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SEASONAL VARIATION IN WATER SOLUBLE POLYSACCHARIDE ALGINIC ACID EXTRACTED FROM *SARGASSUM BOVEANUM* J. AGARDH (PHAEOPHYTA, SARGASSACEAE) ALONG THE DIFFERENT SHORES OF KARACHI COAST, PAKISTAN

Fozia Khan and Rashida Qari

Institute of Marine Science, University of Karachi, Karachi 75270, Pakistan

ABSTRACT

Seaweeds are found in great abundance in Pakistan. The present studies deal with survey of perennial brown alga, *S. boveanum*, along Karachi coast for its alginic acid and other biochemical constituents. Round the year studies with monthly interval indicated alginic acid concentration in this alga to be high in winter (December to February; 15.85 to 24.68 %) and low in summer (May to September; 11.12 to 17.63 %). Moisture contents of the sample dried at 70 ^{0}C for 24 hours range from 5.89 to 13.9 %. Ash content varied from 23.54 to 41.35 % and carbohydrates from 28.51 to 46.98 %. Biomass availability of this alga was ranged from 570 to 1150 g.m^{-2}; varying temporally along the coast.

Key Word: Polysaccharides, Alginic Acid, Extraction, Phaeophyta and Karachi Coast, Pakistan.

INTRODUCTION

The alginic acid is the most abundant water soluble gelling polysaccharides of brown seaweeds especially the members of Fucophyceae and has considerable technological importance for both its solution properties and as a gelling agent. Alginic acid contents are generally varied from 40% to 80% of dry weight defatted algal biomass; depend on the environment and some other factors **(**Zvyagintseva *et al.*, 2003, Honya *et al*., 1999**).** Structurally brown algal thallus depends primarily on alginates (alginic acid and its salts), and its ability to form gels and viscous solutions contributes to the flexibility of the algae. Among the macromolecules of the *Sargassum*, alginic acid was identified as the most important constituent. (Davis *et al*., 2004)

Polysaccharide products on account of their physical properties have been used in a variety of practical applications (Chapman and Chapman, 1980). Alginates are widely demanded in food, pharmaceutical, textile, and paint and paper industries for their thickening, stabilizing, gel forming and film forming properties.

In Pakistan, large number of seaweeds are found; many of which occur in great abundance (Saifullah, 1973; Qari and Qasim, 1988; Qari and Qasim, 1994; Qari 2002). These seaweeds go in waste and are not utilized as a source of alginic acid in Pakistan. Amongst Phaeophyceae, *S. boveanum* is an attached perennial species (Qari and Qasim, 1988; Qari and Qasim, 1994; Qari, 2002) which is found nearly all year round at Karachi coast. This study is initiated to assess this species as a potential source of alginic acid for industrial use.

MATERIALS AND METHODS

Seaweeds were collected from four different shores (Hawks Bay, Buleji, Paradise Point and Manora) of Karachi coast on monthly interval for the period of one year. These shores are sandy, rocky and swampy wetlands. The coastal water of these shores shows a luxuriant growth of marine algae along offshore and inshore water. The sampling method of Chapman (1964) was followed for estimating the seaweed biomass. A quadrate of one-meter square was used for sampling. Each month ten quadrates (3 m apart) from low tidal mark to high tidal mark were randomly sampled in the intertidal zone. For biochemical estimation seaweed samples were dried at 70^{o}C for 24 hours and were ground in fine homogenized powder. Moisture and ash contents were estimated by standard method of A.O.A.C. (1990). The carbohydrate was estimated by the phenol-sulphuric acid method reported by Dubois *et al*. (1956).

