MINERAL NUTRITION OF *ANACYSTIS MONTANA* (LIGHTF.) DR. AND DAILY*

by G. S. VENKATARAMAN, *Indian Agricultural Research Institute, New Delhi*

(Communicated by M. S. Randhawa, F.N.I.)

(*Received April 5, 1961*)

ABSTRACT

The mineral nutrition of the blue-green alga, *Anacystis montana* (Lightf.) Dr. and Daily is discussed. The minimum concentrations of the various major elements to produce a maximum growth under the present experimental conditions have been found to be (in ppm) : 13·6 N, 0·45 P, 0·825 S, 0·45 K, 0·125 Mg, 1·12 Fe and 1 ml of Chu's micronutrient solution. An N/P ratio of about 30/1 has been indicated. The potassium requirement is low and the omission of calcium has not been found to decrease the yield of the alga. All the three sources of iron, viz. ferric chloride, ferric citrate and Fe-EDTA, have been found to be equally effective at higher concentrations, while at lower concentrations ferric chloride gives better yield.

INTRODUCTION

The recent comprehensive reviews on the growth physiology and metabolism of algae by Brunnel *et al.* (1950), Blinks (1951), Myers (1951) and Fogg (1956); on the inorganic nutrition of algae by Ketchum (1954) and Krauss (1953); on the physiology of freshwater algae by Krauss (1958) and on the mass culture of algae by Tamiya (1957) have given increasing coherence to the field of algal nutrition and metabolism. The role of various elements in green plants with a major emphasis on algae has been discussed succinctly by Pirson (1955). The assimilation and function of individual elements have been studied by various workers (Harvey 1940; Chu 1942, 1943; Crammer and Myers 1948; Pearsall and Loose 1937; Pratt and Fong 1940; Gerloff *et al.* 1950; Pearsall 1932; Schrift 1954*a*, *b*; Matudaira 1942; Allen and Arnon 1955; Pirson 1937, 1939; Hopkins and Wann 1925, 1926, 1927; Myers 1951; Kratz and Myers 1955; Natarajan 1960).

The present investigation was undertaken to collect basic data on the nutritional requirements of *Anacystis montana* (Lightf.) Dr. and Daily, a blue-green alga belonging to the order Chroococcales (Fig. 1).

---

* The present communication forms a part of the thesis approved for the Degree of Doctor of Philosophy in Botany of the Banaras Hindu University.

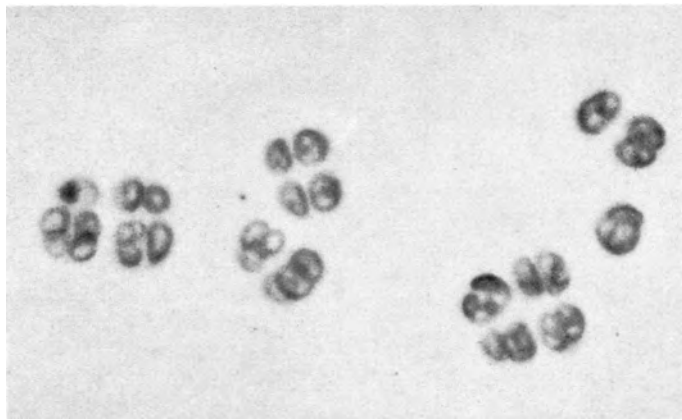

FIG. 1.

Material and Methods

Source of the material: The alga was isolated from the barks of Neem trees (*Melia azaderach*) and was determined by Dr. Francis Drouet. The alga was repeatedly subcultured on silica gel plates before finally maintaining in stock cultures.

Culture media: The greater growth in Chu 10 medium in contrast to that obtained in other media like Fogg's (1949) *Anabaena cylindrica* medium with 0·02 per cent potassium nitrate, *Chlorella* medium of Pearsall and Loose (1937) and Myers and Clark (1944) led to the selection of this medium as the basic medium. However, Chu 10 medium was found to be unsuitable for the nutritional studies, since the individual salts used in this medium provided more than one essential element for growth (Gerloff *et al.* 1950; Natarajan 1960). Hence *Coccochloris* medium of Gerloff *et al.* (1950) was chosen for the experiments described here. In this medium, the cations were provided as chloride salts and the anions as sodium salts, thus permitting independent variation of each essential element (Gerloff *et al.* 1950). The sodium carbonate and sodium silicate were also included and the iron source was provided as ferric-citrate-citric acid. The basic solution was supplemented by 1 ml of Chu's micronutrient solution (Chu 1942). In the experiments where iron was supplied as complex of EDTA (ethylene-diamine-tetra-acetic acid), the iron complex was prepared according to the formula of Jacobson (1951).

