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Influence of Environmental Factors on Algae in Rice-Field Soil from the Iraqi Marshes

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ABSTRACT. A study is reported of the algae in one sample of rice field soil from the Iraqi marshes. 43 taxa were revealed by enrichment culture techniques as opposed to 11 by direct microscopy. *Microcoleus chthonoplastes* and *Nostoc muscorum* were the dominants in the field, but the latter was more successful in laboratory culture (which was carried out only under fully aerobic conditions). The effects of temperature, nitrogen source, phosphate and NaCl were tested. Substantial growth occurred at 45°C, but all algae cultured at 48°C eventually died. Species which were heterocystous and therefore presumed nitrogen-fixers were an important component of the algae crop in the field and dominated laboratory cultures lacking combined nitrogen. 6 taxa showed good growth in medium enriched with 0.5 M NaCl; 3 taxa grew in 1 M NaCl, but growth was very slow. It is suggested that field research on these algae might indicate ways of modifying cultivation practices to increase the nitrogen status of the soil without the need for introducing nitrogenous fertilizers to this region.

It has long been recognized that algae can play an important role in the maintenance of fertility in rice-fields. This is especially true of the blue-green algae, since many of the widespread rice-field species are capable of fixing atmospheric nitrogen (Singh 1961, Roger and Kulasooriya 1980). In spite of the many studies, however, it is still difficult to comment on the importance of different environmental factors and any selective effect these may have on the occurrence or abundance of particular species. The majority of studies have been descriptive (see Roger and Kulasooriya 1980) and few concern the rice-fields of the Gulf States, in spite of the fact that rice is grown in Saudi Arabia, Iraq and Iran.

By far the most detailed study in the region is a floristic account of rice-fields in southeastern Iraq by Al-Kaisi (1976), who reported that algae were widespread and abundant there during the months of July and August when the survey was made; blue-green algae were overwhelmingly dominant, forming up to 86% of the algal vegetation (although details are not given of the sampling methods used to make this estimate). Hamdi *et al.* (1978) summarized the results of a survey of nitrogen-fixing organisms in various Iraqi soils. For the seven rice-field soils sampled, counts (established by the most probable number method) showed mean values per gramme of 3.1×10^5 for *Azotobacter* spp. and 98 for blue-green algae. Rice-fields showed the lowest *Azotobacter* and highest blue-green algal count of all the types of crop studies. Khoja (1973) reported the isolation in laboratory culture of a strain of *Anabaena* from the soil surface of a rice-field in Al-Hassa, Saudi Arabia. In addition to phototrophic growth, growth occurred in the dark with sucrose and glucose and fructose but not maltose (Khoja and Whitton 1975).

In contrast to the broad survey of Al-Kaisi (1976), the present study was planned to be an intensive account of the algae in soil from one small area. The particular soil sample taken was from a relatively mature rice-field with moist soil, but no standing water. The site was not far from an area of marsh whose algal flora has been described by Maulood *et al.* (1981).

Environmental Background

A brief general account of the rice-field environment in the southern marshes of Iraq will be given before giving details of the site on which the present study is based. The area of the southern marshes in Iraq is very extensive, though the exact size given by various authors differs, presumably due to the difficulty of defining a 'marsh'. According to Buringh (1960), more than 35000 km^2 is covered by water at the time of peak flood. Thesiger (1964) describes the marshes as occupying an area of more than 15000 km^2. However, permanent marshes cover only about 10000 km^2 (Buringh 1960). Inside the marshes, rice is grown on land which is under water during the peak period of floods from the Tigris and Euphrates, March-Arpil, but which requires earth dams to maintain the water level in the fields during summer. Rice is planted in the fields in late spring and standing water typically remains until late August or September, when the rice is approaching the stage of panicle initiation. The soils gradually dry out during the period of flowering and grain ripening. Immediately after the harvest, the land is sometimes ploughed (Buringh 1960). The same land may also be planted with other crops during the winter season, such as wheat, barley or oats (Al-Kaisi 1976). It is unclear just what percentage of the marshes which could be used for rice cultivation is in fact cultivated, but the importance of the crop is evident from the fact that about 80% of Iraqi rice production comes from the south, especially the marshes area (Chakravarty 1976).