Alginic acid extraction described here is based on the method described by Haug (1964) and Whyte (1998).The sample of seaweeds (10 gram) and 1% aqueous Calcium Chloride (300 ml) were taken in a 1 L beaker and kept on a stirred hot plate with stirring the contents continuously at 60 ^{o}C for 15 minutes. The mixture was then centrifuged at 2000 rpm for 5 minutes and re-extracted with calcium chloride; combined the residues and washed with water and dilute HCl (0.05M) and retuned the residue of above sample and treated with aqueous sodium carbonate, stirred contents continuously and heated at 45^{o}C for 30 minutes. The mixture was centrifuged and poured the alginate solution slowly into the 100 ml aqueous calcium chloride (25%) in a 2L beaker. The calcium alginate first washed

with ethanol and then cold hydrochloric acid. For confirmation of Ca alginate tested the filtrate with one drop of silver nitrate solution until no formation of chloride precipitate is evident. Finally washed with ethanol, acetone and diethyl ether for solvent exchange, and then dried, weighed and calculated percentage yield of alginic acid.

For the determination of purity and the physical properties i.e., relative density and viscosity of alginic acid the methods of Whyte *et al*. (1981) and Whyte (1998) were followed.

Table 1. Analysis of variance (ANOVA) of alginic acid, biomass and biochemical composition in *Sargassum boveanum* from different shores (Hawks Bay, Buleji, Paradise Point and Manora) of Karachi .

Alginic acid						
Source	DF	Seq SS	Adj SS	Adj MS	F	P
Shore	3	70.729	70.729	23.576	4.51**	0.009
Month	11	317.050	317.050	28.823	5.52***	0.000
Error	33	172.426	172.426	5.225		
Total	47	560.205				
Relative density						
Source	DF	Seq SS	Adj SS	Adj MS	F	P
Shore	3	0.008930	0.008930	0.002977	0.65	0.590
Month	11	0.046663	0.046663	0.004242	0.92	0.532
Error	33	0.151836	0.151836	0.004601		
Total	47	0.207428				
Viscosity						
Source	DF	Seq SS	Adj SS	Adj MS	F	P
Shore	3	730.59	730.59	243.53	4.55**	0.009
Month	11	513.70	513.70	46.70	0.87	0.575
Error	33	1766.97	1766.97	53.54		
Total	47	3011.26				
Biomass						
Source	DF	Seq SS	Adj SS	Adj MS	F	P
Shore	3	73472	3472	24491	1.52	0.229
Month	11	627914	627914	57083	3.53**	0.002
Error	33	533284	533284	16160		
Total	47	1234670				
Moisture						
Source	DF	Seq SS	Adj SS	Adj MS	F	P
Shore	3	205.039	205.039	68.346	68.32***	0.000
Month	11	17.482	17.482	1.589	1.59	0.148
Error	33	33.014	33.014	1.000		
Total	47	255.536				
Ash						
Source	DF	Seq SS	Adj SS	Adj MS	F	P
Shore	3	104.80	104.80	34.93	3.18*	0.037
Month	11	128.59	128.59	11.69	1.07	0.417
Error	33	362.16	362.16	10.97		
Total	47	595.55				
Carbohydrates						
Source	DF	Seq SS	Adj SS	Adj MS	F	P
Shore	3	105.054	105.054	35.018	5.32 **	0.004
Month	11	390.621	390.621	35.511	5.39 ***	0.000
Error	33	217.307	217.307	6.585		
Total	47	712.982				

RESULTS AND DISCUSSION

For the seasonal variation in water soluble polysaccharide alginic acid *S. boveanum* was collected from the four different shores (Hawks Bay, Buleji, Paradise Point and Manora) of Karachi coast in period of January 2005 to December 2005. The concentration of alginic acid in the alga was found to vary from 11.13- 24.69 % at Hawks Bay, 11.12-21.6 % at Buleji, 11.63-24.68 % at Paradise Point and 12.53-21.54 % at Manora shore (Fig. 1). At Hawks Bay the alginic acid content was found highest in February (24.69 %) and lowest in July (11.3 %) and at Buleji the content was found highest in March (21.6 %) and low in May (11.12 %) (Fig.1). Where as at Paradise Point alginic acid content was found highest in February (24.68 %) and lowest in September (11.63 %) and at Manora coast it was highest in January (21.54 %) and lowest in September (12.53 %) (Fig.1).

