Phykos 44 (2): 44-53 (2014)

Choice of culture media for isolation of algae from soils of some rice-fields

Madhavi Latha.D, Raj Kumar.B[*] and Sai Krishna.T[**]

* Department of Botany, Osmania Univ.Hyderabad, India. .
** Department of Biotechnology, Chaitanya Bharathi Institute of Technology, Hyderabad, India.
e-mail: kml75@rediffmail.com

Abstract

In this study soil algae inhabiting rhizosphere of paddy fields in Hyderabad were studied using different culture media. In all 54 algal species belonging to 28 different genera viz., Cyanophyceae, Chlorophyceae, Bacillariophyceae and Euglenophyceae were identified of which 36 species of 21 genera came up in the soil plates. The distribution and diversity of soil algal species was shown to be strongly dependent on availability of nutrients in the soil. High number of algal species were isolated using different culture media and compared to direct soil method. Various environmental edaphic factors appeared to have influenced taxonomical diversity of the soil algae..

Key words: Cyanophycea, Chlorophyceae, Bacillariophyceae, Euglenophyceae and Physico-chemical factors

Introduction

Several methods and basic culture media were developed in the late 1800 and early 1900s. Algal culture techniques have been described in several earlier books and articles (Moore 1903, Kuster 1907, Chodat 1913, Richer 1913, Pringsheim 1924, Kufferath 1928/29, Lwoff 1932, Meier 1932,Vischer 1937, Bold 1942, Chu 1942, Pringsheim 1946, Brunel et al.1950, Lewin 1959, Fogg 1965, Venkataraman 1969, Stein 1973, Guillard and Richmond 1986). Soil is the most important non-aqueous substrate for algae and cyanobacteria (Zenova et al.,1995). Due to their high capacity for morphological and physiological adaptations to different environments, both algae and cyanobacteria often act as pioneer microorganisms in terrestrial ecosystems (Metting,1981; Hoffmann,1989). Soil fertility is generally is improved by the organic matter produced by these organisms (Mishra, 2004). Soil algae excrete growth-promoting substances such as hormones, vitamins, amino acids, and organic acids that affect other organisms in their survival and multiplication in many ways (Roger and Reynaud, 1982; Wilson, 2006).

Over a century ago algae were first studied in culture by Beijerinck, (1890) and he was the first person to work with axenic cultures. Beijerinck, Knop and Bristol made some modification in culture media that helped in improved growth of algae in artificial culture media. Compared to Bristol's and Tamiya's media, Chu-10 media has been successfully used for culturing of many algal species. Medium 3.07 was indicated that the algal populations in the soil varied between 10^5-10^6 cells per ml of soil in solution. Optimization of Media in order to isolate different algal species from the soil and also to isolate pure cultures was done by several workers from time to time. Pringsheim (1946) suggested that the selection of a suitable culture media for isolation and successful cultivation of certain species of algae. The algal biotypes which are endemic to different fields have different adaptability and can have differences in producing different useful secondary metabolites qualitatively and quantitatively. Earlier several studies were carried to isolate different endemic algal species for taxonomic importance and for their important secondary metabolites (Ahmed 1994; Faith and Zaki, 2003; Shanab, 2006), The present study aims at isolation of species or strains from soils of some rice fields to know their biodiversity and their adaptability and abundance, using different growth media and their importance.

Materials and methods:

Sampling and morphological observations:

Rhizosphere soils from cultivated paddy fields at three different areas in Balapur, Hyderabad, were collected over the period from September to November 2010. About 500gms of each soil sample was placed in sterile plastic bags and transported to the laboratory immediately and were subjected for the isolation and identification of algal species. 10gm of each soil sample was placed in a flask and diluted 100-fold with distilled sterile water. After shaking for 1hr, the soil suspensions were fixed with formaldehyde solution (1%) and incubated for 90-days at room temperature (Table 3). In another part, 5gm of each soil sample without formaldehyde were placed in flasks containing 100ml of different liquid culture media respectively and incubated for 90-days at room temperature. For each media six replicates were maintained to study the population dynamics at intervals of every 15-days. 0.1ml of

10^{-2} soil dilution was streaked on solid agar medium (1.5% Agar). Algae were identified by using under Trinocular Meopta research microscope. The isolates were microscopically observed and sub-cultured and preserved for further study.

