# EFFECT OF DIFFERENT CHEMICAL COMPOUNDS ON THE GROWTH OF TWO SOIL ALGAL SPECIES *NOSTOC HUMIFUSUM* AND *OSCILLATORIA EARLEI*.

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## *Abstract*

The current study was performed on a farm land at El-Khanka District, Kalyoubia Governorate where the land showed symptoms of increase in its salt content. Some of the physical and chemical characteristics of the collected soil samples were determined. Only two halotolerant species of algae were isolated from the soil samples containing lower total soluble salt contents. Purification of algae from bacteria was done then subjected to a series of experiments for determination of the effect of different chemical constituents on their growth. The results showed that the change in salinity (NaCl contents) as well as changing in carbon and nitrogen sources may have a considerable effect on the growth of the two algae species. In addition, also the major biochemical contents of the algal species were affected by the previous changes.

## *Introduction*

The world of microorganisms can be classified according to their response to salt concentration, into two major groups, the halophiles and the non-halophiles. The halophiles are the salt-loving organisms and they survive in relatively high concentrations of salts and the degree of their halophilism is related to the salt concentration of their environment (Larsen, 1962).

According to Larsen, (1962) halophiles are classified into: slightly halophilic species which grow best in the medium containing 2-5% NaCl; moderately halophilic species, which grow best in the medium containing 8-20% NaCl, and extremely halophilic species which grow best in the medium containing 20-30% NaCl. Larsen, (1962) also grouped halophiles into 3 categories: First group, consisting of microorganisms which have specific requirements for $Na^+$ and $Cl^-$. The second group, containing microorganisms which have specific requirements for $Na^+$ but not for the anion; and the third group, included microorganisms with no specific requirements for the cation or the anion. Brown (1964) stated that the extremely halophilic microorganisms can tolerate and grow well at low water activity, which, he defined as the value for saturated solution of sodium chloride. He also pointed out, that the sodium chloride is the only readily soluble salt, which is known to be able to support

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some form of life over its entire concentration range. No other salts of monovalent cation are known even to approximate that capability.

Meanwhile, some microorganisms can tolerate other divalent salts such as calcium chloride at moderate concentration in the presence of sodium chloride. Whereas, Kushner, (1978) identified halotolerant microorganisms as "those that apparently require no salt for growth", but may grow in NaCl concentrations of 10% or more. In order to grow and reproduce in such high-salt, low-water activity environments, these organisms have made basic biochemical adaptations in their proteins, osmoregulation mechanisms, nucleic acids, and lipids (Litchfield, 1998). Algal activity in soils in which moisture is not a limiting factor is governed in large measure by range and fluctuation of temperature (Metting, 1981; Raven and Geider, 1988). It is generally accepted that neutral and alkaline soils are more favorable to development of algae (Fogg *et al*., 1973; Friedman and Galun, 1974). Certain algae also display a greater range of pH tolerance than required in the environments where they occur (Schulle, 1968). Some elements have been suggested as either limiting or inhibitory to algae as salt stresses were investigated (Chauhan *et al*., 2000; Allakhverdiev *et al*., 2001). The examination of amino acids, proteins and their relation of salt tolerant were studied (Thomas and Shanmuga, 1991; Bhargava *et al*., 2003). Also, photosynthesis in some cyanobacteria was shown to be stimulated by $Na^+$ concentrations (Reinhold *et al*., 1984).

On the other hand, the studies of salinity as controlling factor for blooms of $N_2$-fixing cyanobacteria was investigated by Moisander *et al*., (2002) and Pia *et al*., (2002). Also, studies were made on the investigation of photosynthesis and chlorophyll 'a' and the relation of salt stress by Singh and Kshatriya, 2002 and Demergasso, *et al*., (2003). So several attempts had been carried out to isolate and identify the halotolerant microorganisms from saline soils (Teaumroong *et al*., 2002; Michelle-Anne, 2003 and Krumbein *et al*., 2004).

The aim of this work is to study the effect of changes in some physical and chemical parameters on the growth of algal species isolated from soil of high salt content and clarify their metabolic activities under salts stress conditions.

## *Materials and Methods*

**Soil:** The soil samples were collected during April 2001 at two different localities chosen to represent two different saline habitats located at El-Khanka District, Kalyoubia Governorate. Soil samples were collected in sealed sterile plastic bags and transported immediately to the laboratory.

**Grain Size Analysis**: Fifty grams of each soil sample were used for analysis. The soil air dried and subjected to manual sieves analysis. Particle size analysis of different soil samples were accomplished according to Piper (1950).

**Total Soluble Salts** : Another fifty grams of soil were suspended in 250 ml distilled water and mixed well using magnetic stirrer for 30 mins. The measuring of total soluble salts (TSS) was adopted according to A.O.A.C. (1975).

**Conductivity and pH**: Electrical conductivity (EC) was determined using electrical conductivity meter model (TDS / temperature meter WD-35607-20) and pH was measured using Hanna digital pH meter.

**Determination of metals:** Sodium and potassium were determined in the soil pastes photometrically as described by Stewart (1974). Meanwhile, calcium and magnesium were estimated according to Page *et al*. (1982) using atomic absorption spectrophotometer Perkin-Elmer, Model 2380. Chlorides were determined in the soil extract according to the method described by Jackson (1967). Sulphates were estimated applying the turbidometric methods as described in the Standard Methods (1989). Carbonates percentage were determined according to Jackson (1967) and the total organic carbon percentages were determined as described by Piper (1950).

