GROWTH REQUIREMENTS OF A HALOPHILIC BLUE-GREEN ALGA, *PHORMIDIUM TENUE* (MENEGH).

By V. Krishna Pillai

(Central Marine Fisheries Research Station, Mandapam Camp)

CONTENTS

I. Introduction

In order to account for the negligible growth of algæ observed in the saline lagoons at Mandapam a complete knowledge of the normal nutritional and general physiological factors controlling the growth of at least the dominant species of the algal association is necessary. This information will also have applied significance in the maintenance of adequate algal growth, a prerequisite for successful fish culture in these lagoons, regardless of whether or not the fish grown feeds directly on the algæ.

In the course of a study of the chemical compositions of the waters in the lagoons and their effect on the growth of algæ, several interesting factors were observed. During high salinity periods the bulk of the algal mass consisted of the species of blue-green alga, *Phormidium tenue* (Menegh). The other species of blue-greens present were found to occur in extremely small quantities during these periods. Even in the case of *P. tenue* the growth rate was found to decrease considerably with rise in salinity beyond 8 per cent. When the salinity of the outside water reached 10 per cent the algæ began to die and disintegrate giving out hydrogen sulphide.

The present study was undertaken to collect as much data as possible on *P. tenue* as a representative of the very few species that can withstand wide fluctuations in the salinity of the outside water. Though the collections

taken periodically from the Palk Bay lagoons consisted mainly of the above species they could not be utilised for detailed investigations, as it was found very difficult to separate the alga from fine sand particles attached to it. Hence the necessity arose for resorting to culture of the alga.

According to Pringsheim (1949) only very few species of Cyanophyceæ have been studied in cultures. The work on the Cyanophyceæ so far has been mainly in taxonomy and general ecology and very little information is available on their nutritional requirements. Fritsch (1945), summarising the work done by previous workers on Cyanophyceæ, states that the majority of these algæ that have been studied from the point of view of nutrition are capable of growth in mineral solutions devoid of organic substances and that a slight alkaline reaction of the culture medium is most suitable; he also refers to the fact that these forms require considerable amounts of calcium and magnesium for growth, in general that they grow better on agar and that some of them show better growth in the presence of nitrate-N rather than of ammonia-N or nitrite-N. These observations, though of basic importance, deal with only a few of the numerous factors controlling the growth of these algæ.

The present study enquires into the effects of the major nutrient elements, Na, K, Ca, Mg, S, P, N and Cl as well as some of the trace elements like Ba, B, Mn, Mo, Cu, I, Zn, Sr, Fe, Br, F, etc., on the growth of *P. tenue* and aims at discovering a suitable combination of the elements that will produce the maximum growth of the alga.

II. Experimental

(*a*) *Preparation of pure cultures.*—The first attempt was to prepare a pure culture of the alga for purposes of inoculation. Chemical treatment of the cells with dilute chlorine water and hydrogen peroxide as suggested by Pringsheim (1949) was found unsuccessful. When the pH of the media was slightly on the acid side a fungus began to grow and cover up the entire surface of the culture tubes; whereas in an alkaline pH only the bacteria grew. To destroy the contaminants the same algal cells were inoculated successively to sterile media adjusted to the contrasting pH ranges. By repeating the above process a number of times an almost pure culture of the alga could be obtained. The possibility of using antibiotics like Penicillin and Streptomycin in preparing bacteria-free cultures of the alga, as in the case of phytoplankton (Spencer, 1952) was, therefore, not studied.

A reference to the work carried out by previous workers on other species of algæ and diatoms, indicates the importance of some trace elements in

their growth. Even when the elements, Ca, Mg, K, Fe, S, P and N, considered to be the most indispensable, are provided in the right proportions at suitable pH, almost all the algæ failed to grow, or only exhibited a very limited growth (Pringsheim, 1945). As early as 1914 Allen found that certain diatoms can be grown in artificial sea water if a few c.c. of normal sea water is added to it. Similarly Harvey (1933) mentions the use of sterilised garden soil extract for the best growth of *Nitzschia closterium*. The explanation for all these may lie in the necessity for the presence of certain elements, other than those mentioned above, in the media. This has been confirmed by Hayrve's later work (1939) with the diatom *Ditylum* where he obtained an enhanced growth rate when small quantities of manganese, ranging from 1-2 mg./m.^{3} were added to the culture media. His general conclusion was that the lack of manganese in sea water could render it infertile to *Ditylum*.

In the present study two basic media were made use of—one (stock solution No. 1) containing Na, Ca, Mg, Cl and SO_4 and the second containing all the components for artificial sea water (stock solution No. 2) according to the formula of Lyman and Fleming (1940). The salts used in the media were recrystallised from B.D.H. analar reagents. Solid agar media were prepared by dissolving 1·5 gm. of refined Gelidium agar in 10 c.c. of the stock solutions. After being dissolved under pressure and filtered, the agar was transferred (10 c.c. each) to clean test-tubes and sterilised.

