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OBSERVATIONS ON THE IONIC COMPOSITION OF BLUE-GREEN ALGAE GROWING IN SALINE LAGOONS

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INTRODUCTION

The capacity of certain Myxophyceae to withstand marked alterations in salinity of the medium as well as the presence of some of them in salt marshes have been reported by various authors, viz., Cavara (1902) (*vide* Brooks and Brooks, 1947), Ercevogic (1930), Prat (1925) (Fritsch, 1945), and Carter (1933). The abundant occurrence of some of the species of blue-green algae in solar salt marshes has been noticed by Hansgirg (1887) and Hof and Fremy (1933) (*vide* Fritsch, 1945), chief among these being *Microcoleus chthonoplastes*, a species often associated with *Lyngbya aestuarii*. Hof and Fremy have distinguished the algae as halotolerant and halophilic species, the latter term denoting those which can grow and multiply in solutions more concentrated than three molar NaCl, applicable to a few Chroococcales (esp. *Aphanocapsa littoralis*) and forms like *Spirulina subsalsa* and *Phormidium tenue*. The vertical expansion and contraction of filaments of blue-green algae esp. *Oscillatoria jenensis* and their relationship to osmo-regulation in the algae have been studied in detail by Schmid (1923). In their monograph Brooks and Brooks (1947) have discussed the degree of permeability in blue-green algae which can withstand relatively concentrated brines whose osmotic pressures are about 150 atmospheres. They observe that these cells are permeable to water in both directions in response to the osmotic forces. But no quantitative data are given to substantiate the statement and to explain the various physiological changes taking place in the algal cells under varying salinity conditions.

Nakamura (1938) discusses the process of carbon assimilation of lower algae in the presence of hydrogen sulphide. Instead of water, H_2S is the source of hydrogen for the reduction of CO_2, the evolution of oxygen in this case being replaced by the deposition of sulphur within the cells.

As the salinity rises in the waters of saline lagoons, corresponding changes in the other constituents naturally follow, but in degree and rate of change they need not necessarily be proportional to the changes in the chloride content. Howes (1939) observes that the relative proportion of salts in estuaries and salt marshes need not be the same as in sea water. He has collected data from a saline lagoon

in East Essex and shows that there is a significant rise in the sulphate content of the water. Also there is a slow decrease in the ratios K/Na, Ca/Na and Mg/Na, whereas the ratio $\frac{Ca+Mg}{K+Na}$ remains almost the same as that of normal sea water. Such differences in the relative proportions of salts between lagoon waters and normal sea water are explained as being due to the differential precipitation during evaporation (Beadle, 1943).

The differences in the ionic proportions will be accentuated in inland salt water lagoons, and the survival of plant and animal species under these conditions will depend upon special adaptations, the fauna and flora being subjected to the wide fluctuations in the ionic composition of the waters.

Apart from the major constituents mentioned above several other minor elements also may contribute to the healthy growth of algae in waters. In recent years a few attempts have been made to study the growth requirements of a number of blue-green algae. Gerloff, Fitzgerald and Folke Skoog (1950*a*, *b*) describe procedures employed in the continuous culture of about 22 species of blue-green algae including species of *Microcystis*, *Aphanizomenon* and *Lyngbya* and some others which have not previously been maintained in cultures. These studies bring to light the importance of several minor elements especially iron in the metabolism and growth of these algae. Excellent summaries of the nutritional requirements of other forms, especially among the green algae, have been given by Mainx (1929), Molisch (1895, 1896) (*vide* Stiles, W. 'Trace elements in plants and animals', 1948), etc., which bring to light the importance of a number of minor elements such as manganese, iron, copper, iodine, boron, etc., in the metabolism and distribution of algae. The manganese and iron requirements of *Chlorella* have been studied by Hopkins (1930, 1938) and Hopkins and Wann (1927) respectively, and of the diatom *Ditylum brightwelli*, by Harvey (1939). Roberg (1932) found that autotrophic chlorophyceae could not grow in the absence of iron and reports increased growth of two unicellular green algae, *Coccomyxa simplex* and *Chlorella vulgaris* as a result of small additions of salts of iron, zinc and copper to the normal nutrient solutions. Uspenski (1924 and 1925—*vide* Bold, 1942) has worked on iron as a factor in the distribution of algae in the Russian waters. As regards the lagoon waters there is every possibility of an almost perpetual shortage in some of the essential minor elements as supplies of sea water are cut off periodically, and even when the lagoons are in contact with the sea the entry of sea water is often insufficient for adequate irrigation and replenishment of nutrients.

