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Biochemical Composition of Marine Macroalgae from Gangetic Delta at the Apex of Bay of Bengal

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Abstract: Variations in protein, lipid, carbohydrate and astaxanthin content of *Enteromorpha intestinalis, Ulva lactuca* and *Catenella repens* were documented over a 10 months period from September 2007 to June 2008. The macroalgal species were collected from six sampling stations of Indian Sundarbans, a Gangetic delta at the apex of Bay of Bengal. On dry weight basis, the protein content varied from 4.15±0.02% (in *Catenella repens*) at Lothian to 14.19±0.09% (in *Catenella repens*) at Frasergaunge. The lipid content was low and varied from 0.07±0.02% (in *Enteromorpha intestinalis*) at Lothian to 1.06± 0.12% (in *Ulva lactuca*) at Gosaba. The level of carbohydrate was very high compared to that of lipid and protein and varied from 21.65± 0.76% (in *Catenella repens*) at Gosaba to 57.03± 1.63% (in *Enteromorpha intestinalis*) at Lothian. Astaxanthin values ranged from 97.73± 0.32 ppm (in *Catenella repens*) at Gosaba to 186.11± 2.72 ppm (in *Enteromorpha intestinalis*) at Frasergaunge. The values varied over a narrow range in the remaining stations. The results of biochemical composition of macroalgae seem to be strongly influenced by ambient hydrological parameters (surface water salinity, temperature and nitrate content) in the present geographical locale.

Key words: Marine macroalgae % Biochemical composition % Indian Sundarbans

INTRODUCTION

Macroalgal resources of the oceans and estuaries are found attached to the rocks, corals and other submerged strata in the intertidal and shallow subtidal zones. They are clearly sighted during low tide condition on the pneumatophores and trunks of mangroves. In marine ecosystems, macroalgae are ecologically and biologically important and provide nutrition and an accommodating environment for other living organisms [1-6]. Because of these properties, macroalgae are considered as one of the most important biotic components maintaining the ecosystem's stability [7].

The industrial applications of macroalgae are also varied. Their polysaccharides are used in food, cosmetics, paint, crop, textile, paper, rubber and building industries. In addition, they are used in medicine and in pharmacology for their antimicrobial, antiviral, antitumor, anticoagulant and brinolytic properties [8-10, 1, 11-16]. According to FAO, the annual global aquaculture production of marine algae is 6.5×10^6 tonnes [1]. Macroalgae have been harvested since long period in the Far East, where they are used in the food industry [7]. Regarding the economic importance of macroalgae, a wide variety of essential amino acids has been recorded in *Enteromorpha intestinalis*. It also has a good capacity for fermentation, which is shown by methanisation tests [17]. Moreira-da-Silva *et al*., [18] studied the possibility of using marine macrophytes as a substrate for biogas generation. Special research attention has been given to *Enteromorpha intestinalis* and *Ulva lactuca* as a source of methane generation through anaerobic digestion, the time involved in gas production and the possibility of using the depleted biomass as a fertilizer for phytoplankton cultures. Finally, the possibility of using algae for other energetic options has been analyzed and their potential was compared with that of the water hyacinth *Eichornia crassipes* [19]. The dominancy of *Enteromorpha* spp. in areas of high sewage pollution speaks in favour of utilizing this macroalgae as agent of bioremediation.

Macroalgae are unique sources of protein although the content varies with the types. Protein content of brown seaweeds are generally small (average: 5-15% of

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dry weight), whereas, higher protein content are recorded in green and red seaweeds (average: 10-30% of dry weight). The protein levels of *Ulva* and *Enteromorpha* spp. generally range between 5-20% of dry weight. Because of their high protein content, protein concentrates (PCs) of seaweeds have become more important for the food industry, especially in developed countries [20]. The recent utilization of macroalgae as a fish feed is also gaining momentum.

Lipids represent only 1-5% of algal dry matter and exhibit an interesting polyunsaturated fatty acid composition particularly omega 3 and omega 6 acids which play an important role in the prevention of cardio vascular diseases, osteoarthritis and diabetes. The red and brown algae are rich in fatty acids with 20 carbon atoms: cicosapentanoic acid (EPA, T3 C 20:5) and arachidonic acid (AA, T6 C 20:4).