For liquid culture 10 c.c. portions of the sterile stock solutions were transferred to sterile petri-dishes. The pH of the medium was adjusted to the desired range. The weight of the various elements present in 10 c.c. of the artificial sea water was as given below:—

Name of element	Weight in mg.	Name of element	Weight in mg.
Na	108·132	NO_3-N	1·64
Ca	3·970	N in other forms	0·00004
K	4·496	SO_4	26·4947
Mg	12·720	HCO_3	1·394
B	0·045	Cl	189·880
Sr	0·130	P	0·570
Fe	0·00014	F	0·0014
		Br	0·065

The vessels used in the experiments were all of the best pyrex type. The amount of silica dissolved by salt solutions from this type of glass is considered to be the minimum (Harvey, 1933).

Standard solutions of each of the above elements as well as those of Cu, Mo, B, Zn, Mn, I, Ba and Fe were prepared from the respective salts at different dilutions, the concentrations of each ranging from 0·001 g. per c.c. to 0·00004 g. per c.c. A litre or 100 c.c. of standard solutions containing a high concentration of each of the elements was prepared first and the smaller standards were then prepared from them by dilution. These solutions were sterilised and kept in a cool chamber.

To tubes containing agar in stock solution No. 1 were added known quantities of potassium, nitrogen (as nitrate), phosphorus as phosphate, bromide, fluoride, etc., separately and in combinations; these tubes of agar media were sterilised at 15 lb. pressure for 20 minutes and made into slopes. A small strand of *P. tenue* from the stock culture was inoculated on to the agar slope in each tube. The tubes were placed near a glass window having thick ground glass shutters and facing west. For promoting photosynthesis during night a 100 watts bulb was used as recommended by Pringsheim and Pringsheim (1949). It was observed that there was absolutely no growth in any of the tubes; even though the composition in some of the tubes was identical with that of stock solution No. 2, which is artificial sea water. The alga died two or three days after inoculation.

Another series of agar tubes were prepared with the full artificial sea water (stock solution No. 2), and they were treated with the above trace elements separately and in combinations. Comparatively very high concentrations of trace elements ranging from 0·001 g. to 0·0001 g. to each tube were used in this series. The alga, though at first showing multiplication to some extent in a limited number of tubes, died off after four or five days.

Two fresh series with stock solution No. 2 (the pH of the first being 7·8 and that of the second being 8·3) were prepared and treated with trace elements. Smaller concentrations of the trace elements, *viz.*, 0·00004 to 0·000004 g. per tube, were used this time. The tubes were inoculated with tiny strands of the alga as before. The effect was sudden and marked; from the second day onwards growth could be observed in some of the tubes, and soon the algal strands were seen creeping over the entire surface of the tubes. The intensity of multiplication varied with treatment; in tubes treated with copper and barium either singly or in combination with other elements there was practically no growth at all and the alga died. The variation in the intensity of multiplication in some of the most successful

tubes is reproduced in Fig. 1 (Plate III). During the first week the alga crept up the sides of the tubes facing the light and completely filled the surface. During the second week it began to spread throughout the agar, penetrating it and going deep down to the bottom of the tubes (Fig. 1, Tube Nos. 33, 36, 53, etc.). After 21 days, at which stage the alga seemed to have attained the maximum growth, the culture medium with the alga was taken out and the chlorophyll extracted by dissolving it in acetone following the method of Harvey (1934), the final estimation being done with the aid of a Hilger-Spekker Photo-electric Absorptiometer using green filters.

(*b*) *Liquid culture.*—10 c.c. each of stock solution No. 2 was measured out into sterile petri-dishes. As in the case of the agar tubes different combinations of trace elements were added to the media. Equal quantities of *P. tenue* from stock culture were transferred to the dishes and left near the window as before. All the liquid cultures were maintained at pH 8·2. Growth was very quick and noticeable even 12 hours after inoculation. In the case of successful treatments an algal mat covering the entire bottom of the petri-dishes was formed within 10 to 15 days (Fig. 2, *a* and *b* in Plate IV). There was good agreement between duplicates of the same treatment. A peculiarity noticed in the case of cultures grown in the liquid media was that the trichomes of the algæ were individually free.

After 15 days the crop from each was taken out, dried between filter-papers and the weight determined. Half of the alga from each set was used for chlorophyll extraction and the other half was used for the estimation of total organic nitrogen by the microkjeldahl method. The values obtained with a typical series of liquid cultures, with details of treatment, are given in Table I. During the course of the experiment it was observed that there was a reduction in the volume of the media; hence the final volume as well as the salinity of the fluid left behind in the dishes were also determined. Further, the solutions left behind were analysed for their nitrate content by reducing the nitrate with Davarda's alloy and estimating as ammonia in the microkjeldahl.