Experimental

The present experiment is a study on some of the problems indicated above and is calculated to gather information on the following points:

(1) Variation in the intensity of the growth of lagoon algae with rise in salinity and their limits of salinity tolerance,
(2) The variation in the chemical composition of lagoon waters and the possible effect of the abnormal rise in the chloride content on the ratios between other ions,
(3) Ionic changes taking place in the cell fluid of certain algal species as well as of the algae as a whole growing in the lagoons, and
(4) Differences in the amounts of some of the minor elements present in the water and in the algae.

Two stations which represented two different conditions were selected from Palk Bay Lagoons at Mandapam Camp. The first station was selected from the major lagoon extending over several hundred acres. From October till the end of

March it had connection with the sea and was under tidal influence, while the second one was separated from the first even in the beginning of March, though it originally formed part of the main lagoon. Naturally the rate of concentration of salt was greater in station II than in station I from March.

Samples of bottom algae and water were separately collected from the above stations at definite intervals. The algal collections did not contain any other types of algae other than blue-greens though almost all the collections showed presence of the diatom *Nitzschia vitrea* (Krishna Pillai, 1954). The water samples were analysed for Na, K, Ca, Mg, Fe, Mn, Cu, I, B, Zn, N (both protein and non-protein nitrogen) and S contents. The cell fluids were extracted from the fresh algae by grinding them well with acid-washed sand and pressing out the fluid at high pressure. The fluid thus obtained was centrifuged and used for analysis.

Method of Analysis

The methods employed for the analysis of Na, K, Ca, Mg, Cu and Cl contents of the water and of the algae and their cell fluid were those employed by Robertson and Webb (1939) for biological materials. B.D.H. micro analytical reagents were used in the estimations. In the case of the estimation of potassium a slight modification has been made; instead of dissolving the precipitate of potassium cobalti nitrite in ceric sulphate, the Kramer and Tisdall's volumetric method has been used.

The total iron in the algae was estimated by first igniting accurately weighed quantities of air-dried samples, digesting with excess of concentrated HCl and applying the potassium thiocyanate method of colour comparison, the final comparison being made in Hilger Spekker Photo-electric Absorptiometer (A.O.A.C., 1945, p. 157).

Iodine was determined by the method specially adapted for biological materials and used by Von Fellenberg (1924).

Sulphate in water was determined by the usual method of precipitation and weighing as $BaSO_4$. Total sulphur in the algae was determined by the method employed by Aitken (1930).

Zinc was estimated by the method suggested by Hibbard (1934), while manganese in sea water and in the algae was determined by the periodate method given by Snell and Snell (1949).

Total as well as non-protein nitrogen were estimated by the micro-kjeldahl technique (Hawk, Oser and Summerson, 1947) absorbing the ammonia in 2% boric acid and finally titrating against standard $N/70$ sulphuric acid.

After each collection the total wet weight of the algae from a unit area of each station was estimated so that the variations in the algal production at these stations could be studied. The values are tabulated in Table I. An attempt was also made to study the variations in the amount of particular species growing under varying salinities, which, however, was not successful owing to the difficulty in isolating each species. A complete qualitative analysis of the algal samples was done and the component species with their relative amounts noted (Krishna Pillai, 1954). The results of the chemical analysis of the algae and water are given in Tables I and II respectively. In the case of water samples values are given only for the Fe, Mn and Cu contents, as the presence of other minor elements, viz., B, I and Zn could not be detected owing either to their complete absence or to insufficient sensitivity of the methods employed.

