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Biochemical and Energy Dispersive Spectroscopic Studies of *Sargassum SP*.

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Abstract- Biochemical studies on five species of *Sargassum* have been made to understand which is the most mportant and useful for mariculture, the species studied was *Sargassum wightii*, *Sargassum linearifolium*, *Sargassum polycystum*, *Sargassum plagiophyllum* and *Sargassum illicifolium*. Biochemical studies such as the amount of protein, amino acid, nd iodine have been made for comparison. The amount of iodine (211.7 ± 3.99) in *S. wightii* was higher than the other species. The amount of protein (47.5 ± 2.02 mg g^{-1} fr. wt.) in *S. wightii* was higher whereas the amount of amino acid (0.52 ± 0.13 mg/g fr. wt.) was high is *S. wightii.* But the total sugar (0.70 ± 0.17 mg/g fr. wt.) in *S. wightii* showed maximum value. Vegetative plants of *Sargassum* sp. were collected from the natural habitat at Nochurani coast 1-2 μm sections of the alga were subjected to SEM Energy Dispersive spectroscopic (EDS) studies revealed that localized accumulation of different chemical elements such as Na, Zn, P, Ca, Mg and S in the cell-wall; Na, Zn, P, Ca, S and Mg in cortical region, Mn, Pb, Zn, Ca, P, Mg and S in medullary region. The composition of different chemical elements present in three different structure determine the chemical interaction between them and further it gives clue for algal growth requirements. The biochemical studies of the five species showed better in *S. wightii* and this may be a good species for mariculture programme.

Keywords – Biochemical, Elemental composition, SEM-EDS, *Sargassum* sp., Nochurani coast

I. INTRODUCTION

Principle of Energy Dispersive spectroscopic analysis (EDS)

The EDS system is under control of sophisticated software that, as a simple command from the operator, automatically collects an energy-dispersive X-ray spectrum, identifies the elements present, obtains X-ray count data, and presents all of the pertinent data in an appropriate display format. The spectrum can be stored in disk memory and recalled as desired. Flexibility is central to the performance of the EDS software. It easily adapts to fit virtually any user's needs. While peaks are identified land labelled automatically a manual mode is available to allow the operator to identify peaks by elemental symbol, atomic number, or cursor position. This may be done exclusively by the operator or in conjunction with automatic mode. The operator may also modify search parameters to find tune the search for a particular type of analysis. Elements that are not relevant to the analysis may be excluded from the search.

Energy dispersive X-ray microanalysis system works in the principle of detection and characterization of characteristic X-ray generated in Electron Microscope. Whenever electron beam interacts with any elements or metals, X-ray are detected by a solid state detector. They are digitized, amplified and fed to a multichannel analyser, which sorts the X-ray signal depending upon their energy. The data generated were compared with existing data stored in computer programme and the computer provides the possibility of presence of various elements present in the samples.

Seaweeds are macroscopic algae which form an important component of the marine living resource. They have been harvested by man for centuries particularly in Japan and China where they form a part of the staple diet. The uses of seaweeds as food, fodder and manure are well known in many countries. Marine algae contain more than 60 trace elements in a concentration much higher than in terrestrial plants. They also contain protein, iodine, bromine, vitamins and substances of stimulatory and antibiotic nature. From the specimens biochemical studies such as total sugars, reducing and non-reducing sugars, total protein, amino acids, iodine, elements such as Na, Mg, Al, Cl, Ca, Cu, Mn and Zn were estimated and compared. Seaweeds are the only source for the production of agar, alginate and carrageenan. These phycocolloids are extensively used in various industries such as food, onfectionary, textile, pharmaceutical, dairy and paper mostly as gelling, stabilising and thickening agents. The brown seaweed *Sargassum* has been exploited for its alginate, since it is abundantly occurring along the south east coast of India. In the present study five species of *Sargassum* such as *S. wightii*, *S. linearifolium, S. polycystum, S. plagiophyllum* and *S. illicifolium* have been chosen for biochemical and elemental composition were subjected to South East Coast of Tamil Nadu, South India. From Nochurani coast.

