1st Int. Symp. Phycol. (ISP).
(21-22) Oct. 2000.

Egypt. J. Phycol. Vol. 1, 2000

ROLE OF SOME CYANOPHYTA(CYANOBACTERIA) IN ENHANCEMENT OF SOIL CHARACTERISTICS

Ahmed D. El-Gamal[1]; Mokhtar S. Ammar[1]; Usama M. Abd El-Raouf[2] and Taher M. Taha[2]

[1]*Botany & Microbiology Dept.,Fac.of Sci., Al-Azhar Univ., Cairo.*
[2]*Botany Dept., Fac. of Sci., Al-Azhar Univ., Assuit.*

Abstract:

The current study was performed to assess the effect of nitrogen-fixing (Cyanobacteria), isolated from Egyptian soil to improve and enhance natural poorly sandy soil. Four cyanobacterial species were isolated & identified as *Anabaena variabilis*, *Nostoc paludosum*, *N. entophytum* & *N.* sp. Z-medium proved the satisfied results giving the best growth within 50 days among other media. Bacteria free cultures were obtained, as the combination of chlorine water & mercuric chloride method was the most effective method used. Cyanobacterial growth was followed by determination of dry weight, acetylene reduction activity and total nitrogen. Algal species were inoculated separately to sandy soil allowed them growing .Physical and chemical characteristics of treated soil were determined. The results showed that the inoculation of different algal species to soil caused a significant enhancement in both physical and chemical properties of soil.

Introduction:

Cyanobacteria may be the first organisms developed on volcanic and other rock substrates. Algae consolidate the soil surface leading to crust formation, as well as they improve soil infiltration and afford substrate upon which seeds and spores germinate (Sheilds and Durrell, 1964). Algalization leads to improve the aggregation, hydraulic conductivity and organic carbon percentage for alkaline soil under field conditions (Kaushik and Muriti, 1981 and Ahmed and Ahmedunsia, 1984).

Fogg and Stewart (1965) reported that the nitrogen fixing blue-green algae play an important role in fertility of many types of habitats ranging from sand dunes, rocky shores and rice fields. Under natural habitats, algae normally grow as mixed communities including different genera and species, so algae must be cultivated under laboratory conditions to isolate a single algal species from other types of organism life (Bunt, 1936; Pringsheim, 1949; Taha, 1963 and Khadr, 1975).

The aim of the present work was focused towards the possibility of using cyanobacteria as a biological conditioners, where cyanobacterial use has the ability to improve soil properties.

Materials & Methods:

4 species of nitrogen-fixing cyanobacteria were isolated & identified according to Geitler (1932) and Desikachary (1959) as *Anabaena variabilis*, *Nostoc paludosum*, *N. entophytum* and *N. species*. The first two genera were isolated from Cairo - Ismailia desert road, while the latter two species were isolated from El-Fayoum governorate. Different media were used for testing their potentiality upon cyanobacterial biomass production. The

(ISSN: 1110-8649)

