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CHEMICAL STUDIES ON INDIAN SEAWEEDS

III. Partition of Sulphur and its Relation to the Carbohydrate Content

BY

V. KRISHNA PILLAI

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III. Partition of Sulphur and its Relation to the Carbohydrate Content

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INTRODUCTION

SEAWEEDS in general derive their importance from their carbohydrate content. Part of the carbohydrate is in the form of cellulose which cannot be hydrolysed by ordinary means; and the rest in the form of polysaccharides either as agar or as algin, the latter being considered as a polymerised form of *d*-mannuronic acid. Kylin (1913) is of the opinion that simple reducing sugars constitute the first products of photosynthesis and that they occur in very small quantities. Haas and Hill (1929) have found a pentose in *Pelvetia canaliculata* though it could not be detected in *Fucus serratus* and *Ascophyllum nodusum*. Recently much work has been done on the fucoidin content—a polysaccharide sulphate ester, of Phæophyceæ (Percival and Ross, 1950). The isolation of a polysaccharide, xylan, which gives only xylose on hydrolysis from the red seaweed, *Rhodymenia palmata*, has been reported by Percival and Chanda (1950). Dextrine like polysaccharides such as laminarin are a common feature of the Phæophyceæ while mannitol is said to be universal in them. In red seaweeds, on the other hand, mannitol is absent while other sugar alcohols like dulcitol and sorbitol have been detected (Haas and Hill, 1931 & 1932; Hassid, 1933).

The earlier work also indicate the importance of sulphur in the carbohydrate metabolism of the seaweeds. The composition of agar, the chief polysaccharide constituent of red seaweeds, as given by Jones and Peat (1942), itself indicates that SO_4 forms an integral part of the agar molecule. Barry and Dillon (1944) assume that there are as many as 53 galactose units to each SO_4 group with at least 140 such units to each non-reducing end group. Percival and Ross (1950) found a fixed percentage of sulphate in the polysaccharides isolated from a few species of brown seaweeds, *viz.*, *Fucus vesciculosus*, *F. spiralis*, *L. cloustoni* and *H. lorea*. Ross (1953) in his analysis of the red seaweeds of Great Britain observed wide variations in the sulphate content of the various species, the minimum figure being associated with a low galactose content or with none. The sulphate increases to a figure representing approximately one sulphate group per hexose unit. It was further noticed that species containing any appreciable amount contained galactose as the primary sugar showing that these two are interrelated.

Takagi and Susuki (1952) have shown that sulphur containing amino acids are present in the seaweeds investigated by them. The presence of cystine—a sulphur containing amino acid—was recognised in all marine algæ which they studied—the quantity being largest in brown algæ, next in greens and least in reds.

In the present communication results of an attempt to study the partition of sulphur in eleven common seaweeds of the Indian coast in relation to their polysaccharide content and distribution are presented.

The species studied, the details of collection and the method of preparation of the sample for analyses have been dealt with in an earlier paper (Krishna Pillai, 1956).

METHODS OF ANALYSES

Total sulphur.—The method of Aitken (1930) was employed in the estimation of total sulphur in the seaweeds, Benedict-Denis reagent being used and the sulphur precipitated as $BaSO_4$. Dried seaweed samples, not more than 0·5 g. for each estimation, are accurately weighed and used.

Total sulphate.—One gram of the air-dried material was ashed carefully in a silica crucible without allowing the material to melt. After cooling the ash was taken up with water and filtered. The solution was heated to boiling point and sulphate was precipitated as barium sulphate with boiling barium chloride.

Ionic sulphate.—One gram each of the sample was taken and powdered with acid-washed sand. The material was taken up with glass distilled water and stirred continuously for an hour with a stirrer. The mixture was centrifuged and the clear extract used for the precipitation of sulphate.

Invert sugar.—One gram of the air-dried sample was macerated with glass distilled water and transferred to a beaker. The contents were stirred continuously for sometime and the resultant mixture centrifuged. To the clear solution was added 5 c.c. of 38% hydrochloric acid and the flask was placed in a water-bath maintained at 70° C. The solution was kept at 69° C. for 8 minutes. After cooling quickly the solution was neutralised with sodium hydroxide and treated with Fehling's solution. The Fehling's solution was prepared by mixing 5 c.c. of copper sulphate and 5 c.c. of alkaline tartrate solution. The mixture was diluted to about 50 c.c. and heated in a regulated flame so that boiling began in four minutes. The boiling was continued for three more minutes and the precipitated copper oxide filtered through a weighed scintered crucible lined on the inside with asbestos mat which was previously treated. The precipitate that collected on the asbestos was washed with 95% alcohol three times followed by three washings with ether. The washed precipitate was dried at 105° C. for 2 hours and weighed.

Other cold water-soluble polysaccharides.—The sample was treated as before for the estimation of invert sugar. After removal of the water-soluble proteins by precipitation the extract was hydrolysed with 6N hydrochloric acid for 4 hours. After hydrolysis the solution was cooled, neutralised with sodium hydroxide and filtered. The filtrate was treated with 10 c.c. of Fehling's solution as indicated above and boiled. The precipitated copper oxide was filtered, washed and weighed.

Hot water-soluble polysaccharides.—One gram of the sample was ground well with water and heated for four hours on a boiling water-bath. This was filtered hot and the residue was extracted again. The clear extracts are combined and hydrolysed with 6N hydrochloric acid. As before the reducing sugars are estimated on aliquotes of the hydrolysates.

Total carbohydrate.—One gram of dried sample was ground well with acid-washed sand and the entire mixture was taken up with distilled water, and transferred to the apparatus for hydrolysis. The requisite quantity of acid was added and the hydrolysis allowed to proceed for about 24 hours. The solution was filtered, cooled and the reducing sugars estimated on a i-quotes as indicated before.

The values for the total, cold water-soluble and hot water-soluble carbohydrates and that for the fraction of sulphur in organic combination in the form of SO_4 and other forms for the different seaweeds during the 12 months of collection are given in Tables I to VIII. The seasonal variations in the ionic sulphur, total sulphate and total sulphur are represented in Figs. 1 to 11. The total agar content, the invert sugar corresponding to the hot water-soluble carbohydrate and the fraction of SO_4 that has gone into organic combination are plotted in Figs. 12 to 17.

Discussion of Results

Ionic sulphate in the algæ represents the first stage of absorption of the element sulphur from water. It may be seen form Figs. 1 to 11 that the quantity of ionic sulphate varies within wide range depending on the type of the alga and the time of collection, and sometimes it constitutes more

TABLE I

Sulphur and sugar contents in seaweeds collected during April, 1952

Name of Species	Sugar Content			Hot water-soluble	Sulphur	
	Total Sugar	Cold water-soluble Simple	Cold water-soluble Total		SO_4 in organic combination	S in other forms of combination
C. linum ..	16·16	1·10	2·00	6·86	1·11	1·09
G. lichenoides ..	18·96	0·80	2·48	13·66	2·91	0·76
C. dasyphylla ..	14·00	0·18	2·16	10·98	1·86	1·62
E. intestinalis ..	14·70	Nil	1·20	6·36	2·01	1·95
A. spicifera ..	14·30	1·00	3·45	7·15	1·55	0·58
L. papillosa ..	12·12	0·54	2·70	5·00	1·61	1·85
H. musciformis ..	12·00	Nil	2·40	8·00	0·96	1·26
S. filiforme ..	16·89	0·90	2·60	9·80	1·95	1·32
S. furcellatum ..	11·40	0·75	2·00	8·90	1·30	0·75
R. intricata ..	13·70	0·88	3·60	3·80	0·35	0·07
P. australis ..	10·75	1·50	2·40	2·45	0·32	0·93