Cultures: 250 ml 'Pyrex' conical flasks, each containing 100 ml of the culture solution, were used as culture vessels. The complete medium except phosphate was autoclaved at 20 lb pressure for 30 minutes and allowed to cool. The phosphate solution was autoclaved separately, cooled and then added to the culture solution. The *p*H of all the cultures, except in the *p*H and nitrate experiments, was adjusted to *p*H 10 by a Backman glass-electrode *p*H meter. Each flask was inoculated with 1 ml of the algal suspension from an actively growing culture. The cultures were maintained under identical conditions of illumination and temperature. In all experiments, each element was uniformly varied from three to one-fortieth times the concentration as that in the basic medium.

Growth determinations: The growth of the alga was estimated in terms of its dry weight. The dry weight determinations were made on algal material sedimented by centrifugation and washed twice by resuspension in double distilled water, followed by centrifugation and drying to a constant weight at 60°C. The yield from three flasks was placed in one weighing bottle to provide sufficient material for an accurate dry weight determination. The values reported are the means with their standard deviations based upon the results of three determinations, using a correction value for the salts.

RESULTS

(i) pH experiments:

In the *p*H experiments frequent adjustments were made with dilute hydrochloric acid and sodium hydroxide. The sodium carbonate in the basic medium was omitted in these experiments, unless otherwise stated, to facilitate the maintenance of *p*H at desired levels. The *p*H adjustment was made every twelve hours in the vessels where there was considerable growth, so that the variation in *p*H did not exceed half a unit from the required levels, while in the vessels with little or no growth adjustments were made every twenty-four hours. This is the only experiment to be reported here where the cultures were not kept sterile.

TABLE I

Effect of pH and the presence or absence of sodium carbonate and sodium silicate on the growth of Anacystis montana *(growth after 10 days; values are means with their standard deviations based on three determinations)*

	Treatments	Dry wt. Mg/300 ml
1.	Basic medium with 3 times nitrate; Na_2CO_3 omitted; *p*H adjusted daily to 5	Nil
2.	As (1), *p*H adjusted daily to 6	Nil
3.	As (1) ,, ,, to 7	6·68 ± 0·029
4.	As (1) ,, ,, to 8	10·78 ± 0·029
5.	As (1) ,, ,, to 9	48·65 ± 0·082
6.	As (1) ,, ,, to 10	71·98 ± 0·066
7.	As (1) ,, ,, to 11	38·28 ± 0·085
8.	Basic medium with 3 times NO_3; *p*H not adjusted; initial *p*H 8·4, final *p*H 10	29·75 ± 0·085
9.	As (8) except Na_2CO_3 and Na_2SiO_3 omitted; *p*H adjusted daily to 8·4	26·81 ± 0·077
10.	As (9) except *p*H not adjusted; initial *p*H 6·5; final *p*H 7·5 ..	4·06 ± 0·039

The results of the *p*H experiments are summarized in Table I, which shows that a comparatively high *p*H is required by this alga for its maximum growth. At *p*H 5 and 6 there was absolutely no growth and only slight growth was obtained at *p*H 7 and 8. There was a steady increase in the total yield of the alga above *p*H 8, reaching a maximum at *p*H 10. The yield was more or less the same in treatments 8 and 9, but when both sodium carbonate and sodium silicate were omitted and the *p*H was also left unadjusted the growth was negligible (Table I, treatment 10).

(ii) Strength of the culture medium:

Before studying the effects of the individual elements on the growth of the alga, an attempt was made to find out whether the basic medium contained the optimum amounts of all elements, so that only the element under study could

be the limiting factor. Culture solutions were prepared in which all the constituent salts were varied from two to three times of that present in the basic medium. When the concentration of the salts in the basic medium was increased there were heavy precipitates which made the dry weight determinations rather difficult. The concentrations of individual salts like nitrate and phosphate were also doubled and trebled and the results are shown in Table II.