employed media were Z-medium (Staub, 1961), where the composition of this medium was made from the following stock solutions, macronutrients, EDTA $FeCl_3$ solution and trace elements solutions as follow: $NaNO_3$, 46.7 g ;$CaNO_3$. $4H_{2O}$, 5.9 g ; K_2 HPO_4, 3.1 g ; $MgSO_4$. 7 H_2 O, 2.5 g and Na_2CO_3 ,2.1 g / 100 ml of dist. water EDTA $FeCl_3$ solution was prepared by mixing 5 ml of 0.1 N $FeCl_3$. $6H_2$ O in 0.1 N HCl solution with 5 ml of 0.1 N EDTA solution, this 10 ml was made to 500 ml with distilled water and 10 ml of EDTA $FeCl_3$ solution are taken for preparation of 1 liter medium . Microelements composed of the following ingredients that were dissolved in 100 ml distilled water: H_3 BO_3 , 310 mg ; $MnSO_4$. $4H_2$ O , 223 mg ; Na_2 SO_4. $2H_2$ O , 3.3 mg ; $(NH_4)_6$ Mo_7 O_{24} . $4H_2$ O , 8.8 mg ; KBr,11.9 mg ; KI,8.7 mg ; $ZnSO_4$. $7H_2$ O, 28.7 mg ; $Cd(NO_3)_2$. $4H_2$ O, 15.4 mg ; $Co(NO_3)_2$. $6H_2$ O, 14.6 mg ; $CuSO_4$. $5H_2$ O , 12.5 mg; $NiSO_4$ $(NH_4)_2$ SO_4 . $6H_2$ O,19.8 mg ; $Cr(NO_3)_2$. $7H_2$ O, 3.7 mg ; V_2 O_4 $(SO_4)_3$. 16 H_2O, 3.5 mg and Al_2 $(SO_4)_3$ K_2SO_4. 24 H_2O. 0.08 ml of microelements solution was required for preparation of one liter of Z medium ; Chu`s medium No. 10 (Chu, 1942), Allen`s medium (Allen`s, 1968, modification of Hughes *et al.*, 1958), modified Watanabe medium (Watanabe, 1951), soil extract medium (the Botany School Cambridge, 1966) and BG13 medium (Ferris and Hirsch, 1991) nutrient agar medium (Oxoid, 1965) and enrichment medium (the Botany School Cambridge, 1966).The latter two media were used for testing the purity of cyanobacterial species from bacteria. Cyanobacteria were cultured and isolated according to the following techniques described by Esmarsch (1914) and El-Ayouty and Ayyad (1972). Cyanobacteria were purified from bacteria by using the combination of two methods: the chlorine water method (Fogg, 1942) and mercuric chloride method (Gupta *et al.* 1956). The growth of the studied cyanobacteria was followed within 50 days(at 6 days intervals) by determination of dry weight, determination of nitregenase activity by acetylene reduction assay (Stewart *et al.* 1967) and total fixed nitrogen (Kjeldahl method). The soil model used for algal application was collected from Cairo - Belbis desert road (Km 5). It was characterized by its sandy nature and poorly content of nutrients. A pot experiment was conducted at botanical garden, Botany and Microbiol. Dept., Fac. of Sci., Al-Azhar Univ., Nasr City, Cairo.The planned experiment was divided into 2 groups. The first group was considered the control of experiment (received no cyanobacteria) and the second group which is inoculated with each of cyanobacterial species (0.136 g fresh weight of algae / 1 kg autoclaved soil). Each treatment was in triplicates. The pots were irrigated daily with distilled water and the experiment was conducted for 40 days. Then the soil was air dried and kept in plastic bags till soil analysis. Maximum water holding capacity, gravitational and capillary water were determined according to the standard methods. Total nitrogen (Kjeldahl method), ammonia nitrogen (Tan, 1996), nitrate nitrogen (Markus *et al.* 1982), organic carbon (Piper, 1950), exopolysaccharides (Lowe, 1993), total carbonate (Hesse, 1994), pH (Jackson, 1958) and phosphorus contents (Chapman and Pratt, 1961) were determined in all soils under study.

Results and Discussion:

The four algal species were got in pure culture and be free from bacteria Trials of blue-greens purification were very difficult as a result of similarities between bacteria and cyanobacteria as well as the nature of gelatinous sheath, which fails the purification

process, and bacteria can be imbedded easily inside such sheath. Any way, the difficulty of purification process was also reported by many authors (Khadr, 1975; Castenholz 1988; and Ferris and Hirsch, 1991). Furthermore, some authors failed to purify algae from bacteria (El-Borollosy, 1972). In our case, the combination of chlorine water and mercuric chloride was the most effective method for obtaining bacteria-free culture, although some other methods revealed some success for killing bacteria in one side and some failure in the other side as they might be lethal for algae themselves.

Algae were identified according to Geitler (1932) and Desikachary (1959) as *Anabaena variabilis*, *Nostoc paludosum*, *N. entophytum* and *N. species*. The first two genera were isolated from Cairo - Ismailia desert road (Km 76), while the latter two species were isolated from Al-Fayoum governorate.