TABLE II

Sulphur and sugar contents in seaweeds collected during May, 1952

Name of Species	Sugar Content			Hot water-soluble	Sulphur	
	Total Sugar	Cold water-soluble Simple	Cold water-soluble Total		SO_4 in organic combination	S in other forms of combination
C. linum ..	15·00	1·00	1·21	6·49	1·89	0·36
G. lichenoides ..	17·80	Nil	2·32	11·60	3·15	0·92
C. dasyphylla ..	12·94	0·50	1·61	9·20	2·02	0·14
E. intestinalis ..	21·40	1·00	1·87	7·15	1·88	0·87
A. spicifera ..	14·66	0·54	2·86	6·70	1·65	1·15
L. papillosa ..	12·94	Nil	3·59	4·96	1·89	1·81
H. musciformis ..	8·72	Nil	1·25	8·25	1·65	1·08
S. filiforme ..	13·74	0·75	1·65	8·40	..	..
S. furcellatum ..	16·48	Nil	1·34	8·18	1·60	1·00
R. intricata ..	15·20	Nil	4·69	5·20	0·05	0·82
P. australis ..	5·43	Nil	2·77	3·32	0·01	0·65

TABLE III

Sulphur and sugar contents in seaweeds collected during June, 1952

Name of Species	Sugar Content			Hot water-soluble	Sulphur	
	Total Sugar	Cold water-soluble			SO_4 in organic combination	S in other forms of combination
		Simple	Total			
C. linum ..	14·55	0·40	1·07	5·89	0·91	0·05
G. lichenoides ..	..	Nil	..	..	2·72	1·31
C. dasyphylla ..	11·15	0·50	1·52	5·89	1·49	0·92
E. intestinalis ..	10·08	0·75	0·63	5·60	0·67	0·49
A. spicifera ..	19·70	0·61	1·95	8·25	1·23	1·51
L. papillosa ..	8·63	Nil	2·23	4·50	1·46	1·67
H. musciformis ..	9·40	Nil	1·83	6·80	1·41	1·92
S. filiforme ..	11·45	0·44	1·49	7·20	1·20	1·57
S. furcellatum ..	18·90	Nil	1·96	9·88	0·47	0·69
R. intricata ..	16·54	Nil	3·50	3·68	0·51	0·07
P. australis ..	9·67	Nil	3·13	4·32	Nil	1·27

TABLE IV

Sulphur and sugar contents in seaweeds collected during July, 1952

Name of Species	Sugar Content			Hot water-soluble	Sulphur	
	Total Sugar	Cold water-soluble			SO_4 in organic combination	S in other forms of combination
		Simple	Total			
C. linum ..	16·17	0·43	1·00	6·30	0·42	0·51
G. lichenoides ..	15·17	Nil	0·89	9·20	2·15	1·07
C. dasyphylla ..	13·24	0·60	0·70	9·01	1·60	0·88
E. intestinalis ..	14·76	0·42	1·23	8·00	0·84	0·40
A. spicifera ..	13·14	Nil	0·89	8·01	1·29	1·16
L. papillosa ..	13·20	Nil	1·20	7·80	1·38	1·67
H. musciformis ..	12·34	0·62	0·80	6·70	1·96	1·57
S. filiforme ..	15·00	0·60	0·76	9·40	0·99	0·89
S. furcellatum ..	17·00	Nil	2·41	10·08	1·63	0·95
R. intricata ..	7·00	Nil	1·20	1·46	0·29	0·71
P. australis ..	9·20	Nil	2·30	2·68	0·36	1·15

TABLE V

Sulphur and sugar contents in seaweeds collected during August, 1952

Name of Species	Sugar Content			Hot water-soluble	Sulphur	
	Total Sugar	Cold water-soluble			SO_4 in organic combination	S in other forms of combination
		Simple	Total			
C. linum	12·37	1·08	1·80	6·40	0·25	1·62
G. lichenoides	14·00	0·80	1·64	8·20	2·00	1·35
C. dasyphylla	8·25	0·50	1·34	3·59	2·71	1·14
E. intestinalis	12·08	1·18	2·61	8·00	0·51	1·21
A. spicifera	13·14	0·80	1·00	7·30	1·49	0·78
L. papillosa	10·37	1·08	2·23	6·17	1·44	1·46
H. musciformis	12·00	1·20	2·68	8·25	1·59	2·14
S. filiforme	13·00	0·70	1·20	7·00	1·46	0·63
S. furcellatum	17·88	1·47	3·13	8·25	1·39	0·24
R. intricata	11·30	1·68	2·80	3·10	0·11	0·62
P. australis	12·70	Nil	3·60	3·65	0·66	0·82

TABLE VI

Sulphur and sugar contents in seaweeds collected during September, 1952

Name of Species	Sugar Content			Hot water-soluble	Sulphur	
	Total Sugar	Cold water-soluble			SO_4 in organic combination	S in other forms of combination
		Simple	Total			
C. linum	13·56	1·00	2·00	6·00	0·06	0·28
G. lichenoides	14·10	1·20	2·50	8·70	1·54	0·89
C. dasyphylla	8·34	1·60	4·41	6·80	1·01	1·63
E. intestinalis	12·48	0·98	2·70	8·30	0·41	0·22
A. spicifera	12·00	0·90	2·30	6·80	1·18	0·88
L. papillosa	12·54	0·64	0·89	4·80	0·24	2·84
H. musciformis	14·76	0·43	0·89	5·40	1·82	0·31
S. filiforme	12·36	1·00	1·23	5·40	0·85	0·79
S. furcellatum	7·30	1·12	2·68	6·93	0·95	0·67
R. intricata	13·14	1·64	3·59	4·40	0·16	0·44
P. australis	15·17	0·80	3·89	4·20	0·70	0·69

TABLE VII

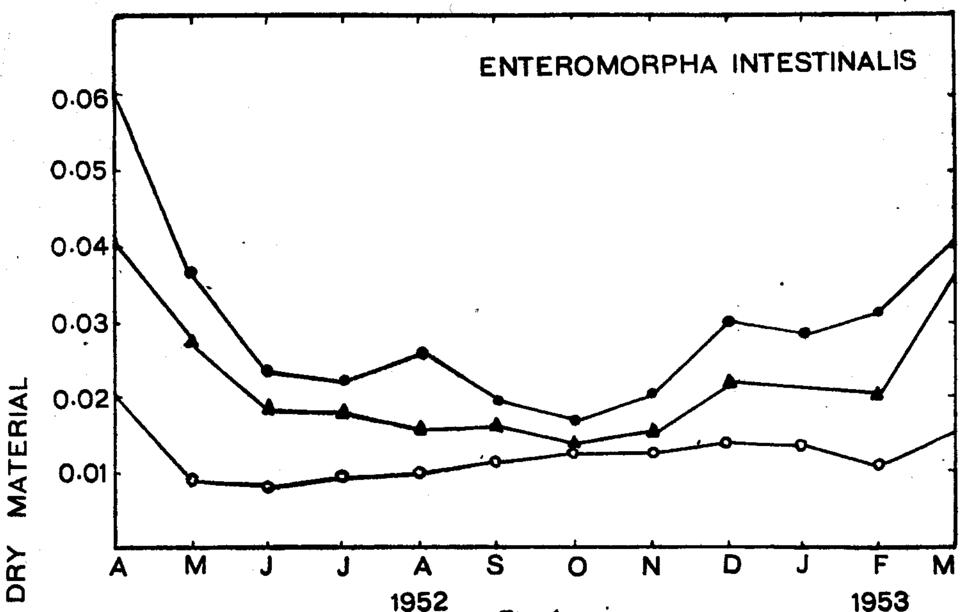
Sulphur and sugar contents in seaweeds collected during December, 1952

