Carrageenan Content in Three Species of Hypnea (H. musciformis Wulfen J. V. Lamouroux, H. pannosa J. Agardh and H. valentiae Turner Montagne) of Karachi Coast

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Abstract Seaweeds are marine algae commonly found in marine environment. The red seaweed (Rhodophyta) is commonly exploited for the production of polysaccharides. Carrageenan is one of the commercial polysaccharide. It is complex of D-galactose 3, 6-anhydro D-galactose and monoesterified sulphuric acid found in cell wall of red seaweeds, used as an emulsifier, a binder. It is also used for suspension and stabilization in pharmaceutical, cosmetic and food processing. Hypnea is a red algal genus and well known carrageenan producing marine plant. Therefore three species of Hypnea: H. musciformis, H. pannosa and H. valentiae were collected from the four different coastal areas (Buleji, Hawks Bay, Manora and Paradise Point) of Karachi coast for the extraction of carrageenan in present study. The highest carrageenan extracted from H. valentiae (18-46.93%) as compared with the other two studied species. The high carrageenan concentration was mostly found in winter season. The total biomass 6846.79 g/m² of three species of Hypnea was recorded during the study period and the highest biomass was recorded in January and the lowest in August. The highest biomass was obtained from Manora coast as compared to other three coastal areas. There was significant correlation was found in between carrageenan and biomass. From the present investigation it is concluded that studied seaweeds species of Hypnea have good quantity of carrageenan and can be used in industries for the manufacturing and production of carrageenan.

Keywords Carrageenan; Gelling agent; Biomass; Karachi coast; Red seaweeds

Background Rhodophyta is the division name for red algae. Most of the world seaweeds, over 10,000 species belong to this group. The cell wall of red algae contains polygalactose esters and cellulose, some time impregnated with calcium carbonate. There are several red species of seaweeds have great demand due to their products as food items and hydrocolloids carrageenan and agar used in manufacturing of industrial products. The family Cystocloniaceae of order Gigartinales has the number of carrageenan producing species.

Carrageenan are a collective family of linear, sulfated galactans found in a number of commercially important species of red seaweeds (Sangha et al., 2011). Carrageenan is commonly used as a thickening, suspending, gelling and stabilizing agents for food products and other industrial applications as gelatin (Renn, 1997). It differs from agar mainly in its higher sulfated fraction and higher ash content (Ramalingam et al., 2003). Carrageenophytes were commercially grown in Philippines significantly for last four decades (Anicia et al., 2012). Pereira et al. (2003) have studied eight species of carrageenophytes collected from west Portuguese coast. Mtolera and Buriyo (2004) studied seasonal variation in content and quality of kappa-carrageenan of Hypnea musciformis from Western Indian Ocean. Seaweeds are still leading producer of carrageenan globally (Anicia et al., 2012). Hoffmann et al. (1995) studied the effect of isolation procedures on the molecular composition and physical properties of Eucheuma cottonii carrageenan.

The studies related to biomass have also been conducted in develop countries such as, Central Puget Sound Washington, USA (Thom, 1980), Dodger channel of Canada (Whyte, 1981) and North Carolina (Peckol, 1982). Chock and Mathieson (1983) quantified the standing crop and biomass variation of estuarine seaweed at Cedar point, Dover New Hampshire Maine, UK whereas Jagtap (1992) described the marine flora of Nicobar group of
island in Andaman Sea. Pedrini et al. (1989) reported the marine algae of Trindade Island, Brazil. Gavino and Trono (1999) studied diversity of the seaweeds flora of the Philippines and its utilization. More recently for the exploitation of seaweed resources Faveri et al. (2010) studied the temporal changes in the seaweeds flora in Southern Brazil and its potential causes.