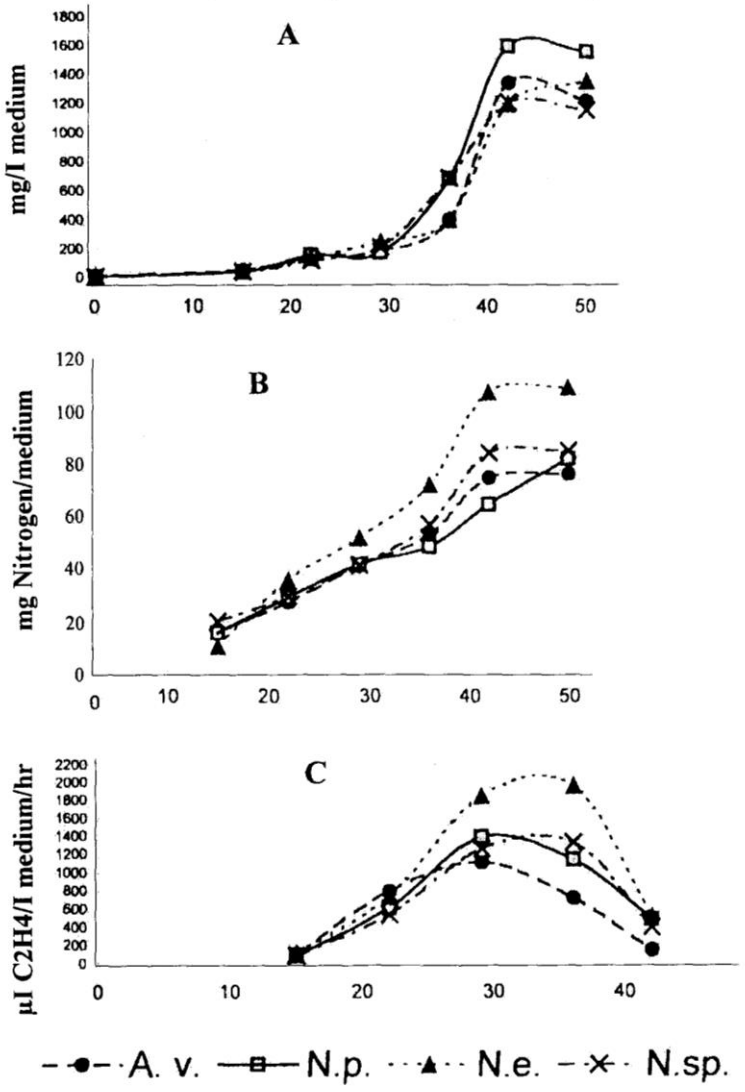
Five different media were used for estimation their potentiality upon cyanobacterial biomass production. Table (1) showed that Z medium proved the satisfied results as it was the best growth medium within 50 days among other media. Dry weights were 1890, 2390, 2385 and 2060 mg/l media for *Anabaena variabilis*, *Nostoc paludosum*, *N. entophytum* and *N. species*, respectively.

Table (1): Effect of different media on cyanobacterial growth after 50 days.(growth expressed as dry weight).

Organism / Media	*Anabaena variabilis*	*Nostoc paludosum*	*Nostoc entophytum*	*Nostoc sp.*
Z	1890 ± 0.1	2390 ± 445	2385 ± 20	2060 ± 350
Allen	1062 ± 75	1960 ± 120	2270 ± 680	1355 ± 60
BG_{13}	890 ± 205	2090 ± 75	2305 ± 165	1900 ± 50
Watanabe	1475 ± 190	2000 ± 4785	1085 ± 290	1955 ± 485
Soil extract	1205 ± 285	1315 ± 290	945 ± 150	1080 ± 90

