

Seasonal variations in Na, K, Mg and Ca and charge balance in marine brown algae from Saurashtra coast (NW coast of India)

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Seasonal variation of Na, K, Mg and Ca were estimated in *Cystoseira indica*, *Sargassum tenerrimum* and *S. johnstonii*. Abundance of these elements were higher during the rapid growth stage but decreased subsequently. Monovalent cations (Na + K) strongly correlated with Cl content suggesting that these cations were balanced by chloride ions. Divalent cations (Ca + Mg) seem to be balanced by the anion sulphate. Ash content of the algae was accounted by these major cations and anions. Charge equivalent among these cations and anions was not balanced in the ash of the algae. The cations charge equivalence could be balanced by chloride and sulphate in addition to the anions of C, N and P origin (OH^- , RCOO^- , H_2PO_3^- etc.). A significant correlation ($P < 0.001$) between per cent of negative ions deficit and per cent of excessive unbalanced cations was observed. Perhaps 9-32% (on large data base upto 60%) of the organic matter participate in charge balance of cations. Intertidal position seemed to affect the osmoregulation process.

Though ash can be determined through a simple method, by nature it is a complex matrix of various salts. During ashing process organic matter gets oxidised, some salts form oxides and certain amount of volatile elements are lost¹. Most of the salts in ash might be present as ionic compounds. The ionic pairing of Na, K, Ca and Mg with respect to sulphate, bicarbonate and carbonate is well understood in seawater medium², whereas, in ash or tissue of marine macro algae the extent of ion pairing, if any, is not known. Marine algae exhibit high content of ash¹ mainly due to the presence of Na, K, Ca and Mg cations (Fe, Sr will be of minor importance) and Cl and SO_4 anions (P, Si are of minor importance), in order to grow in the high ionic strength medium of seawater. Osmoregulation in marine algae is mainly maintained by intrusion and extrusion of Na, K and Cl ions respectively, from seawater and cell sap; or by turgor pressure changes by variable ionic composition of vacuoles³. It has also been reported that dissolved carbohydrates such as polyols [$\text{R} - (\text{OH})_n$] play an important role in the osmoregulation process when alga is under stress⁴. Since the algae are electrically neutral, positive ions charge should be balanced by the negative ions charge. The chemical composition of marine macro algae with respect to major cations and anions is well documented^{1,5}. However, information on charge balancing between anions and cations in marine algae is scanty^{6,7}. In the present study an attempt has been made to study the mass balance between major positive ions (Na, K, Ca

and Mg) and negative ions (Cl and S) to an approximation in marine macro algal tissues. Since more than 95% of inorganic matter^{1,5} is constituted by Na, K, Ca, Mg, Cl and S only, other minor cations and anions were not considered in the present study.

Materials and Methods

Three species of marine brown algae *Cystoseira indica* (Thivy *et* Doshi) Mairh, *Sargassum tenerrimum* J. Ag. and *S. johnstonii* Setchell and Gardner (Phaeophyceae/Fucales) were collected from the intertidal regions of Porbandar (lat. $21^\circ 38''\text{N}$; long. $69^\circ 37''\text{E}$) and Okha (lat. $22^\circ 28''\text{N}$; long. $69^\circ 05''\text{E}$) over a period of one year (Oct. 1982 to Sept. 1983). A detailed description of the sampling sites, method of algae collection including their status of phenology, sample processing and analytical methodology have been discussed elsewhere⁸⁻¹¹. In brief, the estimation of alkali and alkaline metals involves the destruction of organic matter with acid digestion (nitric acid + perchloric acid + sulphuric acid) followed by flame photometric (Na and K) and atomic absorption spectrophotometric (Ca and Mg) analysis.

Sulphur was not analysed in the present study. However, it was calculated from the generalised value of S in Indian algae by using the C, N, P atomic ratio (Redfield ratio). The calculation was done through the estimation of expected C : N : P atomic ratio (here after atomic ratio as AR) which was calculated from observed⁹ and generalised⁵ C : N : P_{AR} as follows:

Expected $C_{AR} = [(Generalised C_{AR} - Redfield\ coefficient\ for\ C) / (Observed\ C_{AR} - Redfield\ coefficient\ for\ C)] \times Observed\ C_{AR}$

