2:** Effect of L-proline (a.a\*) and DL-B-phenylalanine (a.a\*\*), amino acids on the pigment contents of salinized culture of *Nostoc linckia* (mg/ml).

Age (days)	Treatments	Chl"a"	Car.	PC. (X10 <sup>-3</sup> )	PE. (X10 <sup>-3</sup> )	APC.(X10 <sup>3</sup> )
3	Control	0.233	0.002	1.0	0.4	1.8
	0.2 M NaCl	0.318	0.001	0.1	0.4	1.2
	0.2 M NaCl + 5mM (a.a*)	0.146	0.002	1.5	0.7	1.9
	0.2 M NaCl + 5mM (a.a**)	0.205	0.002	1.0	1.0	1.5
6	Control	0.485	0.011	1.0	1.2	2.1
	0.2 M NaCl	0.521	0.006	1.1	0.4	2.2
	0.2 M NaCl + 5mM (a.a*)	0.601	0.012	1.9	0.7	2.6
	0.2 M NaCl + 5mM (a.a**)	0.503	0.11	1.6	0.8	2.2
9	Control	1.380	0.011	2.4	1.7	3.6
	0.2 M NaCl	1.105	0.008	2.0	1.1	3.6
	0.2 M NaCl + 5mM (a.a*)	1.923	0.12	2.3	1.4	4.1
	0.2 M NaCl + 5mM (a.a**)	1.600	0.011	1.9	1.0	3.1
12	Control	1.974	0.012	3.2	2.3	4.2
	0.2 M NaCl	1.672	0.009	2.7	1.6	3.2
	0.2 M NaCl + 5mM (a.a*)	2.231	0.015	209	1.7	5.2
	0.2 M NaCl + 5mM (a.a**)	1.821	0.116	2.3	1.6	4.1
15	Control	1.431	0.023	3.8	3.4	5.2
	0.2 M NaCl	1.983	0.014	2.9	2.0	6.3
	0.2 M NaCl + 5mM (a.a*)	2.622	0.027	3.1	2.6	5.0
	0.2 M NaCl + 5mM (a.a**)	2.100	0.026	2.9	2.3	

chlorophyll "a" = Chl"a" and caroten = car. Phycocyanin = PC. Phycoerythrin = PE, .Allophycocyanin = APC.

**Table 3:** Effect of two concentrations of CaCl<sub>2</sub> on some metabolites activities of salinized culture of *A. constricta*.