**Isolation of Algal Species**: Z-Medium as described by Staub (1961) was used for cultivation. The most salt tolerant algal isolates were isolated from saline and semi – saline soils on high sodium containing medium 1, 2, 3 and 4% NaCl as described by El-Ayouty and Ayyad, (1972). For obtaining uni-algal and then axenic cultures, the following procedures were used De, 1939; Fogg, 1942; Gupta *et al*., 1956 and Hoshaw and Rosowski, 1973. Algal identification was carried out according to Geitler (1932) and Desikachary (1959).

**Biochemical Analysis :**The Determination of total soluble carbohydrates by anthrone technique was used according to Umbriet *et al*., (1969).The method of Lowry *et al*., (1951) was used for protein determination. Lipid determination was done by kits. Amino acid analyzer was used for determination and identification of combined amino acids. Dry weight to the nearest mg was also determined.

**Experimental Work;**

**Testing the Halo -Tolerance of Algae**

Plain Z-liquid media was used for growing the isolated algal species as control media. For testing the Halo-Tolerance of the isolated species the control media was supplemented with different NaCl concentrations; 1, 2, 3 and 4% (w/v). In addition several salts including $MgCl_2$, KCl, $NaNO_3$, $Na_2SO_4$, and $CaCl_2$ were added to Z- liquid media supplemented with NaCl at ratio of (1:1 w/w). The initial pH value of the medium was adjusted to 7.5. In conical flasks of 250 ml capacity sterilized at 1.5 atms for 20min, 100ml media were then inoculated with 3 ml algal suspension (of each algal isolate) and incubated at 28 ± 2ºC, 2500 Lux for 15 days. At the end of the experiment the algal dry weights were determined to the nearest mg.

**Effect of certain parameters on algal growth**

Also, the effect of different parameters on the growth of the isolated algae was measured including: the effect of different light intensities; the effect of different incubation periods; the effect of different temperatures; and the effect of different pH values.

In addition, the effect of different carbohydrate compounds as carbon source and the effect of different nitrogenous compounds were also examined for each algae species.

## *Results*

The mechanical analysis of soil samples was carried out to evaluate the possible effects of soil texture on maintenance of microorganism population. Two types of samples were recognized in the current study. The results of soil physical and chemical analysis of the two types of soil samples are presented in Table (1) It is clear from the data presented in Table (1) that the two soil types showed no detectable differences in the percentages of clay and sand. Soil sample B is characterized by higher percentage of silt. From chemical point of view, the pH value was alkaline for both soil samples.

**Table (1): The percentage of soil components of the two studied soil types and their chemical analysis. Data expressed as mean ± S.D.**

| Soil fractions | Soil samples | |
|---|---|---|
| | A | B |
| Sand % | 21.5 ± 0.3 | 20 ± 0.46 |
| Silt% | 32.5 ± 1 | 35 ± 0.22 |
| Clay% | 46 ± 0.25 | 45 ± 0.67 |
| Chemicals Characteristics | | |
| pH | 7.87 ± 0.6 | 8.17 ± 0.2 |
| E.C. (Mils/Cm) | 51.76 ± 0.45 | 14.21 ± 0.12 |
| T.S.S. % | 20.8 ± 0.2 | 7.95 ± 0.9 |
| Organic carbons % | 0.57 ± 0.3 | 0.56 ± 0.38 |
| Carbonates % | 7.15 ± 0.41 | 6.95 ± 0.73 |
| Chlorides % | 1.73 ± 0.1 | 0.603 ± 0.28 |
| Sulphates % | 8.2 ± 0.05 | 8.79 ± 0.57 |
| Moisture content % | 8.42 ± 0.47 | 8.1 ± 0.26 |
| Sodium (ppm) | 1280 ± 0.1 | 885 ± 0.12 |
| Potassium (ppm) | 30 ± 0.51 | 20 ± 0.44 |
| Calcium (ppm) | 106.24 ± 0.22 | 83.88 ± 0.76 |
| Magnesium (ppm) | 130 ± 0.7 | 169.86 ± 0.79 |

Data in Table (1) also indicated that the percentage of total soluble salts (T.S.S.) was higher in type A soil as well as the electrical conductivity being 20.8

± 0.2 % w/v 51.76 ± 0.45 Mils/Cm, respectively. Soil sample type A contained a higher content of chlorides, sodium, potassium and calcium. On the other hand, soil sample type B was characterized by high magnesium content. Also, it is clear from Table (1) that carbonates, sulphate and organic carbon contents of the soil in the two studied types were almost similar.

Algal isolation from both types of soil led to isolation of two cyanobacterial species from soil B. The two isolated microorganisms belong to order Nostocales. The two organisms were identified as: Isolate No. I *Nostoc humifusum* Carmichael ex Born. et flah and Isolate No. II *Oscillatoria earlei* Ganrdner. Plate (I, 1&2).