The results of analysis of the solutions left behind in the vessels are given in Table II.

(*c*) *Growth at higher salinities.*—To study the effect of the treatment in media of high salt content two more sets of stock solutions (Nos. 3 and 4) were prepared. No. 3 contained 23·477 g. more of sodium chloride per litre than No. 2, and chlorinity of this solution was 33·2444‰. Solution No. 4 contained 46·954 g. more sodium chloride per litre, the chlorinity becoming 47·50 parts per thousand. The treatment combinations with these media

TABLE I

Details of Treatment in Liquid Media (Stock Solution No. 2)

	Weight of trace elements added to 10 c.c. of media in mg.											
	Mo	B	Zn	Mn	I	Ba	Fe	N	P	Weight of alga (g.)	Total N in alga (mg.)	Chloro-phyll (pig-ment unit)
Normal sea water	..	..	..	..	..	..	..	..	..	Nil		
1 (Control)	..	..	..	..	..	..	..	..	..	0·0074	0·11	25·0
2	0·004	..	..	0·04	..	..	..	..	..	0·0062	0·10	25·0
3	..	..	..	0·08	0·04	..	0·10	..	..	0·0640	0·57	150·0
4	..	..	..	0·08	..	..	0·10	..	..	0·0520	..	70·0
5	..	0·02	..	..	..	..	..	..	..	0·0400	0·38	90·0
6	..	0·02	..	0·08	..	..	0·10	..	..	0·0880	0·84	210·0
7	..	0·02	..	0·08	0·04	..	0·10	..	..	0·0900	0·84	210·0
8	..	0·01	..	0·08	0·04	..	0·05	5·00	0·75	0·1488	1·32	345·0
9	..	..	..	0·04	..	..	0·05	..	0·75	0·1080	..	250·0
10	..	..	..	0·08	..	..	0·05	5·00	0·75	0·0512	0·97	70·0
11	0·004	0·01	..	0·04	..	..	0·05	..	..	0·1280	1·15	300·0
12	0·004	0·01	0·04	..	..	..	0·05	..	..	0·0180	0·16	25·0
13	0·004	0·01	0·04	0·04	0·04	..	0·05	..	0·75	0·1150	1·04	270·0

TABLE II

Analysis of Water Left in the Vessels (From Stock Solution No. 2)

	Total Vol. c.c.	Total NO_3-N in mg.	NO_3-N originally present in media	Salinity ‰
			(mg.)	
1 (Control) ..	5·6	1·01	1·68	55·98
2	6·3	1·01	1·68	48·76
3	6·7	0·29	1·68	45·15
4	6·0	..	1·68	..
5	6·0	0·69	1·68	..
6	6·0	0·28	1·68	50·57
7	6·5	0·27	1·68	50·57
8	7·3	2·72	6·68	46·96
9	6·5	..	..	41·54
10	6·5	3·63	6·68	46·96
11	6·0	..	1·68	50·57
12	6·7	1·00	1·68	45·15

were the same as before in order to examine the effect of the same treatment under higher salinity conditions. A further aim of these experiments, since the alga is known to be one of the few species of blue-greens that can withstand a relatively high salinity of the outside water, was to ascertain if the intensity of growth under the higher degree of salinity is equal to that attained under normal salinity conditions, when the necessary nutrient elements are available.

As with the previous series both solid and liquid cultures were tried. In media having double chloride content there was practically no difference in the growth rate of the alga. In liquid cultures growth was quicker, to a certain extent, than in the previous series, for within 12 to 13 days the algal mat covered the bottom of the petri-dishes in the most successful treatments. As before the crop in each treatment was estimated and analysed. The results are given in Table III. The solutions left behind in the treatment vessels were again analysed for salinity and total nitrate-nitrogen contents (Table IV).

The cultures in solution No. 4, containing three times the normal chloride concentration, behaved in a different manner; growth did not start in any of the treatment vessels till the seventh day; thereafter slow growth began in the four vessels treated with excess of SO_4. All the other cultures