The seaweed resources are abundant around the rocky shores of Karachi. Many studies have been made on the taxonomy (Shameel and Nizamuddin, 1972; Nizamuddin and Begum, 1978; Shameel, 1987), standing crop and biomass (Saleem, 1965; Saifullah, 1973; Qari and Qasim, 1988; 1994; Qari, 2002) at different coast of Karachi. Recently Qari et al. (2014) and Qari (2017) studied seaweeds diversity and distribution at the beach of Nathia Gali and Paradise Point of Karachi coast. Coastal waters around Hawks Bay, Sandspit, Buleji, Paradise Point, Nathia Gali, Manora and Cape Monze inhabit a variety of marine benthic algae (Qari and Qasim, 1988; Shameel and Tanaka, 1992; Qari and Qasim, 1994; Qari et al., 2014; Qari, 2017). In Pakistan still no any benefits get from the resources of seaweed and no any manufacturing industries or unit which is being used for making carrageenan from seaweeds. It may be due to very limited knowledge of carrageenan processing technology.

The present study is the first investigation in Pakistan. Hence the main objective of this work was to find out the yield of carrageenan and gel properties of three species of Hypnea which occur abundantly at the coast of Karachi and may be used for the exploitation of carrageenan on commercial scale. Therefore in present study worked on abundance and distribution (Biomass) of three species of Hypnea (carrageenophytes) and extracted carrageenan from them, along with prevailing hydrographic conditions of four different coastal areas of Karachi coast (Buleji, Hawks Bay, Manora and Paradise Point).

1 Materials and Methods

The monthly fresh samples of Hypnea species (H. musciformis, H. pannosa and H. valentiae) were collected from four exposed shores of Karachi coast Buleji, Hawks Bay, Manora and Paradise Point at low tide by the quadrat method (Chapman, 1964). All three species were placed individually in prelabeled plastic bags and brought to the laboratory where all plants were carefully cleaned from mud debris and other epiphytes with filtered seawater.

The kappa carrageenan was extracted according to a method adopted from Vandermeulen (1988). The gellan temperature, melting point, relative density, viscosity and strength determined by the method of Whyte et al. (1981). Temperature of seawater was read using a mercury thermometer. Water samples of pH were measured with the digital pH meter (Model PM-65) and salinity was measured by hand-hold Refractometer.

2 Results and Discussion

During the study period the total one hundred four samples (104) were collected from four sites: Buleji (31 samples), Hawks Bay (19 samples), Manora (29 samples) and Paradise Point (25 samples). Total 39 numbers of individuals of Hypnea musciformis, 28 H. pannosa and 37 H. valentiae were recorded in the whole study period. The present data reveals high variability in the content of carrageenan in between all three species, sampling sites and collection time (Figure 1; Figure 2; Figure 3). The concentration of carrageenan was mostly decreasing from January to August and then again increased till December (Figure 1). The high carrageenan concentration was mostly found in winter season. The highest content of carrageenan was obtained from Buleji samples as compared to other three coasts samples (Figure 2). The highest carrageenan extracted from H. valentiae (18-46.93%) as compared to other two species H. musciformis (18-31%) and H. pannosa (20-30.4%) (Figure 3).

The total biomass 6846.79 g/m² was recorded during the study period and the highest biomass was recorded in January (919.38g/m²) and the lowest (97.8 g/m²) recorded in August (Figure 4). The highest biomass was obtained from Manora coast as compared to other three coastal areas (Figure 5). The highest contribution to the total biomass was observed by H. musciformis 37.63% as compared to H. pannosa (34.87%) and H. valentiae (27.49%) (Figure 6).
Figure 1 Monthly variation in carrageenan content (% dry wt) of Hypnea musciformis, H. pannosa and H. valentiae at Karachi coast

Figure 2 Variation in carrageenan content (% dry wt) at four different sites (Buleji, Hawks Bay, Manora and Paradise Point) of Karachi coast

Figure 3 Variation in carrageenan content (% dry wt) of Hypnea musciformis, H. pannosa and H. valentiae at Karachi coast
Figure 4 Monthly variation in biomass (g/m²) of *Hypnea musciformis*, *H. pannosa* and *H. valentiae* of Karachi coast

Figure 5 Variation in biomass (g/m²) at four different sites (Buleji, Hawks Bay, Manora and Paradise Point) of Karachi coast