Name of Species	Sugar Content			Hot water soluble	Sulphur	
	Total Sugar	Cold water-soluble			SO_4 in organic combination	S in other forms of combination
		Simple	Total			
C. linum ..	13·06	..	1·89	5·83	0·32	0·23
G. lichenoides ..	13·10	1·23	3·20	6·40	0·98	1·16
E. dasyphylla ..	7·30	1·05	4·50	7·20	1·19	2·13
C. intestinalis ..	15·17	1·03	4·04	6·80	0·83	0·80
A. spicifera ..	17·00	0·83	1·34	5·56	1·36	0·80
L. papillosa ..	14·23	0·94	1·79	5·00	0·96	0·36
H. musciformis ..	5·43	Nil	Nil	2·40	0·32	0·94
S. filiforme ..	12·00	1·86	3·00	6·20	0·52	1·36
S. furcellatum ..	12·14	2·00	3·04	7·80	0·40	0·78
R. intricata ..	16·18	0·93	3·80	3·90	0·09	0·18
P. australis ..	16·00	1·00	3·70	4·20	0·20	0·29

TABLE VIII

Sulphur and sugar contents in seaweeds collected during March, 1953

Name of Species	Sugar Content			Hot water-soluble	Sulphur	
	Total Sugar	Cold water-soluble			SO_4 in organic combination	S in other forms of combination
		Simple	Total			
C. linum ..	14·16	1·09	2·20	7·09	1·18	1·06
G. lichenoides ..	22·48	0·73	2·40	15·35	0·94	1·44
C. dasyphylla ..	17·64	1·00	2·70	13·70	1·58	2·12
E. intestinalis ..	20·60	Nil	0·41	5·06	2·05	0·50
A. spicifera ..	11·86	0·50	1·06	6·08	1·20	0·66
L. papillosa ..	11·74	0·81	3·32	4·96	1·10	1·34
H. musciformis ..	10·76	0·80	1·05	8·82	1·13	0·37
S. filiforme ..	25·90	1·00	2·32	14·55	1·37	0·47
S. furcellatum ..	19·75	0·50	2·41	12·50	1·28	1·40
R. intricata ..	11·74	Nil	2·59	2·60	0·32	0·68
P. australis ..	11·15	Nil	1·58	1·64	0·07	0·28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0.05
0.04
0.03
0.02
0.01
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1952
1953

Fig. 1

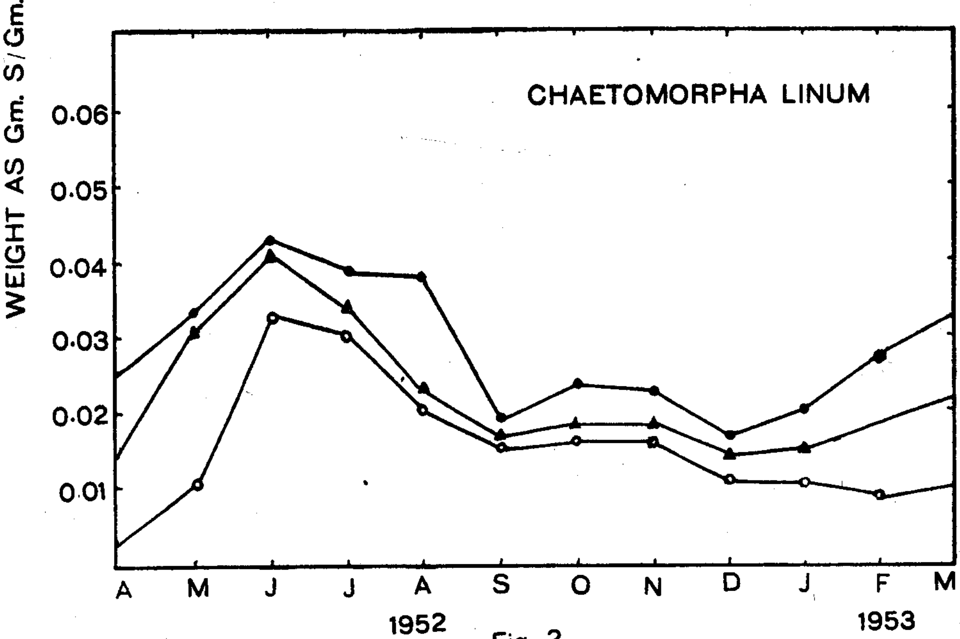
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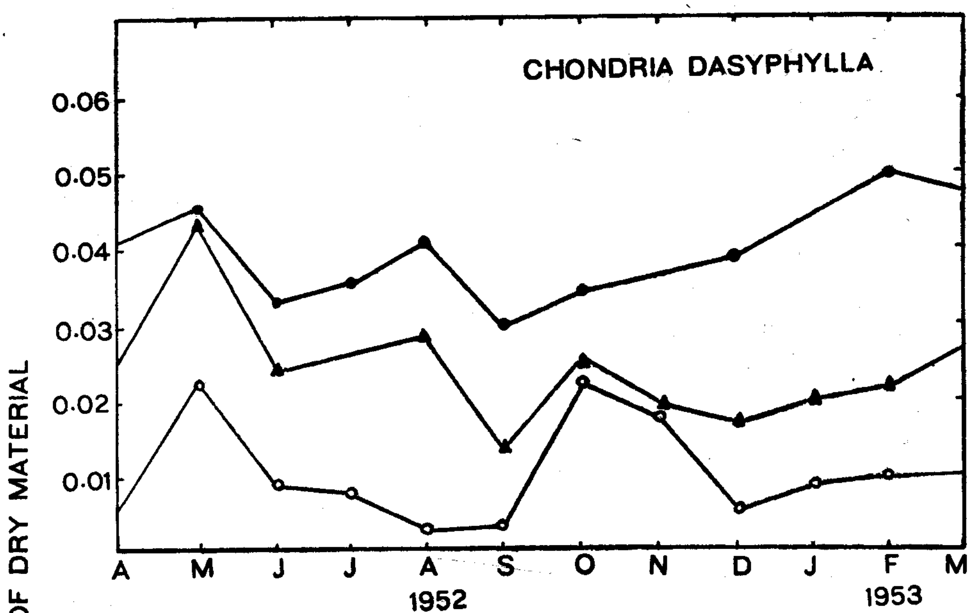
Fig. 2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0.01
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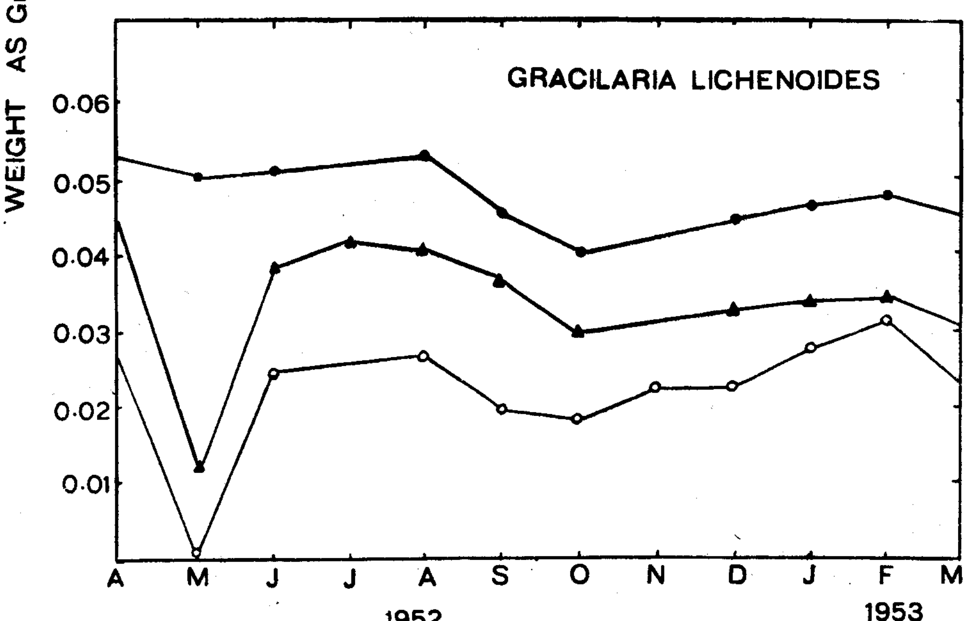
1952
1953