TABLE II

Effect of different concentrations of the basic medium, nitrate and phosphate on the total yield of Anacystis montana *after 10 days' growth*

Treatment	Dry wt. Mg/300 ml		
	Basic medium	Nitrate	Phosphate
Basic medium ..	50·42 ± 0·026	51·25 ± 0·082	50·33 ± 0·047
2× Basic medium ..	66·30 ± 0·047	65·89 ± 0·052	50·42 ± 0·026
3× Basic medium ..	75·11 ± 0·055	76·25 ± 0·095	50·37 ± 0·026

It was observed that high nitrate concentration alone increased the growth of the alga, while the phosphate concentration had no effect (Table II). Hence in all subsequent experiments the nitrate concentration was kept three times that in the basic medium, so that a deficiency of nitrate might not limit the yield of the alga.

(iii) Effect of different concentrations of various elements:

(a) Nitrate: The total yield of the alga after 10 days' growth at various levels of nitrogen from three times to one-fortieth times of that in the basic medium is tabulated in Table III. The initial *p*H of the cultures was adjusted to 8.

TABLE III

Effect of different concentrations of nitrate on the growth of Anacystis montana *(initial pH 8·0)*

Concentration of nitrogen	Dry wt. Mg/300 ml	Final *p*H
1. 3× Basic medium (20·4 ppm N) ..	74·25 ± 0·033	10·2
2. 2× ,, (13·6 ppm N) ..	67·56 ± 0·499	10·0
3. × ,, (6·8 ppm N) ..	52·13 ± 0·017	9·2
4. × 1/2 ,, (3·4 ppm N) ..	31·54 ± 0·036	8·0
5. × 1/4 ,, (1·7 ppm N) ..	13·19 ± 0·054	8·0
6. × 1/10 ,, (0·68 ppm N) ..	7·09 ± 0·081	8·0
7. × 1/20 ,, (0·34 ppm N) ..	5·22 ± 0·026	8·0
8. × 1/40 ,, (0·17 ppm N) ..	5·15 ± 0·046	8·0
9. Without nitrate	Nil	8·0

The results indicate that for the maximum growth of this alga, a relatively high concentration of nitrate is required (Table III, treatment 1). The 6·72 Mg increase in treatment 1 over treatment 2 is not very significant, but the 22·15 Mg increase over treatment 3 attaches greater importance, which means that at least 13·6 ppm N is necessary for the maximum growth of this alga under the present experimental conditions. The media in treatments 4 to 9 gave a negative test for nitrate (diphenylamine test), indicating that at lower concentrations of nitrate the growth of the alga was limited by nitrogen deficiency. In the absence of any added nitrate, there was no growth (treatment 9).

Table III also shows that the final *p*H of the medium in treatments 1 and 2 has increased, which is probably due to the absorption of bicarbonate and nitrate ions from the medium. In view of the results obtained with *p*H experiments, this shift in *p*H is desirable for the growth of the alga (cf. Table I).

(*b*) *Phosphate:* Table IV shows a surprisingly low requirement for phosphorus as compared to nitrogen (cf. Table III). The optimum yield of the alga

TABLE IV

Effect of different concentrations of phosphorus on the growth of Anacystis montana

	Concentration of phosphorus	Dry wt. Mg/300 ml
1.	3× Basic medium (5·4 ppm P) ..	74·32 ± 0·02646
2.	2× ,, (3·6 ppm P) ..	72·46 ± 0·06558
3.	× ,, (1·8 ppm P) ..	72·46 ± 0·06558
4.	× 1/2 ,, (0·9 ppm P) ..	70·93 ± 0·02646
5.	× 1/4 ,, (0·45 ppm P) ..	72·92 ± 0·02646
6.	× 1/10 ,, (0·18 ppm P) ..	34·90 ± 0·05000
7.	× 1/20 ,, (0·09 ppm P) ..	31·15 ± 0·08370
8.	× 1/40 ,, (0·045 ppm P) ..	25·73 ± 0·26460
9.	Without phosphorus	25·62 ± 0·07071

was little affected even at a concentration as low as 0·45 ppm P, whereas for the maximum yield 13·6 ppm N was required. It thus appears that a maximum harvest of this alga can be obtained with an N/P ratio of about 30/1, under the present experimental conditions. The increase in the concentration of phosphorus above that present in the basic medium did not have any appreciable effect on the yield of the alga.