Figure 6 Variation in biomass (g/m²) of *Hypnea musciformis*, *H. pannosa* and *H. valentiae* at Karachi coast
The range of carrageenan content in *Hypnea musciformis* was 18-31% with mean concentration value of 23.72%. In *H. musciformis* Carrageenan concentration varied throughout the years. At Buleji it was high (31%) in May and August whereas at Hawks Bay and Paradise Point in February (28% and 24% respectively) and at Manora (29.7%) in May (Figure 7). In *H. musciformis* the range of biomass value were 20-120 g/m² with mean value of 63.45 g/m² for the four sites Buleji, Hawks Bay, Manora and Paradise Point (Figure 8). At Buleji biomass was high (112 g/m²) in May whereas at Hawks Bay and Manora in January (58 g/m² and 120 g/m² respectively) and at Manora (91.32 g/m²) in November (Figure 8).

**Figure 7** Seasonal variation in carrageenan content of *H. musciformis* at four different sites of Karachi coast

**Figure 8** Seasonal variation in biomass (g/m²) of *H. musciformis* at four different sites of Karachi coast
The extract of carrageenan in *H. musciformis* had density in a range of 0.95-1.34 g/cm³ in all sites samples. In Buleji samples it was high in May (1.34 g/cm³) and in Hawks Bay and Manora samples it was high in December (1.2 g/cm³) whereas in Paradise Point samples it was high (1.1 g/cm³) in November (Table 1). The gel viscosity of *H. musciformis* carrageenan extract was in the range of 7-18 cP. In Buleji samples viscosity was high in January and September (16.57 cP and 16.0 cP respectively) and in Hawks Bay samples it was high (18 cP ) in March. Whereas in Manora samples viscosity was high in January and September (17 cP) and in Paradise Point samples it was high (18 cP) in December (Table 2). The gel boiling point of *H. musciformis* carrageenan was in the range of 30-56.5°C. In Buleji, Hawks Bay and Manora samples boiling point was high in June (55°C, 56.5°C and 52°C respectively) whereas in Paradise Point samples it was high (43°C) in March (Table 3). The gel melting point in *H. musciformis* carrageenan samples were in the range of (42-77°C). The highest melting point was found in January in both Buleji (67°C) and Paradise Point (68°C) samples. Whereas in Hawks Bay samples it was high in March (77°C) and in Manora samples it was high in May (71°C) (Table 4). The gel strength of *H. musciformis* carrageenan extract was 100 g/cm² in all sites samples except Buleji sample has high gel strength (105 g/cm² ) (Table 5).

Table 1 Gel density (g/cm³) of Carrageenan extracted from *Hypnea musciformis*, *H. pannosa*, *H. valentiae* at four different sites of Karachi coast

| Month | Buleji | Hawk Bay | Manora | Paradise Point | Buleji | Hawk Bay | Manora | Paradise Point | Buleji | Hawk Bay | Manora | Paradise Point |
|-------|--------|----------|--------|----------------|--------|----------|--------|----------------|--------|----------|--------|----------------|
| J     | 1.07   | 1.00     | 1.00   | 1.00           | 1.02   | 0.97     | 1.05   | 1.00           | 1.00   | 1.00     | 1.00   | 1.03           |
| F     | 1.20   | 0.98     | 0.98   | 1.00           | -      | -        | 1.10   | 0.98           | 1.00   | 1.09     | 1.00   | 1.00           |
| M     | 1.00   | 0.97     | 1.00   | 0.98           | 1.02   | 1.00     | -      | 0.99           | 0.96   | 1.00     | 1.00   | 1.12           |
| A     | 1.00   | -        | 1.00   | 1.09           | 1.09   | 1.00     | 1.17   | 1.00           | -      | -        | 1.00   |                |
| M     | 1.34   | -        | 1.09   | 1.00           | 1.00   | -        | -      | 1.00           | 1.00   | -        | 1.00   |                |
| J     | 1.20   | 0.97     | 1.00   | -              | -      | -        | -      | 1.11           | 1.10   | 1.00     | -      |                |
| J     | 1.00   | 1.00     | 0.98   | -              | -      | -        | -      | 1.00           | 1.00   | 1.00     | 1.02   | 1.20           |
| A     | 1.10   | -        | -      | -              | -      | -        | -      | -              | 1.00   | -        |        |                |
| S     | 1.00   | -        | 1.10   | 1.00           | 1.00   | 0.98     | 1.00   | -              | 1.00   | -        | 1.00   | 1.00           |
| O     | 1.00   | -        | 1.00   | 1.00           | 1.1    | -        | 1.05   | -              | 0.95   | 1.21     | 1.00   | 1.00           |
| N     | 1.00   | 1.00     | 1.10   | 1.10           | 1.04   | 0.97     | 1.00   | 1.00           | 1.10   | 1.00     | 1.00   | 1.04           |
| D     | 1.00   | 1.20     | 1.20   | 1.00           | 1      | -        | 1.00   | 1.00           | 1.10   | 1.00     | 1.00   | 1.04           |