**Factors effecting growth of both isolates:**

Several experiments were conducted in order to determine the optimum growth conditions of both algal species isolated from the soil. The results of these experiments are presented in Figure (1, a-d). Results in Figure (1 a) indicated that *Nostoc humifusum* had the best growth under light intensity of 2000 Lux. Increasing light intensity up to 5000 Lux resulted in obvious decrease in growth. On the other hand, *Oscillatoria earlei* growth rate increased markedly with increasing of light intensity from 1000 to 3000 Lux then decreased to the minimum growth at 5000 Lux.

Data in Figure (1 b) show that Z liquid medium containing 2% NaCl achieved the maximum growth during the period from $9^{th}$ and $12^{th}$ days for *Nostoc humifusum* and *Oscillatoria earlei*, respectively. In addition, the yield of *Nostoc humifusum* was significantly higher than that of *Oscillatoria earlei* during the same period.

The data in Fig (1c) demonstrated that both algal species achieve the maximum yield at temperature around 30ºC. However, data in Figure (1d) showed that the optimum pH value for growth of the isolated species differ from one to the other. For *Nostoc humifusum* it was clear that it attained maximal growth at pH 7.5. Meanwhile *Oscillatoria earlei* has an optimum pH value at 8.5.

**Testing the Halo -Tolerance of Algae**

The results of testing the effect of different salts combinations with NaCl on the growth of the two isolated algal species is presented in Table(2). From Table (2) it was clear that in case of *Nostoc humifusum*, the maximum growth was achieved in the presence of 2% NaCl in the Z-media meanwhile the increase of NaCl concentration to 3% and 4% caused retardation then inhibition of growth respectively. The substitution of NaCl concentration by equal amount of KCl showed a significant increase in growth at 1% concentration. However when the concentration reached 2% the growth of this species almost doubled to reach 124.7 mg / 30 ml media. The presence of $CaCl_2$ in the media in addition to NaCl showed even more increase in growth but also with the maximum achieved at 2% salt concentration being 257.3 mg/30ml media. Meanwhile the use of $MgCl_2$ did not have the same impact in growth as the maximum growth occurred at concentration 1% being 252 mg/30ml media. The presence of $NaSO_4$ or $NaNO_3$ with sodium chloride in the media showed that the maximum growth occurred at concentration not more than 1% and the increase in concentration of both salts led to inhibition of growth at 2 and 3% respectively (Table 2).

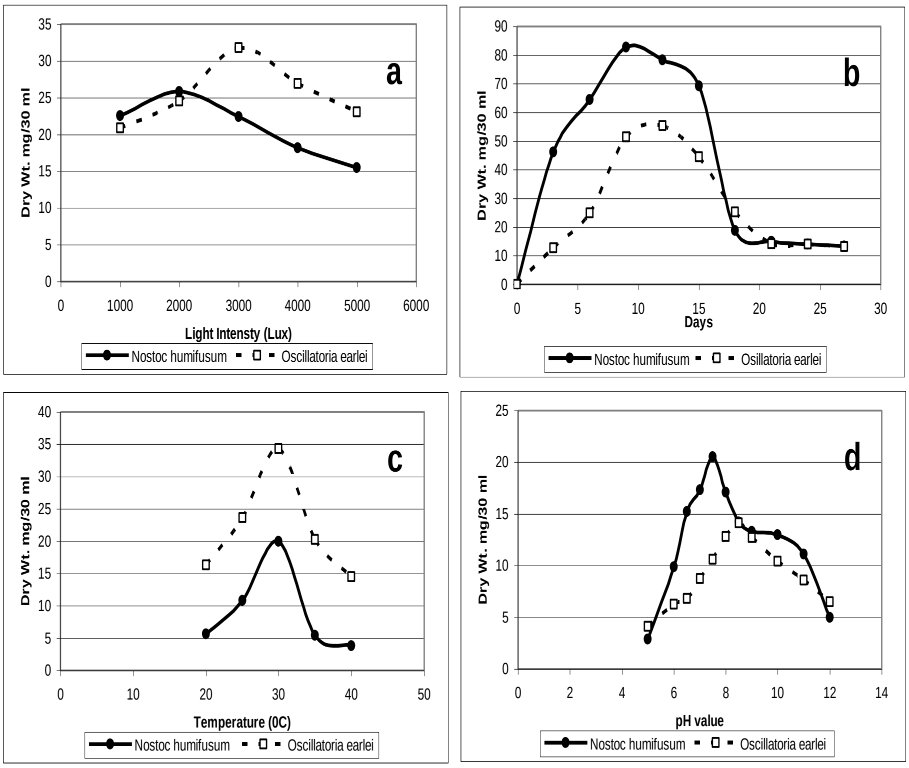


**Figure (1): the effect of different factors on the growth of the two isolated algal species a-Light intensities (Lux); b- Incubation periods with 2% NaCl; c- Temperatures; d- pH values.**

*Oscillatoria earlei* showed to be more halotolerant than *Nostoc humifusum*, it achieved the maximum growth at 3% NaCl being 91 mg/30 ml. However, it could also grow moderately on Z medium supplemented with 4% NaCl with fewer yields.

Adding KCl to NaCl in the medium resulted in maximum growth at only 2% concentration being 200 mg/30 ml media. Data also showed that the increase in concentration in this case caused inhibition in growth of *Oscillatoria earlei*.