Table 2. Correlations (Pearson) between Alginic acid, biomass and biochemical constituents from different shores of Karachi coast.

	AAH	**AAB**	**AAP**	**AAM**
AAB	0.578			
AAP	0.638	0.340		
AAM	0.694	0.445	0.573	
BH	0.524	0.852	0.491	0.510
BB	0.699	0.548	0.829	0.754
BP	0.546	0.273	-0.150	0.407
BM	0.759	0.492	0.653	0.771
MH	0.497	0.078	-0.053	0.247
MB	0.670	0.628	0.501	0.310
MP	0.366	0.123	0.339	0.403
MM	0.642	0.433	0.599	0.925
AH	0.510	0.654	-0.071	0.450
AB	0.047	0.075	0.093	-0.146
AP	0.703	0.626	0.141	0.476
AM	0.282	0.537	0.640	0.413
CH	0.296	0.454	-0.101	0.408
CB	0.814	0.518	0.541	0.546
CP	0.791	0.763	0.383	0.687
CM	0.795	0.688	0.705	0.665

AAH= Alginic acid at Hawks Bay, AAB= Alginic acid at Buleji, AAP = Alginic acid at Paradise Point, AAM = Alginic acid at Manora; BH = Biomass at Hawks Bay, BB = Biomass at Buleji, BP = Biomass at Paradise Point, BM = Biomass at Manora; MH = Moisture at Hawks Bay, MB = Moisture at Buleji, MP = Moisture at Paradise Point, MM = Moisture at Manora; AH = Ash at Hawks Bay, AB = Ash at Buleji, AP = Ash at Paradise Point, AM = Ash at Manora; CH = Carbohydrate at Hawks Bay, CB = Carbohydrate at Buleji, CP = Carbohydrate at Paradise Point, CM = Carbohydrate at Manora.

The relative density (RD) of alginic acids was found to vary from 0.69-0.96 units at Hawks Bay, 0.68-0.94 units at Buleji, 0.78-0.98 units at Paradise point and 0.86-0.94 units at Manora shore (Fig. 2). At Hawks Bay shore the highest relative density was recorded in the month of September (0.96 units) and lowest in the month of April (0.69 units) and at Buleji it was highest in the month of February (0.94 units) and low in March (0.68 units) (Fig.2). At Paradise point shore the relative density was high in February (0.97 units) and low in January (0.78units) where as at Manora shore the highest value of relative density was found in May (0.94 units) (Fig. 2). These places are not far distant so the variation of RD in the place and time of collection may be dependant upon the rate of plant material collected and degree of decomposition.

The viscosity of the alginic acid extracted varied from 88.2-106.3 cp at Hawks Bay, 69.54-98.14 cp at Buleji, 66.9-100.3 cp at Paradise point and 85.6-101.6 cp at Manora shore (Fig. 3). At Hawks Bay the high viscosity of alginic acid was found in March (106.3 cp) and low in September (88.2 cp) where as at Buleji shore viscosity of alginic acid was high in April (98.1 cp) and low in March (69.5 cp) (Fig. 3). At Paradise Point viscosity was high in December (100.3 cp) and low in January (66.9 cp) and from Manora shore the value of viscosity was high in the month of September (101.6 cp) and low in January (85.6 cp) (Fig. 3).

Biomass of *Sargassum boveanum* varied from 590 -970 g.m^{-2} at Hawks Bay, 580-1150 g.m^{-2} at Buleji, 590-1150 g.m^{-2} at Paradise point and 510-1000 g.m^{-2} at Manora coast (Fig 4). Obviously, Buleji and Paradise Point were richer shores in biomass of *S. boveanum* as compared to Hawks Bay and Manora.