The climate of the marshes is arid sub-tropical and characterized by a long dry sum-

mer, with an air temperature often exceeding 40°C during July and August, and a rainfall of only 200-300 mm restricted to autumn, winter and spring. There are sometimes slight frosts in cold winters in the marshes area (General Establishment for Studies and Design 1979). In spite of the semi-desert climate, the relative humidity is relatively high. At Basrah airport, just south of the region, the maximum relative humidity lies in the range 46-48% in summer and 70-75% in winter; the values are presumably considerably higher inside the marshes. Evaporation is very high from a free water surface and irrigated land, often exceeding 16 times the rainfall (General Establishment for Studies and Design 1979).

The upper part of the soil consists of very fine loamy sand, silt and clay deposited by the river at time of flood, with particle sizes getting smaller the further from major channels; these soils are extremely calcareous (Buringh 1960), and have moderate to high salinity. Crystals of gypsum and sodium chloride are present in considerable amounts, even covering the soil surface in rice-fields in the south-east (Al-Kaisi 1976). There is apparently no use of commercial fertilizers or insecticides and nearly all manure is taken for fuel.

The location of the site used for the preşent study is shown in Fig. 1. It lies near site D (Um al Schwaich) used in a study (Maulood *et al.* 1981) of the algal ecology of the permanent marshes. The exact location was from the side of a field about 200 m from the main navigation channel. The sample was taken on 21 September 1979, when standing water inside the fields was restricted to a few scattered pools. The soil had in most places started to dry out, with obvious cracks in the surface near the small

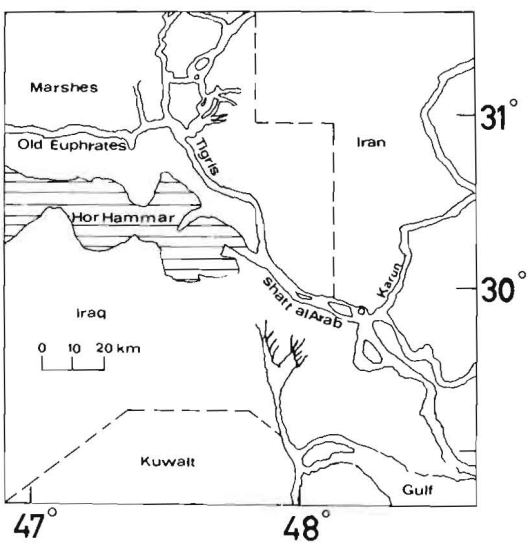

Fig.1. Map of southern marshes, showing location of site from which rice-field soil collected.

channels running through the fields (Photo 1). There was an almost continuous algal cover over the surface of the soil, but no evidence of dried algal fragments on the rice plants themselves. Growths of other plants such as *Marsilea* inside the fields were only moderate, presumably due to previous weeding.

Photo 1. Soil at edge of rice-field with algal cover.

Material and Methods

Collection of Sample

The sample was collected by B.A.W. on 21 September 1979 by pooling materials from microhabitats ranging from wet to dry within an area of 2 m^2 similar to that shown in Photo 1. Soil was included to a depth of 3 mm.

Soil analysis

The sample was dried for 48 hr at 105°C, lightly ground and put through a 80 mesh (210 μm) sieve; the material passing through the mesh was used for analysis. Part was

muffled at 550°C to give the ash weight (neglecting loss of CO_2 from carbonates). Part was digested with atomic absorption spectroscopy grade HNO_3 and boiled for 30 minutes. Metal analyses were carried out on a Perkin-Elmer model 403 atomic absorption spectrophotometer (Holmes and Whitton 1981).

Culture

The air-dried sample of soil was ground to a fine powder with a porcelain mortar and 5 mg aliquot used as a standard inoculum. A total of 0.715 g was used for all the experiments described here. All experiments were carried out in batch liquid culture, though agar was used to grow organisms for taxonomic purposes and to aid isolation. The basal medium was a modification of that due to Allen and Arnon (1955); its composition is shown in Table 1. The effect of nitrate was tested using sodium nitrate; variation in the concentration of sodium ion had relatively little effect over the concentrations needed as controls for these experiments. The effect of phosphate was tested by varying the level of K_2HPO_4 and adding KCl to the K level already present at the highest phosphate concentration. This experiment was buffered to pH 7.6 with HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulphonic acid), using NaOH to make the final adjustment. Experiments were carried out in 100 ml conical flasks with 50 ml medium.