Table 2 Gel Viscosity (cP) of Carrageenan extracted from *Hypnea musciformis*, *H. pannosa*, *H. valentiae* at four different sites of Karachi coast

| Month | Buleji | Hawk Bay | Manora | Paradise Point | Buleji | Hawk Bay | Manora | Paradise Point | Buleji | Hawk Bay | Manora | Paradise Point |
|-------|--------|----------|--------|----------------|--------|----------|--------|----------------|--------|----------|--------|----------------|
| J     | 16.57  | 15.00    | 17.00  | 13.61          | 14.00  | 12.00    | 12.00  | 12.00          | 12.00  | 12.00    | 19.00  | 14.00         |
| F     | 13.00  | 13.00    | 12.00  | 12.00          | -      | -        | 11.00  | 12.60          | 13.00  | 13.50    | 13.00  | 11.00         |
| M     | 12.00  | 18.00    | 11.00  | 12.00          | 8.65   | 11.80    | -      | 14.00          | 9.00   | 10.00    | 14.00  | 12.00         |
| A     | 17.00  | 11.45    | 8.72   | 8.70           | 9.00   | 7.00     | 8.56   | 12.00          | -      | -        | -      | 12.00         |
| M     | 9.54   | 9.60     | 7.00   | 12.00          | -      | -        | 11.24  | 8.80           | -      | 9.00     | -      |               |
| J     | 13.00  | 13.00    | 11.00  | -              | -      | -        | -      | 12.60          | 10.00  | 11.00    | 9.60   |               |
| J     | 12.00  | 12.00    | 12.00  | -              | -      | -        | -      | 12.00          | 12.70  | 17.00    | 12.80  |               |
| A     | 14.00  | -        | -      | -              | -      | -        | -      | -              | -      | -        | 11.00  |               |
| S     | 16.00  | 17.00    | 13.00  | 13.60          | 11.00  | 11.50    | 11.50  | 11.00          | 16.42  | 12.84    | -      |               |
| O     | 12.00  | 12.00    | 11.64  | 12.00          | -      | -        | 9.00   | 10.00          | 15.00  | 10.00    | -      |               |
| N     | 10.00  | 10.00    | 10.00  | 9.00           | 11.00  | 10.00    | 10.00  | 10.00          | 12.00  | 11.00    | -      |               |
| D     | 12.34  | 12.00    | 13.70  | 18.00          | 12.00  | -        | 11.00  | 12.64          | 13.50  | -        | 10.00  | 10.00         |
Table 3 Gel boiling point (°C) of Carrageenan extracted from *Hypnea musciformis*, *H. pannosa*, *H. valentiae* at four different sites of Karachi coast