The growth rate of cyanobacteria depends on many factors such as the nature of the organism, quality and quantity of light available, carbon dioxide concentration, nutrient status, metabolites, etc. (Venkataraman, 1969). In this respect, the growth curves of *Anabaena variabilis* and *Nostoc entophytum* expressed as dry weight, were found to have the nearly similar pattern of growth, as they had long lag phase and short exponential phase. In contrast, *N. paludosum* and *N. sp.* had a relatively shorter lag phase and longer exponential phase. Such resultant difference might be returned to the nature of standard inoculum. The most active growth period of the four isolates was restricted between 36-42 days. Parallel to dry weight, nitrogen fixed by the four isolates behaved similarly and had nearly the same pattern obtained by dry weight method (Fig. 1a). The cyanobacteria nearly reached the maximum values within 42 days. The mean amount of total nitrogen were 72.6,62.5,105 and 82 mg N/l media for *A. variabilis*, *N. paludosum*, *N. entophytum* and *N. sp.*, respectively (Fig. 1b). Cell growth was probably limited by the shortage of

nutrients. Tam 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.



A.v. = Anabaena variabilis *N.p.= Nostoc paludosum*
N.e.= Nostoc entophytum *N.sp.= Nostoc* species

Fig. 1: Growth curves of the four cyanobacterial isolates using dry weight method (A): total fixed nitrogen method (B): and nitrogenase activity assay (C).

Regarding the nitrogenase activity, the results showed that their variation in relation to the growth curve (Fig. 1c) in such a way that it increased up to 33 days, whereafter the value decreased. The organisms grow logarithmically up to 15 days and enter stationary growth after about 30 days. Such findings are in agreement with those recorded by (Hardy, *et al.* 1973 and Granhall, 1989).

It is evident from the present study that soil properties under cyanobacterial inoculations differ significantly from those received no algal treatments (controls). Such results supported the opinion that cyanobacteria incorporation to soil as a biofertilizer plays an important role in improving soil properties. In this respect, the data, concerning the maximum water holding capacity, the gravitational water and capillary water of soil showed significant increase in water holding capacity and capillary water, while showed significant decrease in gravitational water of soil due to cyanobacterial inoculation (Table 2). These results were parallel with those reported by Shields and Durrel, (1964); Metting (1981) and Metting and Rayburn, (1983) who stated that algal incorporation to soil stabilized the surface crust, improve infiltration and increased water retention and soil particles aggregation. Singh (1961) also recorded an increase in water holding capacity of usar lands estimated as much as 40% after one or two years when algae were used.

Table (2): Changes in some physical and chemical soil parameters according to algal inoculation.

Parameters	Control	*Anabaena variabilis*	*Nostoc paludosum*	*Nostoc entophytum*	*Nostoc* sp.
Maximum water holding capacity %	17.59 ± 0.72	24.16 ± 0.88	22.86 ± 0.8	24.81 ± 1.44	25.16 ± 1.87
Soil gravitational water (ml from 100ml water)	92.76 ± 0.22	91 ± 0.1	91.77 ± 0.75	91.0 ± 0.1	90.93 ± 0.12
Soil capillary water %	36.1 ± 1.15	44.94 ± 0.03	41.02 ± 3.76	44.75 ± 0.1	45.185 ± 0.5
Soil total nitrogen (mg / kg soil)	22.7 ± 2.3	152 ± 8.0	114.7 ± 12.2	98.7 ± 25.7	125.3 ± 16.7
Soil ammonium nitrogen mg ammonia N/ kg soil)	1.14 ± 0.116	4.6 ± 0.5	4.8 ± 0.29	9.0 ± 1.7	5.67 ± 0.5
Soil nitrate (mg NO_3^-N / kg soil)	33.2 ± 2.7	335 ± 81	119 ± 24	128 ± 16.0	42.0 ± 7.0
Total organic carbon (mg C / kg soil)	230 ± 50	400 ± 120	470 ± 70	430 ± 20	360 ± 30.0
Exopolysaccharides (mg glucose / kg soil)	16 ± 2.0	59 ± 7.0	62.0 ± 5.0	112 ± 7	112 ± 11
Soil carbonate (mg CO_3^{--} / kg soil)	6800 ± 1200	4500 ± 300	5300 ± 800	6400 ± 1700	5500 ± 600
pH	8.26 ± 0.06	7.97 ± 0.06	7.82 ± 0.02	7.7 ± 0.03	7.84 ± 0.05
Soil phosphorus (mg / kg soil)	24 ± 0.6	28.0 ± 5.0	23.0 ± 1.0	29 ± 2.0	22 ± 0.9