Age (days)	Treatments	Total Glucose ug/ml	Total Nitrogen mg N/100 ml	Total Protein mg/100 ml
3	Control	33.0 ± 0.580	0.84 ± 0.065	7.066 ± 0.033
	0.2 M NaCl	28.3 ± 0.750	0.35 ± 0.006	5.666 ± 0.033
	0.2 M NaCl + 5 mM (a.a*)	24.0 ± 0.577	0.61 ± 0.006	7.767 ± 0.033
	0.2 M NaCl + 5 mM (a.a**)	22.0 ± 0.577	0.54 ± 0.006	6.233 ± 0.033
6	Control	44.80 ± 0.561	1.400 ± 0.057	7.60 ± 0.650
	0.2 M NaCl	36.00 ± 0.577	0.750 ± 0.057	7.10 ± 0.351
	0.2 M NaCl + 5 mM (a.a*)	63.00 ± 0.577	1.100 ± 0.028	9.100 ± 0.100
	0.2 M NaCl + 5 mM (a.a**)	45.00 ± 0.577	0.86 ± 0.033	8.166 ± 0.033
9	Control	62.45 ± 0.42	1.90 ± 0.057	13.20 ± 0.057
	0.2 M NaCl	57.00 ± 0.577	1.20 ± 0.057	8.20 ± 0.077
	0.2 M NaCl + 5 mM (a.a*)	97.00 ± 0.577	1.80 ± 0.057	12.23 ± 0.033
	0.2 M NaCl + 5 mM (a.a**)	76.00 ± 0.577	1.40 ± 0.057	11.23 ± 0.44
12	Control	93.00 ± 0.577	2.40 ± 0.057	16.33 ± 0.120
	0.2 M NaCl	84.00 ± 1.154	1.40 ± 0.057	10.00 ± 0.577
	0.2 M NaCl + 5 mM (a.a*)	132.00 ± 1.154	2.30 ± 0.057	15.43 ± 0.166
	0.2 M NaCl + 5 mM (a.a**)	112.00 ± 1.15	1.90 ± 0.057	12.26 ± 0.176
15	Control	120.27 ± 0.273	3.10 ± 0.057	18.166 ± 0.120
	0.2 M NaCl	113.00 ± 1.00	1.70 ± 0.057	13.400 ± 0.057
	0.2 M NaCl + 5 mM (a.a*)	156.00 ± 0.577	3.36 ± 0.08	17.70 ± 0.057
	0.2 M NaCl + 5 mM (a.a**)	140.00 ± 0.577	2.50 ± 0.057	17.53 ± 0.033
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 4:** Effect of two amino acids, L. protine (a.a\*) and DL-B-phenylallanine (a.a\*\*) 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	32.6±0.058	0.610±0.058	3.80±0.058
	0.2 M NaCl	29.00±0.058	0.350±0.058	4.20±0.115
	0.2 M NaCl + 5 mM (a.a*)	40.00±0.58	0.640±0.058	4.21±0.121
	0.2 M NaCl + 5 mM (a.a**)	28.0±0.577	0.400±0.058	4.00±0.577
6	Control	48.33±0.88	0.800±0.057	4.23±0.033
	0.2 M NaCl	44.00±0.577	0.610±0.005	3.20±0.057
	0.2 M NaCl + 5 mM (a.a*)	62.22±0.577	0.940±0.025	6.10±0.057
	0.2 M NaCl + 5 mM (a.a**)	55.00±0.577	0.810±0.005	5.16±0.088
9	Control	65.23±0.088	65.23±0.088	7.53±0.031
	0.2 M NaCl	58.00±0.577	58.00±0.577	4.56±0.033
	0.2 M NaCl + 5 mM (a.a*)	76.00±0.577	76.00±0.577	8.83±0.066
	0.2 M NaCl + 5 mM (a.a**)	68.88±0.577	68.8±0.577	7.166±0.088
12	Control	95.30±0.115	95.30±0.115	11.3±0.05
	0.2 M NaCl	82.00±0.577	82.00±0.577	6.30±0.057
	0.2 M NaCl + 5 mM (a.a*)	103.33±0.333	103.33±0.33	10.16±0.033
	0.2 M NaCl + 5 mM (a.a**)	98.00±0.577	98.00±0.577	8.40±0.152
15	Control	136±0.577	136.00±0.577	14.20±0.057
	0.2 M NaCl	115.00±0.577	115.00±0.577	9.10±0.057
	0.2 M NaCl + 5 mM (a.a*)	136.00±0.333	136±0.333	12.00±0.577
	0.2 M NaCl + 5 mM (a.a**)	121.00±0.577	121.00±0.577	10.53±0.218
Significance		**	**	**

\*= Significant difference at P £ 0.05 , \*\* = Significant difference at P £ 0.01,

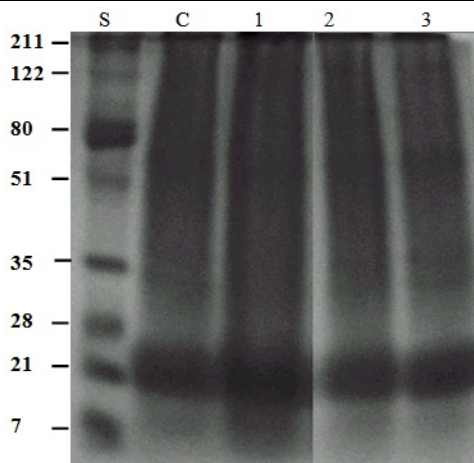
\*\*\*= Significant difference at P £ 0.001 and N.S.= non significant according to F-test

**Table 5 :** The percentage of intensity of molecular weights of protein bands of salinized culture of *A. constricta* treated by to amino acid L – proline (a.a\*) and DL-B- phenylalanine (a.a\*\*) after 15 days incubation.