TABLE I

Trace element content of blue-green algae

(On oven-dry basis)

	Total wet weight of algae g./sq.metre	Dry weight %	Ash %	Protein-N %	Non-protein-N %	Fe	Mn	Cu	I	B	Zn
						(parts per million)					
STATION I—											
(i) ..	930·0	18·3	55·4	0·60	0·52	73·0	1·2	12·5	48·6	Trace	5·0
(ii) ..	635·0	20·7	54·0	0·41	0·49	15·5	<0·1	7·3	41·0	Nil	<1·0
(iii) ..	520·0	46·2	60·3	0·23	0·40	30·8	<0·1	Trace	47·6	Nil	<1·0
STATION II—											
(i) ..	690·0	19·3	68·5	0·47	0·41	55·0	0·4	12·5	24·3	8·0	Nil
(ii) ..	380·0	23·6	70·9	0·21	0·40	2·0	<0·1	Trace	Nil	Nil	Nil
(iii) ..	250·0	49·5	71·0	0·19	0·39	7·8	<0·1	Nil	Nil	Nil	Nil

TABLE II

Chemical composition of water from the two stations

	*p*H	Na mg./litre	K mg./litre	Ca mg./litre	Mg mg./litre	Cl mg./litre	SO_4 mg./litre	Fe μg./litre	Mn μg./litre	Cu μg./litre
STATION I—										
(i)	8·5	9,430	60	910	791	19,880	3,460	14	27	31
(ii)	8·2	26,430	90	1,360	2,740	47,860	4,020	Trace	..	10
(iii)	8·4	71,230	1,510	1,550	2,705	111,720	11,840	Nil	..	10
STATION II—										
(i)	8·4	8,800	40	1,090	1,660	17,320	3,430	8	Nil	10
(ii)	8·2	27,190	80	1,280	5,600	60,670	3,790	Trace	Trace	Nil
(iii)	8·4	56,230	1,520	2,760	7,160	105,120	11,210	Trace	Trace	10

The ionic composition of the cell fluids and the external medium have been tabulated in Tables III and IV.

TABLE III

Ionic composition of water and algal cell fluid from Station I

(Expressed in mM concentrations)

	I		II		III	
	Water	Cell fluid	Water	Cell fluid	Water	Cell fluid
Na	410·0	123·0	1,149·2	130·8	3,097·0	Nil
K	1·5	11·0	2·3	26·0	38·8	36·4
Ca	22·8	45·3	34·2	64·5	38·8	83·7
Mg	32·5	50·3	112·7	68·7	111·0	90·0
Cl	560·7	37·4	1,350·0	45·1	3,151·5	37·0
SO_4	35·0	98·2	42·0	180·3	123·3	244·8

TABLE IV

Ionic composition of water and cell fluid from Station II

(Expressed in mM concentrations)

	I		II		III	
	Water	Cell fluid	Water	Cell fluid	Water	Cell fluid
Na	382·0	112·6	1,182·2	Nil	2,444·8	Nil
K	1·1	8·2	2·1	29·0	39·0	33·6
Ca	27·3	24·6	31·9	65·7	69·0	80·0
Mg	67·2	23·2	230·0	65·0	294·0	79·0
Cl	488·7	16·6	1,711·0	40·1	2,965·0	27·8
SO_4	35·7	156·4	39·4	218·4	116·8	274·2

The changes in the ratios between Na and K, Ca and Mg, and (Ca+Mg) and (Na+K) in the waters in the two stations are presented in Table V.

TABLE V

Ratios between cations in water

Observations	STATION I			STATION II		
	K/Na	Ca/Mg	$\frac{Ca+Mg}{Na+K}$	K/Na	Ca/Mg	$\frac{Ca+Mg}{Na+K}$
1	0·0065	1·15	0·178	0·0046	0·67	0·300
2	0·0034	0·50	0·150	0·0029	1·60	0·250
3	0·0210	0·55	0·57	0·0190	1·98	0·173

Discussion of Results

The production of algae shows a definite decrease from the first collection to the third in both the stations. It is noteworthy that none of the stations contains any varieties of algae other than the blue-greens. At station I during the first collection when it had connection with the sea oscillatorians constitute the major part of the algal population while *Phormidium, Aphanothece* and *Spirulina* are present only in very small quantities. In station II which is isolated and detached from the major lagoon, *P. tenue* constitutes the bulk of the algae throughout the period of the experiments. This suggests that the conditions offered by this pond is not favourable to any of the above blue-greens except *P. tenue*. But even in this case in the second and third collections the bulk of the algae was decomposing with the liberation of hydrogen sulphide.