The changes in soil total nitrogen obtained by the four isolates showed that *Anabaena variabilis* was the most effective organism for enriching the soil with nitrogen (152.00 mg N/kg soil), while *N. entophytum* had the lowest effect (98.70 mg N/kg soil) (Table 2). Allison and Moris (1930) stated that blue-green algae may be the most important nitrogen-fixing agent in many agricultural soils. Fletcher and Martin (1948) found that the nitrogen of semi-desert increased 400% when algal growth of nitrogen

fixers was extensive. In most cases, it is generally accepted that the incorporation of organic carbon via photosynthesis and of organic nitrogen via nitrogen fixation are the most important contributions that algae add to the fertility of soil (Metting, 1981).

Where, the highest value in soil ammonia content (9.0 mg ammonia N/kg soil) was recorded from soil inoculated with *Nostoc entophytum*.The lowest value (4.6 mg ammonia nitrogen/kg soil) was, however, recorded from soil inoculated with *Anabaena variabilis* (Table 2).

Our data in Table (2) clearly prove the importance of algae, to a certain extent, as the sole active soil reformer, where the highest value of soil nitrate was recorded (335.00 mg nitrate N/kg soil) from soil inoculated with *A.variabilis* whilst, the lowest one (42.00 mg nitrate-nitrogen/kg soil) from soil inoculated with *N. sp.*

The maximum value of soil organic carbon content (470.00 mg/kg soil) was detected from soils inoculated with *N. paludosum*, while the minimum value (360.00 mg/kg soil) was recorded from soil inoculated with *N. sp.* The data cleared that the successful introduction of an efficient isolate in an area depends largely on its ability to survive and compete with other complex of interacting factors of soil. Any way, our data agree with those of Ghazal (1980) and De Cair *et al.* (1997) who reported the role of blue-green algae in improving the soil organic content.

Also, the data clearly confirmed that all soils inoculated with cyanobacteria had significant results over controls, as the exopolysaccharides are unique organic substances produced by cyanobacteria and released into soil. *N. entophytum* &N.sp. were the most effective organism for producing exopolysaccharides (112.00 mg glucose/kg soil for both algae), on the other hand, *A. variabilis*, had the lowest value (59.00 mg glucose/kg soil).

Regarding the changes in soil carbonate, results generally revealed that the significant reduction of soil carbonate for soils inoculated with cyanobacteria over control (Table 2). Also, results given in table (2) confirmed that pH values were reduced as a result of cyanobacterial inoculation compared with the control. Singh(1950, 1961)reported the same result.

The highest value of phosphorus (29.00mg/kg soil) was recorded from soil inoculated with *N.entophytum*, while *N.*sp. had the lowest value (22.00mg/kg soil).

The use of cyanobacteria as biological conditioners is possible, as the application of cyanobacteria positively stimulate the surrounding conditions which in turn improve the soil and plant properties. Any way, the cyanobacterial application to the field required many laboratory studies to penetrate deeply into their behavior and to magnify their role.

References

Ahmed, S.I. and **Ahmedunsia, A.** 1984. Utilization of blue-green algae as biofertilizer for paddy cultivation.*Pakistan J. Sci. Indust. Res.*, **27(6):355-358.**

Allen, M.M. 1968. Simple conditions for growth of unicellular blue-green algae on plates. *J. Phycol.*, **4:1-4.**

Allison, F.E.and **Moris, M.J.**1930. Nitrogen fixation by blue-green algae.*Sci.*, **71:221-223.**

Bunt, J.S. 1936. Nitrogen fixing blue-green algae in Australian rice soils. *Nature* **192:479-480.**