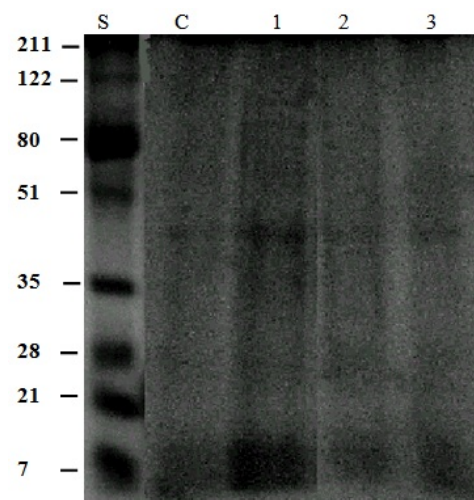
Bands	Control		0.2 M NaCl		0.2 M NaCl(a.a*)		0.2 M NaCl(a.a**)		M.W Standard Kda
	AMT%	M. W	AMT%	M. W	AMT%	M. W	AMT%	M. W	
1	46.98	77	4.03	171	42.28	159	3.15	146	211
2	4.22	37	65.84	70	12.82	79	17.17	76	122
3	3.19	33	5.79	36	5.65	62	4.19	62	80
4	45.61	19	3.2	36	0.78	38	1.06	37	51
5			21.11	19	4.85	35	6.16	35	35
6					3.77	33	3.94	33	28
7						17	64.32	21	21
8									

**Table 6:** The percentage of intensity of molecular weights of protein bands of salinized culture of *Nostoc linckia* treated by to amino acid L – proline (a.a.\*) and DL-B- phenylalanine (a.a.\*\* ) after 15 days incubation

Bands	Control		0.2 M NaCl 0		.2 M NaCl(a.a*)		0.2 M NaCl(a.a**)		M.W Standard KDa
	AMT%	M. W	AMT%	M. W	AMT%	M. W	AMT%	M. W	
1	32.44	44	16.34	44	30.85	43	86.25	44	211
2	67.56	6	83.66	6	69.15	6	13.71	6	122
3									80
4									51
5									35
6									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<sub>2</sub> and Lane 3= 0.2M NaCL+0.06M.



**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<sub>2</sub> and Lane 3= 0.2M NaCL+0.06M ]

**Discussion**

In the course of our experiment, we found that salinity induced significant decrease in the values of the parameters tested as growth, pigment contents and metabolic products. The treated culture of *Anabaena constricta* or *Nostoc linckia* by 0.2 M NaCl and by 5mM L-proline or 5 mM DL-B phenylalanine induced a significantly increase in growth parameters. The recorded increase in the values of growth of *A. constricta* and *N. linckia* induced by the interactive effect between L. proline and DL-B phenylalanine and salinity stress (0.2 M NaCl ) is in accordance with the results obtained by Heikal and shaddad (1982). They found that the

exogenous application of the L-proline and DL-B phenylalanine (100 ppm) can alleviate the inhibitory effect of salinity on kidney bean plant, however, the rate of either proline or phenylalanine in ameliorating (mitigative) the effect of salinity.

The data obtained in the present study with cyanobacteria species indicate that the protein synthesis is significantly suppressed in the test organisms when the cells are subjected to elevated levels of salinity with L-proline or DL-B phenylalanine. The degree of suppression depends on the severity of the stressor. It has been documented that most organisms investigated to date respond to shock treatment by synthesizing a new set of proteins, Bhagwat and Apte (1989), Thomas, et al (1990) and Schubert, et al (1993). Our results indicate that cyanobacterium *A. constricta* response in the same manner, while *N. linckia* very little in respond to synthesize a new set of protein.

The present results indicated that the salinized culture of *A. constricta* when treated by 5 mM of L proline showed a number of new proteins at around 17, 62 and 159 KDa, while addition of 5mM of DL-B phenylalanine showed a number of new proteins at around 21, 62 and 146 KDa. This result in agreement with the data obtained by (Rajeshwar and Donat 1996). Application of 5 mM L proline and 5 mM DL-B-phenylalanine alleviated the inhibitory effect of salinity stress on *A. constricta* and on *N. Linckia*.