| Month | Buleji | Hawks Bay | Manora | Paradise Point | Buleji | Hawks Bay | Manora | Paradise Point | Buleji | Hawks Bay | Manora | Paradise Point |
|-------|--------|-----------|--------|---------------|--------|-----------|--------|---------------|--------|-----------|--------|---------------|
| J     | 43.0   | 44.0      | 30.0   | 41.0          | 38.0   | 40.0      | 52.0   | 44.0          | 40.0   | 53.0      | 48.6   | 42.0          |
| F     | 38.7   | 47.6      | 37.0   | 38.6          | -      | -         | 48.0   | 48.5          | 47.0   | 50.0      | 45.0   | 51.0          |
| M     | 44.0   | 42.5      | 36.8   | 43.0          | 42.0   | 37.0      | -      | 46.0          | 48.5   | 50.5      | 45.0   | 46.0          |
| A     | 46.4   | -         | 38.0   | 40.5          | 40.0   | 45.0      | 45.0   | 44.0          | 48.0   | -         | -      | 44.0          |
| M     | 41.0   | -         | 43.0   | 40.0          | 53.0   | -         | -      | 54.0          | 50.0   | -         | 51.0   | -             |
| J     | 55.0   | 56.5      | 52.0   | -             | -      | -         | 44.5   | -             | 50.0   | 50.0      | 43.0   | -             |
| J     | 48.3   | 46.2      | 41.7   | -             | -      | -         | -      | -             | 52.0   | 52.5      | 40.0   | 45.0          |
| A     | 43.0   | -         | -      | -             | -      | -         | -      | -             | -      | 46.0      | -      | -             |
| S     | 37.8   | -         | 44.0   | 36.0          | 51.0   | 50.0      | 50.0   | -             | 48.0   | -         | 44.0   | 44.0          |
| O     | 40.0   | -         | 38.0   | 40.5          | 54.0   | -         | 46.0   | -             | 44.0   | 53.5      | 51.5   | 44.0          |
| N     | 44.8   | 45.4      | 42.0   | 40.0          | 44.0   | 44.0      | 40.0   | 40.0          | 48.0   | 53.0      | 44.0   | -             |
| D     | 51.2   | 44.0      | 42.0   | 40.0          | 40.0   | -         | 41.5   | 44.0          | 48.0   | -         | 44.0   | 44.0          |

Table 4 Gel melting point (°C) of Carrageenan extracted from *Hypnea musciformis*, *H. pannosa*, *H. valentiae* at four different sites of Karachi coast

| Month | Buleji | Hawks Bay | Manora | Paradise Point | Buleji | Hawks Bay | Manora | Paradise Point | Buleji | Hawks Bay | Manora | Paradise Point |
|-------|--------|-----------|--------|---------------|--------|-----------|--------|---------------|--------|-----------|--------|---------------|
| J     | 67.0   | 51.5      | 50.0   | 68.0          | 43.0   | 52.0      | 48.5   | 51.5          | 48.5   | 41.5      | 53.0   | 44.0          |
| F     | 48.0   | 42.2      | 45.0   | 43.0          | -      | -         | 50.0   | 55.0          | 46.0   | 43.0      | 51.5   | 53.5          |
| M     | 51.4   | 77.0      | 67.0   | 42.0          | 50.0   | 46.0      | -      | 54.0          | 47.0   | 51.0      | 51.0   | 55.0          |
| A     | 45.0   | -         | 51.0   | 50.0          | 50.0   | 44.5      | 42.0   | 46.0          | 47.5   | -         | 47.0   | 46.0          |
| M     | 44.0   | -         | 71.0   | 53.0          | 50.5   | -         | 51.0   | 51.0          | 46.0   | -         | -      | -             |
| J     | 46.0   | 67.0      | 63.0   | -             | -      | -         | 47.0   | -             | 50.0   | 48.0      | 44.5   | -             |
| J     | 54.0   | 60.0      | 42.0   | -             | -      | -         | -      | -             | 53.0   | 52.0      | 45.0   | 41.0          |
| A     | 60.5   | -         | -      | -             | -      | -         | -      | -             | -      | 47.0      | -      | -             |
| S     | 61.0   | -         | 60.0   | 51.0          | 50.0   | 52.0      | 44.0   | -             | 55.0   | -         | 52.0   | 44.0          |
| O     | 56.5   | -         | 53.8   | 50.0          | 52.0   | -         | 51.0   | -             | 51.0   | 53.0      | 55.0   | 44.5          |
| N     | 50.0   | 50.5      | 55.0   | 50.0          | 50.0   | 48.0      | 50.0   | 52.0          | 51.0   | 55.0      | 45.0   | -             |
| D     | 53.0   | 54.0      | 55.0   | 52.0          | 50.5   | -         | 50.0   | 48.0          | 50.0   | -         | 48.5   | 50.5          |