Physico-chemical characteristics of the soils were also investigated to assess the impact of edaphic factors on population dynamics of soil algae. Soil particles size analysis of the different air dried soil samples was determined according to Shubert and Starks (1980) method. The percentage of soil moisture was estimated on the same day by following the protocol proposed by El-Gamal (1995)**.** The pH of the soils were measured using standard digital pH meter. Electrical conductivity of the soil was estimated by Jackson(1977) method using digital electric conductivity meter method in ratio of 1:2 soil and water ratio. For estimation of organic carbon the method suggested by Walkley and Black, (1934) was used. Nitrogen was estimated by Subbaiah and Asija (1956) method, Phosphorus by Olsen *et al* method (1954) and Potassium by Muhr *et al*.,(1965) using Flame Photometer.

The determination of nomenclature of the organisms were carried out with reference to the relevant texts (Fritsch 1945, Desikachary 1959; Prescott 1962; John et al.,2002; Komarek and Anagnostidis 2005; Kobayasi et al.,2006).

Culture media used for isolation of algae:

The following media were used for isolation of algae: 1. Pringsheim's medium (1946), 2. Modified Bristol's medium (1949), 3.Chu10 medium (1942), 4. PAAP medium (1969), 5. 3.07 medium (1949), 6. Tamiya medium (1956), 7. Ishimura & Iwasa medium (1979), 8. Sager & Granick medium (1953), 9. Beijerink medium (1904), 10. Knop medium (1865), 11. Beneck's medium (1898)and 12. Fogg's medium (1965).

Results

Physico-chemical properties of the soil:

The paddy fields studied were slightly alkaline, with pH ranging between 7.2 to 7.3 (Table-1). Eclectrical conductivity for the soils studied showed very low for site-2 compared to site-3and 1. Organic carbon content was found to be high for site-1 and moderate for site-3and 2.Available Nitrogen also showing low in all three sites. Available Potassium also shows moderate in all three different soil samples.

Table 1: Showing average values of Physico-chemical parameters of rhizosphere soils of paddy during three months period from some localities of Hyderabad.

	Moisture(%)	Temp(°C)	pH	EC(dsm-1)	OC (Kg/ha)	N (Kg/ha)	P (Kg/ha)	K (Kg/ha)
Site -1	**7.21**	**29.08**	**7.23**	**0.27**	**2.26**	**239.26**	**33.51**	**226.13**
Site-2	**5.4**	**31.58**	**7.28**	**0.17**	**1.52**	**211.42**	**31.03**	**268.37**
Site-3	**5.32**	**32.25**	**7.3**	**0.23**	**1.24**	**207.12**	**30.95**	**235.13**

Table-2: Standard range of General nutritional status of the soil

	EC(dsm^{-1})	OC (Kg/ha)	N (Kg/ha)	P (Kg/ha)	K (Kg/ha)
Low	Below 0.8	Below 0.5	Below 280	Below 10	below 108
Medium	0.8 to 1.6	0.5 to 0.75	280 to 560	10 to 24.6	108 to 280
High	Above 1.6	Above 0.75	Above 560	Above 24.6	Above 280

The percentage of moisture in all the soils of the rice fields was found to be above the standard range. This is expected because rice fields require standing water for their growth. The temperature was average prevailing during the sampling season. The pH was found to be slightly alkaline. The Electrical conductivity (EC) of the soils tested was below the standard range indicating that it correlates strongly to soil particle size and texture under investigation (Table 2). High organic carbon (OC) availability was recorded in rice fields than the standard range. Similarly available contents of nitrogen, phosphorus and potassium levels ranged from moderate to high level suggesting that in general all the three sites are rich in nutritional status. These findings also reflect the richness of Algal population over the growing season and their population dynamics reaching optimum levels in mid season and maximum toward end of the season indicating build up of algal population densities growth period of rice fields.