Similarly, expected N_{AR} was calculated. Expected S_{AR} from expected C_{AR} was calculated by

$$(10.6/108) \times Expected\ C_{AR}$$

Similarly, expected S_{AR} was also calculated from expected N_{AR} values by

$$(10.6/15.6) \times Expected\ N_{AR}$$

Percentage of S (average of C and N derived) was calculated from S_{AR} . In the above calculations Redfield coefficients^{12,13} were calculated from the C : N : P of 22.5 : 3.8 : 0.54. Generalised⁵ values of C, N, S, P_{AR} considered for the marine algae were 555 : 35.5 : 10.6 : 1. Since P and S positively correlated^{14,15} and in Redfield ratio C and N values are normalised to P, our attempt to estimate the expected S from C, N, P_{AR} is justified.

Results and Discussion

Contents of Na, K, Mg and Ca in algae were

maximum during Oct./Nov. in early growth stage of the algae (Fig. 1). Algae in the second fruiting stage (*C. indica* and *S. johnstonii*) exhibited relatively higher values for these metals during May. Whereas no definite trend was evident in other months. Data on S are comparable with that of Maru *et al.*¹⁶ for *Cystoseira* sp. and *Sargassum* spp. Sulphur did not vary much (Fig. 1E) whereas P varied widely⁹ in all the algae. However, wide variations in P and S were noted in *Nereocystis leutkeana*¹⁷ and *Laminaria digitata*¹⁸ but their seasonal pattern of S in relation to P is similar as it was noted in the present study. A positive correlation between P and S was observed ($P < 0.1$) in *Ulva* sp.¹⁴ and *S. ilicifolium*¹⁵. In the present study S correlated significantly with P in all the algae ($P < 0.1$).

Ash content was greater than the total sum of Na, K, Mg, Ca, Cl and S. The net deficit ranged from 5.2 to 21.5% (Table 1). For brown algae it was a maximum of 6% in Oct. during early growth period, and to a minimum of 3.5% in July, during senescent period. This is in agreement with the reported data for brown

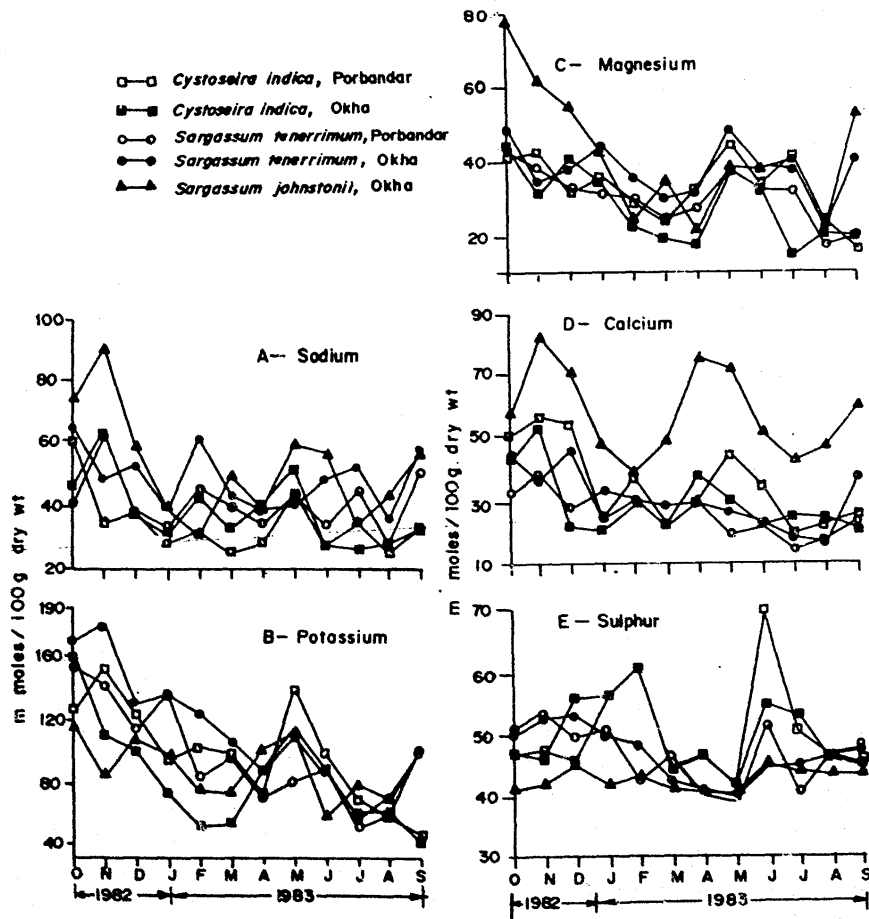


Fig. 1—Major cations and anions in marine brown algae: A) sodium, B) potassium, C) magnesium, D) calcium and E) sulphur