II. MATERIALS AND METHODS

Vegetative plants of *Sargassum* species were collected at Nochurani coast during summer (April – June, 2019). The collection was made during the morning low tide. They were transported to the laboratory in plastic packets, brushed off the epiphytes and washed several times in filtered seawater followed by distilled water. These five alga species of 2-3 mm were fixed in 3% glutardehyde for scanning electron microscopic and Energy Dispersive Spectroscopic studies. Then they were dehydrated through a graded series of alcohol with 12-15 m interval at 4°C upto 70%. They were further dehydrated in 90% and 100% of alcohol and kept at room temperature for 2-3 h. Finally the dehydrated samples treated with critical point drier (CPD). Then they were mounted on a stub and the specimens were coated [1]. They were examined with JOEL JSM-56010 LV with INSA-EDS and photomicrographs were taken selectively from the computer screen at central sophisticated instrumentation laboratory, Department of Physics, Annamalai University, Annamalainagar, Tamil Nadu, India.

III. EXPERIMENT AND RESULT

Biochemical composition

Protein

The quantitative estimation of protein in the five species of *Sargassum* showed wide range in mg/g fresh weight. In *Sargassum wightii* showed the value of 47.5 ± 2.02 mg/g fr. wt. in *S. linarifolium* it was 34.8 ± 1.13 mg/g fr. wt. *S. polycystum* was noted 21.2 ± 1.52 mg/g fr. wt. *S. plagiophyllum* 30.5 ± 0.73 mg/g fr. wt. and *S. illicifolium* recorded 44.4 ± 2.13 mg/g fr. wt. The maximum amount of protein was observed in *S. wightii* 47.5 ± 2.02 mg/g fr. wt. and the minimum value was in *S. polycystum* (21.2 ± 1.52 mg/g fr. wt.) (Table 1).

Amino acid

When compared the amount (Table 1) of amino acids in *Sargassum* species it showed a close correlation them *S. wightii* (0.52 ± 0.13 mg/g fr. wt.), *S. linarifolium (*0.39 ± 0.17 mg/g fr. wt.), *S. polycystum* (0.37 ± 0.14 mg/g fr. wt.), *S. plagiophyllum* (0.34 ± 0.10 mg/g fr. wt.) and *S. illicifolium* 0.45 ± 0.21 mg/g fr. wt.. The maximum mount of amino acid was observed in *S. wightii* 0.52 ± 0.13 and the minimum was in *S. plagiophyllum* (0.34 ± 0.10 mg/g fr. wt.) (Table 1).

Iodine

The amount of iodine present in the *Sargassum* species was maximum in *S. wightii* 211.7 ± 3.99 mg/g fr. wt., *S. linarifolium* 116.24 ± 2.63 mg/g fr. wt., *S. polcystum* 105.35 ± 2.08 mg/g fr. wt., *S. plagiophyllum* 135.71 ± 3.06 mg/g fr. wt. and *S. ilicifolium* 206.80 ± 1.90 mg/g fr. wt. was observed maximum amount in iodine *S. wightii* (211.7 ± 3.99) and minimum amount in *S. polycystum* (105.35 ± 2.08) (Table 1).

Carbohydrate

The quantitative estimation of carbohydrates from the *Sargassum* using Nelson technique showed in *S. wightii* 0.70 ± 0.17 mg/g fr. wt. showed on maximum value total sugar. Minimum value of total sugar on *S. iliccifolium* 0.60 ± 0.11 mg/g fr. wt. The maximum value of reducing sugar *S. linarifolium* 0.63 ± 0.10 mg/g fr wt.

minimum values *S. ilicifolium* 0.60 ± 0.11 mg/g fr. wt. and the maximum non reducing sugar values of *S. ilicifolium* 0.24 ± 0.06 mg/g fr. wt. minimum values on *S. linarifolium* 0.02 ± 01 mg/g fr. wt. (Table 2).

Analysis of the different elemental composition of the five seaweeds using SEM-EDS was carried during the different climatic conditions of the period January 2018 post monsoon to December 2018 monsoon. The experiment carried out for four seasons *i.e.* summer, pre monsoon, post monsoon and monsoon. The results of the study showed a distinct amount of each element.