Biodiversity of Algal species:

A total of 36 algal species belonging to 21 genera were isolated and identified from preserved specimens of three soil samples under the investigation using 1% formaldehyde solution, where as 54 algal species belonging to 28 genera were isolated using different culture media. Most of the algal species identified belongs to Cyanophyceae, Chlorophyceae, Bacillariophyceae and Euglenophycea. Algae belonging to Euglenophycea were not observed in soil preserved in 1% formaldehyde solution. These findings indicate the importance of using different culture media in isolation and identification of different algal species.

Role of different culture media for isolation of Algal species from different soils:

The average numbers of colonies isolated using different media are presented in Table 3. Among the media used the highest number of algal colonies and species were isolated using modified Bristol's medium followed by Beneck's medium and Tamiya medium. The other media used were able to isolate moderate number of algal colonies where as PAAP medium was least preferred for the algal growth. Over all 40 species per 5grams of soil were isolated and identified using different media. The data also reflects that different soils harbored different algal populations and the species. It also indicates all the soils analyzed are rich in algal populations. This study also indicates the importance of using different media for isolation and study of different algal species from the soil.

Table-3: Table showing average number of algal colonies using different culture media

	Name of the algal media	15th day	30th day	45th day	60th day	75th day	90th day	Total	Average
1	Pringsheim's medium , (1946)	32	38	42	5	52	61	272	45.33
2	Modified Bristol's medium, (1949)	34	41	52	68	72	78	345	57.5
3	Chu#10 medium, (1942)	27	28	39	58	63	68	283	47.16
4	PAAP medium, (1969)	3	5	16	22	31	38	115	19.16
5	3.07 medium, (1949)	22	28	40	51	59	67	267	44.5
6	Tamiya medium, (1956)	30	32	46	57	61	65	291	48.5
7	Ishiura & Iwasa medium,(1979)	28	30	45	59	63	63	288	48
8	Sagar & Granick medium, (1953)	6	14	19	26	33	37	135	22.5
9	Beijerink medium,(1904)	20	27	43	49	55	60	254	42.33
10	Knop medium,(1865)	20	24	39	46	50	54	233	38.83
11	Beneck's medium,(1898)	30	36	48	57	63	68	302	50.33
12	FOGG's medium, (1965)	26	28	43	53	60	66	276	46
	Total number of colonies	278	331	472	593	662	725	3047	
	Average	23.16	27.58	39.33	49.71	55.16	60.42		

	Name of the Soil Algal species	*Algal culture media											
		1	2	3	4	5	6	7	8	9	10	11	12
	Cyanophyceae												
1	***Aphanocapsa biformis***	✓	✓	✓		✓	✓	✓					✓
2	***Aphanocapsa pulchara***		✓				✓	✓		✓	✓	✓	
3	***Aphanothece bullosa***	✓	✓	✓			✓	✓					✓
4	***Aphanothece castagnei***		✓	✓		✓	✓	✓	✓	✓		✓	✓
5	***Aphanothece pallida***	✓	✓	✓			✓	✓		✓		✓	✓
6	***Aphanothece stagnina***	✓	✓	✓			✓	✓				✓	
7	***Chroococcus minor***	✓	✓	✓		✓	✓	✓	✓	✓		✓	✓
8	***Chroococcus minutus***	✓	✓	✓			✓	✓		✓	✓		✓
9	***Chroococcus pallidus***	✓	✓	✓		✓	✓	✓		✓		✓	
10	***Chroococcus turgidus***	✓	✓	✓	✓	✓	✓	✓		✓	✓	✓	✓
11	***Gloeocapsa gelatinosa***	✓	✓	✓			✓	✓		✓		✓	✓
12	***Gloeocapsa Punctata***	✓	✓	✓			✓	✓				✓	✓
13	***Gloeothece rupestris var.maxima***	✓	✓	✓			✓	✓				✓	✓
14	***Gloeothece samoensis***		✓	✓		✓	✓	✓		✓		✓	✓
15	***Merismopedia convolute***	✓	✓	✓		✓	✓	✓		✓		✓	✓
16	***Merismopedia tenuissima***	✓	✓	✓	✓	✓	✓	✓		✓	✓	✓	✓
17	***Microcystis aeruginosa***	✓	✓	✓		✓	✓	✓	✓			✓	✓
18	***Microcystis elongata***	✓	✓	✓			✓	✓			✓	✓	✓
19	***Microcystis flos-aquae***	✓	✓	✓		✓	✓		✓		✓	✓	
20	***Microcystis viridis***		✓	✓		✓	✓			✓	✓	✓	✓
21	***Anabaena ambigua***	✓	✓	✓		✓	✓					✓	✓
22	***Anabaena oryzae***	✓	✓	✓		✓	✓				✓	✓	✓
23	***Anabaena variabilis***	✓	✓	✓			✓					✓	✓
24	***Aulosira aenigmatica***	✓	✓	✓		✓	✓						✓
25	***Nostoc carneum***	✓	✓	✓		✓	✓			✓		✓	✓
26	***Nostoc commune***	✓	✓	✓		✓	✓		✓	✓		✓	✓
27	***Nostoc linckia***	✓	✓	✓		✓					✓	✓	✓
28	***Nostoc punctiforme***	✓	✓	✓	✓	✓				✓	✓	✓	✓
29	***Lyngbya birgei***	✓		✓		✓		✓			✓		✓
30	***Lyngbya laxespiralis***	✓	✓		✓	✓	✓		✓	✓		✓	
31	***Oscillatoria acuta***	✓	✓	✓		✓		✓		✓	✓	✓	✓
32	***Oscillatoria chlorina***	✓	✓	✓				✓	✓				✓
33	***Oscillatoria limosa***	✓	✓	✓	✓			✓		✓	✓		✓
34	***Oscillatoria princeps***	✓	✓	✓		✓		✓			✓	✓	✓
35	***Oscillatoria sancta***	✓	✓	✓		✓	✓	✓		✓		✓	✓