TABLE III

Details of Treatment in Media of Higher Salinities

Treatment	Weight of trace elements added to 10 c.c. of solution (mg.)										Weight of alga (g.)	Total Nitrogen in alga (mg.)	Chlorophyll (pigment units)
	SO_4	Mo	B	Zn	Mn	I	Ba	Fe	N	P			
Salinity of media 54·4 ‰—													
1 (Control)	..	..	..	..	..	..	..	..	..	..	0·0050	..	20·0
2	..	..	..	..	0·08	0·04	..	0·10	..	..	0·1000	0·98	266·6
3	..	..	0·01	..	0·08	..	..	0·10	..	..	0·1000	0·78	213·8
4	..	..	0·01	..	0·08	0·04	..	0·10	..	..	0·0800	0·93	264·0
5	..	..	0·01	..	0·08	0·04	..	0·05	..	..	0·1200	1·08	333·0
6	..	..	0·01	..	0·08	0·04	..	0·05	5·00	0·75	0·1200	0·97	264·0
7	..	0·04	0·01	..	0·08	..	..	0·05	..	..	0·1050	0·71	269·0
8	..	0·04	0·01	..	0·08	..	..	0·05	5·00	0·75	0·0900	0·63	272·0
9	5·0	..	0·01	..	0·08	0·04	..	0·05	5·00	0·75	0·0600	1·04	191·0
10	5·0	..	0·01	..	0·08	0·04	..	0·05	..	..	0·1000	1·00	305·0
11	5·0	0·04	0·01	..	0·08	..	..	0·05	..	..	0·1200	1·12	591·0
Salinity of media 85·7 ‰—													
1 (Control)	..	..	..	..	..	..	..	..	..	..	Nil	..	..
2	..	..	..	..	0·08	0·04	..	0·10	..	..	0·0200	0·18	..
3	..	..	0·01	..	0·08	0·04	..	0·10	..	..	0·0200	0·20	..
4	..	..	0·01	..	0·08	0·04	..	0·05	..	..	0·2200	0·24	..
5	..	..	0·01	..	0·08	0·04	..	0·05	5·00	0·75	0·0250	0·25	..
6	..	0·04	0·01	..	0·08	..	..	0·05	5·00	0·75	0·0150	0·16	..
7	..	..	0·01	..	0·08	0·04	..	0·05	10·00	0·75	0·0250	0·25	..
8	5·0	..	..	..	0·08	0·04	..	0·10	..	..	0·0600	0·73	..
9	5·0	..	0·01	..	0·08	0·04	..	0·10	..	..	0·0500	0·69	..
10	5·0	..	0·01	..	0·08	0·04	..	0·05	..	..	0·0350	0·59	..
11	5·0	..	0·01	..	0·08	0·04	..	0·05	5·00	0·75	0·0350	0·59	..
12	5·0	..	0·01	..	0·08	0·04	..	0·08	10·00	0·75	0·0300	0·50	..

TABLE IV

Analysis of Solution from Cultures in Media of Higher Salinity

Treatment No.	Stock solution No. 3 (S ‰ = 54·4)				Treatment No.	Stock solution No. 4 (S ‰ = 85·7)			
	Vol. of water left behind c.c.	Salinity ‰	Final Nitrate-N in water (mg.)	Original amount of NO_3-N (mg.)		Vol. of water left behind c.c.	Salinity ‰	Final NO_3-N in water (mg.)	Original NO_3-N in water (mg.)
1 (Control) ..	6·0	83·03	1·16	1·68	1 (Control) ..	6·0	115·2	1·23	1·68
2 ..	7·0	72·20	0·23	1·68	2 ..	6·9	104·4	1·16	1·68
3 ..	6·6	74·01	0·22	1·68	3 ..	7·9	94·0	1·21	1·68
4 ..	6·5	74·01	0·27	1·68	4 ..	6·7	97·2	1·05	1·68
5 ..	6·0	84·64	0·22	1·68	5 ..	7·3	93·6	5·84	6·68
6 ..	7·2	72·20	0·88	6·68	6 ..	7·7	86·4	6·00	6·68
7 ..	6·3	83·03	0·37	1·68	7 ..	8·3	86·0	6·21	6·68
8 ..	7·5	68·59	1·74	6·68	8 ..	7·3	93·6	0·97	1·68
9 ..	7·5	66·88	3·60	6·68	9 ..	8·0	87·0	1·01	1·68
10 ..	8·2	61·37	0·27	1·68	10 ..	8·1	86·0	0·81	1·68
11 ..	7·0	72·01	0·26	1·68	11 ..	7·5	86·5	5·75	6·68
					12 ..	6·2	112·4	10·62	11·68

remained without any marked change. The growth rate in the sulphate-treated vessels was comparatively quicker later, but when compared to the previous cultures the growth was very poor, and the crop did not show the characteristic dark green colour of the healthy alga. The results of these treatments and the results of analysis of the fluid left behind are tabulated in Tables III and IV respectively.