The order of elements in different seaweeds as follows *Sargssum* species *S. wightii:* Na > Mg > Cl > Ca > Cu > Zn, *S. linearifolium:* Na > Mg > Al > Ca > Cu > Zn*, S. polycystum:* Mg> Al > Ca > Cu > Zn*, S. plagiophyllum:* Na > Mg > Al > Cl > Ca > Cu and *S. illicifolium:* Na > Mg > Al > Cl > Ca > Cu > Zn.

Energy Dispersive X-ray Micro-analysis system works on the principle of detection and characterization of characteristic X-rays generated in electron microscope. Whenever electron beam interacts with any elements or metals, X-rays are detected by a solid state detector. They are digitized, amplified and fed to a multichannel analyser, which sorts the X-ray signals depending upon their energy. The data generated were compared with existing data stored in computer programme and the computer provides the possibility of presence of various elements present in the samples.

The order of elements in different seaweeds as follows *S. wightii* Ca > Mg > Cr > Na > Zn > P > S > Fe > Cl > Si > K > Mn; *S. linarifolium* Ca > Si > Zn > Mg > S > Cr > Mn > Cl > P > Na > Fe > K; *S. polycystum* Ca > Mg > Si > Na > Cl > S > Cr > Zn > Mn > Fe > K; *S. plagiophyllum* Ca > Cl > Mg > P > Cr > Zn > Na > Fe > P > Mn > S > K and *S. plagiophyllum* Cl > P > Si > Fe > Cr > Zn > Na > Mg > K (Tables 3-6 and Fig. 1).

Seaweeds collected during post-monsoon *S. wightii* calcium showed maximum values 38.67 ± 0.60 to 33.17 ± 0.23 monsoon season showed minimum contribution of calcium. They were ranging between total weight. Minimum phosphorus values were varied among the five species during monsoon period. least values were obtained for *S. wightii* (3.99 ± 0.08), *S. ilinea* (2.64 ± 0.09), *S. polycystum* (1.10 ± 0.04), *S. plagiophyllum* (4.93 ± 0.07) and *S. ilicifolium* (9.87 ± 0.12) (Tables 3-6).

The pattern of chemical elements in the cell wall, plastid and floridean starch was found to be in the order such as Na, Mg, Si, Al, Cl, S, K, Ca, Cu, Mn, Zn (Tables 3-6). The high values of Ca obtained on the cell-wall rigidity of their structures. The four chemical elements Zn, P, Ca, Mg, Mn and S were common in all the three components. Similarly the cortical and medullary region contained Mg but it was absent in the cell-well. Among the chemical elements, Mn was specific to medullary region.

It's key role is nourishment of the nutritive material. Presence of Mn is needed for catalysing the photolysis of water and 0_2 evolution associated with pigment system II.

Mg is present in the cortical and medullary region because Mg forms the nucleus for the porphyrin ring and hence its presence in the plastid is understandable.

Role of calcium (Ca)

Calcium as calcium pectate is an important constituent of cell wall and required in large amounts of cell division. It is a structural component of chromosomes. It is an essential co-factors (or) an activator of number of enzymes. It plays an important role in flocculation of soil aggregates and it content of soil. It acts as a buffer in plant system and ameliorates the toxic effects of other nutrients.

Role of magnesium (Mg)

Magnesium is an essential constituent of chlorophyll several photosynthetic enzyme present in chlorophyll require magnesium as an activated. Magnesium helps in the formation of chlorophyll imparts dark green colour to leaves and it is indispensable for photosynthesis by seaweeds, It plays a part in the production of carbohydrates, proteins, fats and vitamins. It helps in the translocation of carbohydrate and fats.

Role of copper (Cu)

Copper is a constituent of chlorophyll and thus helps the synthesis of chlorophyll. Copper is capable of acting as electron carrier in enzyme synthesis, which brings about oxidoreductase, nitrate reductase, cytochrome oxidase and ascorbic oxidase *etc.* It is essential for synthesis of vitamin A and other compounds in seaweeds.