(*c*) *Sulphur:* Table V summarizes the dry weight values at various sulphate levels in the medium. The sulphate concentration also could be lowered up to 0·825 ppm S without appreciably affecting the total yield of the alga.

(*d*) *Potassium:* The concentration of potassium could be considerably lowered and above 4·5 ppm K the growth of the alga was found to be appreciably inhibited (Table VI).

TABLE V

Effect of different concentrations of sulphur on the growth of Anacystis montana

Concentration of sulphur	Dry wt. Mg/300 ml
1. 3× Basic medium (9·9 ppm S) ..	77·03 ± 0·02916
2. 2× ,, (6·6 ppm S) ..	81·13 ± 0·00518
3. × ,, (3·3 ppm S) ..	76·25 ± 0·09486
4. × 1/2 ,, (1·65 ppm S) ..	75·09 ± 0·03802
5. 1/4 ,, (0·825 ppm S) ..	72·08 ± 0·06442
6. × 1/10 ,, (0·33 ppm S) ..	59·29 ± 0·09649
7. × 1/20 ,, (0·165 ppm S) ..	34·33 ± 0·02249
8. × 1/40 ,, (0·0825 ppm S) ..	31·87 ± 0·06671
9. Without sulphur	30·20 ± 0·02550

TABLE VI

Effect of different concentrations of potassium on the growth of Anacystis montana

Concentration of potassium	Dry wt. Mg/300 ml
1. 3× Basic medium (13·5 ppm K) ..	43·07 ± 0·05191
2. 2× ,, (9·0 ppm K) ..	48·15 ± 0·02000
3. × ,, (4·5 ppm K) ..	74·07 ± 0·04743
4. × 1/2 ,, (2·25 ppm K) ..	72·12 ± 0·06246
5. × 1/4 ,, (1·125 ppm K) ..	68·23 ± 0·06205
6. × 1/10 ,, (0·45 ppm K) ..	68·61 ± 0·02550
7. × 1/20 ,, (0·225 ppm K) ..	68·15 ± 0·04062
8. × 1/40 ,, (0·1125 ppm K) ..	58·07 ± 0·05291
9. Without potassium	39·70 ± 0·01732

(*e*) *Magnesium:* Table VII shows that the magnesium concentration can be as low as 0·125 ppm Mg, which is one-twentieth of that present in the basic medium. However, concentrations above 5 ppm Mg and below 0·125 ppm Mg inhibited the growth (treatments 1 and 8).

TABLE VII

Effect of different concentrations of magnesium on the growth of Anacystis montana

Concentration of magnesium	Dry wt. Mg/300 ml
1. 3× Basic medium (7·5 ppm Mg) ..	68·35 ± 0·04062
2. 2× ,, (5·0 ppm Mg) ..	76·16 ± 0·02636
3. × ,, (2·5 ppm Mg) ..	72·51 ± 0·00678
4. × 1/2 ,, (1·25 ppm Mg) ..	71·17 ± 0·27040
5. × 1/4 ,, (0·625 ppm Mg) ..	72·51 ± 0·06708
6. × 1/10 ,, (0·25 ppm Mg) ..	70·41 ± 0·02000
7. × 1/20 ,, (0·125 ppm Mg) ..	69·35 ± 0·24910
8. × 1/40 ,, (0·0625 ppm Mg) ..	45·62 ± 0·09324
9. Without magnesium	23·61 ± 0·03606

(*f*) *Calcium:* The yield of the alga was little affected even when calcium was completely omitted (Table VIII). However, the medium could not be said to be completely free from calcium, since this element might be present as impurities in the salts or might have been introduced along with the inoculum. Nevertheless, the results indicate that this alga requires calcium only in traces.

TABLE VIII

Effect of different concentrations of calcium on the growth of Anacystis montana

Concentration of calcium	Dry wt. Mg/300 ml
1. 3× Basic medium (29·4 ppm Ca) ..	54·61 ± 0·03604
2. 2× ,, (19·6 ppm Ca) ..	58·71 ± 0·02121
3. × ,, (9·8 ppm Ca) ..	74·26 ± 0·06138
4. × 1/2 ,, (4·9 ppm Ca) ..	64·51 ± 0·02121
5. × 1/4 ,, (2·45 ppm Ca) ..	60·83 ± 0·02000
6. × 1/10 ,, (0·98 ppm Ca) ..	62·56 ± 0·03082
7. × 1/20 ,, (0·49 ppm Ca) ..	64·81 ± 0·02000
8. × 1/40 ,, (0·245 ppm Ca) ..	76·61 ± 0·09082
9. Without calcium	72·53 ± 0·03999

(*g*) *Iron:* The total yield of the alga increased with increasing concentrations of iron within the limits investigated (Table IX) and the maximum yield was obtained at 1·68 ppm Fe (treatment 1).