Astaxanthin is a naturally occurring carotenoid pigment with unique antioxidant property, which is present in both micro and macroalgae. It is an important feed ingredient both in pisciculture and animal husbandry sector owing to its wide application as an antioxidant.

Enteromorpha intestinalis, *Ulva lactuca* and *Catenella repens* are the dominant macroalgae found in Indian Sundarbans (a World Heritage Site and a Biosphere Reserve), which is a Gangetic delta in the north east coast of Indian Sub-continent. In the intertidal zone of this mangrove dominated ecosystem, a distinct zonation is often visualized with respect to distribution pattern of seaweeds. The members of Chlorophyceae (*Enteromorpha intestinalis* and *Ulva lactuca*) occupy higher level in comparison to *Catenella repens* (belonging to Rhodophyceae) when the section of vertical zonation on a substratum is considered. Despite the abundance of macroalgae in the mangrove dominated Gangetic delta, literature on this community is meager. Very few studies have been undertaken to document the role of *Enteromorpha* spp. and *Ulva lactuca* as primary producers in Sundarbans ecosystem [21], but no study has yet been undertaken on the ecology and biochemical characteristics of macroalgae in this part of Indian sub-continent.

The main objective of the present study was to determine the nutritional value of *Enteromorpha intestinalis*, *Ulva lactuca* and *Catenella repens* by analyzing their biochemical composition (protein, lipid, carbohydrate and astaxanthin). Another aim of the study was to determine the spatial variation of the biochemical components of these three commonly available macroalgae with respect to relevant hydrological parameters.

MATERIALS AND METHODS

Macroalgal Collection: Each species of the macroalgae (*Enteromorpha intestinalis*, *Ulva lactuca* and *Catenella repens*) distributed throughout the entire stretch of Indian Sundarbans (within the latitude 21°30′N to 22°30′N and longitude 87°25′E to 89°10′E) were collected at monthly intervals during September 2007 to June 2008, from six different sampling sites namely Stn. 1, Gosaba (22°08'53.66"N; 88°56'34.20"E), Stn.2, Chotomollakhali (22°10'21.74"N; 88°53'55.18"E), Stn.3, Bali (22°04'35.17"N; 88°44'55.70"E), Stn.4, Bony Camp (21°54'34.56"N; 88°35'42.13"E), Stn.5, Lothian (21°42'45.73"N; 88°18'12.82"E) and Stn.6, Frasergaunge (21°36'55.72"N; 88°12'33.15"E) (Figure 1). The algal samples on block jetties and hard substrata (like boulder, mangrove trunk etc) were hand-picked from shallow littoral water; washed in the field with ambient seawater to remove epiphytes, sediments and organic matter; rinsed with distilled water, dried with tissue paper and brought to the laboratory to store at -20°C.

Analytical Methods: The collected species were subjected to biochemical analysis as per the standard protocol. For each species, triplicate analyses were averaged for each of the samples for soluble carbohydrate, total protein, total lipid and astaxanthin. The total carbohydrate content was assayed by the phenol-sulphuric acid method [22] after extraction with 2.5N HCl. The results were calculated from a glucose standard curve. Total lipid was determined by Soxhlet method as described by Folch *et al.*, [23]. The total protein content was determined with Folin reagent with bovine albumin serving as standard [24]. Astaxanthin was analyzed by standard spectrophotometric method after organic solvent extraction with appropriate dilution and expressed in ppm unit [25]. Lipid, protein and carbohydrate contents were expressed as the percentage dry weight. The results are given as a mean with standard deviation (±SD) as quality assurance to the data.

Surface water were collected simultaneously from all the six sampling sites to monitor salinity, temperature and nitrate of the ambient water as per the method of Strickland and Parsons [26] to pinpoint the hydrological parameters to which the vegetation are exposed in natural condition.

Statistical analysis: Mean values of each biochemical component was subjected to one-way ANOVA followed by Duncan's multiple range test at $p<0.05$ [27] to detect significant differences among groups (selected species).