In station I also the percentage of *P. tenue* in the collection increased with the rise in chloride content of the water and all the other algae originally present in the collections disappeared.

The total nitrogen content of the algae shows a definite decrease. This decrease is more evident in the protein nitrogen rather than in the non-protein (Table I) indicating probably that the protein is being disintegrated. The total nitrogen and the ash content of the algae are comparatively lower than the values reported by Schuster (1949) for blue-green algae. Among the minor elements only Fe, Mn, Cu, B, Zn and I have been studied.

Table II gives the variations in the chemical composition of the water including those in the minor elements. The samples do not contain zinc, iodine and boron. Their absence cannot be ascribed to the lack of sensitivity of the methods adopted, which can detect these elements even in low concentrations (10 μg. in a litre). Iron, manganese and copper found in the water samples are very low when compared to the values reported for normal sea water by Black and Mitchell (1952). Thus it is clear that the water in the lagoons is deficient in the above minor elements and to this extent cannot provide the true and natural conditions in sea water.

It may be seen from Table I that as the salinity of the outside water increases the water content of the algae slowly goes down (represented by a rise in dry matter), which would naturally lead to a concentration of the ions in their cell fluids. But this increase in the ionic concentration is most marked, among cations, only with K, Ca and Mg. As these ions increase, the concentration of Na ion does not show any significant change until the chloride concentration of the external medium rises to 1,711·0 mM, when all the Na from the cell fluid is given out (Table IV). It may be seen from Tables III and IV that the variation in the concentration of Na ion in the cell fluid of algae from the two stations is only between 112·6 mM and 130·8 mM, while the concentration of Na ion in water varies from 383 mM to 1,182 mM.

Fig. 1 drawn from the actual weights of the various ions in 1 c.c. each of the solutions shows that when the chloride content of the outside water is 560·7 mM in station I, Na constitutes 45% of the cation content of the cell fluid. With rise in salinity to 1,350 mM chloride the percentage of Na ion falls to 36 in station I. But in station II when the chloride content reaches 1,711 mM the percentage of Na in cell fluid falls to zero the cation composition of the last being Na = nil; K = 20%; Ca = 48% and Mg = 22%. This means that between these chloride concentrations (i.e. between 1,350 mM and 1,711 mM) complete elimination of Na must have taken place.

Subsequent observations on the cell fluid of the algae from both stations show that the percentage of cation remains the same even when the salinity of the outside water goes up to a chloride content of 3,151·5 mM. This may indicate that beyond a certain salinity at which complete elimination of Na from the cell fluids takes place, no cation inter-change occurs between the external medium and the cell

fluids of the algae or, in other words, that some sort of plasmolysis of the algal cells takes place when the chloride content of the external medium reaches 1,711 mM and that the cell fluids remain passive afterwards. The high values for the cations in the cell fluids during the third set of observations can only be due to loss of water from the cells during plasmolysis. The percentage composition of the cations remains the same after plasmolysis (Fig. 1) even though the composition of the external medium alters considerably indicating probably that no selective absorption of ions has taken place after plasmolysis.