Fig. 3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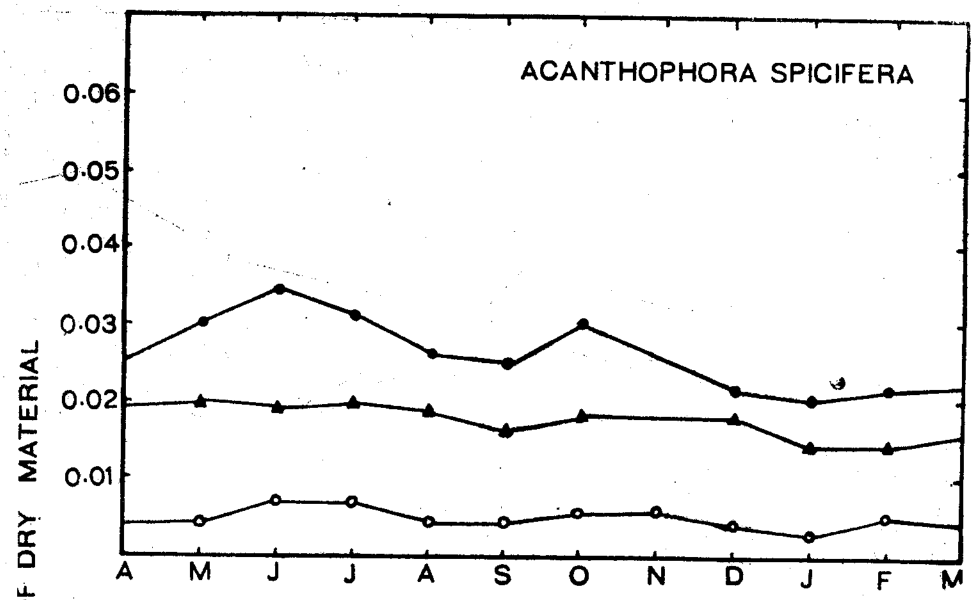
0.03
0.02
0.01
A M J J A S O N D J F M
1952
1953

Fig. 4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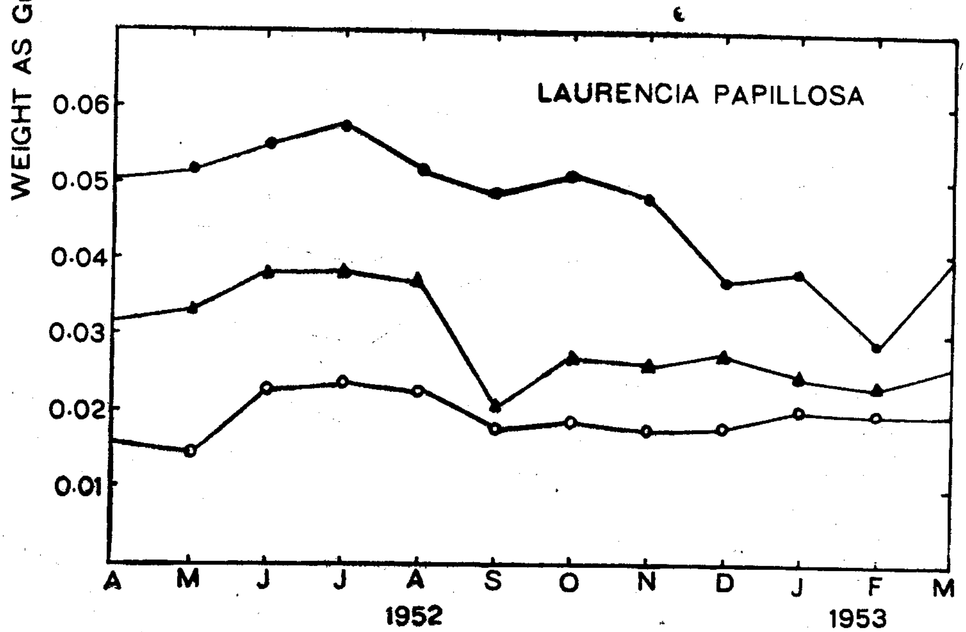
0.01
A M J J A S O N D J F M
1952
1953

Fig. 5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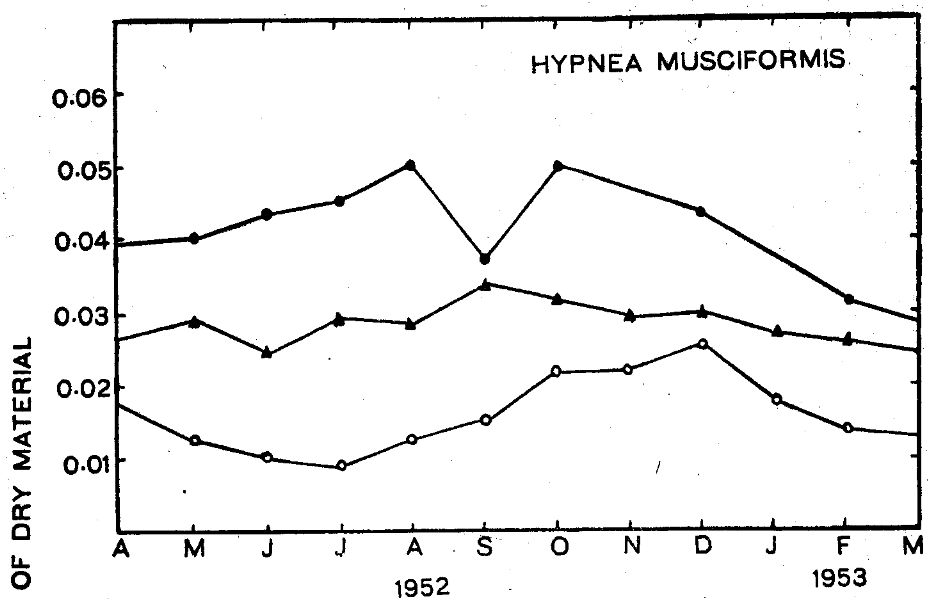
0.04
0.03
0.02
0.01
A M J J A S O N D J F M
1952
1953