III. Discussion of Results

The results of experiments conducted with stock solution No. 1 reveal that the presence of the major elements Na, K, Ca, Mg, S, P and N alone, even if present in excess, will not be sufficient to promote the growth of *P. tenue*. Likewise in full artificial sea water the alga could not grow and multiply. The only conclusion that could be drawn from these preliminary experiments is that the presence of some other element or elements in traces is required for the growth of *P. tenue*. The initial culture trials in both solid and liquid media disclose that in those cultures which have been treated with copper the alga died; even in as low a concentration as 0·16 mg. per litre the effect was evident. These trials also prove that when copper is present along with other trace elements growth of the alga is either completely prevented or retarded. The growth inhibiting effect of such concentrations of Cu present along with other elements, can best be understood when the individual and combined effects of other elements are taken into consideration. For example in a treatment with a combination of B, Mn and Fe, the crop after 15 days corresponds to 78 pigment units of chlorophyll; whereas in another treatment with the same combination of the above elements together with 0·16 mg./litre of copper, the total chlorophyll corresponds to only 24 pigment units.

The trials conducted with media (both solid and liquid) of different pH, ranging from 7·8 to 8·3 indicate that the alga favours the higher pH values. This is in conformity with observations made by previous workers that Cyanophyceæ generally favour an alkaline pH (Fritsch, 1945).

Molybdenum, zinc and barium do not seem to be very essential for the growth of the alga. The cultures which were enriched with these elements either singly or in combinations did not produce any increase in the growth of the alga; on the other hand in most of the vessels the alga died. Several dilutions of the elements, ranging between 0·04 mg./litre to 4·0 mg./litre, were tried in different cultures with the same result. But unlike in the case of copper small concentrations do not appear to affect growth adversely if the other essential elements are present; thus in a treatment, where Mo, Zn, B, Mn, I, Ba, P and N were added, a very good growth was observed.

However, in another treatment containing only the three trace elements B, Mn and Fe the growth was found to be slightly higher as shown by the chlorophyll content.

The individual effect of the elements B, Mn, I and Fe is quite evident from the tables. In the treatments where these elements are added alone or in combinations the growth increases considerably. This effect is manifested both in the solid and the liquid media. Comparing the various treatments it may be seen that a combination of B, Mn, I and Fe gives the best growth, provided sufficient nitrogen and phosphorus are present in the media in available forms. Examination of Tables I and II gives an idea of the quantities of the various elements to be added to the culture. There was no increase in the growth rate if the amount of any particular trace element was increased beyond a certain limit. By conducting a number of experiments it has been found that for most favourable growth the maximum concentrations of the four elements B, Mn, I and Fe are 1 mg./litre, 8 mg./litre, 0·4 mg./litre and 5·0 mg./litre respectively as may be seen from Tables I and II, where the weights and total organic nitrogen content are tabulated. The total organic nitrogen and the chlorophyll content are maximum in the above treatments with slight variations where the amount of one essential element is altered. It can also be seen that the most essential of these trace elements are Mn and Fe. Table III gives the weight of algæ produced in different treatments in two media of higher salinity. In treatments made with stock solution No. 3, where the salinity of the water is about 8·5 per cent., the same combination of B, Mn, I and Fe gives the best growth. Thus high salinity ranging from 5·4 per cent. to 8·5 per cent. does not seem to affect the growth if the elements mentioned above are present in the right proportions.

It is interesting to note that the alga does not absorb nitrogen from any source other than the media. The total organic nitrogen in the algæ and the total nitrogen in the media left behind together do not exceed the total nitrate-nitrogen added to the media. In all the liquid cultures where detailed analysis could be done on the nitrogen distribution this fact was observed. Thus the experiments give no evidence that *P. tenue* fixes atmospheric nitrogen. This is in agreement with the observation made by De (1939) with respect to another species of the alga, *viz.*, *P. foveolarum*. But some explanation has to be found for the unaccountable nitrogen which was neither found in the solution nor in an organic form in the algæ. The only possible explanation is that some of the nitrogen is present in an inorganic form in the alga. This leads us to believe that the alga absorbs nitrogen in inorganic form itself which, however, has to be substantiated by further experiments.

The importance of silica in the growth of the alga cannot be overlooked. Since the media have been found to dissolve certain amount of silica from the glass vessels (this amount being very low with salt solutions according to Harvey, 1933) it was impossible to maintain controlled conditions with respect to silicate contents. However, since the volume of the media and the size, and quality of the vessels were the same, it can be presumed that the amount of silica dissolved from the vessels was uniform.

As the trace elements are added to the media in the form of soluble salts various other ions are also introduced. Table V gives the amount of other ions added to the media corresponding to known weights of the trace elements. It may be seen that the amount of extraneous elements thus added is too small to affect the results.