TABLE IX

Effect of different concentrations of iron (as Fe-citrate) on the growth of Anacystis montana

Concentration of iron	Dry wt. Mg/300 ml
1. 1·68 ppm Fe	79·56 ± 0·04061
2. 1·12 ppm Fe	75·34 ± 0·06442
3. 0·56 ppm Fe (Basic medium) ..	73·09 ± 0·05999
4. 0·28 ppm Fe	55·61 ± 0·03604
5. 0·14 ppm Fe	55·24 ± 0·06243
6. 0·056 ppm Fe	55·09*
7. 0·028 ppm Fe	55·23 ± 0·04523
8. 0·014 ppm Fe	50·22 ± 0·04582
9. Without iron	33·23 ± 0·04242

* Single determination only.

(*iv*) *Effect of different sources of iron:*

Table X summarizes the results obtained with three different sources of iron, viz. ferric chloride (freshly prepared and one-year-old solution), ferric citrate-citric acid and Fe-ethylene-diamine-tetra-acetic acid (Fe-EDTA).

At higher concentrations all the three sources of iron were equally effective, although Fe-EDTA proved to be superior to the ferric chloride and ferric citrate. At lower concentrations, however, particularly below 0·28 ppm Fe, the ferric citrate and Fe-EDTA gave more yields than ferric chloride (Table X). The one-year-old ferric chloride solution was inferior to the freshly prepared solution, which might be probably due to a gradual conversion of iron into a form unavailable to the alga.

TABLE X

Effect of different sources of iron on the growth of Anacystis montana

Treatment	Dry wt. Mg/300 ml			
	Fe-citrate	Fe-EDTA	Ferric chloride	
			Fresh	One year old
1. 3× Basic medium	80·23 ± 0·04	99·12 ± 0·06	80·2 ± 0·86	29·24 ± 0·02
2. 2× ,,	76·32 ± 0·03	91·13 ± 0·03	75·6 ± 0·02	30·43 ± 0·04
3. × ,,	73·2*	82·22 ± 0·03	70·5 ± 0·04	33·12 ± 0·02
4. × 1/2 ,,	54·60 ± 0·05	72·06 ± 0·05	54·6 ± 0·03	30·06*
5. × 1/4 ,,	55·21 ± 0·03	71·53 ± 0·06	50·5 ± 0·02	35·12 ± 0·01
6. × 1/10 ,,	53·07 ± 0·03	68·30 ± 0·03	47·6 ± 0·03	35·22 ± 0·02
7. × 1/20 ,,	55·16 ± 0·05	64·42 ± 0·04	43·1 ± 0·03	36·04 ± 0·03
8. × 1/40 ,,	50·11 ± 0·02	63·33 ± 0·05	42·8 ± 0·05	38·64 ± 0·07

* Single determination only.

The above results indicate that a maximum yield of the alga can be obtained, if the minimum concentrations of various major elements are provided in the following proportions in ppm: 13·6 N, 0·45 P, 0·825 S, 0·45 K, 0·125 Mg, 1·12 Fe and traces of calcium. Based on this, an experimental solution was prepared with the minimum concentrations of each major element (Solution E1) and with double the minimum concentrations of each element (Solution E2) and the growth of the alga in these solutions was compared with that in the basic medium. The results are shown in Table XI.

TABLE XI

Comparative yields of Anacystis montana *in the experimental and basic media with three times nitrate*

Solution	Dry wt. Mg/300 ml
Solution E1 ..	58·07 ± 0·05291
Solution E2 ..	91·30 ± 0·02646
Basic medium ..	76·25 ± 0·09486

It was found that in the Solution E1 the yield of the alga was only 58·07 ± 0·05291 Mg/300 ml of the medium as compared to 76·25 ± 0·09486 Mg in the basal medium. However, when the minimum concentrations of these elements were doubled (Solution E2) the yield increased to 91·3 ± 0·02646 Mg/300 ml of the medium.