Table 5 Gel strength (g/cm³) of Carrageenan extracted from *Hypnea musciformis*, *H. pannosa*, *H. valentiae* at four different sites of Karachi coast

| S.NO | Seaweeds Species | Collection Sites | Gel Strength |
|------|-----------------|-----------------|--------------|
| 1    | *H. musciformis* | Buleji          | 105          |
|      |                  | Hawks Bay       | 100          |
|      |                  | Manora          | 100          |
|      |                  | Paradise Point  | 100          |
| 2    | *H. pannosa*     | Buleji          | 98           |
|      |                  | Hawks Bay       | 120          |
|      |                  | Manora          | 105          |
|      |                  | Paradise Point  | 100          |
| 3    | *H. valentiae*   | Buleji          | 98           |
|      |                  | Hawks Bay       | 100          |
|      |                  | Manora          | 105          |
|      |                  | Paradise Point  | 102          |
In *H. pannosa* the range of carrageenan was 20-30.4% with mean concentration 24.24%. Carrageenan concentration in *H. pannosa* was high 28% in September at Buleji, 26% in January at Hawks Bay, 30% in April and November at Manora and 30.4% in February at Paradise Point (Figure 9). The range of biomass value was 51.7-121 g/m² with mean value of 84.4 g/m² for the four sites Buleji, Hawks Bay, Manora and Paradise Point in *H. pannosa* (Figure 7). At Buleji biomass was high (112 g/m²) in March whereas at Hawks Bay, Manora and Paradise Point in January (96 g/m², 121 g/m² and 100 g/m² respectively) (Figure 10).

The relative density of carrageenan samples of *H. pannosa* was in a range of 0.97-1.17 g/cm³ in all sites samples. In Buleji samples it was high in October (1.1 g/cm³) and in Hawks Bay it was high in November whereas in Manora samples it was high in April (1.17 g/cm³) and in Paradise Point samples it was 1.0 g/cm³ except February and March (Table 1). The gel viscosity of *H. pannosa* carrageenan extract was in the range of 7-14 cP. In both Buleji (14 cP) and Hawks Bay (12 cP) samples viscosity was high in January and in Manora samples it was high (12.60 cP) in June, whereas in Paradise Point samples viscosity was high (14 cP) in March (Table 2). The gel boiling point of *H. pannosa* carrageenan was in the range of 37-54°C. In Buleji samples boiling point was high in October (54°C) and in Hawks Bay samples it was high in September (50). In Manora samples boiling point was high (52°C) in January and in Paradise Point samples high (54°C) in May (Table 3). The gel melting point in *H. pannosa* was high (52°C) in January and in Paradise Point samples high (54°C) in May (Figure 10).
H. pannosa carrageenan samples were in the range of (42-55°C). The highest melting point was found in October in both Buleji (54°C) and Manora (51°C) samples. Whereas in Hawks Bay samples it was high (52°C) in January and September and in Manora samples it was high in February (55°C) (Table 4). The gel strength of H. pannosa carrageenan extract samples was in the range of 98-120 g/cm² and the high value was found in Hawks Bay sample (120 g/cm²) (Table 5).

In H. valentiae the range of carrageenan was 18-46.93% with mean concentration 35.41%. Carrageenan concentration in H. valentiae was high in April (46.93%) and September (46%) at Buleji, in October (42.50%) at Hawks Bay, in March (35.60%) at Manora and July and September (33.6 % and 33% respectively) at Paradise Point (Figure 11). In H. valentiae the range of biomass value was 31.8-64.75 g/m² with mean value of 50.86 g/m² for the four sites Buleji, Hawks Bay, Manora and Paradise Point (Figure 7). At Buleji and Manora biomass was high in October (62.8 g/m² and 60.4 g/m², respectively) and at Hawks Bay and Paradise Point in January (61.46 g/m² and 64.75 g/m² respectively) (Figure 12).