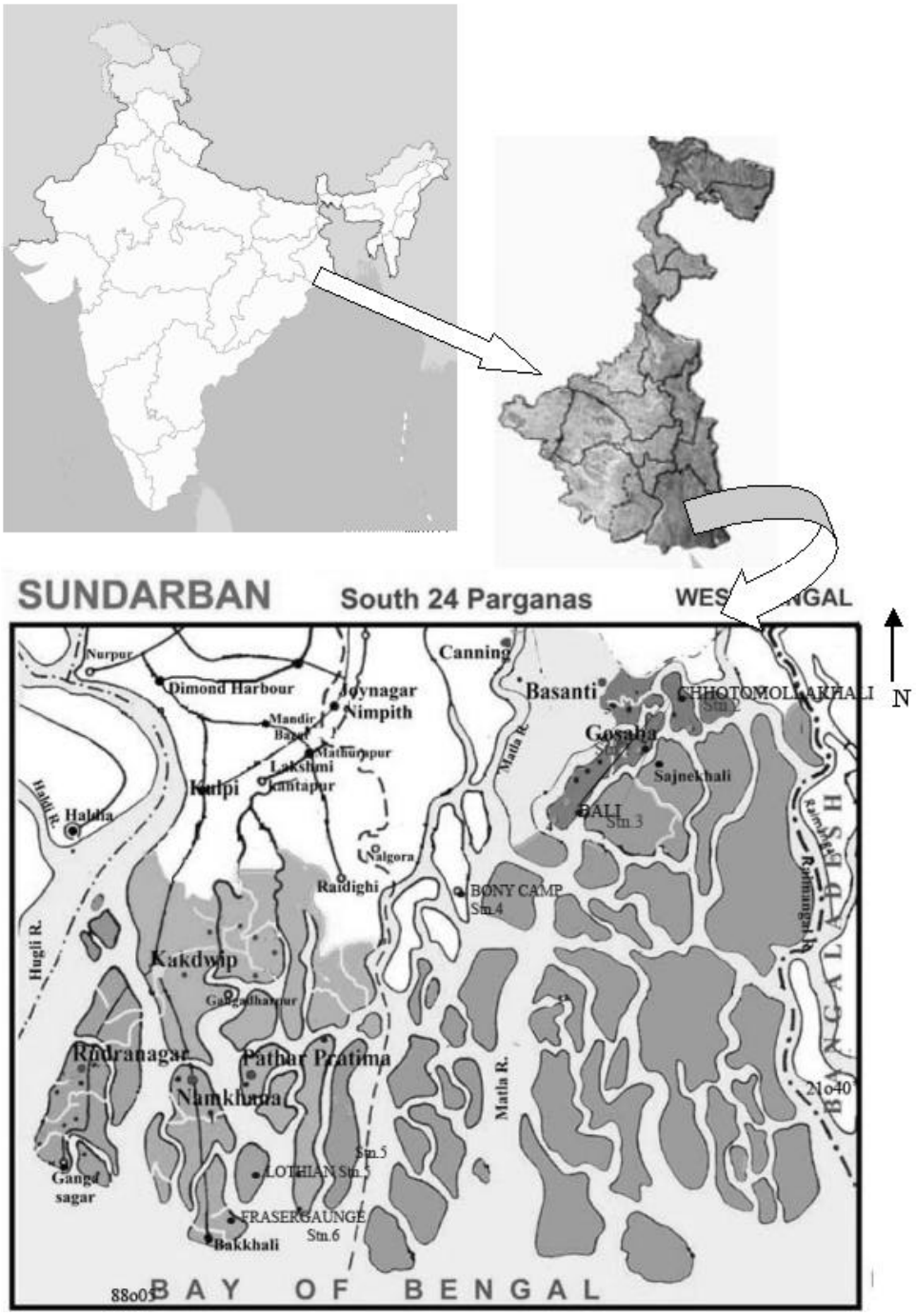


Fig. 1: The Gangetic Delta and The Indian Sundarbans (map showing sampling stations)

RESULTS AND DISCUSSION

The average lipid, protein, carbohydrate and astaxanthin values of *Enteromorpha intestinalis*, *Ulva lactuca* and *Catenella repens* are presented in Tables 1, 2 and 3.