Castenholz,R.W.1988.Culturing method for cyanobacteria.*Meth.Enzymol.***167: 68-92**

Chapman, H.D. and **Pratt, P.F.** 1961. Methods of analysis for soil, plant and water. *Calif. Univ., USA.*

Chu, S.P. 1942. The influence of the mineral composition of the medium on the growth of planktonic algae. I. Methods and culture media. *J. Ecol.* **30:284.**

De Caire, Z.G.; de Cano, M.S.; de Mule M.C.Z.; Palma, R.M.and **Colombo,K.**1997. Exopolysaccharides of *Nostoc muscorum*(cyanob- acteria)in the aggregation of soil particles.*J.App.Phycol.*,**9:249-253.**

Desikachary, T.V. 1959. Cyanophyta. *Monograph I.C.A.R. New Dalhi. India.*

El-Ayouty, E.Y. and **Ayyad, M.A.** 1972. Studies on blue-green algae of the Nile Delta.1-Description of some species in a wheat field. *Egypt. J. Bot.*, **15:283-321.**

El-Borollosy, M.A.1972. Studies on nitrogen-fixingblue-green algae in A.R.E., M.Sc. Thesis, *Faculty of Agriculture, Ain Shams Univrsity.*

Esmarsch, F. 1914. Untersuchungen uber die verbreitung der Cyanophyceen auf invershiedenen Boden. *Hedwigia.* **55:224-327.**

Ferris, M.J. and **Hirsch, C.F.** 1991. Method for isolation and purification of cyanobacteria. *Applied and Environmental Microbiology,* **57(5):1448-1452.**

Fletcher,J.E.and **Martin,W.P.**1948.Some effects of algae and mold in the rain crust of desert soil. *Ecol.*, **29:95-100.**

Fogg, G.E. 1942. Studies on nitrogen fixation by blue-green algae. I. Nitrogen fixation by *Anabaena cylindrica* Lemm. *J. Exp. Biol.*, **19:78.**

Fogg, G.E. and **Stewart, W.D.P.** 1965. Nitrogen fixation in blue-green algae. II- Nitrogen fixation by *Mestigoclads laminosus. J. Expt. Bot.*, **2:117-120.**

Geitler, L. 1932. Cyanophyceae. Rabenhort's Kryptogamen flora von Europa. *Acad. Verlages - geselschaft. Leipzig.*

Ghazal, F.M.A.1980.Studies on enzymatic activity in rice soil inoculated with blue-green algae .M.Sc.Thesis, *Fac.of Agric., Al-Azhar Univ., Cairo, Egypt.*

Granhall, U.1989.Nitrogenase activity in *Anabaena inaequalis* in relation to growth and light-dark cycles. *Phykos,* **28(1&2):80-88.**

Gupta, U.S.; Maheshwari, K.L. and **Sen Gupta, S.R.** 1956. A method for obtaining algal cultures free from associated microorganisms. *Curr. Sci.* **25-91.**

Hardy, R.W.F.; Burns, R.C.and **Holsten, R.D.**1973.Application of acetylene-ethylene assay for measurement of nitrogen fixation.*Soil Bio.Biochem.***5:47-81.**

Hesse, D.R. 1994. A textbook of soil chemical analysis, *CBS Publishers & Distributors, Delhi, India.*

Hughes, E.O.; Gorham, P.R. and **Zehnder, A.** 1958. Toxicity of unialgal culture of *Microcystis aeruginosa. Can. J. Microbiol.*, **4:225-236.**

Jackson, M.L. 1958. Soil chemical analysis. *Constable and Co. Ltd. London.*

Kaushik, B.D. and **Murti, K.G.** 1981. Effect of blue-green algae and gypsum application on physico-chemical properties of alkali soils. *Phykos,* **20 (1 & 2) 91-94.**

Khadr, M.S. 1975. Studies on nitrogen-fixing blue-green algae in Egyptian soil. Ph.D. Thesis. *Fac. Agric. Al-Azhar Univ.*

Lowe, L.E. 1993. Total and labile polysaccharide analysis. *Canadian Society of soil Science.*