The density of carrageenan samples of H. valentiae was in a range of 0.95-1.21 g/cm³ in all sites samples. In Buleji samples it was high in June (1.11 g/cm³) and in Hawks Bay it was high in October (1.21 g/cm³). Whereas in
Manora samples it was high in January (1.03 g/cm³) and in Paradise Point samples it was 1.2 g/cm³ in July (Table 1). The gel viscosity of *H. valentiae* carrageenan extract was in the range of 8.8-19 cP. In both Buleji (19 cP) and Hawks Bay (14 cP) samples viscosity was high in January and in Manora samples it was high (17 cP) in July whereas in samples of Paradise Point viscosity was high (16 cP) in February (Table 2). The gel boiling point of *H. valentiae* carrageenan was in the range of 40-53.5°C. In Buleji samples boiling point was high in July (52°C) and in both Hawks Bay (53.5°C) and Manora (52.5°C) samples it was high in October. In Paradise Point samples it was high (51°C) in February (Table 3). The gel melting point in *H. valentiae* carrageenan samples were in the range of (41-55°C). The highest melting point (55°C) was found in September, November, October and March in all studied sites samples i.e., Buleji, Hawks Bay, Manora and Paradise Point. The gel strength of *H. valentiae* carrageenan extracts was in the range of 98-105 g/cm² and the high value was found in Manora sample (105 g/cm²) (Table 5).

Table 6 showed the temperature of seawater ranged from 18-34°C and the mean was 25.44°C during the study period. The high temperature in seawater was recorded in summer (June to August) and the lowest temperature was recorded in winter (December to February). The salinity of seawater ranged from 34.5-38‰ with the mean of 36.33‰. The high salinity (38‰) was recorded in April and January in the sample of Buleji and Hawks Bay respectively (Table 6). The pH of surface seawater ranged from 6.5-7.5 with the mean of 7.0 (Table 6). In the present study it is noted that physical factor temperature affect on the biomass and carrageenan content.

Table 6 Variation in hydrographic conditions of seawater collected from four different sites of Karachi coast

| Month | Temperature | pH | Salinity |
|-------|-------------|----|----------|
|       | Buleji      | Hawks | Manora | Paradise Point | Buleji | Hawks | Manora | Paradise Point | Buleji | Hawks | Manora | Paradise Point | Buleji | Hawks | Manora | Paradise Point |
| J     | 18.00       | 18.50 | 18.00 | 18.00     | 7.00   | 7.50  | 6.40   | 7.00     | 37.00  | 38.00 | 35.50 | 37.00          |
| F     | 20.50       | 24.00 | 23.00 | 23.00     | 7.00   | 7.00  | 7.00   | 7.00     | 36.80  | 36.00 | 36.00 | 37.00          |
| M     | 22.00       | 26.00 | 26.00 | 23.50     | 7.00   | 7.00  | 7.00   | 7.00     | 37.00  | 36.00 | 36.00 | 36.50          |
| A     | 23.00       | 27.00 | 27.50 | 25.00     | 7.50   | 7.00  | 6.85   | 6.80     | 38.00  | 35.00 | 34.50 | 36.00          |
| M     | 28.50       | 30.40 | 28.00 | 26.00     | 7.50   | 6.50  | 6.50   | 6.80     | 36.00  | 37.00 | 36.00 | 37.00          |
| J     | 30.00       | 32.00 | 30.00 | 28.00     | 7.50   | 7.00  | 7.00   | 7.00     | 36.50  | 36.00 | 36.00 | 37.00          |
| J     | 28.00       | 34.00 | 29.50 | 28.00     | 7.50   | 6.50  | 7.00   | 7.30     | 36.00  | 36.00 | 36.00 | 37.00          |
| A     | 28.00       | 30.00 | 30.00 | 27.00     | 7.00   | 7.00  | 6.60   | 7.00     | 36.00  | 36.00 | 36.50 | 37.50          |
| S     | 26.50       | 29.50 | 29.00 | 25.00     | 7.00   | 7.00  | 7.00   | 7.00     | 36.50  | 36.50 | 36.00 | 36.00          |
| O     | 25.00       | 29.00 | 28.50 | 24.50     | 7.00   | 7.00  | 7.00   | 7.00     | 36.00  | 36.50 | 36.00 | 36.00          |
| N     | 23.00       | 26.00 | 26.00 | 24.00     | 7.20   | 7.50  | 7.50   | 7.00     | 36.50  | 36.00 | 36.00 | 36.00          |
| D     | 18.00       | 18.00 | 18.50 | 20.00     | 7.00   | 7.00  | 7.00   | 7.00     | 37.00  | 37.00 | 36.00 | 35.00          |