Continued…

36	***Phormidium ambiguum***	✓	✓	✓	✓			✓		✓	✓	✓	✓
37	***Phormidium favosum***	✓	✓					✓	✓	✓		✓	✓
38	***Spirulina major***	✓	✓	✓				✓			✓		✓
39	***Calothrix braunii***	✓	✓	✓		✓			✓		✓	✓	✓
40	***Scytonema simplex***	✓	✓	✓		✓				✓	✓	✓	✓
41	***Tolypothrix nodosa***	✓	✓	✓		✓			✓	✓	✓	✓	✓
	Chlorophyceae												
42	***Chlorella vulgaris***		✓			✓	✓	✓		✓		✓	
43	***Chlorococcum humicola***		✓		✓	✓	✓	✓	✓	✓		✓	
44	***Eudorina elegans***		✓			✓	✓	✓				✓	
45	***Pandorina morum***		✓				✓	✓				✓	
46	***Scenedesmus obliquus***		✓			✓	✓	✓				✓	
47	***Closterium archerianum***		✓			✓	✓	✓		✓		✓	
48	***Cosmarium leave***		✓			✓	✓	✓		✓	✓	✓	
49	***Navicula cuspidata var.ambigua***		✓				✓	✓		✓	✓		
50	***Navicula mutica* var.*elliptica***		✓		✓	✓	✓	✓	✓	✓	✓	✓	
51	***Nitzschia amphibian***		✓				✓	✓			✓		
52	***Nitzschia palea***		✓		✓	✓	✓	✓			✓	✓	
	Euglenophyceae												
53	***Euglena polymorpha***		✓			✓	✓	✓		✓	✓	✓	
54	***Phacus orbicularis***		✓		✓	✓	✓	✓		✓	✓	✓	
	Frequency of occurance	37	53	38	10	36	41	40	12	31	25	45	36

1. Pringsheim's medium, 2. Modified Bristol medium, 3.Chu-10 medium, 4. PAAP medium, 5. Trainor's3.07 medium, 6. Tamiya medium, 7. Ishimura & Iwasa medium, 8. Sagar & Granick medium, 9. Beijerink medium, 10. Knop medium 11. Beneck's medium, 12. FOGG's medium.