Role of zinc (Zn)

It is involved in the biosynthesis of seaweeds growth hormone (IAA) and reproduction of certain marine algae. It plays a vital role in photosynthesis and nitrogen metabolism. It helps in carbohydrate transformation and sulphur metabolism. It is required for enhanced rate of growth, production and synthesis of RNA.

[2] studied the biochemical contents of *Ulva lactuca* from Port Okha in relation to ecological factors and presented the month-wise protein, fat, carbohydrate, crude fibre, sodium, potassium, calcium and phosphorus contents of these species. Seasonal variations in biochemical composition of some seaweeds from Goa coast was made by [3]. She found that the carbohydrate contents in all marine algae more or less closely correlated.

The iodine content of the Indian *Sargassum* was studied by[4]; [5] estimated in a more elaborate way the iodine contents of eleven species of algae growing around Mandapam. The quantity of iodine present in many green, brown and red algae of the Gujarat coast was determined by [6], [7], [8] [9].

Distribution of minerals such as Ca, Mg and Cl was high in the selected species of seaweeds in the study area. Significantly higher concentration of elements such as Ca, Mg and K were encountered in the various type of seaweeds during summer and post-monsoon periods which reflect the capacity of these seaweed to accumulate more amount of elements during these seasons. The concentration of Na was also found to be high during summer and pre-monsoon seasons which coinciding with peak period of growth [10]. Moreover, differences in element concentration of seaweeds in the study area, during the various seasons might be related not only to different mineral level in water but also to different ecological conditions such as tidal range, temperature and salinity [11].

Generally, bioaccumulation of the elements in the seaweeds depends upon the pH, salinity, dissolved O_2 and the osmotic potential of the system [12]. Marine algae exhibit high content of ash [13] mainly due to the presence of Na, K, Ca and Mg cations (Fe and S will be minor importance).

Higher accumulation of Mg and Fe was mostly observed in seaweeds during summer season, it may be explained here that the accumulation elements *in situ* was more due to the reduction in osmoregulation activities usually affected by the increase in salinity. Though the silicon and Cl concentrations in ambient water exceeded those of other elements, the accumulation in seaweeds was very low and the most preferred being Ca, Mg and Na. This probably due to the fact that Ca and Mg are the predominant occupants of the uptake binding sites of the seaweeds which would inhibit accumulation of silicon and Cl by their competition of binding sites [14].

The enhanced bioaccumulation of most of the elements in seaweeds during summer and pre-monsoon seasons could perhaps be due to the following reasons: (i) ambient concentration of these elements was high during these seasons thereby facilitating their uptake by the seaweeds, (ii) seasonal variation in mineral content in seaweed may be related to growth rates and metabolic process [15]. [16] reported quick uptake of elements during summer and slow uptake during winter and (iii) ecological implications are important in metal uptake by seaweeds . This was evident as dissolved oxygen and pH of the water samples during various seasons in the present study showed the variations of correlation between various elements in the seaweeds. Our results showed maximum values of oxygen during summer (April-June) are associated with the rise in seaweeds population [17]. These observations are in agreement with those of Chan (1989) who reported that the seasonal variations of the mineral concentration in aquatic biota may be due to seasonal fluctuation in tissue mass and changes in physico-chemical characteristics of the surrounding water.