Discussion

The present investigation describes a method which permits an evaluation of the relative proportions of the essential major elements required for the maximum yield of the blue-green alga, *Anacystis montana* (Lightf.) Dr. and Daily, under the present experimental conditions. The minor elements were provided by shotgun procedure (1 ml/1) and no attempt was made to find out the actual requirement.

Although the blue-green algae have been found to prefer an alkaline medium for their growth, only a few accurate determinations of the effect of *p*H on their growth have been made (Martens 1914; Gerloff *et al.* 1950; Allison and Hoover 1935; Allison *et al.* 1937; Kratz and Myers 1955; Venkataraman 1961; Singh 1942; cf. *also* Harder 1917; Allen 1952; Walp and Schopbach 1942). A maximum growth rate for *Anacystis nidulans* was supported in the *p*H range 7·4–9 (Kratz and Myers 1955). In the present investigation, *Anacystis montana* has been found to require a high *p*H for its optimum growth (*p*H 10, Table I) (*see also* Gerloff *et al.* 1950). This indicates that, for the optimum growth of this alga, the medium must not only contain the various essential elements but also include such salts which will keep the *p*H of the medium in the alkaline range (Gerloff *et al.* 1950). The inclusion of sodium carbonate and sodium silicate in Chu 10 medium accounts for the general suitability of this solution for many blue-green algae. Table I shows a very poor yield in treatment 10, where both sodium carbonate and sodium silicate were omitted and the *p*H was left unadjusted. This indicates that the desirable effects of sodium carbonate and sodium silicate are mainly concerned with the H-ion concentration of the medium (Gerloff *et al.* 1950).

Anacystis montana requires a high level of nitrogen as compared to phosphorus (Tables III and IV) and shows no evidence of growth supported by nitrogen fixation (cf. *also Coccochloris peniocystis*, Gerloff *et al.* 1950; *Anacystis nidulans*, Kratz and Myers 1955). This high nitrogen requirement may be due to the adaptation of the alga to its peculiar habitat. Myers (1947) observed that by keeping the nitrogen level at 168 Mg/1, the major salts in the medium could be diluted to as low as twenty times without affecting the growth of *Chlorella* (cf. Chu 1946; Rhode 1948).

The availability of nitrate ions is closely associated with the H-ion concentration of the medium and conversely their assimilation changes the *p*H of the medium. The final shift in the *p*H from 8 to 10·2 in treatments

1 and 2 in Table III is desirable for the growth of this alga as seen from the *p*H experiments (cf. Table I) (*see also* Pratt and Fong 1940; Gerloff *et al.* 1950).

The range of phosphorus concentration for different species varies from 8·9 to 17·8 Mg P/litre and higher concentrations have been found to inhibit the growth (Chu 1942). However, a surprisingly low requirement of phosphorus is indicated for the present alga (Table IV) which shows that a maximum harvest of this alga can be obtained with an N/P ratio of approximately 30/1 (cf. Gerloff *et al.* 1950). An N/P ratio of 12·5/1 is indicated for *Kirchneriella subsolitaria* (Potash 1958) and 22/1 for *Selenastrum westii* (Natarajan 1960). The sulphate requirement also is very low for this alga (0·825 ppm S).

Of the cations tested, the present alga requires low amounts of potassium (*see also Chroococcus*, Emerson and Lewis 1942; *Selenastrum westii*, Natarajan 1960) and the yield is more or less the same up to 0·225 ppm K (Table VI). *Coccochloris peniocystis*, however, has a high potassium requirement and Gerloff *et al.* (1950) reported that the growth of the alga decreased below a concentration of 2·25 ppm K. Kratz and Myers (1955) observed that the Na : K ratio was not critical and might be varied from 40 : 800 to 800 : 5 without affecting the growth rate of *Anacystis nidulans* and *Anabaena variabilis*. A concentration of less than 7·8 Mg K per litre has been found to depress the growth of *Chlorella* (Scott 1943).