TABLE V

Amounts of Other Ions Introduced during Treatment

Element added	Form in which added	Weight in mg. of the element in 0·1 c.c. of standard solution of the salt	Weight in mg. of other elements or groups of elements
Cu	$CuSO_4, 5H_2O$	0·0016	$SO_4 = 0·0025$
Mo	$(NH_4)_6, Mo_7O_{24}, 4H_2O$	0·0044	$N = 0·00054$
B	H_3BO_3	0·0100	..
Zn	$ZnSO_4, H_2O$	0·04	$SO_4 = 0·06$
Mn	$MnCl_2, 4H_2O$	0·04	$Cl_2 = 0·05$
I	KI	0·04	$K = 0·012$
Ba	$BaSO_4$	0·04	$SO_4 = 0·027$
Fe	$Fe\ NH_4\ (SO_4)_2,\ 12\ H_2O$	0·05	$N = 0·013$; $SO_4 = 0·169$
Ca	$CaCl_2$	1·00	$Cl_2 = 1·750$
P	Na_2HPO_4	0·15	$Na = 0·080$
N	$NaNO_3$	0·6	$Na = 1·000$

In treatments Nos. 9, 10 and 11 with media of salinity 54·4 parts per thousand and treatment Nos. 8, 9, 10, 11 and 12 with media of salinity 85·7 parts per thousand certain peculiarities are noticed. In these treatments SO_4 ion is added along with other elements to bring the condition of the media to that of the naturally occurring lagoon water. In the media with starting salinity 54·4 parts per thousand the presence of sulphate ions does not seem to inhibit the growth of the alga as judged by weight, but there

is an unusual increase in the total organic nitrogen in the algæ. In treatments without the addition of excess of SO_4 the total organic nitrogen in 0·1 g. of wet alga is only 0·7775 mg., whereas in the treatment where excess