The major biochemical component in the selected seaweeds was carbohydrate. The percentage of soluble carbohydrate in rhodophytes, *Catenella repens* (overall mean 27.16%±4.06% of dry weight; range 21.65± 0.76 to 33.67± 0.87% of dry weight) (Table 3) was lower than the chlorophytes (mean 45.32± 8.30% of dry weight;

Table 1: The mean (±SD) contents of lipid, protein, carbohydrate and astaxanthin in *Enteromorpha intestinalis* from Indian Sundarbans during September '07 – June '08

Stations	Protein (% of dry weight)	Lipid (% of dry weight)	Carbohydrate (% of dry weight)	Astaxanthin (ppm of dry weight)
Frasergaunge	13.84±0.17^{a}	0.09±0.02^{cd}	52.09±0.84^{b}	186.11±2.72^{a}
Lothian	5.18±0.52^{f}	0.07±0.02^{d}	57.03±1.63^{a}	185.40±1.83^{a}
Bony Camp	9.57±0.61^{d}	0.24±0.02^{b}	42.36±1.22^{d}	135.81±1.85^{c}
Gosaba	12.79±0.03^{b}	0.30±0.01^{a}	33.53±0.03^{f}	112.72±0.83^{e}
Bali	7.48±0.03^{e}	0.11±0.01^{c}	48.40±0.45^{c}	145.60±1.17^{b}
Chotomollakhali	11.37±0.03^{c}	0.22±0.01^{b}	38.51±0.62^{e}	131.78±1.53^{d}

*means in a whole column with different superscripts (a-f) are significantly different ($p<0.05$, Duncan multiple range test).

Table 2: The mean (±SD) contents of lipid, protein, carbohydrate and astaxanthin in *Ulva lactuca* from Indian Sundarbans during September '07 – June '08*

Stations	Protein (% of dry weight)	Lipid (% of dry weight)	Carbohydrate (% of dry weight)	Astaxanthin (ppm of dry weight)
Frasergaunge	11.64±0.22^{a}	0.38±0.15^{cd}	45.83±1.61^{b}	142.65±3.0^{b}
Lothian	6.92±0.04^{f}	0.28±0.03^{d}	48.34±1.00^{a}	150.68±2.12^{a}
Bony Camp	8.30±0.32^{d}	0.74±0.06^{b}	43.83±1.96^{c}	125.46±2.08^{d}
Gosaba	10.98±0.04^{b}	1.06±0.12^{a}	24.33±0.08^{e}	102.40±0.35^{f}
Bali	7.88±0.01^{e}	0.49±0.01^{c}	42.04±0.15^{c}	130.18±0.90^{c}
Chotomollakhali	9.78±0.02^{c}	0.68±0.03^{b}	38.67±0.17^{d}	115.67±0.12^{e}

*means in a whole column with different superscripts (a-f) are significantly different ($p<0.05$, Duncan multiple range test)

Table 3: The mean (±SD) contents of lipid, protein, carbohydrate and astaxanthin in *Catenella repens* from Indian Sundarbans during September '07 – June '08

Stations	Protein (% of dry weight)	Lipid (% of dry weight)	Carbohydrate (% of dry weight)	Astaxanthin (ppm of dry weight)
Frasergaunge	14.19±0.09^{a}	0.14±0.02^{c}	33.67±0.87^{a}	181.99±3.54^{a}
Lothian	4.15±0.02^{e}	0.17±0.02^{bc}	26.43±0.88^{c}	105.29±0.07^{d}
Bony Camp	10.25±0.15^{d}	0.25±0.02^{a}	28.60±0.18^{b}	135.49±2.12^{b}
Gosaba	12.18±0.24^{b}	0.20±0.02^{b}	21.65±0.76^{e}	97.73±0.32^{e}
Bali	4.58±0.03^{e}	0.19±0.02^{b}	29.04±0.13^{b}	180.44±1.09^{a}
Chotomollakhali	11.47±0.59^{c}	0.21±0.04^{ab}	23.55±0.54^{d}	128.65±0.78^{c}

*means in a whole column with different superscripts (a-f) are significantly different ($p<0.05$, Duncan multiple range test)

Table 4: Physico-chemical variables of aquatic environment of six selected stations during September '07 – June '08

Stations	Salinity ($^{0}/_{00}$)	Temperature (°C)	Nitrate (µgat/l)
Frasergaunge	25.0^{a}	34.0^{a}	23.84^{a}
Lothian	22.3^{a}	35.0^{a}	13.84^{f}
Bony Camp	21.3^{a}	32.0^{a}	16.84^{d}
Gosaba	16.3^{a}	31.3^{a}	21.69^{b}
Bali	19.7^{a}	33.0^{a}	15.02^{e}
Chotomollakhali	17.0^{a}	32.7^{a}	19.53^{c}

*means in a whole column with different superscripts (a-f) are significantly different ($p<0.05$, Duncan multiple range test).

range 33.53± 0.03 to 57.03± 1.63% of dry weight in *Enteromorpha intestinalis* and mean 40.51± 8.12% of dry weight; range 24.33± 0.08 to 48.34± 1.00% of dry weight in *Ulva lactuca*) (Tables 1 and 2).