Table 1. Chemical composition of modified Allen and Arnon (1955) medium used for culturing. (EDTA, ethylenediaminetetra-acetic acid)

salt	mg^{-1}
$K_2HPO_4.3H_2O$	250
$MgSO_4.7H_2O$	200
NaCl	230
$CaCl_2.2H_2O$	66
$FeCl_3.6H_2O$	19.4
$Na_2EDTA.2H_2O$	25.4
EDTA: Fe (molar ratio)	0.94:1
$MnSO_4.4H_2O$	0.5
$NaMoO_4.2H_2O$	0.06
$ZnSO_4.7H_2O$	0.05
$CuSO_4.5H_2O$	0.02
$CoCl_2.6H_2O$	0.01
H_3BO_3	0.07
$NiSO_4.7H_2O$	0.01

The effect of temperature was tested on an aluminium block with a temperature gradient which permitted flask temperatures to be tested over the range 15-50°C. Temperature fluctuation at any particular place was in the range ± 1°C. Continuous light was used; its intensity ranged from 90-150 μE m^{-2} s^{-1}, but the flasks were randomized at each temperature twice a day to reduce any possible effect due to differences in intensity.

Isolation of axenic clonal strains was carried out by the methods suggested by Wiedeman *et al.* (1964) and Bowyer and Skerman (1968).

Extraction of Chlorophyll

Chlorophyll *a* was extracted and analyzed according to the method given by Marker *et al.* (1980), using hot methanol and absorbance measured after passing through a glass-fibre filter.

Microscopy

The algal populations in culture flasks were lightly homogenized before aliquots were removed for microscopy.

Replication

The results shown in Fig. 2-6 are those for individual experiments. However, the majority of experiments were repeated at least once, with closely similar results. These include confirmation of the differing responses of mixed populations to the presence or absence of combined N at 15° versus 40°C (Fig. 2) and in 0.1 M versus 0.5 M NaCl (Fig. 6).

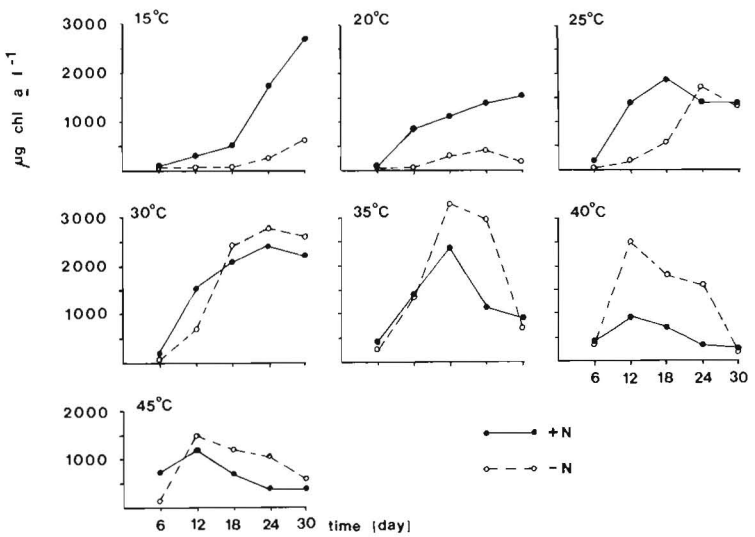

Fig.2 Influence of a) temperature and b) presence or absence of combined nitrogen on growth of algal community developing on inoculation with soil sample.

Taxonomy

The allocation of names to blue-green algae is, for various reasons, difficult. The conventions used here are the same as those used by Potts and Whitton (1980); a justification for using width categories for certain genera rather than binomials is given by Whitton *et al.* (1979).

Results

Chemical Composition of Soil

The rice plants grow in a calcareous soil (Table 2), whose metal composition differs little from that of sediments from the floor of a main channel in the marsh reported by Maulood *et al.* (1981). The main difference is the higher Na content in the rice fields (1720 versus 966 $\mu g\ g^{-1}$ in ashed material).

Table 2. Chemical composition of soil, based on total dry weight and ash weight (n - 4 in each case).