Data in Table (2) indicated that adding of $CaCl_2$ and $MgCl_2$ to the media caused a sharp improvement of the growth to reach the maximum of 453 and 223 mg/30ml media at concentrations 3% of each salt respectively. On the other hand the concentration 4% of $CaCl_2$ and $MgCl_2$ showed a sharp decrease in growth to reach almost half of the yield being 212 and 150 mg/30ml media respectively.

**Table (2):- The effect of different concentrations of NaCl and other salts (KCl, $CaCl_2$, $MgCl_2$, $Na_2SO_4$ and $NaNo_3$) in combination on the growth of *Nostoc humifusum* and *Oscillatoria earlei* grown for 9 days at pH 7.5, 30 ± 2ºC and 2500Lux. Data are expressed as dry weight (mg/30 ml medium) ± SD.**

**C =Control**

| Salts g% | | NaCl | KCl &NaCl | $CaCl_2$ &NaCl | $MgCl_2$ & NaCl | $Na_2SO_4$ & NaCl | $NaNO_3$& NaCl |
|---|---|---|---|---|---|---|---|
| *Nostoc humifusum* | C | 21.8 ±0.3 | 21.8 ± 0.3 | 21.8 ± 0.3 | 21.8 ± 0.3 | 21.8 ± 0.3 | 21.8 ± 0.3 |
| | 1% | 42.46 ±1.4 | 68.3 ± 0.9 | 172.6 ± 0.8 | 252.6 ± 0.3 | 207.6 ± 0.1 | 102.3 ± 0.1 |
| | 2% | 66.86 ± 0.9 | 124.7 ± 0.3 | 257.3 ± 0.5 | 132.3 ± 0.3 | 0 | 43.6 ± 0.12 |
| | 3% | 60.07 ± 0.7 | 48.3 ± 0.7 | 142.3 ± 0.1 | 34.6 ± 0.5 | 0 | 0 |
| | 4% | 0 | 0 | 61.6 ± 0.1 | 0 | 0 | 0 |
| *Oscillatoria earlei* | C | 34.97 ± 0.5 | 34.97 ± 0.5 | 34.97 ± 0.5 | 34.97 ± 0.5 | 34.97 ± 0.5 | 34.97 ± 0.5 |
| | 1% | 54.37 ±0.34 | 67.78 ± 0.5 | 232 ± 0.2 | 60 ± 0.1 | 267.3 ± 0.1 | 160 ± 0.9 |
| | 2% | 62.17 ± 1.0 | 200.2 ± 0.7 | 330.3 ± 0.1 | 99.6 ± 0.1 | 185.6 ± 0.7 | 247 ± 0.3 |
| | 3% | 91.06 ± 0.8 | 43.3 ± 0.4 | 453.3 ± 0.2 | 223.3 ± 0.4 | 0 | 436.6 ± 0.9 |
| | 4% | 73.47 ±1.69 | 23.3 ± 0.4 | 212.3 ± 0.3 | 150 ± 0.3 | 0 | 167.6 ± 0.3 |

Addition of $NaSO_4$ and $NaNO_3$ to the media with NaCl showed an odd behavior in growth where $NaSO_4$ caused a sharp increase in growth at concentration 1% followed by sharp decrease then complete inhibition at concentrations 2 and 3, 4 %. However, the results indicated that *Oscillatoria earlei* is more tolerant to the presence of $NaNO_3$ in the media accompanied with increase in growth up to 3% concentration being 436 mg/30ml media. Also the increase in concentration to 4% caused retardation in growth yielding only 167 mg/30 ml media.

**The Role of Carbon and Nitrogen Sources:**

The effect of adding carbon and nitrogen sources to the media containing NaCl on the growth of the two isolated species calculated as dry weight is presented in Table (3). The results indicated that there is no significant difference between the growth represented as dry weight, of *Nostoc humifusum* and *Oscillatoria earlei* when grown on cellulose, fructose and lactose, as a carbon source, under salt stress. While, there is a significant difference between the growth of both organisms when grown on maltose and mannose under salt stress. The former is favored by *Nostoc humifusum* while the later is favored by *Oscillatoria earlei*.

**Table (3):- Effect of various carbon and nitrogen sources on the growth of *Nostoc humifusum* and *Oscillatoria earlei* grown in Z liquid medium supplemented with 2% NaCl at 30 ± 2ºC, 2000-3000 Lux and pH 7.8-8.5 for 9-12 days. Data are expressed as dry weight ± SD.**