The highest percentage of protein was recorded in *Enteromorpha intestinalis* (mean 10.04± 3.10% of dry weight; range 5.18± 0.52 to 13.84± 0.17% of dry weight) (Table 1) followed by *Catenella repens* (mean 9.47± 3.91% of dry weight; range 4.15± 0.02 to 14.19± 0.09% of dry weight) and *Ulva lactuca* (mean 9.25± 1.75% of dry weight, range 6.92± 0.04 to 11.64± 0.22% of dry weight) (Tables 2 and 3).

The lipid content was highest in *Ulva lactuca* (mean 0.61± 0.27% of dry weight; range 0.28± 0.03 to 1.06± 0.12% of dry weight) (Table 2), followed by *Enteromorpha intestinalis* (mean 0.17± 0.09% of dry weight; range 0.07± 0.02 to 0.30± 0.01% of dry weight) (Table 1) and *Catenella repens* (mean 0.19± 0.04% of dry weight; range 0.14± 0.02 to 0.25± 0.02% of dry weight) (Table 3).

Astaxanthin value was highest in *Enteromorpha intestinalis* (mean 149.57± 28.21 ppm of dry weight; range 112.72± 0.83 to 186.11± 2.72 ppm of dry weight) (Table 1). The next position was occupied by *Catenella repens* (mean 138.27± 33.96 ppm of dry weight; range 97.73± 0.32 to 181.99± 3.54 ppm of dry weight) (Table 3) followed by *Ulva lactuca* (mean 127.84± 16.59 ppm of dry weight; range 102.40± 0.35 to 150.68± 2.12 ppm of dry weight) (Table 2).

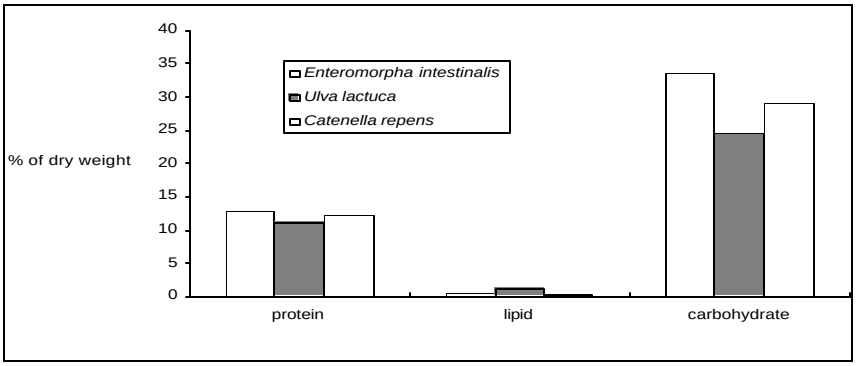
The average surface water salinity of the sampling stations varied as per the order Frasergaunge (25.0 °/$_{00}$) > Lothian (22.3°/$_{00}$) > Bony Camp (21.3°/$_{00}$) > Bali (19.7°/$_{00}$) > Chotomollakhali (17.0°/$_{00}$) > Gosaba (16.3°/$_{00}$). The surface water temperature ranged from 31.3°C to 35.0°C and nitrate content varied from13.84 µgat/l to 23.84 µgat/l (Table 4). The spatial variation of nitrate was significant ($p<0.05$), which may be attributed to the distance of these stations from Bay of Bengal in the south or proximity of these stations to the highly urbanized and industrialized city of Kolkata in the North. The local activities like fish landing, shrimp culture etc. also cause substantial difference between the stations in terms of nitrate load.