The requirement of calcium by various algae has been previously discussed by different authors (Hopkins and Wann 1926; Myers 1944; Pratt and Fong 1940; Trelease and Selsam 1939; Rhode 1948; Gerloff *et al.* 1950; Allen and Arnon 1955). Allison *et al.* (1937) claimed that *Nostoc muscorum* required calcium only when the alga was growing on elementary nitrogen and not when nitrate was the nitrogen source. Allen and Arnon (1955), however, showed an irreplaceable calcium requirement of at least 20 ppm for *Anabaena cylindrica*. However, a comparative study of the rate of growth as a function of calcium concentration showed that the yield of *Anacystis montana* was little affected even when calcium was completely omitted from the medium (*see also* Gerloff *et al.* 1950). Calcium might, however, be present as impurities in the salts used or might have been introduced as carry-overs with the inoculum. Also, the possibility of the calcium requirement of this alga being largely satisfied by magnesium and also partially by the sodium in the medium cannot be ruled out (cf. Scott 1943). The tolerance to high sodium concentrations by the blue-green algae in general accounts for their abundant growth in 'usar' soils where other organisms fail to grow.

The importance of iron for the growth of algae is well substantiated (Hopkins and Wann 1925, 1926, 1927; Hopkins 1930; Rhode 1948; Myers 1951; Hutner *et al.* 1950; Gerloff *et al.* 1950; Goldberg 1952; Eyster 1958; Natarajan 1960). The total yield of *Anacystis montana* increases with the increasing concentrations of iron (Table IX). However, iron supplied as Fe-EDTA has

proved to be more satisfactory than ferric chloride and ferric citrate. The aged ferric chloride solution produced less growth than the freshly prepared solution, which indicates that a solution of ferric chloride solution cannot be kept indefinitely.

The present study indicates that the minimum concentrations of the various major elements required for the maximum yield of *Anacystis montana* are (in ppm): 13·6 N; 0·45 P; 0·825 S; 0·45 K; 0·125 Mg and 1·12 Fe. However, when such a medium was tested, the final yield was poor as compared to that in the original medium with three times nitrate. When the minimum concentrations of the elements were doubled, the yield also increased considerably (Table XI). Although the present study contributes little to an immediate understanding of the exact nutritional requirements of *Anacystis montana*, it indicates the relative proportions in which the elements are required under the present experimental conditions.

Acknowledgements

The author is greatly indebted to Drs. R. Misra, F.N.I., M. S. Randhawa, F.N.I., B. P. Pal, F.N.I., Y. Bharadwaja, F.N.I., A. B. Joshi, R. N. Singh and Professor R. D. Asana for their valuable advice and criticism; to the Indian Council of Agricultural Research for providing all facilities; to Dr. Francis Drouet, Department of Biology, Highlands University, New Mexico, for kindly identifying the alga, and to Dr. A. M. Mayer, Department of Botany, The Hebrew University, Jerusalem, for his valuable discussions.

References

Allen, M. B. (1952). *Arch. Microbiol.*, **17**, 34.

Allen, M. B., and Arnon, D. I. (1955). *Plant Physiol.*, **30**, 366.

Allison, F. E., and Morris, H. J. (1930). *Science*, **71**, 221.

Allison, F. E., and Hoover, S. R. (1935). *Trans. 3rd Int. Congr. Soil. Sci.*, **1**, 145-47. Oxford, England.

Allison, F. E., Hoover, S. R., and Morris, H. J. (1937). *Bot. Gaz.*, **98**, 433.

Blinks, L. R. (1951). Physiology and Biochemistry of Algae: In the Manual of Phycology, p. 263. Ed. G. M. Smith, Chronica Botanica Co., Waltham, Mass.

Brunnel, J., Prescott, G. W., and Tiffany, L. H. (1950). The culturing of Algae : A symposium. (Brunnel *et al.* Ed. The Charles F. Kettering Foundation, pp. 114.)

Chu, S. P. (1942). *J. Ecol.*, **30**, 284.

——— (1943). *Ibid.*, **31**, 109.

——— (1946). *J. Mar. biol. Ass. U.K.*, **26**, 109 (*see also* Science and Technol., China, **2**, 37, 1949).

Craig, F. N., and Trelease, S. F. (1937). *Amer. J. Bot.*, **24**, 233.

Crammer, M., and Myers, J. (1948). ***J. gen. Physiol.*, 32, 93.**

Drouet, F., and Daily, W. A. (1956). *Butler Univ. bot. Stud.*, **12**, 34-70.

Emerson, R., and Lewis, G. M. (1942). *Ibid.*, **25**, 579.

Eyster, C. (1952). *Nature, Lond.*, **170**, 755.