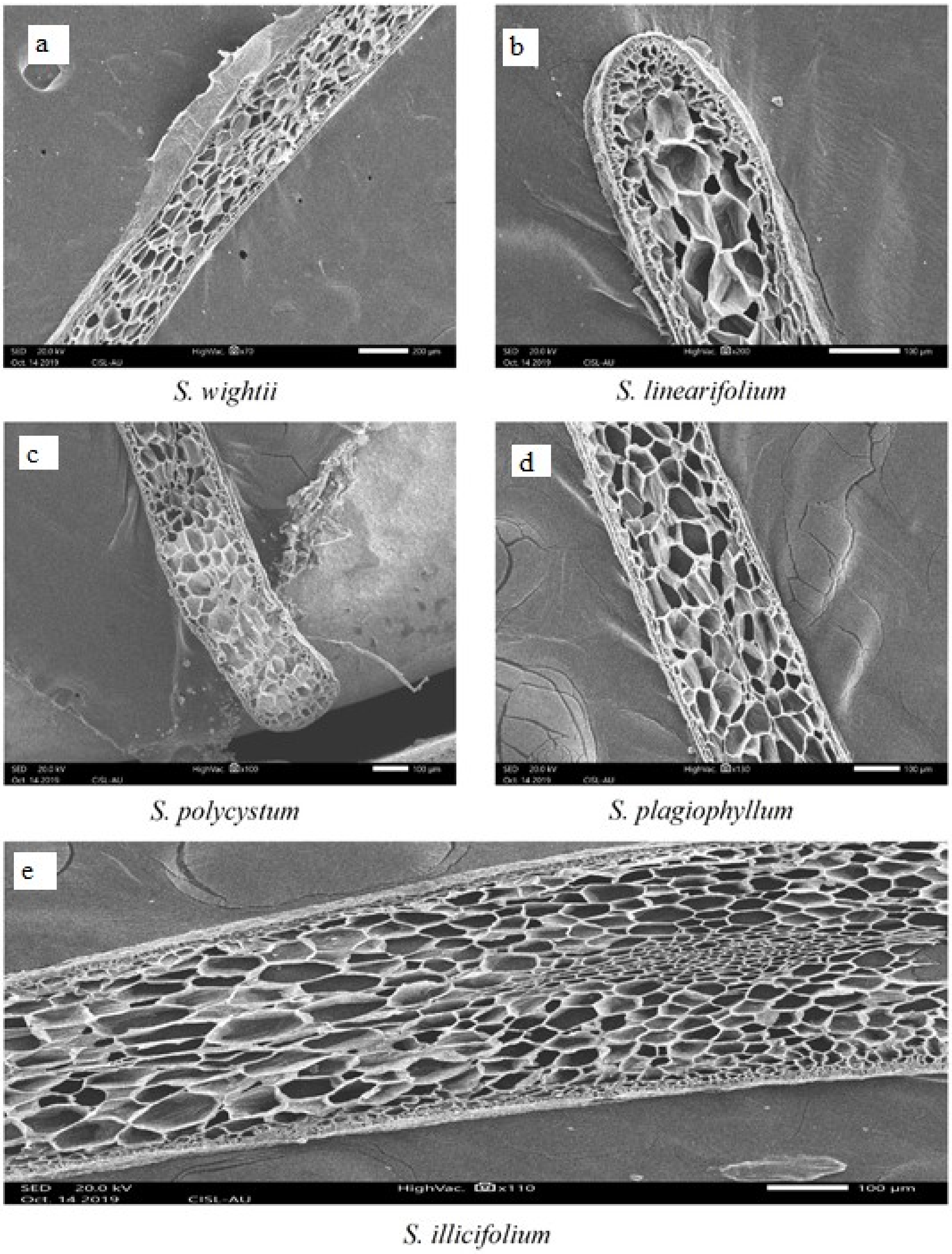

Fig. 1 . Scanning electron microscopic observation of Sargassum species

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Table 1. Biochemical composition of Sargassum species

Name of the species	Protein (mg/g fr. wt.)	Amino acid (mg/g fr. wt.)	Iodine (mg/g fr. wt.)
Sargassum wightti	47.5±2.02	0.52±0.13	211.7±3.99
Sargassuum liniarifolium	34.8±1.13	0.39±0.17	116.24±2.63
Sargssum polycystum	21.2±1.52	0.37±0.14	105.35±2.08
S. plagiophyllum	30.5 ±0.73	0.34±0.10	135.71±3.06
Sargassum ilicifolium	44.4±2.13	0.45±0.21	206.80±1.90