Markus, D.K.; Mckinnon, J.P. and **Buccafuri, A.F.** 1982. Aurmated analysis of nitritc, nitrate and ammonium nitrogen in soils. *New Jersy. Agric. Exp. Stn. Publication no.* **D. 15117-84.**

Metting, B.1981.The systematics and ecology of soil algae.*Bot.Review,* **47:195-312.**

Metting, B.and **Rayburn, W.R.**1983.The influence of a microalgae conditioner on selected Washington soil .An empirical study.*Soil Sci.Soc.Am.J.*, **47:682-685.**

Oxoid Manual of cultural media 1965. Ingredients and other laboratory services. Third Ed. Published by *Oxoid Limited, London.*

Piper, C.S. 1950.Soil and Plant analysis. *Interscience Ins., New York.*

Pringsheim, E.G. 1949. Pure culture of algae, their preparation and maintenance. *Cambridge Univ.*

Shields, L.M. and Durrell, L.M. 1964. Algae in relation to soil fertility. *Bot. Rev.* **30:92-128.**

Singh, R.N.1950.Reclamation of Usar lands in India through blue- green alge.*Nature,***165:325-326.**
Singh, R.N.1961.Role of blue-green algae in nitrogen economy of India.*Indian Council Agric.Res.,New Delhi, India.*
Staub, R. 1961. Ernahrungsphysialogisch autokalogische untersuchengen an der planktischen blualg *Oscillatoria rubescence* D.C. *Scheweiz. Zeitschr. Hydrobiologie,* **23:82-198.**
Stewart, W.D.P.; Fitzgerald, G.P.T. and **Burris, R.H.** 1971.In situ studies on N2 fixation using the acetylene reduction technique.*Proc.Nat.Acad.Sci., USA,* **58:2071-2078.**
Taha, M.S. 1963. Isolation of some nitrogen fixing blue green algae from the rice field of Egypt in pure cultures. *Microbiologya, USSR* : **32(3):493-497.**
Tam, N.F.Y.and **WongY.S.**1995.Waste water treatement with microorganisms.The commercial press (H.K.)Ltd.2D *Finnie St.Quarry Bay,Hong Kong.*
Tan, K.H. 1996. Soil sampling, preparation and analysis, *marcel Dekker, INC. New York, USA.*
The Botany School, Cambridge 1966. Culture collection of algae & protozoa. Lists of strains.
Venkataraman, G.S.1969.The cultivation of algae.*Indian Council Agric.Res.,New Delhi, India.*
Watanaba, A. 1951. Production in cultural solution of some amino acids by the nitrogen fixing blue-green algae. *Arch. Biochem. and Biophys.* **34(1):50-55.**

دور بعض الطحالب الخضراء المزرقة (السيانوبكتريا) في تحسين صفات التربة

أحمد درويش الجمل،مختار صالح عمار،أسامة محمد عبد الرؤوف،طاهر محمد طه
كليتي العلوم (القاهرة وفرع أسيوط) –جامعة الأزهر-قسم النبات والميكروبيولوجى

تهدف الدراسة الحالية إلى تقييم أثر الطحالب الخضراء المزرقة المثبتة للنتروجين ،المعزولة من التربة المصرية في تحسين وتعزيز صفات إحدى عينات التربة الرملية تحت الدراسة.تم عزل وتعريف أربع أنواع من الطحالب هم أنابينا فاريبليس، نوستوك بالودوسم، نوستوك إنتوفيتم، ونوع من النوستوك . أعطى منبت Z احسن النتائج لنمو الطحالب خلال ٥٠ يوم كما أعطت طريقة ماء الكلور وطريقة كلوريد الزئبقيك معا أحسن النتائج لتنقية الطحالب من البكتريا.تم تقدير نمو الطحالب باستخدام طرق الوزن الجاف ، نشاط اختزال الاستيلين وكذلك النتروجين الكلى.أوضحت النتائج الدور المعنوي للطحالب في تحسين كثير من صفات التربة الطبيعية والكيميائية.