Table 1—Proximate mass balance of cations and anions in marine macro algae (I—Percent of excessive ions based on ash; II—Percent of excessive cations; III—Percent of excessive unbalanced cations)

% dry wt. basis							mmoles/100 dry wt.		% -ve ions deficit	Macro algae/ algal groups	Ref.
Total cations	Total anions	(Ash)-(total ions)	*(Corrected ash)-(total ions)	I	II	III	Total cations	Total anions			
6.33	5.65	5.02	1.39	8.21	4.34	0.47	187	164	12	<i>Cystoseira indica</i>	Present study**
7.06	5.37	4.14	0.64	3.86	2.20	0.53	211	156	26	<i>Sargassum tenerrimum</i>	"
7.97	5.59	4.88	1.51	8.18	4.81	1.44	240	162	33	<i>S. johnstonii</i>	"
6.95	5.52	4.64	1.12	6.54	3.64	0.75	207	160	23	Mean marine brown algae	"
18.89	16.10	5.24	2.72	6.75	3.65	0.54	613	457	25	<i>Nereocystis luetkeana</i>	17,19
13.46	9.19	8.57	5.99	19.18	11.40	3.61	435	261	40	<i>Sargassum ilicifolium</i>	18
11.08	10.05	6.27	5.31	19.39	10.17	0.95	326	285	13	<i>Palmaria palmata</i>	21
8.77	4.74	14.21	8.16	29.46	19.13	8.79	250	142	43	<i>Monostroma</i> sp.	22
7.47	3.76	21.52	17.58	53.68	35.71	17.73	275	111	60	Chlorophyceae	5,23***
9.35	5.25	15.33	11.70	39.08	25.03	10.98	283	153	46	Phaeophyceae	5,23***
9.71	5.78	5.86	1.95	9.12	5.72	2.31	321	168	48	Phaeophyceae	1
5.25	2.43	12.60	9.12	44.95	30.73	16.51	176	73	59	Rhodophyceae	5,23***
6.52	3.37	19.49	15.75	53.61	35.34	17.08	215	100	54	Total algae	5,23***

*Corrected ash = (ash)-(oxide portions of Mg and S)

**Ash and chloride values have been taken from ref. Nos 10 and 11 respectively

***Ash content of Chlorophyceae, Phaeophyceae, Rhodophyceae and total algae have been taken from ref. No. 23.

algae^{1,17,19}. The net deficit of ash due to the loss of major ions was greater than that due to minor cations and anions. Therefore, it is possible that during ashing SO_4 would not decompose and Mg might have converted to MgO . When S and Mg are converted to SO_4 and MgO the above deficit reduced marginally, to 1.9-17.6%. The corrected ash contribution to total ions deficit was also maximum in Oct. (2.2%) and minimum in July (0.1%). It seems in the present study that total ions to corrected ash [corrected ash = ash-oxide portions of Mg and S] were almost balanced for *C. indica*, *S. tenerrimum* and *S. johnstonii*, still the gap is wide for major groups of algae (Table 1). Therefore, charge equivalent term was invoked in the mass balance of ions. However, when the cations and anions were converted into equivalent charges, since the reactants participate in equivalents in chemical reactions, a net negative charge deficit of 12-60% was observed (Table 1). However, a minimum (9.5%) was observed in Jan. during the end of reproductive stage, and a maximum (32.9%) was observed in Oct., during the early growth period (Fig. 2).