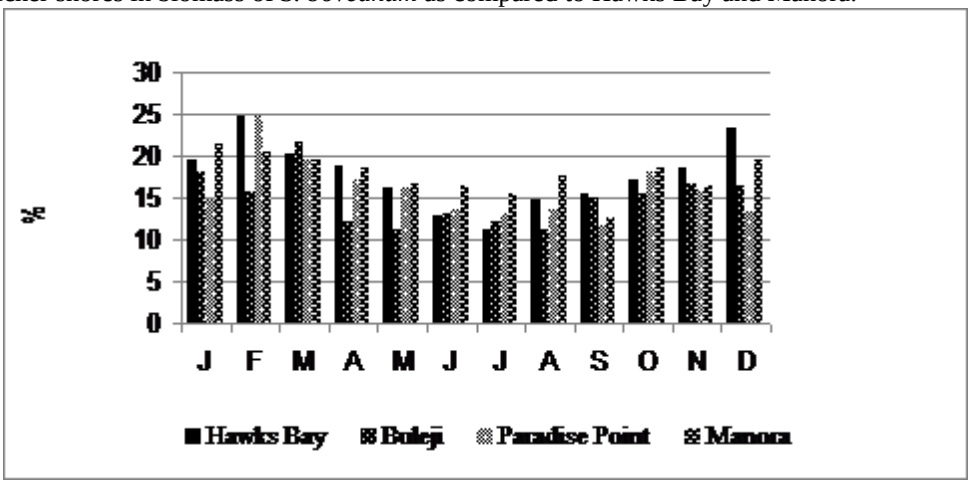

Fig.1. Seasonal variation in alginic acid of *S. boveanum* along the different shores of Karachi coast

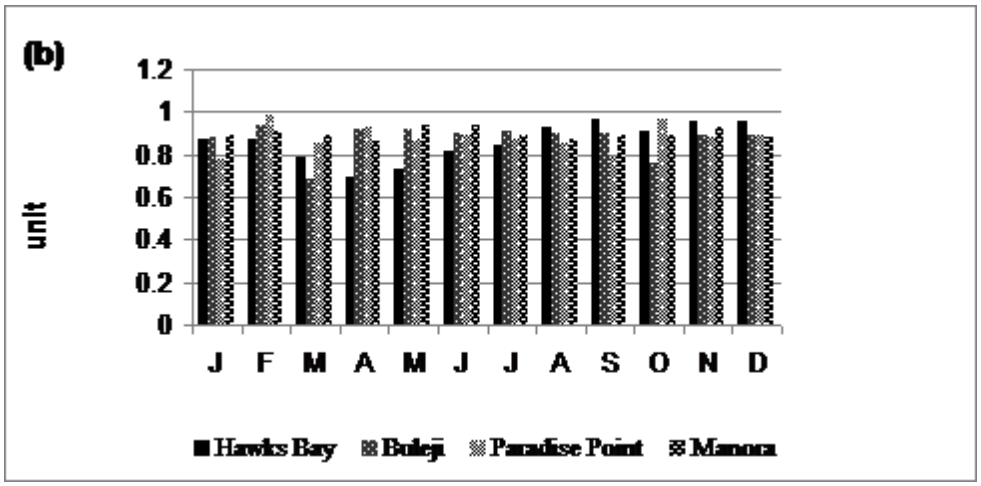

Fig.2. Seasonal variation in relative density of alginic acid in *S. boveanum* along the different shores of Karachi coast.

Fig.3. Seasonal variation in viscosity of alginic acid in *S. boveanum* along the different shores of Karachi coast.

The concentration of moisture was high in December at Hawks Bay (9.36 %) and in November at Buleji (13.9 %) where as in February at Paradise Point (13.67%) and Manora (7.69 %) (Fig. 5-8). Ash concentrations were high in December (41.35%) at Hawks Bay, and in February (37.65%) at Buleji (Fig. 5 and 6). At Paradise Point ash concentrations were high in ash was found high in January (35.02%) and at Manora in March (34.6%) (Fig. 7 and 8). The Carbohydrate concentrations were high in January (39.41%) at Hawks Bay and in February (46.98%) at Buleji where as at Paradise Point it was highest in January (39.65%) and at Manora in February (36.49 %) (Figs. 5-8).