Fig. 6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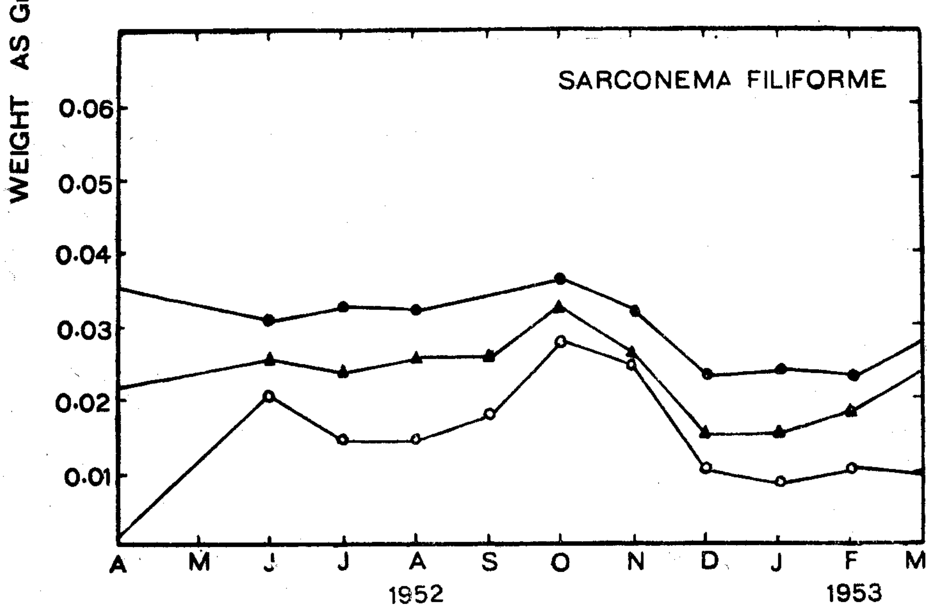
0.01
A M J J A S O N D J F M
1952
1953

Fig. 7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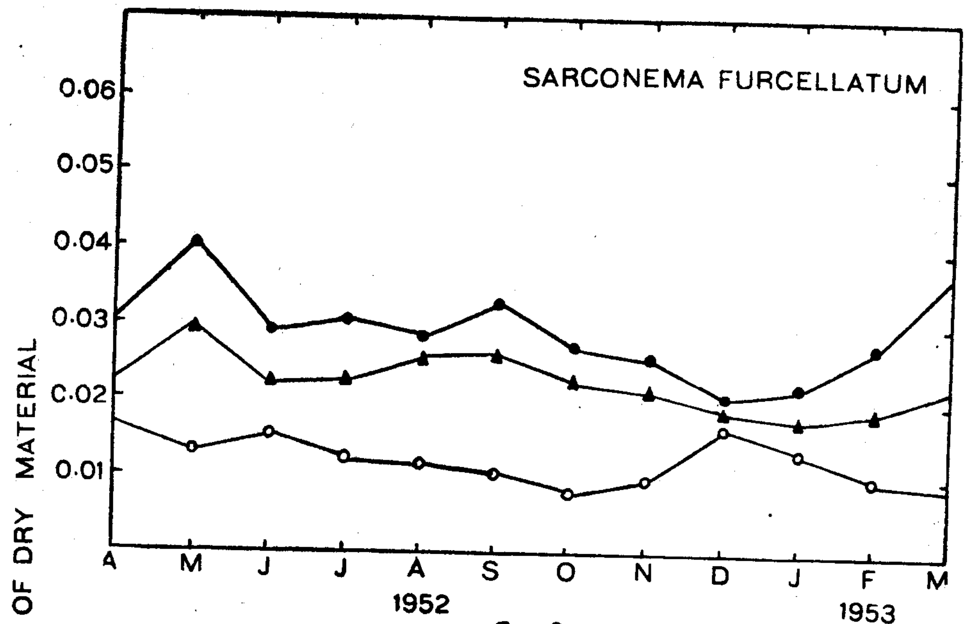
0.02
0.01
A M J J A S O N D J F M
1952
1953

Fig. 8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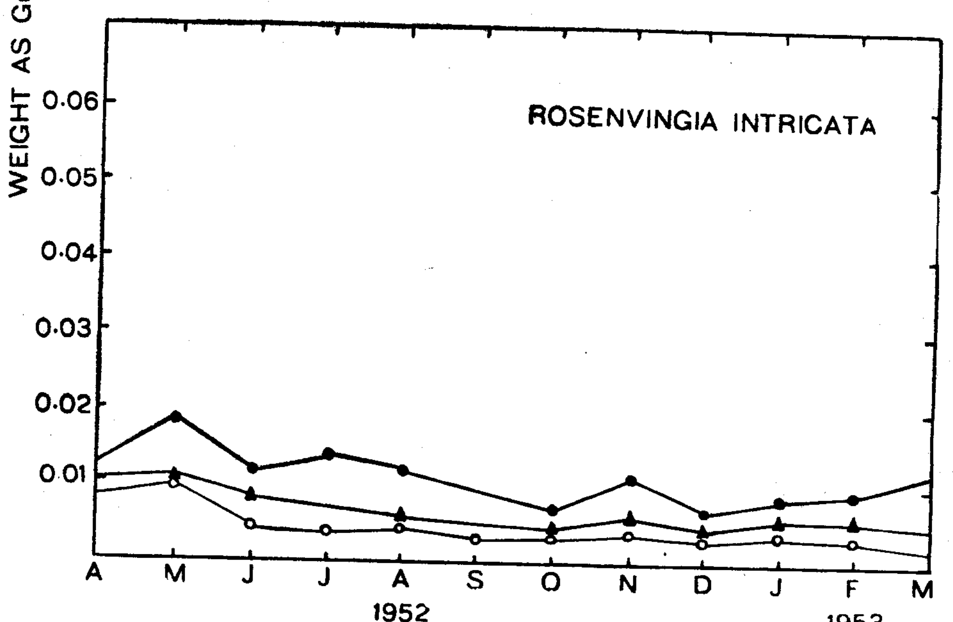
0·02
0·01
A M J J A S O N D J F M
1952
1953

Fig. 9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0·02
0·01
A M J J A S O N D J F M
1952
1953