## REFERENCES

- Bennett, A. and L. Bogorad, 1973. Complementary chromatic adoption in a filamentous blue- green algae. *J. Cell Biol.*, 58: 419 – 435.
- Bhagwat, A.A. and S.K. Apte, 1989. Comparative analysis of proteins induced by heat shock, salinity and osmotic stress in the nitrogen – fixing cyanobacterium *Anabaena* sp. Strain L 31 *J. Bacteriol.*, 171: 5187-5189.
- Erdmann, N., S. Fulda and M. Hagemann, 1992. Glucosylglycerol accumulation during salt acclimation of two unicellular cyanobacteria. *J.Gen. Microbiol.*, 138: 363-368.
- Heikal, M.M. and M.A. Shaddad, 1982. Alleviation of osmotic stress on seed germination and seedling growth of cotton, pea and wheat by proline, phyton (*Aust.* ) 22: 275-287.
- Jacobs, M.B., 1958. The chemical analysis of food and food products, D, Van Nostrand Co., Inc./, New York, pp: 34.
- Jeffrey, S.W. and G.F. Humphrey, 1975. New spectrophotometric equations for determining chlorophyll a, b, c, and c<sup>2</sup> in higher plants, algae and natural phytoplankton. *Biochem. Physiol. Pflanz*, 167-191.
- Jensen, A. and liaen S. Jensen, 1959. quantitative paper chromatography of carotenoids. *Acta. Chem. Scud.*, 13: 1813.
- Kaushik, B.D., 1987. Laboratory methods for blue green algae isolation and purification ( pub Associated publishing co., Nelhi, 17.
- Lamli, U.K., 1970. Cleavage of structural proteins during the assembly of the head of the bacteriophage T4. *nature* 227: 680-685.
- Lowery, O.H. N.J. Raseborough, A.L. Farr and R.J. Randall, 1951. Protein measurement with the follin phenol reagent. *J. Biol. Chem.*, 193: 262-275.
- Naguib, M.L., 1963. colorimetric estimation of plant polysaccharides *Zucher.*, 16: 15-18.
- Rajeshwar, P., Sinha and Donat P. Hader, 1996. Respose of a rice field cyanobacterium *Anabaena* sp. to physiological stressors. *Environ and Experim. Botany*, 36(2): 147 – 155.
- Schubert, H., S. Fulda and M. Hagemann, 1993. Effects of Adaptation to different salt concentrations on photosynthesis and pigmentation of the cyanobacterium *Synechocystis* sp. PCC 6803 K. *Plant Physiol.* 142: 291-295.
- Snedecor, G.W. and W.G.Cochran, 1982. Statistical methods 6<sup>th</sup> ed. Iowa, USA.
- Stanier, R.Y. and G. Cohen- Bazire, 1977. Phototrophic prokaryotes: the cyanobacteria. *Annu. Rev. Microbiol.*, 31: 225 – 274.
- Tandean de Marsac, N. and J. Houmard, 1993. Adaptation of cyanobacteria environmental stimuli: new steps towards molecular mechanisms. *EMS Microbiol Rev.*, 104: 119-190.
- Tel – Or E., 1980. Response of N<sub>2</sub> Fixing cyanobacteria to salt. *Appl. Environ. Microbiol.*, 40: 689-693.
- Thomas, S.P., A. Zaritsky and S. Boussiba, 1990. Ammonia excretion by an L-methionine- DL-Sulfoximine-resistant mutant of the rice field cyanobacterium *Anabaena siamensis*. *Appl. Environ. Microbiol.*, 56: 3499-3504.
- Upchurch, R.G. and G.H. Elkan, 1977. Comparison of colony morphology, salt tolerance, and effectiveness in Rhizobium Japonicum. *Can – J. Microbiol.*, 23: 1118-1122.
- Vonshak, A. and R. Gy, M. Guy, 1988. The response of the filamentous cyanobacterium *Spirulina platensis* to salt stress. *Arch. Microbiol.*, 150: 417-420.