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<sup>-</sup>, RCOO<sup>-</sup>, H<sub>2</sub>PO<sub>4</sub><sup>-</sup> 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 up to 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 lost<sup>1</sup>. 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 medium<sup>2</sup>, 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 ash mainly due to the presence of Na, K, Ca and Mg cations (Fe, Sr will be of minor importance) and Cl and SO<sub>4</sub> 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 vacuoles<sup>3</sup>. It has also been reported that dissolved carbohydrates such as polyols [R - (OH)<sub>n</sub>] play an important role in the osmoregulation process when alga is under stress<sup>4</sup>. 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<sup>1-5</sup>. However, information on charge balancing between anions and cations in marine algae is scanty<sup>6-7</sup>. 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<sup>1-5</sup> 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) Mair h, Sargassum tenerrimum J. Ag. and S. johnstonii Setchell and Gardner (Phaeophyceae/Fucales) were collected from the intertidal regions of Porbandar (lat. 21<sup>°</sup> 38'<sup>°</sup>N; long. 69<sup>°</sup> 37'<sup>°</sup>E) and Okha (lat. 22<sup>°</sup> 28'<sup>°</sup>N; long. 69<sup>°</sup> 05'<sup>°</sup>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 elsewhere<sup>8-11</sup>. 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 observed<sup>9</sup> and generalised<sup>5</sup> C : N : P<sub>AR</sub> as follows:
Expected $C_{AR} = (\text{Generalised } C_{AR} - \text{Redfield coefficient for } C)/(\text{Observed } C_{AR} - \text{Redfield coefficient for } C) \times \text{Observed } C_{AR}$

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

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

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

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

Percentage of $S$ (average of $C$ and $N$ derived) was calculated from $S_{AR}$. In the above calculations Redfield coefficients were calculated from the $C:N:P$ of 22.5 : 3.8 : 0.54. Generalised values of $C,N,S,P_{AR}$ considered for the marine algae were $55:35.5:10.6:1$. Since $P$ and $S$ positively correlated 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. for Cystoseira sp. and Sargassum spp. Sulphur did not vary much (Fig. 1E) whereas $P$ varied widely in all the algae. However, wide variations in $P$ and $S$ were noted in Nereocystis leutkeana and Laminaria digitata 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. and $S. ilicifolium$. 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

![Graphs showing Major cations and anions in marine brown algae: A) sodium, B) potassium, C) magnesium, D) calcium and E) sulphur](image-url)
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)

<table>
<thead>
<tr>
<th>% dry wt. basis</th>
<th>Total</th>
<th>Total</th>
<th>(Ash)</th>
<th>(total</th>
<th>*Corrected</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>Total</th>
<th>Total</th>
<th>% ve</th>
<th>Macro algae/</th>
<th>Ref.</th>
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<tr>
<td></td>
<td>cations</td>
<td>ions)</td>
<td>(total</td>
<td>ions)</td>
<td>ash)</td>
<td></td>
<td></td>
<td></td>
<td>cations</td>
<td>anions</td>
<td>deficit</td>
<td>algal groups</td>
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<tr>
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<td>5.65</td>
<td>5.02</td>
<td>1.39</td>
<td>8.21</td>
<td>4.34</td>
<td>0.47</td>
<td>187</td>
<td>164</td>
<td>12 Cystoseira indica</td>
<td>Present study**</td>
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<tr>
<td>7.06</td>
<td>5.37</td>
<td>4.14</td>
<td>0.64</td>
<td>3.86</td>
<td>2.20</td>
<td>0.55</td>
<td>211</td>
<td>156</td>
<td>26 Sargassum tenerrimum</td>
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<tr>
<td>7.97</td>
<td>5.59</td>
<td>4.88</td>
<td>1.51</td>
<td>8.18</td>
<td>4.81</td>
<td>1.44</td>
<td>240</td>
<td>162</td>
<td>33 S. johnstonii</td>
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</tr>
<tr>
<td>6.95</td>
<td>5.52</td>
<td>4.64</td>
<td>1.12</td>
<td>6.54</td>
<td>3.64</td>
<td>0.75</td>
<td>207</td>
<td>160</td>
<td>23 Mean marine brown algae</td>
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<tr>
<td>18.89</td>
<td>16.10</td>
<td>5.24</td>
<td>2.72</td>
<td>6.75</td>
<td>3.65</td>
<td>0.54</td>
<td>613</td>
<td>457</td>
<td>25 Nereocystis lentillae</td>
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<tr>
<td>11.08</td>
<td>10.05</td>
<td>6.27</td>
<td>5.31</td>
<td>19.39</td>
<td>10.17</td>
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<td>13 Palmaria palmata</td>
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<tr>
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<tr>
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<td>21.52</td>
<td>17.58</td>
<td>53.68</td>
<td>35.71</td>
<td>17.73</td>
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<td>111</td>
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<td>9.35</td>
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<td>15.33</td>
<td>11.70</td>
<td>39.08</td>
<td>25.03</td>
<td>10.98</td>
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<tr>
<td>9.71</td>
<td>5.78</td>
<td>5.86</td>
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<td>5.72</td>
<td>2.31</td>
<td>321</td>
<td>168</td>
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<td>5.25</td>
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<td>19.49</td>
<td>15.75</td>
<td>53.61</td>
<td>35.34</td>
<td>17.08</td>
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<td>100</td>
<td>54 Total algae</td>
<td>5,23***</td>
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</table>

*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.

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 SO₄ would not decompose and Mg might have converted to MgO. When S and MgO are converted to SO₄ 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, (Na + K) : Cl was > 1. Similarly, (Ca + Mg) : S and also (Na + K + Ca + Mg) : (Cl + 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 by Cl ions (P <0.001). The above ratios in different phenological stages of algae are depicted in Fig. 3. (Na + K)/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 and
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 moities 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 Dunaliella tertiolecta grown in hyper saline waters to balance the osmoregulation process due to the intrusion of Na and K ions into cell sap; and alganic acid deposition by complexation with divalent metals through carboxylic groups in brown algae. Bohm reported an excess cations of 0.07 m equivalents/100 mg in a calcareous alga, Halimeda opuntia 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, Munda reported excess anions in Apsophyllum nodosum and Fucus vesiculosus, alganic 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 intertidial region, whereas, Phaeophyceae and Rhodophyceae species occur in 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.

Acknowledgement
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