The algae isolated from soil with different culture media are presented in Table 4. The species *Chroococcus turgidus, Merismopedia tenuissim* and *Nostoc commune* were isolated from all the media used and these appeared to be the most dominant species of soil algae, followed by *Chroococcus minor*, *Merismopedia convolute*, *Oscillatoria acuta* , *O. sancta* and *Phacus orbicularis.* The nature of soil algal communities is the result of the complex influence of different nutrients, soil properties and climatic conditions. This study indicated that substrate media plays a very important role in the distribution and diversity of soil algae (Lin and Wu, 2013). The present study shows that the highest number of species was observed in soil-3 followed by 2 and one. However statistically all the three sampling sites are not having much diversity as per as algal species concerned. All the soils have harbored large number of algal species per 5grams of soil, indicating their richness in algal species. Algal species allows growth of other microorganisms make the soil rich for cultivation of the rice plants thereby helping the plants for nitrogen and carbon source to the rice fields. The general trend is that the soils are enriched by different algal species over the growing season in an experimental manner. The different media used helped in selective isolation of different algal species. Modified Bristol media appears to be best among the 12-media used, followed by Beneck's, Tamiya, Chu#10, Fogg's and Pringsheim's media.

Discussion:

Three rice growing areas were selected to enumerate richness of the algal species in the soil. The results indicate that all the three regions tested were rich in soil algae indicating richness of the soils. The maximum average number of algal colonies was 60.42 using culture media, while it is 43.00 using direct soil cultures. Among soils used, soil-1,2 and 3 appear to be rich in organic carbon, Phosphorus and potassium levels were lesser than nitrogen and electrical conductivity values. The soil profile could also be one of the causes for varied number of algal populations in the soil. These findings were similar to those observed by Salama *et al*. (1973) and Osman *et al*. (2003). In contrast, the results obtained by Fathi and Zaki (2003) indicated that the response of algal biomass at the different investigated sites to soil texture is not reflected in retarded or activated algal growth. The culture method showed significant result with the maximum number (54) algal species of Cyanophyceae, Chlorophyceae, Bacillariophyceae and Euglenophyceae species whereas from direct soil less number of (36) algal species were seen.

Acknowledgement

Authors are thankful to Director of Agricultural University,(Soil testing Lab) Rajendranagar, , Hyderabad for providing necessary laboratory facilities for soil analysis.

References

Ahmed, S.U. 1999. Distributional Pattern of blue-green algae in rice field soils of Nagaon sub-division phykos.38(1&2):101-107

Ahmed, Z.A. (1994). Preliminary survey of soil algal flora in Upper Egypt. Egypt. J. Bot., 34(1): 17-36.

Aiyer R. S, Salahudeen, S, Venkataraman G. S (1972) On a long term algalization field trials with high yielding rice varieties: Yield and Economics. Ind. J Agrc. Sci 42:382

Alexander, M.1971. Microbial Ecology. John Wiley and sons. Inc. New York.511 p.

Amann, R.l, Ludwig W, Schleifer K.H (1995) Phylogenetic identification and in situ detection of individual microbial cells without cultivation. Microbial Rev 59:143-169.

Amita Devi, G.Dorycanta, H and Singh, N.I. 1997. Cyanobacterial flora of rice field soils of Manipur phykos.38(1&2): 13-18.

Barber,D.A. and Lynch, J.M (1977). Microbial growth in the rhizosphere, Soil Biology and Biochemistry. 9, 305-308.

Beijerink, M.W. 1890. Cultureversuche mit *Zoochlorellen*, Lichegonidien anderen niedern Algen. Bot. Zeitung 48: 725-39, 741-54, 757-68, 781-85.

Beijerink, M.W.1904. Das Assimilations product der kohlensaure in den chromatophoren der Diatomeen., Rec.Trav. Bot. Neerl. 1: 28-32.

Bold, H. 1942. The cultivation of algae. Bot. Rev. 8: 69-138.

Bold,H. 1970. Some aspects of the taxonomy of soil algae. Ann. N.Y.Acad..Sci..175:601-16.

Bristol Roach B.M, 1919: On the retention of vitality by algae fro old stored soils. New phytol.18, 92-107

Bristol Roach B.M, 1920: On the algal flora of some desiccated English soils: An important factor of soil biology. Ann.Bot.34:35-80.

Bristol Roach B.M, 1926. On the relation of certain soil algae to some soluble carbon compounds. Ann.Bot.60, 149-200.