Data of protein content in macroalgae from the tropical and subtropical coastal environment frequently show lower concentrations [28, 29]. This is because of predominantly oligotrophic marine environment with low availability of nitrogen as visualized in case of Brazilian marine environment [30, 31]. In the present study, our data of protein concentration in macroalgae are in accordance with the information available in the literature [32-35, 17, 36, 37]. In *Enteromorpha intestinalis* minimum protein was found at Lothian (5.18± 0.52% of dry weight) and the maximum at Frasergaunge (13.84± 0.17% of dry weight). Same trend was also observed for *Ulva lactuca* (minimum at Lothian i.e. 6.92± 0.04% of dry weight and maximum at Frasergaunge i.e. 11.64± 0.22% of dry weight) and *Catenella repens* (minimum at Lothian i.e. 4.15± 02% of dry weight and maximum at Frasergaunge i.e. 14.19± 0.09% of dry weight). Differences in the protein level of the three different species of macroalgae with respect to stations are given in Figures 2 and 3. The significant spatial variation in the protein level of the selected species was confirmed by Duncan multiple range test at 5% level of significance, which may be attributed to the difference in nutrient level (preferably nitrate) of the ambient aquatic phase (Table 4). The significant positive correlation between nitrate level and protein content of three selected macroalgae [$r_{\text{nitrate x protein (}\textit{Enteromorpha}\text{ sp.)}} = 0.976$, $p<0.01$; $r_{\text{nitrate x protein (}\textit{Ulva}\text{ sp.)}} = 0.995$, $p<0.01$; $r_{\text{nitrate x protein (}\textit{Catenella}\text{ sp.)}} = 0.941$, $p<0.01$] statistically confirm our observation. The direct relationship of protein percentage in seaweeds with nitrate of the ambient water was reported by several workers [30, 31]. Frasergaunge being a sewage contaminated zone (due to presence of fish landing station and tourism units) showed maximum nitrate level in the water and subsequently highest percentage of protein in the macroalgae.

Significant variations in carbohydrate content in the seaweeds at the different sampling stations were observed throughout the study period. Carbohydrate is the most important component for metabolism as it supplies the energy needed for respiration and other metabolic processes. Maximum carbohydrate values were observed in stations with high salinity and located in the vicinity of Bay of Bengal. The study area at the apex of Bay of Bengal enjoys bright sunshine and high tropical temperature almost through the year. This may be a reason behind higher carbohydrate value in the seaweeds. The significant positive relationships between ambient water temperature and carbohydrate content of the seaweeds [$r_{\text{temperature x carbohydrate (}\textit{Enteromorpha}\text{ sp.)}} = 0.937$, $p<0.01$; $r_{\text{temperature x carbohydrate (}\textit{Ulva}\text{ sp.)}} = 0.795$, $p<0.01$; $r_{\text{temperature x carbohydrate (}\textit{Catenella}\text{ sp.)}} = 0.489$, $p<0.05$] confirm the view of synthesis of organic carbon (through photosynthesis) under optimum solar radiation and temperature.

In comparison to protein and carbohydrate, lipid exhibited very low proportion in *Enteromorpha intestinalis*, *Ulva lactuca* and *Catenella repens.* Significant variation in lipid percentage was observed between the stations (Table 1, 2 and 3). The mangrove dominated Gangetic delta enjoys a tropical climate and therefore temperature plays a major role in the variation of the lipid content in macroalgae. Significant negative correlation between lipid content and water temperature has also been observed in our study for all the three selected macroalgae [$r_{\text{temperature x lipid (}\textit{Enteromorpha}\text{ sp.)}} = -0.929$, $p<0.01$; $r_{\text{temperature x lipid (}\textit{Ulva}\text{ sp.)}} = -0.952$, $p<0.01$; $r_{\text{temperature x lipid (}\textit{Catenella}\text{ sp.)}} = -0.693$, $p<0.01$]. This observation is in alignment with the works of Jones and Harwood [38] who concluded that temperature increases the level of unsaturation of acyl chains that slows down both metabolism and transport of lipid.