	$\mu g\ g^{-1}$ $\bar{x}$	dry weight ± sd	$\mu g\ g^{-1}$ $\bar{x}$	ash weight ± sd
Na	1470	394	1720	438
K	6370	629	7460	574
Mg	26750	2320	31350	2069
Ca	146800	3060	172300	1990
Al	11100	1340	13000	1410
Mn	730	75	852	86
Fe	16200	1840	19000	1880
Co	42.5	8.6	49.2	10.4
Ni	120	8.1	138	8.6
Zn	315	251	365	290
Cd	1.5	0.5	1.7	0.5
Pb	24.0	8.7	2.7	9.9

Algal Flora

Relatively few algae were seen by direct inspection of the rewetted sample; *Microcoleus chthonoplastes* and *Nostoc muscorum* were the dominants. Laboratory enrichment culture led to a much greater list of forms (Table 3). Of the 43 taxa found, 29 were blue-green algae, 12 green algae, one Euglenophyta and one Xanthophyta. Ten of the blue-green algae were heterocystous. Empty diatom frustules were seen in the soil samples, but no live cells.

Table 3. List of algae identified from soil sample.

Taxon	Taxonomic and morphological notes (see text)	Seen by direct viewing of sample	Seen after various enrichment techniques	Whether obtained in axenic clonal culture
Cyanophyta				
Anabaena catenula	+	+	(+)	
Anabaena cylindrica Lemm.			+	+
Aphanocapsa $> 2 \leq 4\mu$m			+	
Aphanothece stagnina (Spreng) A. Br.		+	+	
Calothrix fusca (Kütz.) Born. et Flah.	+		+	
Calothrix parietina (Näg.) Thuret	+		+	+
Calothrix $> 2 \leq 2$ μm	+		+	+
Chroococcus $> 4 \leq 8$ μm		+	+	
Cylindrospermum muscicola (Kütz.)			+	+
Gloeothece $> 2 \leq 4$ μm			+	
Hapalosiphon welwitschii			+	+
Lyngbya aestuarii (Mert.) Liebm.			+	
Lyngbya < 1 μm			+	
Lyngbya $> 1 \leq 2$ μm			+	
Lyngbya $> 2 \leq 4$ μm		+	+	
Lyngbya $> 4 \leq 8$ μm			+	
Microchaete $> 4 \leq 8$ μm		+	+	
Microcoleus chthonoplastes Thuret		+	+	
Nodularia harveyana (Thw.) Thuret			+	
Nostoc muscorum Ag.	+	+	+	+
Oscillatoria redekei van Goor			+	
Oscillatoria ≤ 2 μm			+	
Oscillatoria $> 2 \leq 4$ μm			+	

Plectonema < 2 μm		+	+	
Plectonema > 2 ≤ 4 μm		+	+	
Schizothrix < 2 μm		+	+	
Schizothrix > 2 ≤ 4 μm		+	+	
Scytonema sp.			+	
Tolypothrix distorta Kütz.			+	
Euglenophyta				
Euglena sp.			+	
Xanthophyta				
Tribonema minus (Wille) Hazen			+	
Chlorophyta				
Chlamydomonas sp.			+	
Chlorella vulgaris Beijerinck			+	
Chlorococcum humicola (Näg.) Rabern.			+	
Draparnaldia sp.			+	
Gonium sociale (Duj.) Warming			+	
Hormidium flaccidum A. Br.			+	
Pandorina morum (Müll.) Bory			+	
Scenedesmus abundans (Kirch.) Chodat			+	
Scenedesmus arcuatus Lemm.			+	
Stichococcus bacillaris Näg.			+	
Stigeoclonium sp.			+	
Ulothrix sp.			+	

Although a detailed taxonomic discussion of the various forms lies outside the scope of this paper, comments will be made on a few genera:

Anabaena

The dominant form of *Anabaena* at the field site was *A. catenula;* an organism with similar trichome width found in many enrichment cultures was probably the same, but akinetes were never seen.

Calothrix

C. fusca and *C. parietina* both formed hairs in enrichment cultures, but when *C. parietina* had been brought into axenic culture, no hairs were noted subsequently. The form listed as *Calothrix* $> 2 \leqslant 4$ μm did not form hairs in enrichment or axenic culture. This strain had trichomes 3-4 μm wide at the base and a sheath which was thin and colourless under all the culture conditions tested, including ones which led to the formation of brown sheaths in *C. parietina*.