Fig. 10

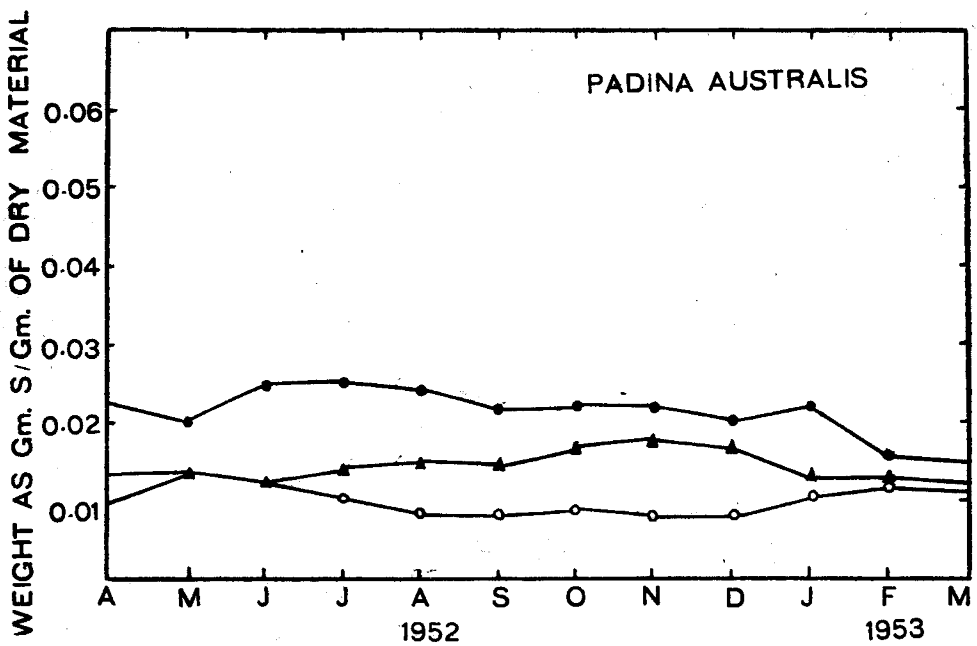
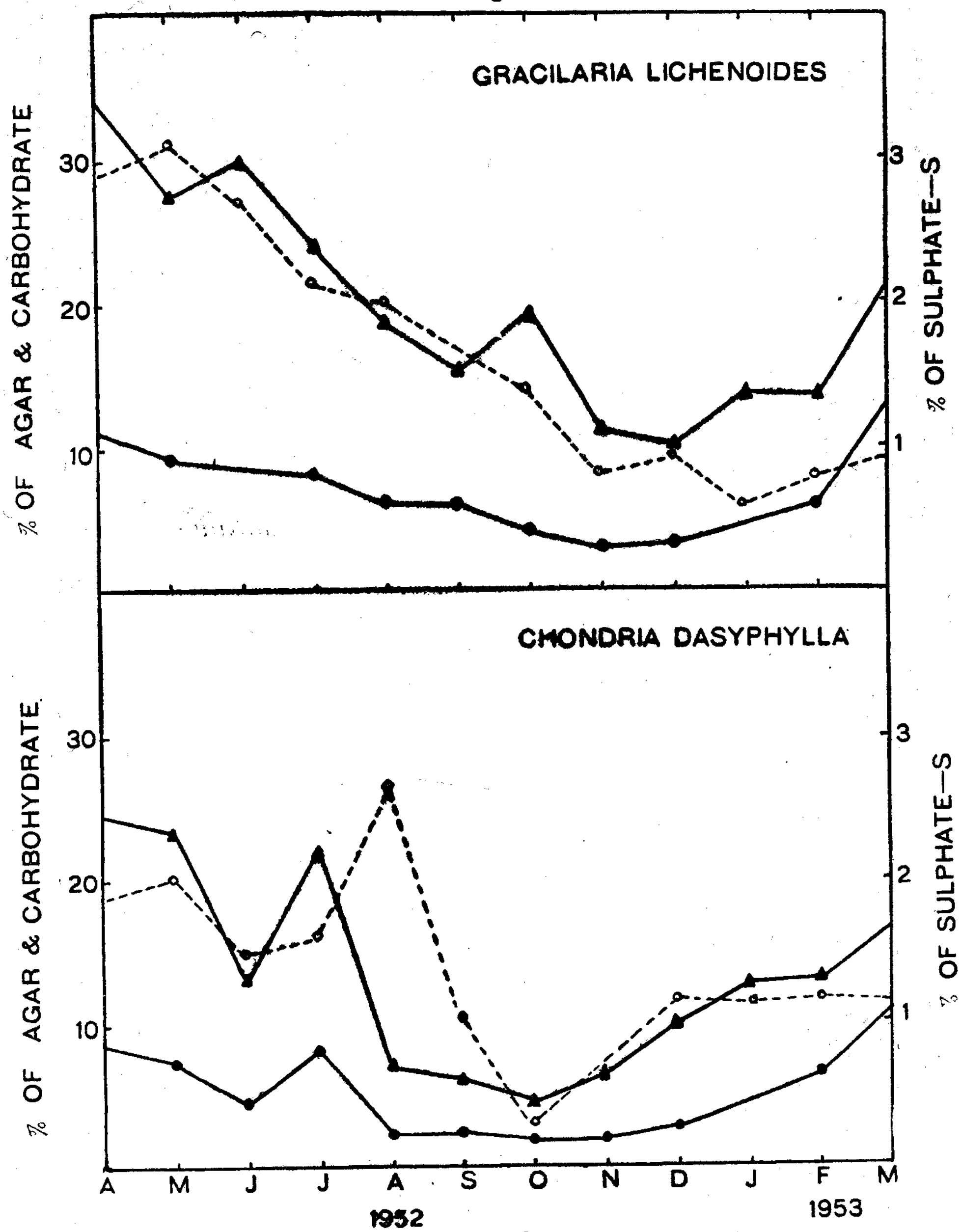


Fig. 11

than 70% of the total sulphur content of the algæ. For example in *C. linum* collected in June ionic sulphate constitutes 76·3% of the total sulphur, while in *G. lichenoides* collected in April and *A. spicifera* collected in May the corresponding figures are 50·7% and 52·2% respectively. Further in the collection made after September when the algæ are in the young stage the major portion of the sulphur is made up of the free ionic sulphate. ncidentally this increase in the absorption of SO_4 may be responsible for the less absorption of Cl, accumulation of which is considered injurious to the algæ. The difference between the ionic and the total sulphate gives the fraction that is in combination with the carbohydrate of the algæ. Similarly the difference between the total sulphate sulphur and the total sulphur gives the amount of sulphur that has gone into other forms of combinations—probably in the protein fraction of the algæ as sulphur-containing amino acids. The variations in these fractions of sulphur are quite distinct and can be correlated with the different growth stages of the plants and their organic constituents. Thus for example in *C. dasyphylla* there is a definite increase in the "protein sulphur" content corresponding to an increase in the total nitrogen content (Krishna Pillai, 1957) reaching a maximum in February, thereafter decreasing suddenly until the difference becomes least in May when the nitrogen content also falls to a minimum. The SO_4