Generally, $(\text{Na} + \text{K}) : \text{Cl}$ was > 1 . Similarly, $(\text{Ca} + \text{Mg}) : \text{S}$ and also $(\text{Na} + \text{K} + \text{Ca} + \text{Mg}) : (\text{Cl} + \text{S})$ were > 1 . Therefore, the predominance of positively charged Na, K, Ca and Mg over negatively charged Cl and S ions in charge balance of algae could be seen. Univalent cations might have been balanced mainly

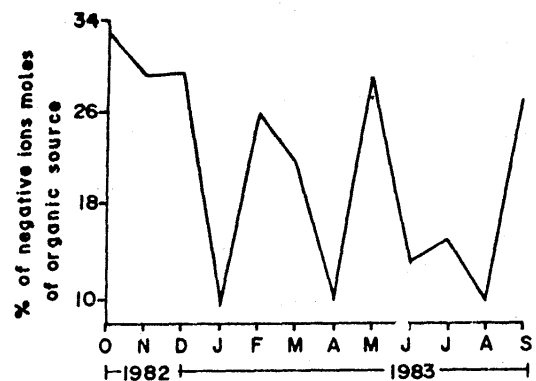


Fig. 2—Monthly variation in per cent moles of negative ions of organic source in association with per cent of excessive unbalanced cations in marine brown algae

by Cl ions ($P < 0.001$). The above ratios in different phenological stages of algae are depicted in Fig. 3. $(\text{Na} + \text{K})/\text{Cl}$ did not vary much during the different life stages in each plant while total cation to anion ratio varied marginally showing higher values during early and senescent stages than that in reproductive stage.

The difference between corrected per cent ash and per cent total ions was termed as per cent excessive ions. The difference between excessive cations and anions presented in Table 1 as per cent of excessive unbalanced cations. A significant relationship ($P < 0.001$) between excessive unbalanced cations

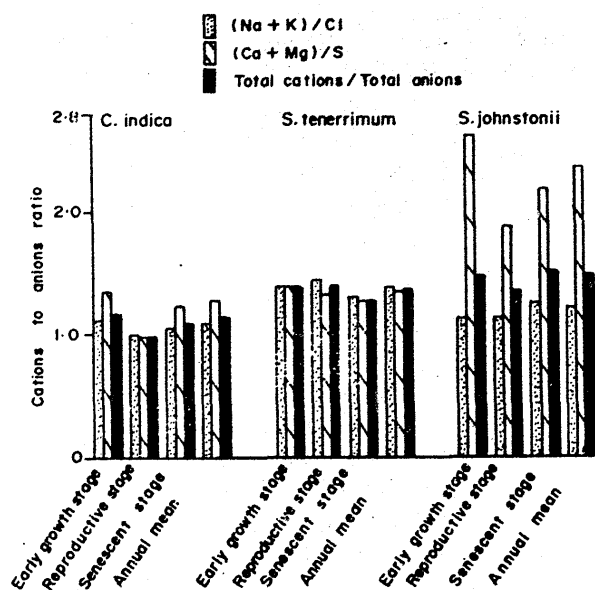


Fig. 3—(Na + K) : Cl, (Ca + Mg) : S and (Na + K + Mg + Ca) : (Cl + S) ratios during different phenological stages of marine brown algae

and the amount of moles deficit negative ions was observed (Fig. 4).

To balance the deficit negative ions with that of positive ions it is presumed that the deficit charge might arise from anionic moieties of dissolved organic constituents such as carbohydrates, amino acids and fatty acids etc. in order to maintain electrical neutrality of algal tissues. This is further supported by studies on high glycerol production in *Dunliella tertiolecta* grown in hyper saline waters to balance the osmoregulation process⁴ due to the intrusion of Na and K ions into cell sap; and alginic acid deposition by complexation with divalent metals through carboxylic groups in brown algae²⁰. Bhom⁷ reported an excess cations of 0.07 m equivalents/100 mg in a calcareous alga, *Halimeda opentia* with respect to cations of Na, K, Ca, Sr and anions of Cl, SO₄, PO₄ and CO₃ whereas, carbonate being the main anion to balance the positive ions. However, Munda⁶ reported excess anions in *Ascophyllum nodosum* and *Fucus vesiculosus*, alginic acid being the major anion in balancing the cations. It is also evident from the present study (Table 1, Fig. 4) that anions contribution from organic source is significant in balancing the cations in seaweed tissues.