Cylindrospermum

This alga formed heterocysts and akinetes in enrichment culture, in spite of the levels of both combined N and P in the medium being very high. When brought into bacterized clonal culture, the alga lost its heterocysts in + N medium and formed akinetes only in − N medium and at P concentrations up to 10 mg l^{-1} (but not 44.5 mg l^{-1}). The bacterial-free clonal culture formed heterocysts in − N medium, but failed to form akinetes even under extreme P deficiency.

Nostoc

Two morphologically very similar strains have been brought into clonal culture, though only one has so far been made axenic. The strains differ, however, in that one can form phycoerythrin, while the other can not do so.

Effect of 1) Temperature and 2) Presence or Absence of Combined Nitrogen

The influence of nitrogen source was tested over the temperature range 15-50°C. No algae grew at 50°C. At 48°C slight growth was evident in both media after 3 days, but subsequently the cultures died. Substantial growth occurred at 45°C and this was chosen as the upper temperature for the detailed experiment. Growth occurred in both − N and + N media over the whole temperature range, but the relative effect of the two media differed according to temperature (Fig. 2). Due to the complexity of the ecosystems inside the culture flasks, too much attention cannot be placed on small differences, but two aspects seem to be established clearly:

(i) Initial growth was always faster in − N medium but the difference was greater the lower the temperature.

(ii) Growth continued to the end of the experimental period of 30 days at 15°C in both media, but at higher temperatures most of these mixed cultures showed a marked

drop in crop (estimated as chlorophyll *a* content) towards the end of the 30 days period, in comparison with earlier peak values. The effect was particularly pronounced at 35-45°C in −N medium.

Microscopic inspection of the samples helped to give more insight into the processes taking place in the mixed cultures. There was a decrease in the number of species observed at 40°C and even more so at 45°C (Fig. 3). Somewhat surprizingly, more species were usually evident in −N medium, even when the maximum crop reached was low, as at 15° and 20°C. The dominant species in + N medium at lower temperatures were *Chlorococcum humicola, Ulothrix* sp. and *Tribonema minus*. Blue-green algae became increasingly important at higher temperatures. Protozoa were apparently the main agents responsible for decreases in the size of crops in old + N cultures. Their sparse occurrence at 15°C is probably the explanation why the maximum crop reached in − N medium was at this temperature. The decrease in chlorophyll *a* content of old cultures at higher temperatures in + N medium is due to several factors. The Oscillatoriaceae often became yellowish. *Cylindrospermum* was sometimes an important component of the population and this formed akinetes. A range of species were present in −N medium but hetereocystous blue-green algae were always the dominants, with *Anabaena cylindrica* more important at 20-25°C and *Cylindrospermum muscicola* and *Nostoc muscorum* more important at the higher temperatures. *Calothrix* spp. were never dominant.

Effect of Phosphate

The influence of phosphate concentration on growth (measured as chlorophyll *a)* at

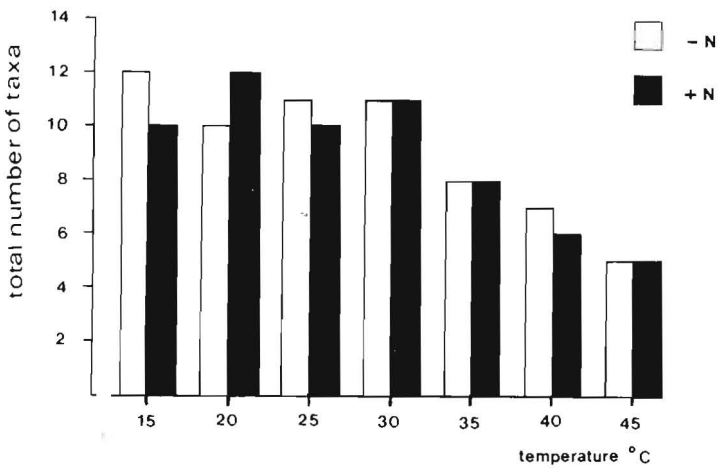

Fig.3 Influence of a) temperature and b) presence or absence of combined nitrogen on total number of taxa noted on all five days at which samples harvested.