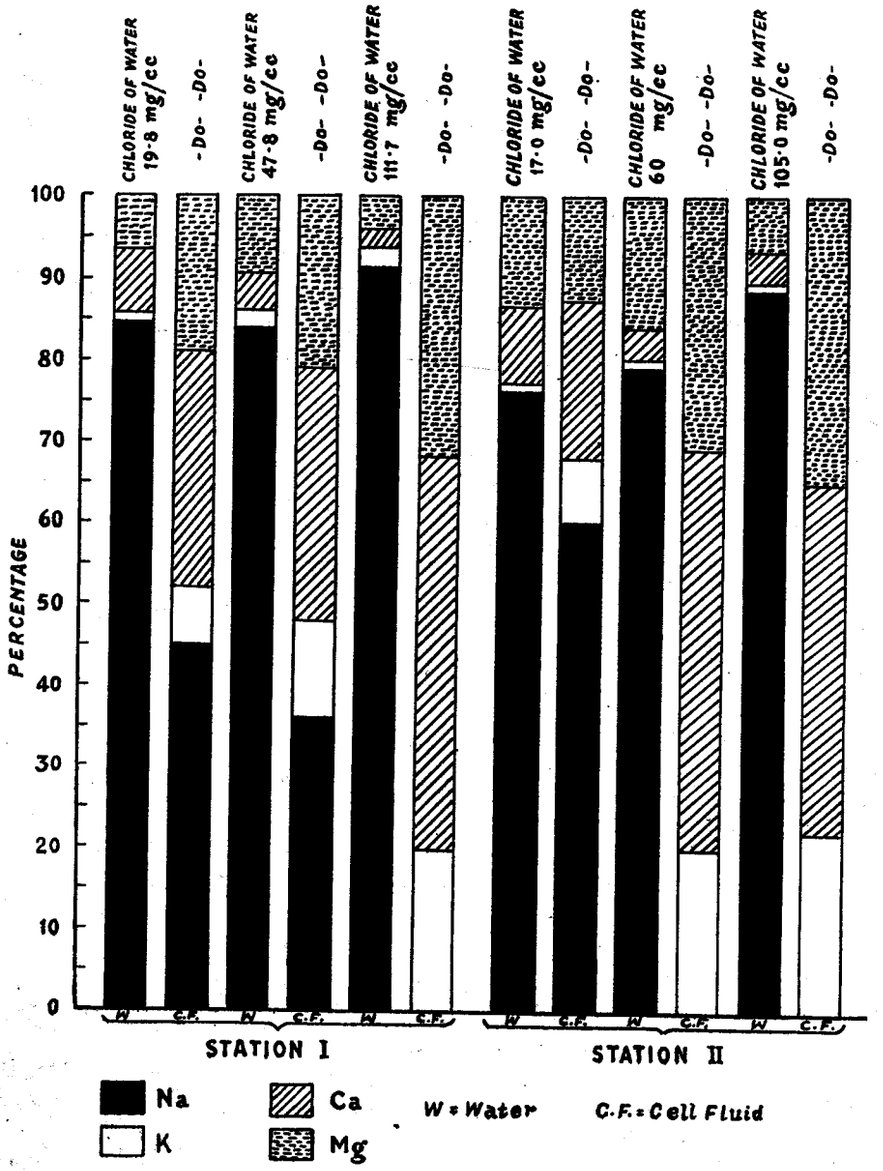
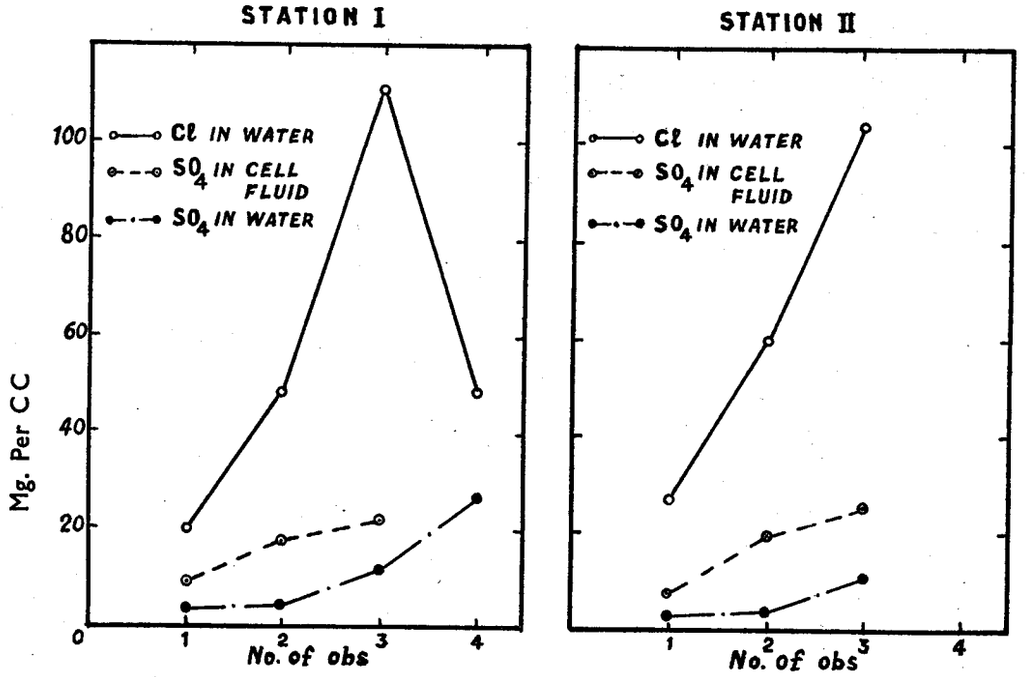