Brook, A.1968. The discoloration of roots in the United States and Canada by algae. J. Phycol.4:250

Brunel , J., Prescott,G.W., and Tiffany, L.N., eds. 1950. The culturing of Algae. Charles F.

Kettering Foundation, Antioch Press, Yellow Springs, Ohio, 114 pp.

Cano, M.S.; Mule,M.C.Z.; de Caire, G.Z.; de Palama, R.M. and Colombok, K. (1997). Aggregation of soil particles by Nostoc muscorum Ag.(Cyanobacteria). International J. of Expermintal Botany.60 (2): 33-38.

Chodat, R. 1913. Monographic dalgues en culture pure. Beitr. Kryptogamenfl. Schweiz 4(2): 1-266.

Chu, S. 1942. The influence of the mineral composition of the medium on the growth of planktonic algae.1. Methods and culture media J. Ecol. 30: 284-325.

Dar by shire, J.F. and Greaves, M.P.(1970). An improved method for the study of the inter relationships of soil microorganisms and plant roots, Soil Biology and Biochemistry. 2: 63-71.

Darley, W.M. (1982). Algal Biology: A Physiological Approach, Blackwell Scientific Publications, Oxford.

Desikachary: T. 1959. Cyanophyta. Indian council of Agricultural Research, New Delhi, monograph on algae,686p.

Droop, M.R. 1967. A procedure for routine purification of algal cultures with antibiotics. Brit. Phycol. Bull. 3: 295-297.

Edwards,P. 1969. Field and cultural studies on the seasonal periodicity of growth and reproduction of selected taxa benthic marine algae. Contrib. Mar. Sci.14:49-114

El-Gamal, A.D. (1995). Systematical studies on the algae isolated from some cultivated areas and laboratory studies on the effect of light, temperature and humidity on three selected soil algae. Ph.D. Thesis, Fac. Of Sci.,Al-Azhar University, Cairo, Egypt.

Fathi, A.A. and Zaki, F.T. (2003). Preliminary survey of edaphic algae in El-Minia region, Nile valley, Egypt. *Egyptian J. of Phycol* 4(2): 131-148.

Fogg,1956. Acomparative physiology and Biochemistry of Blue green algae.Bact.Rev.20:148-165.

Fogg, G. 1965. *Algal Cultures and Phytoplakton Ecology*. Univ.Wisconsin Press, Madison. 126 p.

Fogg. G. 1975 "Algal cultures and Phytoplankton Ecology" University of Wisconsin,Madison.2nd ed.175p.

Fogg.G.W. Stewart, P.Fay, and A. Waisby.1973. The blue green algae.Academic press, London and New york. 469 p.

Fritsch.F.1945. The structure and Reproduction of the Algae.Volume II. The University Press. Cambridge.939p.

Gerwick,W.H. Roberts, M.A. Proteau, P.J. and Chen, J.L.1994. *Screening cultured marinemicroalgae for anticancer-type activity*. J. Appl. Phycol.6: 143-149.

Goyal, S.K. 1997. *Algae and the Soil Environment,* Phykos,36: 1-12.

Guillard R.R.L. 1995. Culture methods. In: Manual on Harmful Marine Microalgae. (Hsllegraeff, G.M., D.M. Anderson, A.D. Cembella, eds.) pp.45-62.

Hoffmann, L.(1989). *Algae of Terrestrial Habitats. The Bot. Rev.* 55 (2): 77-105.

Humphreys .K Jelens's *cost and optimization engineering*, 3rd ed. Mcgraw-Hill;1991.

Jackson, M.L. (1973): Soil Chemical Analysis (Indian edition). Prentice Hall of India Pvt. Ltd. New Delhi.

Jackson, M.L. (1977). Soil Chemical Analysis. Pentice Hall of India. Private Limited-New Delhi.

John, D.M., Whitton B.A., Brook A.J. (2002).The fresh water algal flora of the British Isles. An identification guide to fresh water and terrestrial algae., Cambridge University Press pp.468- 470.

Khan, S.I. 1957. *Cultures of the algae of rice fields of Kashmir*. Biologia. 3: 29-43

Knop.w. 1865. Quantitative untersuchungen uber die ernahrungsporozesse der flanzen. Landwirtschvers stn 7: 93-107.