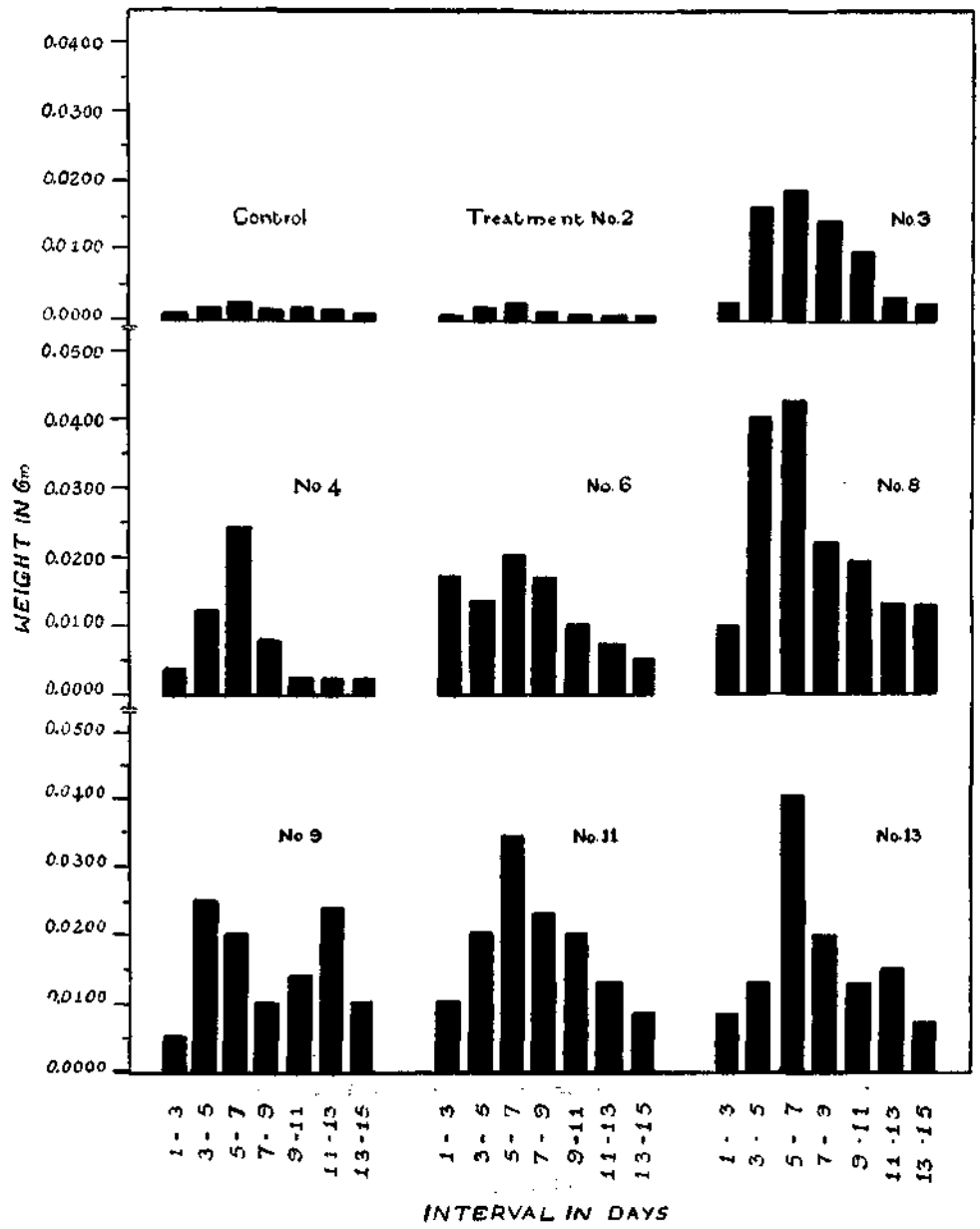


FIG. 3. Histogram showing the actual growth of *Phormidium tenue* during intervals of two days in liquid cultures under different treatments.

SO_4 is added the total organic nitrogen in the same weight of the alga is 1·004 mg. There is a similar increase in all the sulphate-treated cultures.

In the case of media with a starting salinity of 85·7 parts per thousand only those vessels with SO_4 ions show growth. Even though the growth is comparatively poor, here again an unusual increase in the total organic nitrogen was observed. The peculiar behaviour of the cultures in the sulphate-treated vessels probably means that since the chloride content of the medium has gone beyond tolerable limits the presence of SO_4 is necessary for the algæ to absorb the nutrients. This assumption will explain fully well the absence of growth in the other vessels even when the best treatment combinations are given.

The actual growth of the alga at intervals of two days in some of the treatments in liquid culture is represented in Fig. 3. It may be seen that the growth is maximum between the 5th and 13th day after inoculation.

Finally, comparing the liquid cultures and the cultures on agar it has to be pointed out that the alga grows better in the former. This is not in agreement with the observations made on other species of Cyanophyceæ by previous workers (Fritsch, 1945). When grown on agar media the alga retains to a certain extent its habit of fusion of trichomes as observed in nature; while in liquid culture the trichomes tend to remain free.

Although it has been possible to observe that certain combinations of the trace elements promote healthy growth of the alga, the exact role of the elements in the metabolism of the alga is still unknown. Work on this line is in progress.

IV. Summary

The effects of trace elements on the growth of the blue-green alga *Phormidium tenue*, cultured in both agar and liquid media, have been studied; and the complement of trace elements and the amount of each required for the best growth of the alga have been indicated. The growth of the alga in media of different pH ranges has been investigated. It was found that the alga exhibits optimal growth in the presence of B, Mn, I and Fe in a medium having a pH 8·2.

The behaviour of the alga under varying degrees of salinity of the medium and its response to treatments with different trace-element combinations have been investigated. It was observed that the alga responds to treatment with trace elements and exhibits normal growth until the salinity of the medium reaches 8·5 per cent, beyond which range there is a definite fall in the growth rate.

The rate of growth as well as the absorption of nitrogen from the media have been studied in detail. The alga, in all probability, absorbs nitrogen from the media; and no evidence is obtained to show that it fixes atmospheric nitrogen when sufficient quantity of nitrogen is available in the media.

The maximum limit of salinity of the outside water that the alga can tolerate appears to be about 8·5 per cent. Within this range growth is normal and healthy if the trace elements Mn, B, I and Fe are present in a definite concentration and proportion; but beyond this salinity there is very little growth. The presence in excess of SO_4 in the media, however, appears to prevent the toxicity of the high chloride content, to a limited extent, facilitating slow growth of the alga.

V. ACKNOWLEDGEMENTS

My grateful thanks are due to Dr. N. K. Panikkar, Chief Research Officer, Central Marine Fisheries Research Station, Mandapam, for suggesting and guiding the work and to Dr. (Mrs.) F. Thivy for the help she has rendered in the preparation of this paper.

VI. REFERENCES

Allen, E. J. (1914) .. "On the Culture of the Plankton Diatom *Thalassiosira gravida* Cleve in artificial sea water," *Journ. Mar. Biol. Assn. U.K.*, **10**, 417–39.

De, P. K. (1939) .. "The role of blue-green algæ in nitrogen fixation in rice fields," *Proc. Roy. Soc. (Lond.)*, **27 B**, 121–39.

Fritsch, F. E. (1945) .. Structure and Reproduction of the Algae, Vol. II. Cambridge: at the University Press.

Harvey, H. W. (1933) .. "On the rate of diatom growth," *Journ. Mar. Biol. Assn. U.K.*, **19**, 270.

———— (1934) .. "Measurement of Phytoplankton production," *ibid.*, **19**, 771.

———— (1939) .. "Substances controlling growth of diatoms," *ibid.*, **23**, 50.

Lyman, J. and Fleming, R. (1940) "Composition of sea water," *Journ. Mar. Res.*, **3**, 134.