FIG. 1. The ratios between Na, K, Ca and Mg expressed in percentage assuming the total of the four ions to be 100.

In the case of anion exchange also certain peculiarities are noticed. Even though the changes in the chloride content of the external medium are very great, no such high values are noticed in the chloride content of the cell fluid. The changes in the inside chloride are so negligible that the ratio between the external and internal chlorides also increases and is almost directly proportional to the chloride content of the external medium. This fact is highly significant and proves beyond doubt that the blue-green algae, especially *P. tenue*, can withstand wide variations in the salinity of the external medium without allowing free passage of Cl ions into the cell fluids.

The cell fluid of the algae not being affected by the high salinity of the outer medium, the reason for plasmolysis and subsequent destruction cannot be ascribed to the chloride content of the water. Some other factor might be responsible for plasmolysing the algal cells. As mentioned above there is a significant rise in the SO_4 content of the cell fluid as well as of the external medium. Figs. 2 and 3 show



FIGS. 2 and 3. Variation in the Cl and SO_4 of water and cell fluid of the algae at the two stations.

that there is a gradual increase in the SO_4 content of the cell fluids of the algae until the chloride content of the external water reaches the vicinity of 6·0%. Thereafter there is practically no increase in the SO_4 content as the curve tends to droop down in both cases. On the other hand, the changes in the SO_4 content of the external medium is very low at the beginning until the chloride content reaches 6·0%, after which there is a steady increase. At some stage beyond a chloride content of 11·2%, sodium chloride crystallises out from the water and the resultant chloride content of the water falls considerably, but the SO_4 continues to increase. So the behaviour of SO_4 ion in the cell fluid is just the reverse of that of Cl ion, and

TABLE VI

	Station I						Station II					
	Observation 1		2		3		1		2		3	
	Cell fluid mg./c.c.	Algae corresponding to 1 c.c. cell fluid in mg.	Cell fluid mg./c.c.	Algae corresponding to 1 c.c. cell fluid in mg.	Cell fluid mg./c.c.	Algae corresponding to 1 c.c. cell fluid in mg.	Cell fluid mg./c.c.	Algae corresponding to 1 c.c. cell fluid in mg.	Cell fluid mg./c.c.	Algae corresponding to 1 c.c. cell fluid in mg.	Cell fluid mg./c.c.	Algae corresponding to 1 c.c. cell fluid in mg.
Na	2·8	2·8	3·0	2·9	Nil	Nil	2·6	2·5	Nil	Nil	Nil	Nil
K	0·4	0·4	1·0	1·1	1·4	1·4	0·3	0·3	1·2	1·2	1·3	..
Ca	1·8	2·1	1·6	2·7	3·4	3·4	0·8	1·1	2·6	3·8	3·2	..
Mg	1·2	1·7	1·7	2.2	2·2	2·2	0·6	0·9	1·6	1·9	1·9	..
Cl	1·3	1·1	1·4	1·1	1·3	1·1	0·6	0·6	1·6	1·5	0·9	1·7
SO_4	9·4	..	17·3	..	23·7	..	15·0	..	21·0	..	26·3	..
Total S as SO_4	..	13·1	..	23·1	..	31·3	..	18·3	..	26·3	..	32·9