Kobayasi H, Idle M, Mayama S, Nagumo T, Osada K (2006) H, Kobayasi's Atlas of Japanese Diatoms Based on Electron Microscopy. Uchida-rolakuho, Tokyo, Japan, in Japanese.

Komarek J, Anagnostidis K (2005) Cyano-prokaryota, 2. Oscillatoriales,, in Brudel,B.,Gartner, L.

Kratz, W.A. and Myers, J. 1955. *Nutrition and growth of several blue-green algae.* Amer.J.Bot.42:282-287.

Krienitz and M.Schlagerl (eds), Subwasserflora von Mitteleuropa,vol 19/2.Elsevier Gmbh, Munchen,p.760.

Kufferath, H. 1928/29. La Culture des algues. Revue Algol. 4: 127-346.

Kuster, E. 1907. Anleitung zur kultur der Mikroorganismen (1. Auflage). Verlag B.G. Teubner, Leipzig, Germany, 201 pp.

Lewin, R.A. 1959. *The isolation of algae.* Rev. Algol. (new series) 3: 181-197.

Lin CS, Wu JT (2013) Environmental factors affecting the diversity and abundance of soil photo microbes in arid lands of subtropical Tiwan,Geomicrobiol j, doi: 10, 1080 / 01490451. 2013.828135.

Lund, J.W.G. 1945. Observations on soil algae. The ecology size and taxonomy of British soil diatoms. New phytol. 45:196-219.

Lwoff, A. 1932. Recberebs biochimiques sur la nutrition des protozoaires Le pouvoir de synthese. Monographies de J. Institut Pasteur. Masson, Paris, 158 pp.

Madhusoodanan, P.V. and Dominic, T.K. 1995. *Variations in the diversity of Blue-green algae inrice field of Kerala*. Phykos.34 (1&2): 65-69.

Meier, F. 1932. Cultivating algae for scientific research. Ann. Rep. Board Regents Smithsonian Inst. 1932: 373-83

Metting, B. (1981). *The systematic and ecology of soil algae*, *Bot. Rev*., 47: 195-312.

Mishra U, Pabbi S (2004) Cyanobacteria: A potential biofertilizer for rice.Resonanace 9:6-10.

Moore, G.T. 1903. *Methods for growing algae in pure cultures.* J. Appl. Microsc. 6:2309-2314.

Moore, G.T. 1903. Methods for growing pure cultures of algae. J. Appl. Microsc. Labor. Meth. 6: 2309-14.

Muhr, G.R., Datta, N.P., Sankrabamoney, H., Leley, V.K. and Donahue, R.L. (1965). Soil Testing in India. USAID Mission to India, New Delhi.

Newman, E.I.(1978). Root microorganisms: their significance in the ecosystem, Biological Riview 53, 511-554.

Olsen SR et al.1954. Estimation of available Phosphorus in soils by extraction with sodium bicarbonate U.S. Dep. Agric.Washington, D.C. Circ.939.

Osman, M.E.H.; El-Naggar, A.; Omar, H.H.and Esmail, G.H.(2003).Distribution of different soil algal taxa in relation to physico-chemical characteristics of soil at Gharabia governorate.*Egypt.J.Phycol.*,4:36-54.

PAAP. 1969. Provisional algal assay procedure. Joint Ind. Gov. Task Force on Eutrophication. USD1, Corvallis, OR.

Prescott, G.W. (1962). 'Algae of the Western Great Lakes area.' W.C. Brown company Publishers, Dubuque, Iowa,USA.

Pringsheim, E. G.1924. Algenkultur. In: abderhalden, E., ed. Hand buch der biologischen Ar beitsmet boden, Abt. X1 (2/1). Urban and Schwarzen berg, Berlin, pp.377-406.

Pringsheim, E. 1946. *Pure Cultures of Algae*. Cambridge University Press, London. 119 pp.

Pringsheim, E. 1967. Phycology in the field and in the laboratory.J. Phycol.3: 93-5.

Prova soli, L.J., Mc Laughlin, and M. Droop.1957. *The development of artificial media for marinealgae* Arch.Mikrobiol. 25: 392-428.