——— (1958). *Ohio J. Sci.*, **58, 33.**

Fogg, G. E. (1942). *J. expt. Biol.*, **19**, 78.
——— (1949). *Ann. Bot., Lond.*, n. s. **13**, 241.
——— (1956). *Annu. Rev. Pl. Physiol.*, **7**, 51.
Gerloff, G. C., Fitzgerald, G. P., and Skoog, F. (1950). *Amer. J. Bot.*, **37**, 216.
Goldberg, E. D. (1952). *Biol. Bull.*, **102**, 243.
Harder, R. (1917). *Z. Bot.*, **9**, 145.
Harvey, H. W. (1940). *J. Mar. biol. Ass. U.K.*, **24**, 115.
Hopkins, E. F. (1930). *Bot. Gaz.*, **89**, 209.
Hopkins, E. F., and Wann, F. B. (1925). *J. gen. Physiol.*, **9**, 205.
——— (1926). *Bot. Gaz.*, **81**, 353.
——— (1927). *Ibid.*, **83**, 194.
Hutner, S. H., Provasoli, L., Schatz, A., and Haskins, C. P. (1950). *Proc. Amer. phil. Soc.*, **94**, 153.
Jacobson, L. (1951). *Plant Physiol.*, **26**, 411–413.
Ketchum, B. H. (1954). *Annu. Rev. Pl. Physiol.*, **5**, 55.
Kratz, W. A., and Myers, J. (1955). *Amer. J. Bot.*, **42**, 282.
Krauss, R. W. (1953). In algal culture: Frpm Laboratory to Pilot Plant, 85–102. (Burlew, J. S. Ed. Cambridge Institution of Washington, Washington, D.C., 357 pp.)
——— (1958). *Annu. Rev. Pl. Physiol.*, **9**, 207.
Martens, H. (1914). *Beitr. Biol. Pfl.*, **12**, 439 (cited from Ketchum, 1954).
Matudaira, T. (1942). *Proc. imp. Acad. Japan.*, **18**, 107 (cited from Ketchum 1954).
Myers, J. (1944). *Plant Physiol.*, **19**, 579.
——— (1947). *Ibid.*, **22**, 590.
——— (1951). *Annu. Rev. Microbiol.*, **5**, 157.
Myers, J., and Clark, L. B. (1944). *J. gen. Physiol.*, **28**, 103–12.
Natarajan, K. V. (1960). Mineral nutrition of Selenastrum westii. *Proc. Symp. Algology, India*, (1959), pp. 156–161. Publ. I.C.A.R.
Pearsall, W. H. (1932). *J. Ecol.*, **20**, 241.
Pearsall, W. H., and Loose, L. (1937). *Proc. roy. Soc., London*, B**121**, 451.
Pirson, A. (1937). *Z. Bot.*, **31**, 193.
——— (1939). *Planta*, **29**, 231.
——— (1955). *Annu. Rev. Pl. Physiol.*, **6**, 71.
Potash, M. (1958). *Phycological News Bull.*, **11**(35), 64 (Abstr.).
Pratt, R., and Fong, J. (1940). *Amer. J. Bot.*, **27**, 431, III. **27**, 735.
Rhode, W. (1948). *Symb. Bot. upsaliensis*, **10**, 149.
Schrift, A. (1954*a*). *Amer. J. Bot.*, **41**(3), 223–230.
——— (1954*b*). *Ibid.*, **41**(4), 345–352.
Scott, G. T. (1943). *J. cell. comp. Physiol.*, **21**, 327.
——— (1944). *Ibid.*, **23**, 47–58.
Singh, R. N. (1942). *Indian J. agric. Sci.*, **12**, 743.
Tamiya, H. (1957). *Annu. Rev. Pl. Physiol.*, **8**, 309.
Tanada, T. (1951). *Amer. J. Bot.*, **38**, 276.
Trelease, S. H., and Selsam, M. E. (1939). *Ibid.*, **26**, 339.
Venkataraman, G. S. (1961). Studies on nitrogen fixation by blue-green algae. II. Nitrogen fixation by *Cylindrospermum sphaerica* Prasad under various conditions. *Proc. Nat. Acad. Sci., India*, **31***A*, 100.
Walp, L., and Schopbach, R. (1942). *Growth*, **6**, 173.
Witsch, H. von (1948). *Arch. Mikrobiol.*, **14**, 128.