the cell walls do not resist the entrance of the SO_4 ions. It may be that the high concentration of the SO_4 ion in the cell fluid that brings about plasmolysis of the cells. The exact significance and the actual rôle of the SO_4 ions in the metabolism of the blue-green algae remains to be understood. However, it may be seen from Table VI that most of the SO_4 ions that get into the cell fluid do not actually go into effective combination with the cell wall, but remain in the form of ionic sulphate in the fluid itself. The changes in the difference between the ionic sulphate in unit volume of cell fluid and the total sulphur (expressed as SO_4) in the algae corresponding to that volume of cell fluid is very small when compared with the rise in the ionic sulphate in the cell fluid (Table VI). The continuous evolution of hydrogen sulphide from the two stations during high salinities may be due to the bacterial decomposition of the dying algae which contain a high percentage of free SO_4 ions. Part of the SO_4 released by the dying algae remains unreacted by sulphur reducing bacteria which probably accounts for the high SO_4 content of the water after plasmolysis of the algae. It may be noted that the analysis of the cell fluids and total algae from the third collections have been conducted on a mixture of fresh and decaying algae, as it was found extremely difficult to separate the two from the samples which were in the form of a scum.

The ratios between the cations in the water of the two stations show wide variations (Table V). In station I the ratio between the actual weights of potassium and sodium (K/Na) at first decreases from 0·0065 to 0·0034 corresponding to an increase in the chloride content from 19·88 mg./c.c. to 47·87 mg./c.c. Thereafter the ratio increases and the value is 0·021 when the chloride content is 111·72 mg./c.c. The fact that the ratio of K/Na in normal sea water is much higher than 0·0065 shows that lagoon water is deficient in potassium or that all the potassium in the water is being absorbed by and concentrated in the algae. This latter explanation finds support in the increase of the potassium concentration of the cell sap corresponding to a decrease in the K/Na ratio in the water until plasmolysis takes place. After plasmolysis the K from the algae is liberated to the surroundings and consequently the value of the ratio K/Na increases.

A similar change can be noticed in the case of water in the second station also. The ratio decreases till the chloride content reaches 60 mg./c.c. and then increases. The ratio between Ca and Mg also fluctuates within varying limits. As in the case of K/Na, Ca/Mg first decreases in both the stations and then increases showing that there is selective absorption of Ca by the algae before plasmolysis takes place. Afterwards the Ca content of the water increases, probably due to decay of the algae and release of Ca into the water, and the ratio Ca/Mg again increases. The ratio $\frac{Ca+Mg}{Na+K}$ shows a general decrease.

Summary

The growth of blue-green algae in the saline lagoons near Mandapam under varying salinity conditions has been studied. Algal growth decreases with rise in salinity in the lagoons and beyond a chloride concentration of 1,700 mM the algae begin to die and disintegrate. Many of the species, viz., *Oscillatoria, Spirulina* and *Aphanothece* seem to disappear when the chloride content of the surrounding water rises to the vicinity of 1,700 mM. The only blue-green alga that survives this extreme condition appears to be *Phormidium tenue*.

The ionic changes taking place in the cell fluids of the algae collected from two stations have been discussed in detail. The algae resist the very high concentrations of chloride in the surrounding water by not allowing free passage of the chloride ions into the cells. But instead there is great accumulation of SO_4 ions in the cell fluid. As plasmolysis takes place the algae slowly disintegrate and liberate SO_4 into the water.

K, Ca and Mg are accumulated by the algae, the absorption of Ca and K being especially significant. When plasmolysis takes place the Na ion is given out of the cell fluid.

Compared to normal sea water the lagoon waters are found to be deficient in Fe, Mn, B and Zn throughout the year; and this may possibly be one of the many reasons for the fall in the growth rate of algae.

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