35°C in N medium is shown in Fig. 4 and on total number of species observed in Fig. 5. The changes with time at 44.5 mg l^{-1} P in this experiment are closely similar to

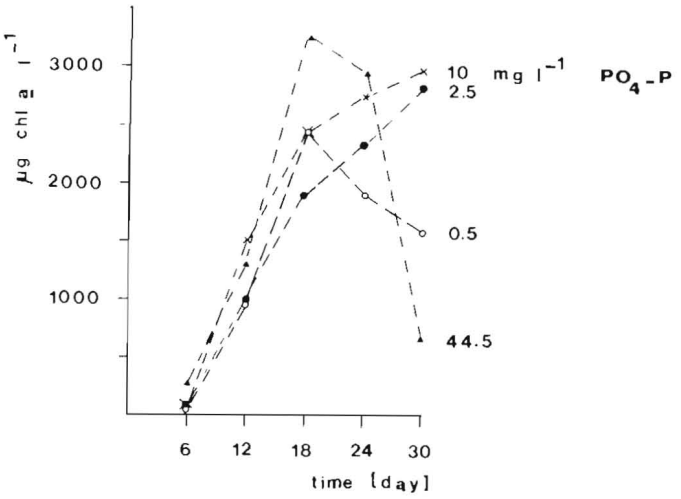

Fig.4 Influence of phosphate on growth of algal community developing on inoculation with soil sample. (Experiment carried out at 35°C, in absence of combined nitrogen).

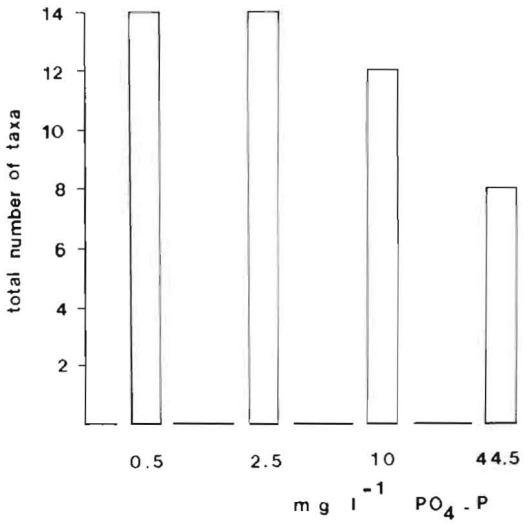

Fig.5 Influence of phosphate (expressed as mg l^{-1} P) on total number of taxa. (Experiment carried out at 35°C in absence of combined nitrogen).

those observed in the previous experiment (compare Fig. 4 with Fig. 2). There was an obvious chlorophyll peak and subsequent decrease at the lowest and highest phosphate concentrations (0.5, 44.5 mg l^{-1} P), but not at the intermediate concentrations. These changes appear to depend largely on the behaviour of *Cylindrospermum* and *Nostoc muscorum*. *Nostoc* was relatively more successful at the lower phosphate concentrations. At 0.5 mg l^{-1} P growth came to a halt by day 18 and akinetes of both species formed from then on. *Cylindrospermum* was relatively more abundant at the higher phosphate concentrations, reaching its peak and differentiating into akinetes earlier. The loss of trichome material is much greater during differentiation of *Cylindrospermum* akinetes than *Nostoc* akinetes, which presumably explains the much greater decrease in chlorophyll *a* content on day 30 with 44.5 mg l^{-1} initial P.

Increasing levels of NaCl brought about a marked reduction in number of species (Table 4), but the effect on overall growth of the culture (Fig. 6) was less marked, at least up to 0.5 M NaCl. The contrast between the abundance of *Nodularia* at 0.5 M and its absence at 0.1 M was striking. This was evident on all five harvest dates. Presumably it was unable to compete effectively with *Anabaena* and *Nostoc* at the lower salinities. (Slight growth of *Nodularia* was found in two flasks at 1 M NaCl, although not in the flask harvested at day 30).

Table 4. Taxa present on day 30 in media enriched with NaCl and grown at 35°C. The two physiological forms of *Nostoc muscorum* are listed separately. (Relative abundance expressed on 1-5 scale)

	0.1 M		0.5 M		1 M	
	− N	+ N	− N	+ N	− N	+ N
Anabaena cylindrica	5					
Cylindrospermum muscicola	4					
Chlorococcum humicola		3		⋆		
Lyngbya ⩽ 1 μm	3	4	2	2	2	3
Lyngbya > 1 ⩽ 2 μm	2	2	4	4	5	4
Lyngbya > 2 ⩽ 4 μm	1	1				
Microcoleus chthonoplastes	1					
Nostoc muscorum (phycocyanin)	2	5	3	2	4	5
N. muscorum (phycoerythrin)	3	2	3	3		
Nodularia harveyana			5	5		

⋆ *Chlorococcum* dead by day 30, but live cells abundant on day 24.