The results of ANOVA (CRD) showed that there were significant variations between shores and months for alginic acid (F= 4.51 and F=5.52 respectively) and carbohydrate (F=5.32 and F=5.39 respectively) (Table 1). Significant variations were also found in between shores for viscosity (F=4.55), moisture (F= 68.32) and ash (F= 3.18) and between months for biomass (F= 3.53) (Table 1).

There was a positive significant correlation between alginic acid of different shores samples for e.g. alginic acid of Hawks Bay with alginic acid of Buleji (r^2 = 0.578), Paradise Point (r^2 = 0.638) and Manora (r^2 = 0.694) and alginic acid of Paradise Point with alginic acid of Manora (Table 2). There was also positive significant correlation found in between alginic acid and biomass at all studied shores except Paradise Point. Table 2 also shows that there was a positive significant correlation between alginic acid and moisture in samples of Buleji (r^2 = 0.628) and Manora (r^2 = 0.925); alginic acid and ash in sample of Hawks Bay (r^2 = 0.510) and alginic acid and carbohydrate in samples of Buleji (r^2 = 0.518) and Manora **(**r^2 = 0.665**).**

The results of the present study show that alginate yield and viscosity were influenced primarily by the season, place of collection and fertility state of the species. The highest alginic acid concentration was found at the shore of Hawks Bay and Paradise Point as compared to other two shores. It was also observed that the alginate yield was highest when *S. boveanum* attained its maximum biomass (Ragaza and Hurtado, 1999 a). The highest concentrations of alginic acid were found during the winter season (December-January). While the smaller percentages of alginic acid in summer season (June- August).

The results for yield of alginic acid extracted from *Sargassum boveanum* obtained during this study were similar to the values reported by Arvizu *et al.* (2007), Jothisaraswathi *et al.* (2006), Chandia *et al.* (2004), Jothi Saraswathi *et al*. (2003) and Mateus *et al*. (1977). The viscosity of alginic acid was also similar to the values reported by Jothisaraswathi *et al.* (2003) and Ragaza and Hurtado (1999 b). The results of the biomass of present study agree with the results recorded by Wong and Phang (2004), Qari and Qasim (1988, 1994), Qari (2002) and Abbas (2006). Moisture percentages are also similar to the results reported by Oliveira *et al.* (2009). The high percentages of ash and carbohydrate in the present investigation do not agree with the results given by Azad *et al*. (2007).