Fig. 12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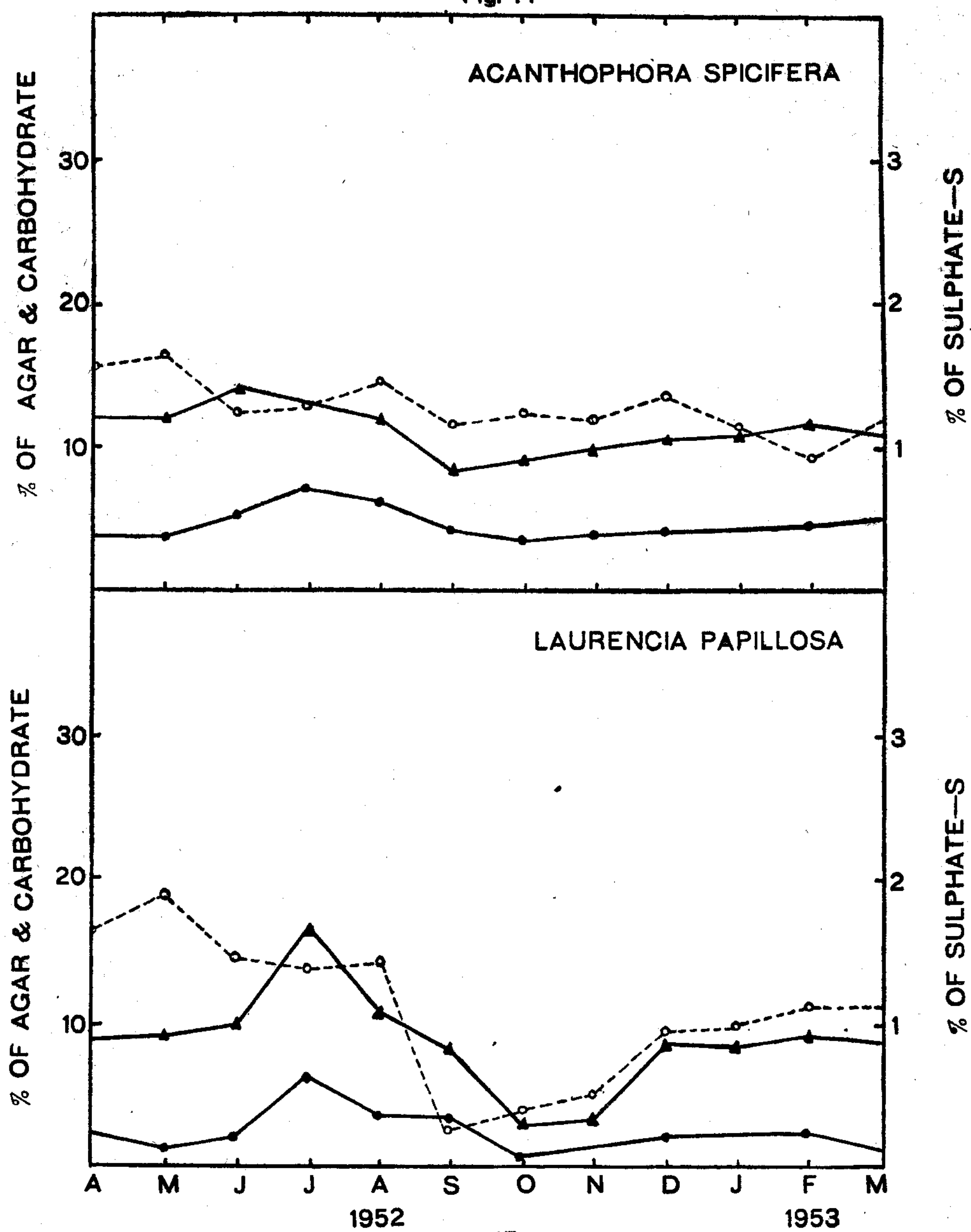
CHONDRIA DASYPHYLLA
% OF AGAR & CARBOHYDRATE
% OF SULPHATE—S
30
20
10
3
2
1
A
M
J
J
A
S
O
N
D
J
F
M
1952
1953

Fig. 13

Fig. 14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% OF AGAR & CARBOHYDRATE
% OF SULPHATE—S
10
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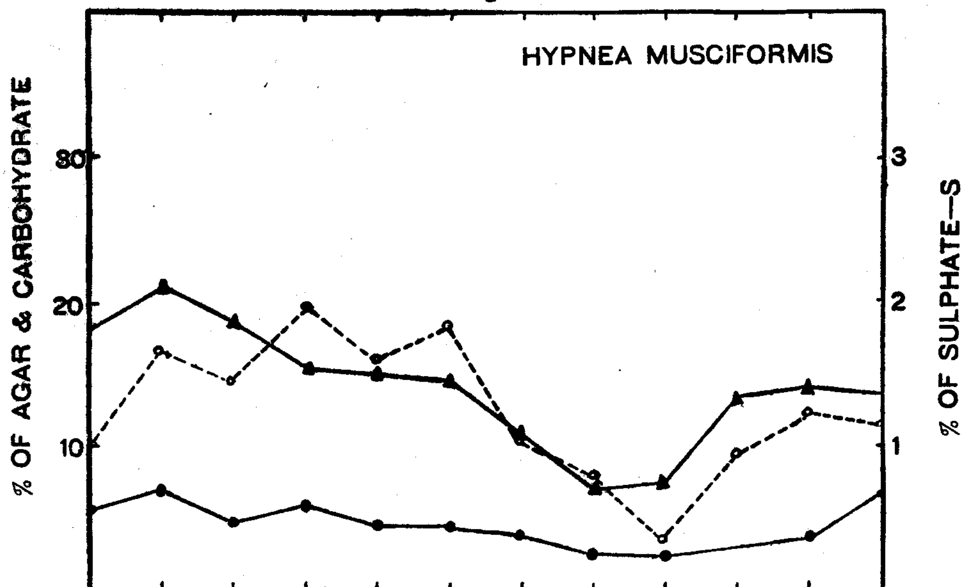
30
1
2
3
LAURENCIA PAPILLOSA
% OF AGAR & CARBOHYDRATE
% OF SULPHATE—S
10
20
30
1
2
3
A M J J A S O N D J F M
1952
1953

Fig. 15

Fig. 16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% OF SULPHATE—S
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2
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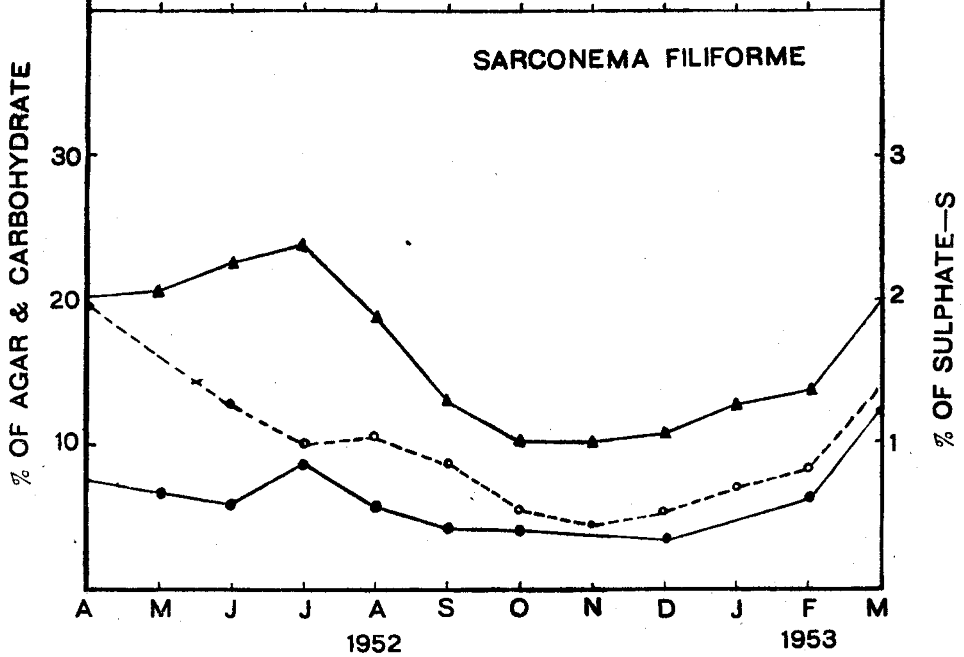
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% OF AGAR & CARBOHYDRATE
30
20
10
% OF SULPHATE—S
3
2
1
A M J J A S O N D J F M
1952
1953