| Dry weight (mg/30ml) | | *Nostoc humifusum* | *Oscillatoria earlei* |
|---|---|---|---|
| Different carbon sources | Galactose | 16.3 ± 1.7 | 14.2 ± 3.2 |
| | Mannose | 6.08 ± 0.63 | 12.17 ± 0.77 |
| | Cellulose | 5 ± 2.5 | 7.57 ± 0.98 |
| | Maltose | 12.57 ± 0.9 | 8.87 ± 0.945 |
| | Fructose | 14.17 ± 1.75 | 12.6 ± 1.3 |
| | Glucose | 19.87 ± 2.36 | 10.07 ± 3.5 |
| | Lactose | 10.47 ± 1.9 | 9.5 ± 2.7 |
| | Sucrose | 7.5 ± 1.7 | 15.9 ± 3.7 |
| Different nitrogen sources | Control | 11.37 ± 0.35 | 16.37 ±0.8 |
| | $(NH_4)_2CO_3$ | 28.5 ± 0.11 | 10.3 ± 0.55 |
| | $(NH_4)_2SO_4$ | 9.87 ± 0.45 | 12.03 ± 0.65 |
| | $(NH_4)_2NO_3$ | 17.7 ± 0.19 | 6.63 ± 0 .17 |
| | $NH_4Cl$ | 14.56 ± 0.98 | 13.1 ± 0.65 |
| | $KNO_3$ | 11.47 ± 0.3 | 14.1 ± 0.88 |
| | $NaNo_2$ | 15.27 ± 0.36 | 8.63 ± 0.99 |
| | Urea | 5.63 ± 0.15 | 10.1 ± 0.46 |
| | L - Prolin | 10.97± 0.17 | 13.8± 0.19 |
| | Peptone | 7.5± 0.11 | 7.4± 0.51 |

***Carbon compounds were added in quantities containing equivalent quantity of C in $Na_2Co_3$ content. Nitrogen compounds were added in quantities containing equivalent quantity of N in $NaNo_3$ content.**

Concerning, dry weight contents in both investigated algae (*Nostoc humifusum* and *Oscillatoria earlei*) it showed totally different behavior when growing on different nitrogen sources. Firstly, *Nostoc humifusum* reached the maximum growth at the best nitrogen source as $(NH_4)_2CO_3$. Also, $(NH_4)_2NO_3$, $NaNO_2$ and $NH_4Cl$ stimulated *Nostoc humifusum* to high growth similarly. However, $KNO_3$, a nitrogen source had similar effect as the control on *Nostoc humifusum*. On the other hand, *Nostoc humifusum* grew with lower growth at both $(NH_4)_2SO_4$ and urea as compared to the control.

Secondly, all the investigated nitrogen sources did not increase the growth of *Oscillatoria earlei* but more likely were inhibitory in compared to the control in the following order; $(NH_4)_2NO_3$, peptone, $NaNO_2$, urea, $(NH_4)_2CO_3$, $(NH_4)_2SO_4$, $NH_4Cl$, L-Proline and $KNO_3$. Meanwhile, from the previous data, unexpectedly $NaNO_2$ increased the growth of *Nostoc humifusum* and *Oscillatoria earlei* at 2% NaCl containing media. This might be attributed to the ability of these algae to metabolize that toxic compound under multiple stresses.

**Effect of Salt Contents on Algal Biochemical Composition:**

Microorganisms differ from one another in their abilities to alter their metabolic activities with the nature and composition of the external environment. Since NaCl is a solute in the external environment, it is expected that cellular contents of solutes will be changed and consequently several other metabolic activities. It was clear from Table (4) that *Nostoc humifusum* contained appreciable amounts of total soluble protein at 2% NaCl. Further increase in NaCl concentration resulted in retardation in the biosynthesis of protein content of *Nostoc humifusum*. On the other hand *Oscillatoria earlei* attained maximum of total soluble protein at 3% NaCl.

**Table (4): Protein, carbohydrates and total lipid contents of *Nostoc humifusum* and *Oscillatoria earlei* cultivated on Z-liquid media supplemented with different NaCl concentrations. Data are expressed as mg/g algal dry weight.**

| NaCl conc. % (w/v) | *Nostoc humifusum* | | | *Oscillatoria earlei* | | |
|---|---|---|---|---|---|---|
| | T. Sol. Prot. (mg/g) | T. Sol. Carb. (mg/g) | Total lipids (mg/g) | T. Sol. Prot. (mg/g) | T. Sol. Carb. (mg/g) | Total lipids (mg/g) |
| Control | 0.61 ± 0.41 | 7.9 ± 0.8 | 10 ± 0.21 | 0.81± 1.2 | 9.4 ± 0.2 | 12 ± 0.5 |
| 1% | 0.78 ± 3.5 | 9.2 ± 2.1 | 12 ± 0.75 | 0.94± 0.1 | 11.2 ± 0.5 | 15 ± 2 |
| 2% | 0.9 ± 0.04 | 11.2 ± 0.9 | 15.5 ± 1 | 1.5 ± 0.8 | 12.1 ± 0.5 | 16 ± 1 |
| 3% | 0.57 ± 0.01 | 8 ± 0.2 | 10.8 ± 0.9 | 1.7 ± 0.07 | 12.5 ± 0.4 | 17.1 ± 0.2 |

**T. Sol. Prot. = Total Soluble Protein; T. Sol. Carb. = Total Soluble Carbohydrates**

Concerning, the amount of total soluble carbohydrate, Table (4) revealed that it varied according to NaCl concentrations. Such effect was apparent in the form of stimulation of higher amounts of total soluble carbohydrates induced at 2% NaCl for *Nostoc humifusum* and remarkable decrease of total soluble carbohydrate at 3% NaCl was also observed for *Nostoc humifusum*, but still the amount of total soluble carbohydrate at 3% NaCl of *Nostoc humifusum* was higher than the amount of the control. On the other hand, *Oscillatoria earlei* had almost the same amount of total soluble carbohydrate at 2% and 3% NaCl.