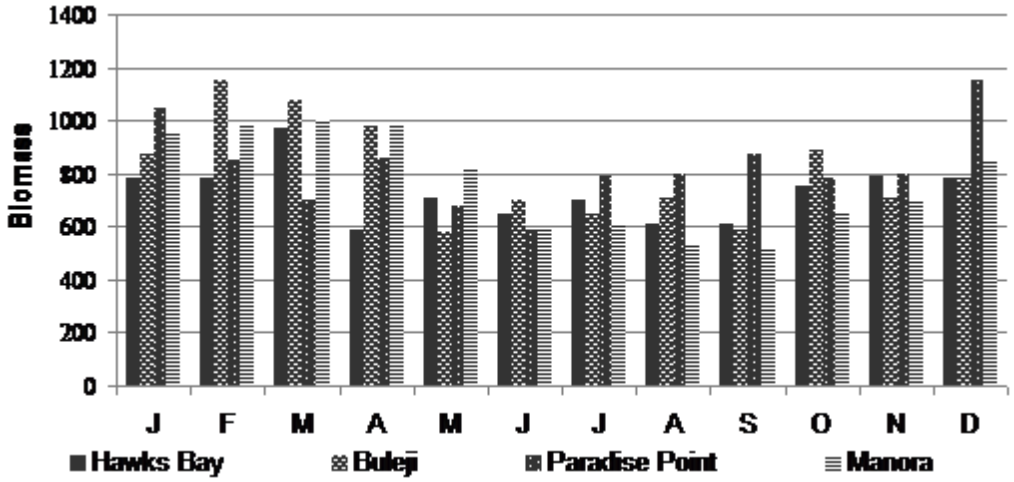

Fig. 4 . Seasonal variation in biomass g.m^{-2} of *S. boveanum* from different shores of Karachi coast.

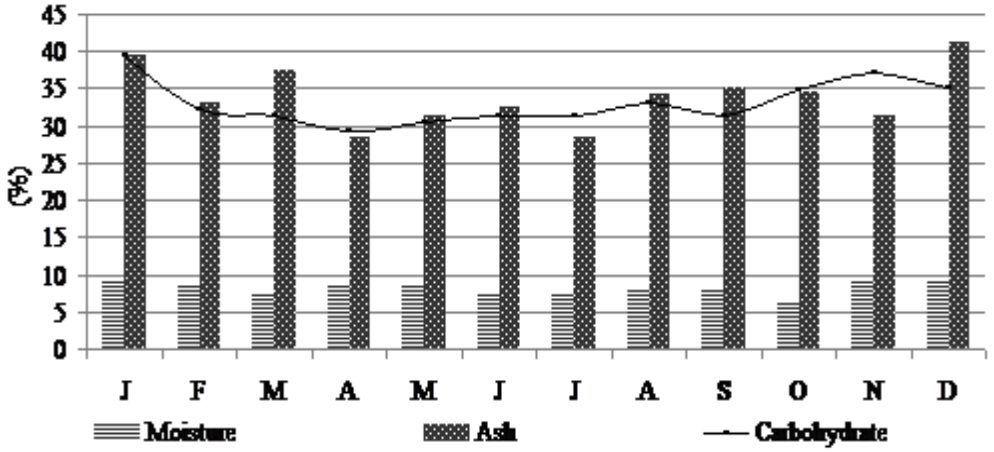

Fig. 5. Seasonal variation in biochemical constituent (%) of *S. boveanum* from Hawks Bay shore.

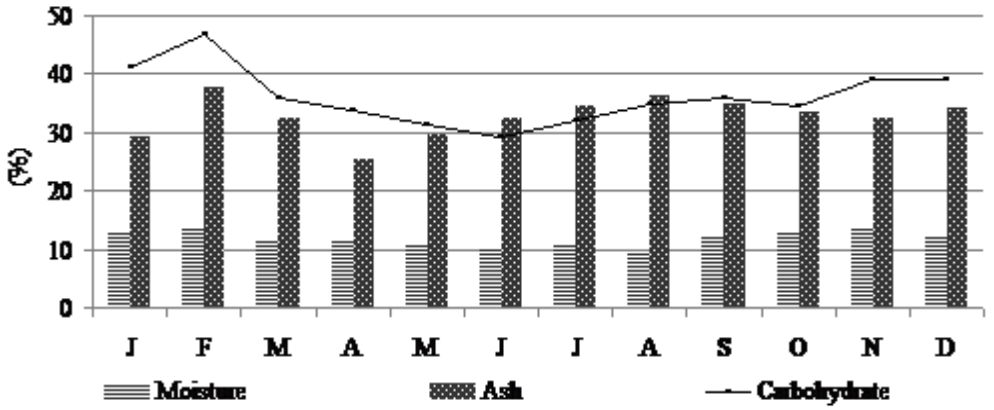

Fig. 6. Seasonal variation in biochemical constituent (%) of *S. boveanum* from Buleji shore.