Table 2. Estimation of carbohydrates from the Sargassum species

Name of the species	Total sugar (mg/g fr. wt.)	Reducing sugar (mg/g fr. wt.)	Non-reducing sugar (mg/g fr. wt.)
Sargassum wightti	0.70±0.17	0.41±0.08	0.28±0.08
Sargassum liniarifolium	0.65±0.14	0.63±0.10	0.02±0.01
Srgassum polycystum	0.67±0.09	0.55±0.7	0.12±0.05
S. plagiophyllum	0.62±0.12	0.51±0.13	0.09±0.03
Sargassum ilicifolium	0.60±0.11	0.36±0.04	0.24±0.06

Table 3. Elemental composition of seaweeds using SEM-EDS during post monsoon (January – March 2018)

I. SEAWEEDS	Mineral (wt%)												
	Na	Mg	Si	S	Cl	K	Ca	Mn	P	Fe	Zn	Cr	Total
S. wightii	10.70± 0.14	26.40± 0.32	3.15±0 .06	3.77±0 .11	3.04±0 .08	2.15± 0.06	38.67± 0.06	1.34± 0.06	–	3.08± 0.11	4.73± 0.08	2.93± 0.09	99.96± 0.24
S. linearifolium	3.73±0 .08	11.41± 0.1	18.68± 0.16	11.81± 0.13	3.20±0 .10	–	39.54± 0.37	5.18± 0.12	2.59±0 .08	–	2.84± 0.06	1.61± 0.07	99.96± 0.31
S. polycystum	12.36± 0.16	16.70± 0.12	10.76± 0.10	5.93±0 .09	9.86±0 .13	1.92± 0.07	30.67± 0.29	1.96± 0.06	1.39±0 .06	1.85± 0.06	4.61± 0.07	1.95± 0.05	99.96± 0.18
S. plagiophyllum	7.10±0 .08	13.64± 0.05	13.65± 0.11	4.75±0 .07	16.03± 0.14	1.82± 0.07	23.92± 0.30	3.18± 0.04	6.09±0 .14	1.94± 0.08	2.73± 0.07	5.10± 0.08	99.95± 0.17
S. illicifolium	6.84±0 .12	4.27±0 .06	9.83±0 .13	2.08±0 .10	19.74± 0.29	3.10± 0.08	29.96± 0.30	–	11.56± 0.19	–	6.69± 0.07	5.90± 0.21	99.97± 0.29

Values are expressed as the mean ± SD; n=3

Table 4. Elemental composition of seaweeds using SEM-EDS during summer (April – June, 2018)

I. Seaweeds	Mineral (wt%)												
	Na	Mg	Si	S	Cl	K	Ca	Mn	P	Fe	Zn	Cr	Total
S. wightii	11.14±0.11	27.96±0.15	4.63±0.09	4.10±0.09	3.60±0.07	2.44±0.06	41.38±0.20	1.50±0.07	–	3.22±0.08	–	–	99.97±0.18
S. linearifolium	5.40±0.14	12.71±0.10	19.20±0.08	11.43±0.13	–	–	41.70±0.20	6.82±0.08	2.73±0.07	–	–	–	99.99±0.23
S. polycystum	13.36±0.52	17.25±0.25	11.66±0.21	6.65±0.14	10.33±0.10	1.71±0.13	34.01±0.22	2.23±0.07	1.16±0.04	1.60±0.17	–	–	99.96±0.24
S. plagiophyllum	8.47±0.11	15.27±0.19	14.84±0.16	5.43±0.07	17.42±0.19	1.48±0.06	25.13±0.20	3.81±0.08	5.64±0.12	2.47±0.75	–	–	99.96±0.12
S. illicifolium	7.94±0.09	4.65±0.07	11.0±0.09	–	20.93±0.14	3.67±0.08	32.85±0.22	–	13.05±0.10	–	–	5.88±0.07	99.97±0.20

Values are expressed as the mean ± SD; n=3

Table 5. Elemental composition of seaweeds using SEM-EDS pre monsoon (July – September 2018)