In Chlorophyceae anions' contribution from organic ligands, i.e. negative ions deficit, was higher than that in Phaeophyceae and Rhodophyceae species (Table 1). In general, habitat of Chlorophyceae species is at upper intertidal region, whereas, Phaeophyceae and Rhodophyceae species occur in

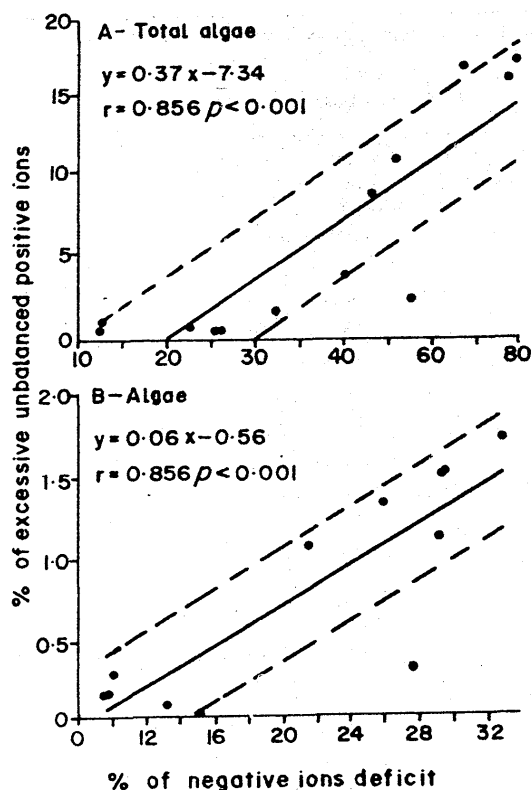


Fig. 4—Correlation between per cent deficit of negative ions and per cent of excessive unbalanced positive ions: A) total algae as in Table 1, B) seasonal mean of brown algae (solid line indicates the linear regression fit and dashed lines at $\pm 1 \sigma$ level about the fitted lines)

mid and lower intertidal regions and they are vulnerable to stress due to desiccation. To resist this environmental stress negative ions of organic nature might play an important role in the osmoregulation process.

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References

- 1 Vinogradov A P, *The elementary chemical composition of marine organisms*, (Sears Foundation for Marine Research, Yale University, New Haven) 1953, 68.
- 2 Garrels R M & Thompson M E, *Am J Sci*, 260 (1962) 57.
- 3 Lobban C, Harrison P J & Duncan M, *The physiological ecology of seaweeds*, (Cambridge University Press, Cambridge) 1985, pp. 242.
- 4 Craigie J S & McLachlan J, *Can J Bot*, 42 (1964) 177.
- 5 Kesava Rao Ch, *Indian J Mar Sci*, 21 (1992) 167.
- 6 Munda I, *Bot Mar*, 6 (1964) 158.
- 7 Bohm E L, *Bull Mar Sci*, 23 (1973) 177.
- 8 Kesava Rao Ch & Indusekhar V K, *Indian J Mar Sci*, 18 (1989) 37.

- 9 Kesava Rao Ch & Indusekhar V K, *Mahasagar - Bull Natn Inst Oceangr*, 22 (1989) 63.
- 10 Kesava Rao Ch, *Indian J Fish*, 41 (1994) 53.
- 11 Kesava Rao CH & Singbal S Y S, *Indian J Mar Sci*, 24 (1995) 137.
- 12 Redfield A C, Ketchum B H & Richards F A, in *The sea*, Vol. 2, edited by M N Hill, (John Wiley & Sons, New York) 1963. 26.
- 13 Bowen H J M, *Environmental chemistry of the elements*, (Academic Press, London) 1979, pp. 333.
- 14 Parekh R G & Chauhan V D, *Seaweed Res Util*, 5 (1982) 1.
- 15 Joshi G V & Gowda C A, *Indian J Mar Sci*, 4 (1975) 165.
- 16 Maru L V, Parekh R G & Lewis E J, *J Mar Biol Ass India*, 17 (1975) 241.
- 17 Whyte J N C & Englar J R, *Bot Mar*, 23 (1980) 19.
- 18 Floch J Y, in *Synthetic and degradative process in marine macrophytes*, edited by L M Srivastava, (Walter de Gruyter, Berlin) 1982, 139.
- 19 Whyte J N C & Englar J R, *Bot Mar*, 23 (1980) 13.
- 20 Percival E & McDowel R H, *Chemistry and enzymology of marine algal polysaccharides*, (Academic Press, London) 1967, pp. 219.
- 21 Black W A P & Woodward F N, *Empire J Exp Agri*, 25 (1957) 51.
- 22 Oza R M, Joshi H V, Parekh R G & Chauhan V D, *Indian J Mar Sci*, 12 (1983) 115.
- 23 Kesava Rao Ch (unpublished data).