Astaxanthin is a powerful antioxidant. A lot of studies demonstrated the antioxidant properties of algal carotenoids and the role they play in preventing many diseases linked to oxidative stress [39, 40]. The synthesis of astaxanthin enhances with the increase in environmental stresses as revealed through a study in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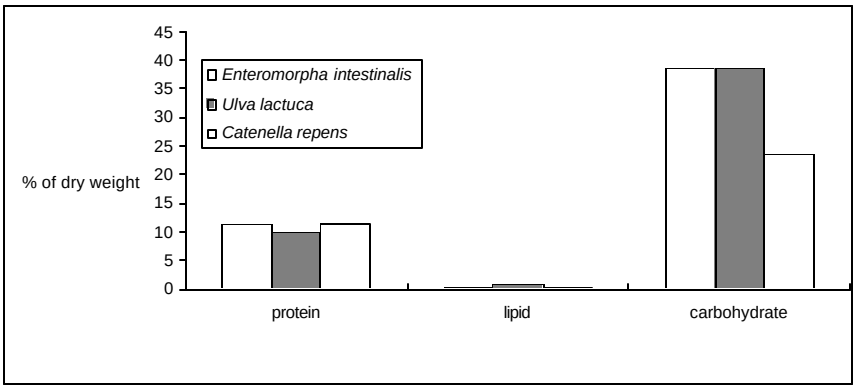
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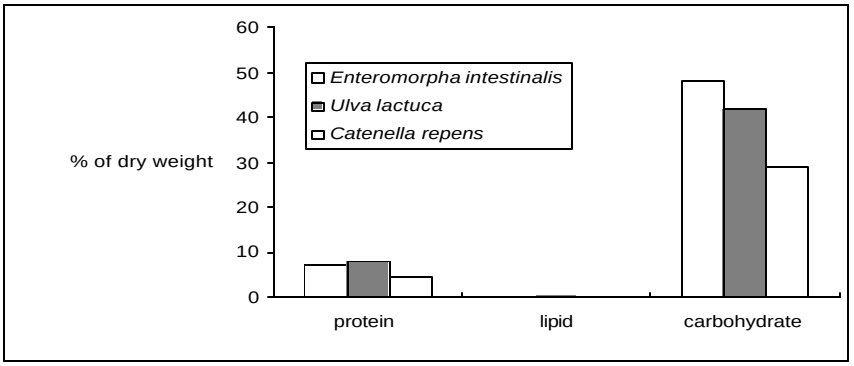
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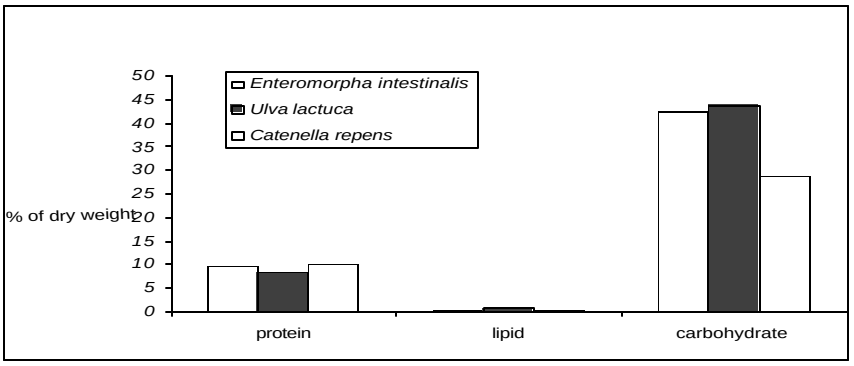
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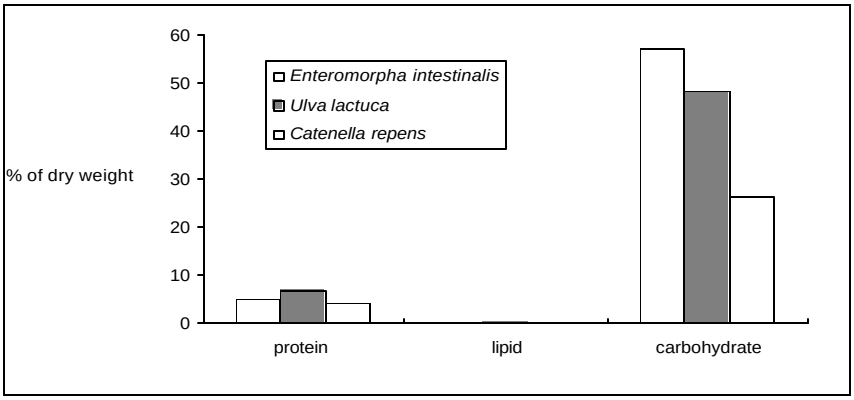