I. Seaweeds	Mineral (wt%)												
	Na	Mg	Si	S	Cl	K	Ca	Mn	P	Fe	Zn	Cr	Total
S. wightii	9.06±0.12	23.13±0.18	2.90±0.09	3.71±0.08	3.04±0.08	–	33.36±0.19	–	3.92±0.08	4.25±0.11	4.83±0.08	11.77±0.11	99.97±0.18
S. linearifolium	2.20±0.07	10.02±0.35	18.79±0.08	8.86±0.11	3.05±0.07	1.54±0.06	31.3±0.29	4.81±0.08	2.94±0.07	1.86±0.06	10.90±0.13	3.85±0.07	99.99±0.23
S. polycystum	10.64±0.11	13.14±0.15	10.95±0.18	8.93±0.08	9.73±0.09	1.73±0.06	29.10±0.22	1.75±0.07	1.93±0.07	1.62±0.06	4.06±0.05	6.38±0.06	99.96±0.24
S. plagiophyllum	6.24±0.13	11.40±0.11	12.42±0.13	3.10±0.04	16.91±0.14	1.90±0.06	21.29±0.19	3.54±0.04	4.93±0.08	6.70±0.14	5.07±0.10	5.86±0.11	99.96±0.12
S. illicifolium	5.92±0.12	4.44±0.15	9.71±0.08	3.57±0.05	19.83±0.19	–	27.33±0.22	6.10±0.10	9.71±0.08	5.60±0.10	7.75±0.08	–	99.97±0.20

Values are expressed as the mean ± SD; n=3

Table 6. Elemental composition of seaweeds using SEM-EDS during monsoon (October – December 2018)

I. SEAWEEDS	Mineral (wt%)												
	Na	Mg	Si	S	Cl	K	Ca	Mn	P	Fe	Zn	Cr	Total
S. wightii	8.95±0.11	22.60±0.17	2.52±0.08	3.67±0.08	2.82±0.03	1.91±0.06	33.17±0.23	1.81±0.06	3.99±0.09	3.86±0.08	4.52±0.09	10.13±0.14	99.95±0.15
S. linearifolium	2.14±0.10	9.53±0.08	17.98±0.13	8.66±0.12	3.82±0.07	1.39±0.08	29.26±0.31	4.34±0.08	2.64±0.09	1.44±0.06	12.46±0.11	6.31±0.04	99.97±0.23
S. polycystum	10.47±0.11	13.08±0.11	10.90±0.08	8.80±0.09	9.06±0.10	1.49±0.05	28.92±0.20	2.53±0.04	1.10±0.04	1.60±0.06	3.96±0.07	8.06±0.07	99.97±0.27
S. plagiophyllum	6.11±0.13	11.32±0.13	10.81±0.09	2.93±0.08	16.41±0.19	2.09±0.05	21.16±0.17	4.50±0.08	4.93±0.07	5.15±0.06	6.61±0.08	7.94±0.12	99.96±0.33
S. illicifolium	5.10±0.11	3.87±0.11	9.14±0.12	2.62±0.06	19.36±0.17	2.80±0.09	25.77±0.23	1.30±0.07	9.87±0.12	8.97±0.07	5.45±0.11	5.71±0.08	99.96±0.19

Values are expressed as the mean ± SD; n=3

IV.CONCLUSION

The biochemical studies with reference to protein, amino acid, iodine, total sugar, reducing sugar, non-reducing sugar, commonly found elemental and composition. Ca > Cu > Zn > Al > Mg > Na and > Cl have been made to understand the interrelationship among these species of the five species of *Sargassum*. *S. wightii* was considered as the elite one of mariculture. On the basis of the biochemical studies it is suggested that *Sargassum wightii* has a special mention in seaweed culture and utilization. The major concerns for elemental analysis with the energy-dispersive X-ray system are the limits of detectability and the area of analysis. The first depends largely on the energy-dispersive system and specimen preparation and the second also involves the microscope performance. In particular, a small, intense electron beam must be used to obtain meaningful results. The combination of a high performance scanning electron microscope with a scanning system and highly efficient energy-dispersive spectroscopic analysis system is the key to useful analysis of small areas of biological samples. This instrumentation is now available and its applications are being explored. Although further refinements are being made in instrumentation and techniques, X-ray microanalysis is now a reality for biological as well as material science applications.

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