Also, the amount of total soluble carbohydrate at 1% is higher than the control. Also, it is clearly demonstrated that lipid content increased by increasing NaCl concentration. Lipid of *Nostoc humifusum* reached its maximum value at 2% NaCl. While lipid content of *Oscillatoria earlei* rapidly increased at 3% NaCl. Data in Table (4) also indicated that total soluble protein, total soluble carbohydrate and total lipid content tend to had higher levels in *Oscillatoria earlei* than *Nostoc humifusum*. This phenomenon may be explained by that *Oscillatoria earlei* had higher tolerance to elevated NaCl concentrations than *Nostoc humifusum*.

The results of the amino acid composition in both normal and 2% NaCl loaded media are presented in Table (5). The results in Table (5) indicated that a total of 16 different amino acids were recognized for the two studied algal species. Also ammonium sulphate was encountered in the two algal isolates as a result of destruction of the bonds between some amino acids. The data showed distinct variations in the percentage of amino acids content of *Nostoc humifusum* before and after exposure to salt stress especially for (tyrosine, histidine, and arginine). It is worthy to mention that the amino acid valine appeared after administration of *Nostoc humifusum* to 2% NaCl. While the rest of the detected amino acids of *Nostoc humifusum* had almost the same percentages when grown in free and 2% NaCl containing medium. On the other hand, the amino acid profile of *Oscillatoria earlei* refers to the presence of the exact same numbers at 2% NaCl and no exposure to NaCl (16 amino acids) but with different percentages.

It is noted from the present investigation that *Nostoc humifusum* had aspartic acid, valine, methionine, leucine, tyrosine and arginine in higher amounts at 2% NaCl while *Oscillatoria earlei* contained higher concentrations of threonine, serine, glutamic acid, proline, glycine, alanine, methionine, leucine, tyrosine and histidine when grow under salt stress as compared with control.

**Table (5):- Amino acid analysis of *Nostoc humifusum* and *Oscillatoria earlei* at 2% NaCl.**

| | Amino acid | *Nostoc humifusum* | | *Oscillatoria earlei* | |
|---|---|---|---|---|---|
| | | Control | 2% NaCl | Control | 2% NaCl |
| Concentration of amino acids % | Aspartic acid | 9.3 | 10.72 | 14.16 | 10.24 |
| | Threonine | 4.51 | 4.89 | 4.02 | 5.16 |
| | Serine | 3.87 | 4.04 | 3.77 | 4.59 |
| | Glutamic acid | 9.27 | 9.7 | 9.78 | 12.07 |
| | Proline | 5.44 | 4.54 | 3.91 | 5.01 |
| | Glycine | 3.85 | 5.04 | 4.61 | 5.43 |
| | Alanine | 4.17 | 3.8 | 3.45 | 4.21 |
| | Cystine | 8.67 | 9.64 | 9.46 | 9.24 |
| | Valine | --- | 0.87 | 1.16 | 0.82 |
| | Methionine | 3.8 | 4.31 | 3.62 | 4.51 |
| | Leucine | 5.16 | 6.15 | 5.17 | 6.5 |
| | Tyrosine | 1.85 | 3.01 | 2.82 | 3.14 |
| | Phenyl- alanine | 3.98 | 4.73 | 3.52 | 4 |
| | Histidine | 4.22 | 1.69 | 3.22 | 5.17 |
| | Lysine | 4.15 | 3.23 | 5.31 | 5.05 |
| | Arginine | 4.75 | 6.12 | 11.58 | 4.22 |
| | Ammonium sulphate | 23.01 | 17.5 | 10.43 | 10.66 |

## *Discussion*

Recently Henley *et al*., (2002) isolated and identified a variety of halotolerant diatoms and cyanobacteria grown on agar plates in 2.5 or 5% salt streaked with soil or water samples. Also, Hua *et al*., (1982) reported that, the metabolic activities of a microorganism vary considerably with changes in their growth environments. Environmental changes are most likely affecting the intracellular concentrations of the low molecular weight pool constituents. Extreme conditions such as high salt concentration of medium had affected the growth and the physiological requirements of microorganisms. So several attempts have been carried on to isolate and identify the halotolerant microorganisms from saline soils (Kelly *et al*., 2003 and Krumbein *et al*., 2004). Kobbia, (1981) concluded that the limitation of species number is a reflection of the salinity status of water even in the presence of other promotive factors.

Many species of algae respond to variations in light intensity by varying their cellular contents of chlorophyll a and or by modifying the activities or concentration of photosynthetic enzymes (Soeder and Stengel, 1974). Tan and Wong, (1995) stated that severe depletion of nutrient supply might lead to progressive cell death and analysis as time proceeded to the end of experiment. From our results, it was clear that both *Nostoc humifusum* and *Oscillatoria earlei* had fast log phase. It was obvious that *Nostoc humifusum* and *Oscillatoria earlei* have nearly the same growth pattern as influenced by different levels of temperatures. Moreover, Nicklisch, (1992) mentioned the importance of the basic environmental factors as temperature to elucidate the essential factors of the growth and decline mass population. Generally, the lower or the higher temperatures inhibited growth of algae.