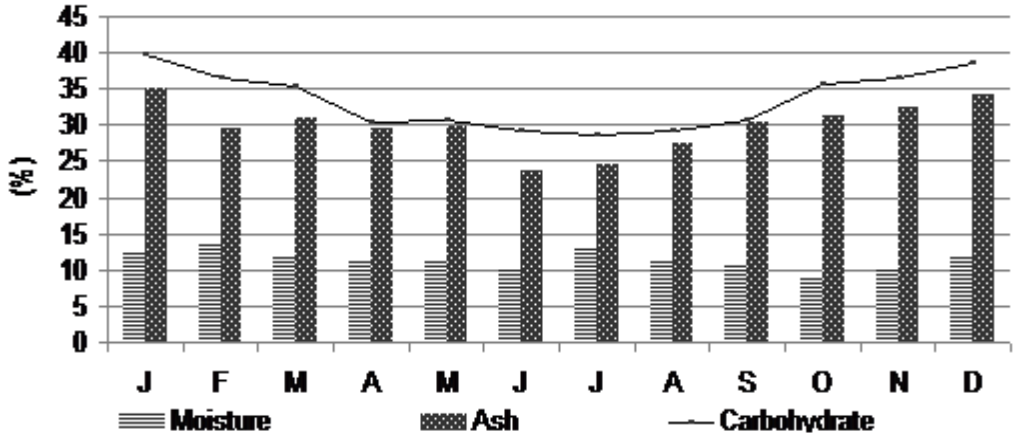

Fig. 7 Seasonal variation in biochemical constituent (%) of *S. boveanum* from Paradise Point shore.

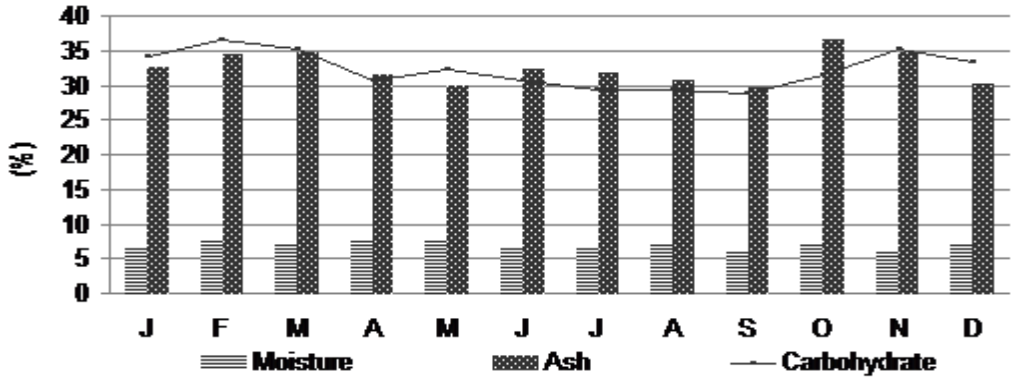

Fig. 8 Seasonal variation in biochemical constituent (%) of *S. boveanum* from Manora shore.

In short *S. boveanum*, abundant species at the shores of Karachi coast, has highly significant relationship between contents of alginic acid and biomass. The high yield of alginic acid (21.54-24.69 g %) was found during the winter period when the biomass show maximum values (780-1150 gm^{-2}). It means that environmental factors play an important role in affecting the growth of algal populations such as temperature (Ragaza and Hurtado, 1999 a). The variation were found in alginic acid concentrations at studied shore in different seasons also due to the environmental factors like temperature, pH, light, salinity and dissolved oxygen (Qari and Siddiqui, 2008, 2005 and 2004 and Qari, 2002).Viscosity of alginic acid from different shores of Karachi coast is variable and depends strongly upon the collection time and drying conditions of seaweeds. To avoid destruction of alginic acid the algae should be collected, transported and dried as quickly as possible due to polymer chain of alginic acid is sensitive to the high insolation temperature. So it is concluded that *Sargassum boveanum* in the area of study is potential source of alginic acid for the food and textile industries. Extraction methodology, however, to be further improved.

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