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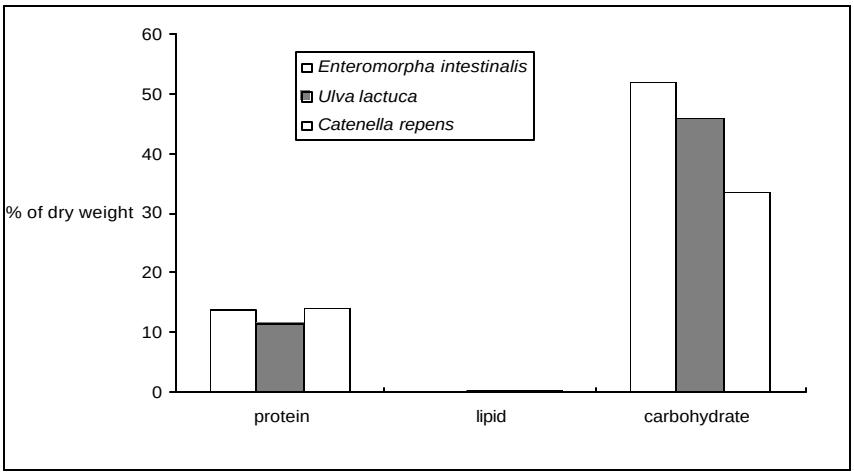

(f)

Fig. 2: Variation in protein, lipid and carbohydrate contents (% of dry weight) at (a) Gosaba (b) Chotomollakhali (c) Bali (d) Bony Camp (e) Lothian (f) Frasergaunge

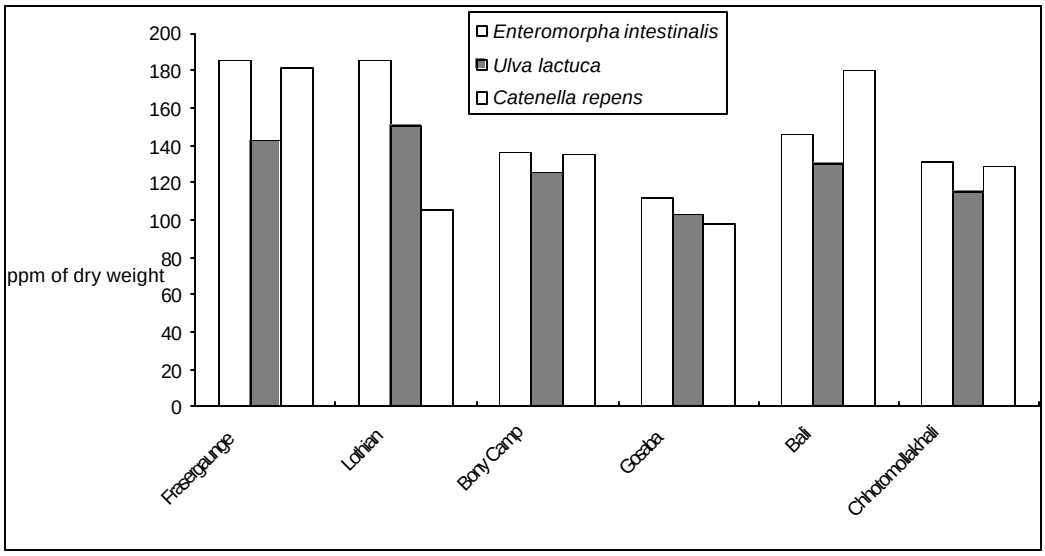

Fig. 3: Variation in astaxanthin contents (ppm of dry weight) in six stations

mangroves by Mitra *et al.*, [41]. In the present study, the astaxanthin load showed significant spatial variation ($p<0.05$) with respect to all the three macroalgae (Tables 1, 2, 3 and Figure 3), which may be due to variation in salinity amongst the selected stations. The significant positive correlation between salinity and astaxanthin level in the selected macroalgal species [$r_{\text{salinity x astaxanthin (}\textit{Enteromorpha}\text{ sp.)}} = 0.886$, $p<0.01$; $r_{\text{salinity x astaxanthin (}\textit{Ulva}\text{ sp.)}} = 0.874$, $p<0.01$; $r_{\text{salinity x astaxanthin (}\textit{Catenella}\text{ sp.)}} = 0.513$, $p<0.05$] speaks in favour of astaxanthin synthesis under stressful condition, posed by high aquatic salinity.

CONCLUSION

The study revealed the members of chlorophyceae as the most nutritionally rich species in terms of carbohydrate, protein, lipid and astaxanthin content. The Gangetic delta is unique in terms of hydrological parameters and several categories of anthropogenic activities that often influence the water quality. The physico-chemical variables of ambient aquatic phase have profound influence on biochemical constituents of the macroalgae.

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