One of the factors that also affect growth of halotolerant microorganism is the pH. The presence of NaCl in relatively high concentrations would certainly affect molecular exchanges in the algal cells as well as cell boundaries. Handley and Michelle, (2003) reported that the general relationship between species richness, pH and salinity; species diversity, pH and salinity were simple and linear; with increasing pH and salinity, species diversity and species richness decreased. Moisander *et al*., (2002) found that each of the studied organisms was able to tolerate with NaCl differently. Also, Garcia-Pichel *et al*., (1999) observed a general pattern of decreasing photosynthesis and oxygen exchange capacity with increasing salinity in cyanobacteria.

The combined effect of NaCl with other salts in our work was tabulated in table (2). $CaCl_2$, KCl, and $MgCl_2$ were less inhibitory on algal growth even at high concentrations. However, $NaNO_3$ was inhibitory at different concentrations. It is quite clear that divalent salts; $Ca^{+2}$ and $Mg^{+2}$ are less harmful than monovalent ones, $Na^+$ and $K^+$. Moreover, the type of acidic radical has an obvious role. For instance $Na_2SO_4$ was more inhibitory than NaCl and $NaNO_3$ at the same concentrations. It could be attributed to the ionization of salts into their radicals.

$Na_2SO_4$ ionizes giving $SO^{-2}$ ions which are more harmful than the other two acidic radicals. This is in agreement with the finding of Gochnaver and Kushner, (1971), who reported that cells of *Hallobacterium halobium* could live longer in 25% NaCl solution if $Mg^{+2}$ and $Ca^{+2}$ ions were present. Also, Wydro *et al.*, (1977) proved that, higher concentrations of monovalent salts inhibited protein synthesis; NaCl was inhibitory more than several other monovalent salts.

Our data indicated that halotolerant algae prefer alkaline conditions more than acidic ones. The presence of high salt concentration in the microbial environment would of course affect the microbial life in two different ways: firstly, by increasing the osmotic pressure of the environment which affects different molecular exchanges. Secondly, was by the direct influence on the biological activities within the organism. To obviate such thought several salts in different concentrations were supplemented to our culture media of the two isolates.

The combined effect of NaCl with other salts was tabulated in table (2). $CaCl_2$, KCl, and $MgCl_2$ were found to be less inhibitory for algal growth even at high concentrations. However, $NaNO_3$ was inhibitory at different concentrations. It is quite clear that divalent salts; $Ca^{+2}$ and $Mg^{+2}$ are less harmful than monovalent ones, $Na^+$ and $K^+$. Moreover, the type of acidic radical has an obvious role. For instance $Na_2SO_4$ was more inhibitory than NaCl and $NaNO_3$ at the same concentrations, which could be attributed to the ionization of salts into their radicals.

Generally, under salt stress conditions microorganisms had especial carbon requirements, Marker (1965) reported that some algae produce extracellular polysaccharides mainly when they are in stationary phase. Guillard and Helleburst, (1971) stated that the amount of carbohydrates released may be represented by a considerable fraction (15 - 90%) of the photo-assimilated carbon of some algae during the active growth period. The results obtained in this investigation are in harmony with those recorded by Incharoensakdi and Wangsupa, (2003**)** who reported that nitrate uptake was dependent on $Na^+$ and that ammonium inhibited nitrate uptake.

Living organisms need several important factors to establish themselves and to have a continued tradition. Of these factors, carbon and nitrogen sources, are necessary and important sources for energy matter ruling such environments. Carbon compounds are the most important factor for energy supply of microorganisms especially under salt stress. Data in Table (3) clearly indicated that *Nostoc humifusum* exposed to 2% NaCl grew best on glucose as a carbon source. Meanwhile, the climax of *Oscillatoria earlei* growth after administration to salt stress was higher on sucrose as a carbon source. On the other hand, *Nostoc humifusum* and *Oscillatoria earlei* favored galactose as second carbon source.

The influence of NaCl on the biosynthesis of lipid, protein and carbohydrates is not characteristically obvious. The total soluble protein content of the investigated species as revealed from our data showed that generally *O. earlei* tend to have the higher levels of soluble protein, and the least value was detected in *N. humifusum*. This might be attributed to the fact that the adaptation of extreme halophiles are associated with change in their proteins, and with other biochemical changes reflected in the chemistry of the cell envelop and cellular lipid as reported by Kushner, (1978). Meanwhile, Salah El Din, (1994) stated that most of the algal species have almost similar physiological functions which are related to either biosynthesis or biodegradation of the same protein macromolecule. However, Dhargalter (1986) found that the changes in the biochemical composition of some green algae may be related to the chemical and morphological changes associated with the various metabolic processes. *Dunaniella salina* synthesizes high concentrations of intracellular glycerol and transferin-like protein to balance the external osmotic pressure (Pick, 1998). Where, Wydro *et al*., (1977) reported that higher concentrations of monovalent salts inhibited protein synthesis. NaCl was found to have stronger inhibition than several other chlorides. In response to osmotic stress, changes in lipids have been studied by Allakhverdiev *et al*., (1999) and Chatterjee *et al*., (2000) who stated that lipid are important in causing cell inactivation and allow high pressure adaptation in halophiles organisms.