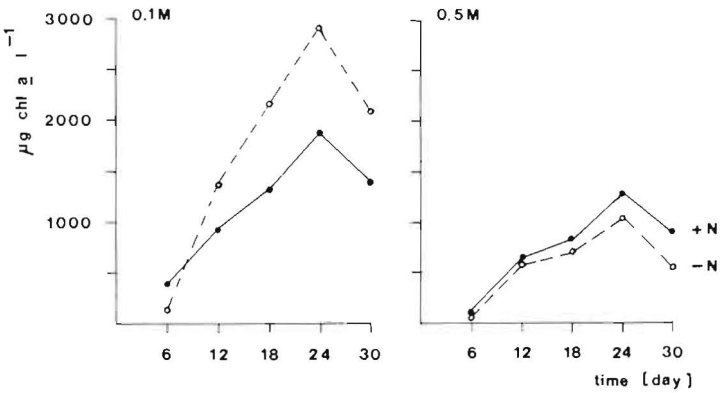

Fig.6 Influence of NaCl on growth of algal community developing on inoculation with soil sample. (Experiment carried out at 35°C in presence and absence of combined nitrogen).

Discussion

The algal flora of the rice-field soil revealed in laboratory culture was much richer than found by direct inspection of the sample. All the algae seen by direct viewing were found also in culture, but *Microcoleus chthonoplastes,* which was the dominant in the field, never became so in the laboratory. Enrichment techniques did, however, lead to marked differences in the flora. Green algae were abundant only at low temperatures in the presence of combined nitrogen. Blue-green algae were the dominants in the presence of combined nitrogen and at all temperatures in the absence of combined nitrogen. Heterocystous blue-green algae dominated cultures free of combined nitrogen during the early stages of growth, but after a period of akinete formation by some of these species, *Lyngbya* forms came to dominate old cultures. Increasing salinity led to a decreasing number of species, but even at salinities well above those of seawater, several species survived.

The abundance of *Nostoc muscorum* in the field sample, and the presence of at least nine other heterocystous blue-green algae in the small sample (0.7 g) used for the present study, together suggest that blue-green algal nitrogen fixation is likely to play an important role at this site. There is an urgent need to study the role of algae in these fields over a whole year to establish whether changes in cultivation practices could be used to increase the nitrogen status of the soil for both rice and winter crops without the necessity of nitrogenous fertilizer. It is hoped that the present laboratory study will be useful as a guide to field observations.

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تأثير العوامل البيئية على الطحالب في تربة حقول الرز في أهوار العراق

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تمت دراسة الطحالب في تربة حقول الرز في أهوار العراق، حيث تم تشخيص ٤٣ نوعاً من الطحالب في المختبر مقابل ١١ نوعاً عند الفحص المباشر للتربة. وكان النوعان: *Microcoleus chthonoplastes* و *Nostoc muscorum* هما السائدان في الحقل، ولكن الأخير فقط نما بشكل جيد في المختبر تحت الظروف الهوائية التامة.

كما تمت دراسة تأثير كل من الحرارة، النتروجين (النترات)، الفوسفور (الفوسفات) وكلوريد الصوديوم. فوجد أن الطحالب تنمو في درجة ٤٨ م لفترة قصيرة تموت بعدها، وأن نمواً جوهرياً يحدث في درجة ٤٥ م.

وقد سادت الطحالب الخضراء المزرقة ذات heterocyst (المثبتة للنتروجين) على الأنواع الأخرى في الحقل والمختبر في وسط النمو الخالي من النتروجين (النترات). ولوحظ نمو ستة أنواع من الطحالب في وسط النمو الحاوي على التركيز ٠,٥ مولار كلوريد الصوديوم، وثلاثة أنواع في الوسط الحاوي على واحد مولار، ولكن النمو كان بطيئاً جداً في التركيز الأخير.

من أجل تطوير زراعة الرز في المنطقة بما يكفل زيادة الإنتاج دون الحاجة إلى استعمال الأسمدة النتروجينية، تبدو الحاجة ماسة إلى إجراء المزيد من البحوث الحقلية حول بيولوجية الطحالب بشكل عام والطحالب الخضراء المزرقة بشكل خاص.