The results of two way analysis of variance (ANOVA) in carrageenan of three different species of *Hypnea* collected from Karachi coast show that there were highly significant variations observed between species (P<0.001), month (P<0.01) and sites (P<0.01). Analysis of variance ANOVA showed that there were highly significant variations observed in biomass between species (P<0.05) and in between months and sites (P<0.001). The differences in species, sites and month in present results reveal that biomass and carrageenan content was different in different species at different sites in different times (Table 7; Table 8). The data for biomass and carrageenan concentrations in different species were analyzed by looking at the relationship in between carrageenan of three different species of *Hypnea* at four different sites found; particular positive significant correlation such as at Hawks Bay in carrageenan of *Hypnea musciformis* and *H. valentiae* correlation was $r^2=0.617$ and at Paradise Point in carrageenan of *Hypnea musciformis* and *H. pammoa* correlation was $r^2=0.717$. There was significant correlation was found in between carrageenan and biomass ($r^2=0.671$). The hydrographic conditions showed insignificant correlation with carrageenan content and biomass of all three studied species.
The results for yield of carrageenan extracted from *Hypnea musciformis* in present work was similar to the values reported by Tuvikene et al. (2006). Dass et al. (1980) obtained high concentration of carrageenan from *Hypnea musciformis* (51.6%) in December when compared with present study. Rodrigueza and Montano (2007) discover in their study that carrageenan yield and biomass were inversely related to each other whereas in present study both carrageenan yield and biomass were found high in the same time i.e., winter season (Figure 1; Figure 4). Qasim and Qari (1988; 1994), Qari et al. (2014), Qari (2017) reported that winter (December to February) was the period of optimal growth and would be the best period for optimum harvesting of seaweeds due to winter period with low air temperature and sun light i.e., low intensity and small duration and high dissolve nutrients are present that affect seaweeds growth, biomass and biochemical composition that results in high biomass of seaweed in winter compared to other seasons. Juneja et al. (2013) described in their study that the relative amounts of carbohydrate product are linked to environmental conditions (temperature, pH and salinity). The present data also showed that seasonal differences between locations and years in the magnitude and timing of the environmental parameters affecting algal growth and polysaccharides content.

The carrageenan is actually natural carbohydrate obtained from red seaweeds (Necas and Bartosikova, 2013). All *Hypnea* species and other carrageenophytes available at Karachi coastal areas in abundant quantities can be utilized for obtaining carrageenan in Pakistan (Qari et al., 2014; Qari, 2017). Today desired and need is the utilization of seaweed in food and medicine. Considering these facts, the present data contributes baseline information about the exploitation of seaweeds as a source of carrageenan for bright future of marine algae or seaweeds.

### 4 Conclusions

The studied shores have rich vegetation of red seaweed. The data of present study reveals high variability in the content of carrageenan in between species, sampling sites and collection time. The highest carrageenan extracted from *H. valentiae* (18-46.93%) as compared with the other two studied species. The highest concentrations of carrageenan were found during the winter season and it was concluded that environmental conditions may play a vital role in the development of these polymers in situ in the red seaweeds. The viscosity measurement showed high molecular weight polysaccharides. The high yield of carrageenan from the above mention species of seaweeds proves their increased market demand and global exploitation. There are also seasonal differences
between locations and years in the magnitude and timing of the environmental parameters affecting algal growth. Considering these facts, the present data contributes baseline information about the exploitation of seaweeds as a source of carrageenan for bright future of marine algae or seaweeds.

Authors’ contributions
Qaisar Abbas and Abdul Rahim Khan carried out the field work for data collection and also conducted the experiment. Rashida Qari designed, interpreted the data, made figures, statistical analysis, and drafted the manuscript. All authors read and approved the final manuscript.

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