Pringsheim, E. G. (1949) Pure Cultures of Algae. Cambridge: at the University Press.

———, and Pringsheim, O. (1949) "Growth requirements of *Porphyridium cruentum* with remarks on the ecology of brackish-water algæ," *Journ. Ecol.*, **37**, No. 1, 57–64.

Spencer, C. P. (1952) .. "On the use of antibiotics for isolating bacteria-free cultures of marine phytoplankton organisms," *Journ. Mar. Biol. Assn. U.K.*, **32**, 97–106.

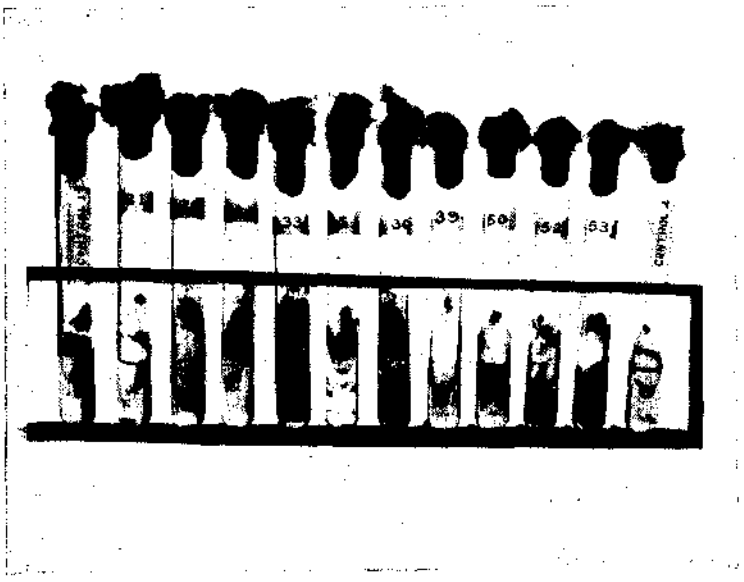
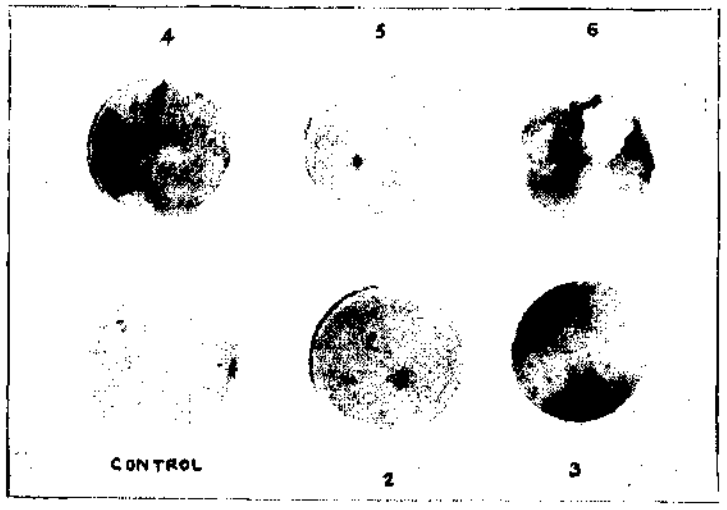
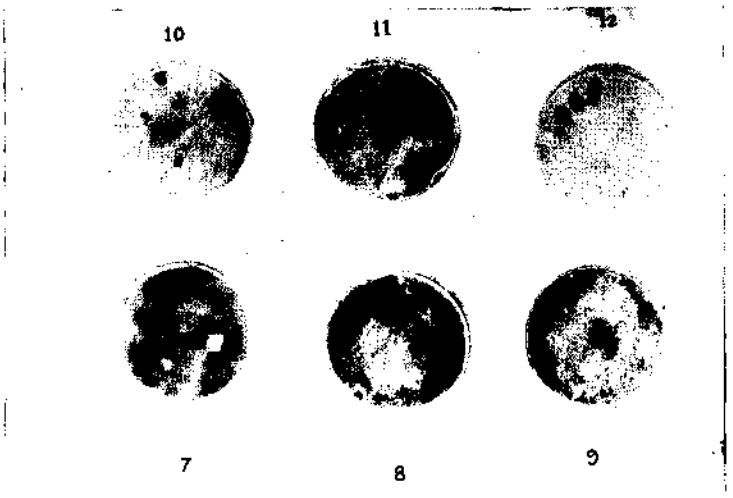


FIG. 1. Differences in the growth rate of *Phormidium tenue* Menegh. under different treatments on agar media.



(*a*)



(*b*)

FIG. 2. (*a*) and (*b*). Formation of algal mat of *Phormidium tenue* Menegh. in liquid cultures.

Details of treatment are given in Table I.