Rai LC, Kumar HD, Mohn FH, Soeder Cl (2000) Services of algae to the environment. J Microbial. Biotechnol 10: 119-136.

Richer, O. 1913. Die Reinkultur und die durch sie erzielten fortschrittee vornehmlich auf botanischem Gebiete. Progressus rei Botanicea 4: 303-60.

Richmond A. (ed.) 1986 CRC Handbook of microalgal mass culture. CRC Press. Boca Raton, Florida, USA.528p.

Roger P, Reynaud P(1976) Dynamics of the algal populations during a culture cycle in a sahel rice field. Rev Ecol Biol Sol 13:545-560.

Roger PA, Reynaud PA (1982) Free-living blue-green algae in tropical soils. Martinus Nijhoff Publisher, La Hague.

Sagar,R. and S.Granick,1954. Nutritional control of sexuality in Chlamydomonas reinhardi. J..Gen.Phyiol. 37: 729-42.

Salama, A.M.; El-Batanouny, K. and Ali, M.I.(1973).Studies on the fungal flora of Egyptian soils. I. West Mediterranean Coast and Lybian Desert.U.A.R.Bot.14:99.

Shanab, Sanna, M.M. (2006).Algal flora of Ain Helwan.II.The soil algae.*Egypt.J.Phycol.*,7:221-235.

Shubert, L.E. and Starks, T.L. (1980). Soil algae relationships from surface mined soils. Br. Phycol. *J.* 15: 417-428.

Skinner, C.E. 1932. *Isolation in pure culture of green algae from soil by a simple technique.* Plant Physiol. 7: 533-537.

Stein J. 1973. *Handbook of Phycological Methods , Culture Methods and Growth Measurements* Cambridge Uni. Press. 448.

Stein, J.R., ed 1973. Handbook of phycological met bods. Cambridge University Press, Cambridge, 448 pp.

Stein,J.R.1966. Growth and mating of Gonium pectoral (Volvocales) in difined media, J. Phycol.2,23-8.

Steven Murray, Jan Scherfig, and Peter S. Dixon, 1971. Evolution of Algal Assay Procedures-PAAP Batch test. Journal WPCF.56: 831-838.

Stewart,W. 1974. Algal physiology and Biochemistry.Univ. Calif.Press,Berkeley.9809p.

Subbaiah, B.V. and Asija, G.L. 1956: A rapid procedure for the estimation of available nitrogen in soils. Current Sci.25: 259-260.

Tamiya,H. 1958. Mass culture of algae. Ann. Rev. Plant Physiol.8:309-34.

Trainor, F.,(1978).Introductory phycology, John wiley & Sons publishers, New York.

Trainor,F. J.Cain, and L. Shubert.1976. Morphology and nutrition of colonial green alga *Scenedesmus* Bot.Rev. 42. 5-2

Trainor,F.1970. Algal morphogenesis: nutritional factors.Proc.N.Y.Acad.Sci.175:749-56.

Venkatraman, G.S. 1969. The cultivation of algae. Indian Council of Agricultural Research, New Delhi. PP:319.

Vischer, W. 1937. Die kultur der heterokonten. In: L., Rabenhort's kryptogamenflora von Deutscbland, Osterreich und der Sch Weiz, voil.II (ed.2). Heterokonten. Akademische verlags gesellos chaft, Leipzig, Germany, pp.190-201.

Walkely, A and Black, I.A. (1934). An examination of the Degtjareff method for determining soilorganic matter and a proposed modification of the chromic acid titration method. Soil Science, 37:29-38.

Warcup, J.H. 1950. The soil-plate method for isolation of fungi from the soil. Nature. London. 166; 117-118.

Warcup, J.H. 1950. The soil-plate method for isolation of fungi from the soil. Nature. London. 166; 117-11
Ward, H. M. 1899. *Some methods for use in the culture of algae*. Ann. Bot., London. 13: 563-566.
Wilson LT (2006) *Cyanobacteria*: A Potential Nitrogen source in Rice Fields, Texas Rice 6: 9-10.

Zenova GM, Shtina EA,Dedysh SN, Glagoleva OB, Likhacheva AA, Gracheva TA(1995) Ecological relations of algae in biocenoses. Mikrobiologia 64:121-133.