Fig. 17

fraction in organic combination along with the carbohydrate also follows a somewhat parallel course but in the reverse order. In October–November the amount of SO_4 in organic combination is a minimum but it increases from that period until it gives the maximum values during the months of May–August. This period of maximum corresponds to the time when the alga becomes mature and when it contains the minimum amount of nitrogen. It may be seen from Figs. 12 to 17 that the agar content of the red seaweeds also follows the same cycle of changes giving minimum values in October–November and maximum in March–July. In the case of *G. lichenoides* the variations in the different fractions are almost similar to that found in *C. dasyphylla* except that the protein fraction is at a maximum in May even though May–August is the period when the total nitrogen content is a minimum. It is interesting to note that the more the absorption of the SO_4 into the carbohydrate molecule the less is the total nitrogen content in the ed seaweeds especially in the agarophytes. It may also be noted that SO_4 fraction in organic combination is at a minimum in *G. lichenoides* during the months of December–February, thereby showing that the agar content during this initial stages of growth of the alga will be low. This is found to be true when the values for the agar content of the species for the above period is examined (Fig. 12). In the case of *A. spicifera,* and *L. papillosa* there is not much of a seasonal cycle in the SO_4 content. The SO_4 in organic combination does not show any significant variations (Fig. 5) and likewise the agar content also stands very low (variation between 8·6 and 14·3% in *A. spicifera* and 3·0 and 16·8% in *L. papillosa*) though maximum values are obtained during may–July. In the case of these two algæ most of the sulphur remains either in the protein fraction or in the water-soluble form itself throughout the period. In *H. musciformis*, *S. filiforme* and *S. furcellatum* also the minimum values for the SO_4 in organic combination is met with in December, when the plants are young while this fraction increases slowly afterwards (corresponding to a decrease in the ionic SO_4) giving maximum values during April–August. During December most of the SO_4 will invariably be in the free, water-soluble form; but the proportion goes down afterwards.

In the two species of Chlorophyceæ studied most of the sulphur is present in the ionic form throughout the different stages of growth. The differences between the total sulphate and the ionic sulphate are comparatively very little. This indicates that although sulphate is accumulated in the green species it does not enter into organic combination as sulphate. But normal accumulation of sulphate may be necessary for the ionic equilibrium in the cells as well as for the synthesis of sulphur-containing amino acids.

The brown species *R. intricata* and *P. australis* stand separate in their sulphur metabolism, as may be seen from the tables and the Figs. (10 and 11). The total sulphur content in these two species is very low compared to the green and the red species mentioned above. Further there is practically no difference between the ionic sulphate and the total sulphate thereby indicating that sulphur does not go into organic combination in the form of SO_4 in these algæ. But a portion of the small quantity of the sulphur seems to undergo other forms of organic combination, probably as amino acids. It may also be seen from the figures that generally the total sulphur is greater in red and green seaweeds, the highest amount observed being 6·0% on dry weight basis in *E. intestinalis*. In *C. dasyphylla* the total sulphur varies between 3·2 and 5·0%, while the corresponding figures for the other species are as follows: *G. lichenoides*, 4·0 and 5·3%; *L. papillosa*, 2·8 and 5·7%; *A. spicifera*, 2·0 and 3·4%; *H. musciformis*, 2·8 and 5·0%; *S. filiforme*, 2·3 and 3·6%; *S. furcellatum*, 2·0 and 4·0%; *R. intricata*, 0·9 and 1·9% and *P. australis* 1·5 and 2·5%.

Tables I to VIII also give the values on the sugar content in the various fractions of the different seaweeds. The data show that the total sugar in all the species gives a maximum in April, May and June, the values decreasing afterwards until they give minimum values in December. In February the values again rise up. The maximum values have been recorded in the agarophytes (giving figures of 22·48% of invert sugar on the dry weight basis). In such of those red species as *A. spicifera* and *L. papillosa* which are known to be poor sources of agar the total reducing sugar in the algal hydrolysates is found to be very low.

The values for the simple sugars are obtained by inversion of the free sugars in the cold water-soluble portion of the algæ. Often very low values (maximum about 1% invert sugar) are obtained with maximum during the young stages of the plants. This indicates that in the young seaweeds simple sugars, the first products of photosynthesis, are present in larger quantities than in the older plants. As the plant grows more and more of the simple sugars are converted into higher polysaccharides.

From the Tables it is evident that a portion of the polysaccharides in the algæ comes out in cold water when the material is ground well with water. This fraction constitutes from 10 to 35% of the total sugars of the algæ. These are neither simple sugars, as they do not reduce Fehling's solution after simple inversion, nor complex molecules like agar, as agar is known to dissolve only in boiling water. However this fraction is a polysaccharide as it resolves into simple sugars on hydrolysis with acid. This fraction can therefore be considered as the second stage of the conversion of simple sugars into complex carbohydrate molecule. Here again the values are found to be maximum during the early statges of the growth of the plants. The minimum values are recorded in May–July when the algæ are mature.

The reducing sugars corresponding to the total hot water-soluble fraction are given in the tables while the amount corresponding to the portion which will dissolve only in hot water is plotted in the graphs (Figs. 12 to 18). The values for the hot water-soluble portion change to a very great extent according to different species, the season and the growth stages of the plants. The maximum values are seen in March–June while the minimum values are found in August–December. The red species generally give the highest values for the aobve fraction constituting nearly 50 to 60% of the total sugar content of the algæ, while in the case of brown species the difference between the cold water and hot water-soluble portions is quite insignificant, thereby showing that the higher carbohydrates formed in the brown seaweeds are insoluble even in hot water. This observation is true because alginic acid which is the major constituent of brown seaweeds is insoluble in hot water.

SUMMARY

Detailed investigations have been conducted on the partition of sulphur in different seaweeds common to the Indian coast. Among the seaweeds studied are two Chlorophyceæ, seven Rhodophyceæ and two Phæophyceæ. The amount of total sulphur, total sulphate and free sulphate in the different seaweeds were estimated periodically by analysing regular monthly collections. From this study it was also possible to follow the changes in the sulphur content of the algæ during the different stages of its growth and during different seasons. An attempt has also been made to bring out possible correlation between the different forms of sulphur and the carbohydrate content of the algæ.

It is observed that in the young plants most of the sulphur is present in the ionic form and that there is practically very little difference between the free sulphate and the total sulphate. As the algæ grow the difference between these two sulphate fractions increases steadily, except perhaps in the brown species, indicating that more and more sulphate passes from the free state into a state of combination with the organic compounds. It is also seen that the variation in the fraction of sulphate which is extracted only in hot water is directly proportional to the variations in the agar content of the seaweeds.

In the two species of Chlorophyceæ examined most of the sulphur exists in the free sulphate form throughout the year. Phæophyceæ contain only comparatively low amounts of sulphur and that practically no difference is noticed between the ionic sulphate and total sulphate.

The studies on the variations in the sugar content in the different fractions of the seaweed bring forth several interesting results. The total sugar content in the hydrolysate of the individual algæ shows a general maximum during the months April–June when the organic constituents are found to be maximum. Young specimens of almost all the seaweeds show varying amounts of simple reducing sugars in their cold water-soluble portions, indicating probably that the first products of photosynthesis in growing young seaweed are the simple sugars. As the algæ grow these simple sugars are converted to higher forms and in the mature algæ they are present only in very minute quantities.

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EXPLANATION OF TEXT-FIGURES

FIGS. 1 to 11 ●——● Total Sulphur.

▲——▲ Total Sulphate—S.

○——○ Free ionic sulphate—S.

FIGS. 12 to 17 ●——● Invert sugar corresponding to hot water-soluble carbohydrate

▲——▲ Agar.

○- - - -○ Sulphate-S corresponding to hot water-soluble portions