Munda and Gubensek, (1976) reported that there were differences in the dominant amino acids between species. Similar conclusion was reported by Mohsen *et al*., (1975). Also, in this respect, Derrien *et al*., (1998) stated that the dominant amino acids mainly depend, on the species, provided as well as cultivation conditions. Moreover, extreme environmental conditions should certainly expect to affect the metabolic pathways. It has been published that the macromolecular composition and metabolic activity of microorganism vary with changes in their growth environment (Neihardt, 1963; Tempest and Meers, 1970). Changes in the growth conditions affected the intracellular concentrations of substances. Amino acid pools are extremely variable and markedly dependent on the nutritional complexity of the growth medium (Holden, 1962). Also, the ability of microorganisms to successfully cope with reduced $a_w$ is related to the accumulation of proline and glutamine in their free amino acids pools (Koujima *et al*., 1978). Our results recorded an increasing in proline and glutamic acid content in *Oscillatoria earlei* under salt stress.

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## تأثير المركبات الكيميائية المختلفة على نمو نوستوك هيمفيوزم و أوسلاتوريا أرليبا من الطحالب المعزولة من التربة.

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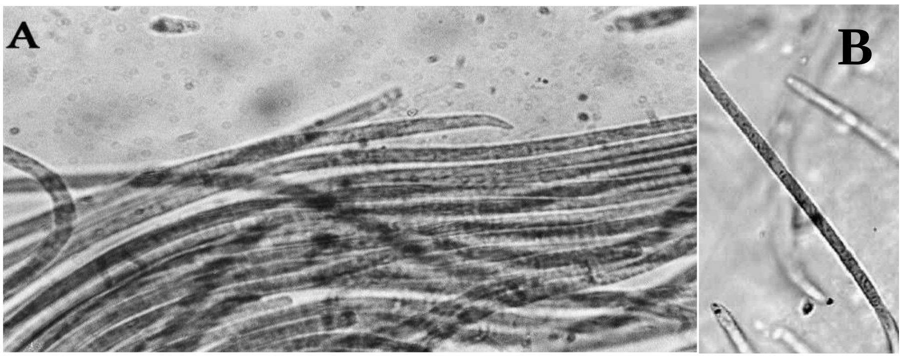
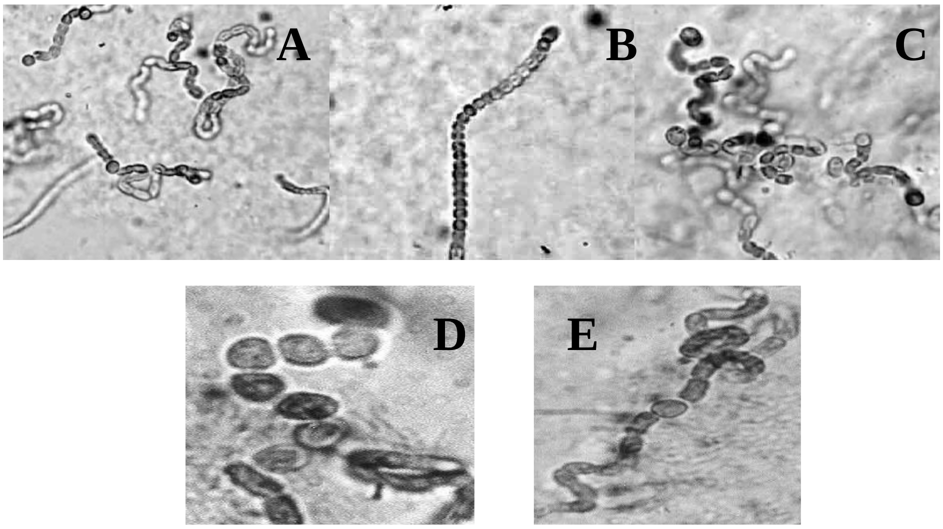
أجريت الدراسه على تربه زراعيه بدى عليها اعراض زيادة الملوحه بمنطقة الخانكة بمحافظة القليوبية. تم تعيين بعض الخصائص الفيزيائية والكيميائية للتربة المختارة. وجد فقط نوعين من الطحالب المقاومه للملوحه من التربه الاقل فى تركيز الأملاح. تم عزل وتنقيه الكائنين (نوستوك هيمفيوزم و أوسلاتوريا أرليبا) محل الدراسة من البكتريا كما تم تعيين أفضل العناصر لتنميتها. أوضحت الدراسه أن الاختلاف فى درجات الملوحه والتغير فى معدلات الكربون والنيتروجين له تأثيره الواضح على نمو تلك الكائنات. وبالاضافه إلى ذلك فقد أثبتت بعض التراكيب الحيوية الموجودة بتلك الكائنات تأثرها بالظروف المختلفة للتجربة.

## Plate I: Isolated Algal Species




***1-Oscillatoria earlei* Ganrdner.**
**A- Free-swimming bundles of trichomes (X 1000).**
**B- A straight attenuated trichome (X 400).**




***2-Nostoc humifusum* Carmichael ex Born. et flah**
**A- Spherical heterocyst or semispherical ones (X 400).**
**B- Short barrel-shaped cells which seen in part as semispherical ones under microscope (X400).**
**C - Contorted or twisted packed filaments (X 400).**
**D- Short chain of compressed spherical or oval shaped-akinetes (X 1000).**
**E- Intercalary sub-spherical heterocyst (X 1000).**