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Bioactive compounds, nutritive properties and adaptation of five seaweeds growing in Cox's Bazar, Bangladesh

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ABSTRACT

The study focused on evaluating the adaptation, nutrient properties, and bioactive compounds of five different seaweed species. Samples were collected in March 2023 from the Rezu Khal estuary, North Nuniachara and St. Martin's Island on the western coast, Cox's Bazar District, Bangladesh. The research found diverse nutrient properties, with varying concentrations of minerals like Na (42.08 to 245.43 mg/kg) and K (71.15 to 445.48 mg/kg) among the seaweeds. *Hypnea musciformis* and *H. spinella* have been found to accumulate more Na than K. Research showed fluctuating levels of heavy metals like Pb, Cr, Ni, and Cd, but they remained within tolerable limits, making these seaweeds non-toxic and suitable for consumption. The seaweed was found to be rich in bioactive compounds, including tannins, saponins, terpenoids, phenols, flavonoids, and steroids having industrial and medicinal applications. *Enteromorpha intestinalis* and *Gracilaria tenuistipitata* exhibited the highest chlorophyll and carotenoid content, respectively. Significant variations ($P < 0.05$) in soluble protein, total phenolic, and flavonoid content were observed, with *G. tenuistipitata*from North Nuniachara standing out for elevated phenols, flavonoids, and protein. These findings highlighted the adaptability of seaweeds to changing marine environments, showcasing their resilience to stress, and microbial attacks, owing to their rich nutrient contents and diverse bioactive metabolites. However, in short, this study underscores the potential of seaweed as valuable natural resources with both nutritional and industrial significance.

Introduction

The Earth's physical and biological systems are being affected by environmental stresses brought on by global warming that are beyond the capacity of nature to restore[1–4]. Marine plants keep the balance of marine environments while also serving as a source of food and chemical compounds[4,5]. Generally, seaweed is a prominent component of ocean ecosystems and photosynthetic autotrophs[4,6–8]. Particularly, seaweeds significantly reduce the quantity of CO_2 entering the aquatic ecosystem and supply different ecological benefits, including the removal of contaminants from the beach and supplying other aquatic creatures a place to live[4,9]. They are often referred to as sea vegetables, which is a highly underutilized crop that has the potential to make a significant contribution to global food security and appears as a nutrient-dense food source when produced and consumed in compliance with safety regulations[5]. They are well known for their nutritional value, which includes vitamins, minerals, proteins, polysaccharides, and dietary fibers in addition to being a low-calorie meal[10]. Bangladesh is the largest deltaic nation in the world, the coastal area divided into three zones (i.e., southwest, central, and southeast coasts), and on the whole coastline of Bangladesh, the Sundarbans Mangrove Forest, St. Martin's Island, and Cox's Bazar have the highest quantities of seaweed because of their sandy and muddy beaches, estuaries, and mangrove swamps, which provide substrate and habitat for various seaweed cultivation[11,12]. The coastal population can obtain very nutritious, naturally cultivated seaweeds in intertidal, tidal, and subtidal zones of coastal waters[3,13,14]. However, aquatic ecosystems are exposed to metals of anthropogenic origin, and seaweeds get overloaded with this metals[15], as they are renowned for their ability to bind to heavy metals[16]. Seaweeds

supply food, shelter, and refuge for various herbivorous grazers such as crustaceans, gastropods, echinoderms, and fishes, as a wide range of marine herbivores are heavily reliant on them[17,18]. They typically generate secondary metabolites in reaction to adverse environmental circumstances, displaying resilience in their metabolism is attributed to the presence of defensive chemicals and mechanisms within their cells[19]. According to[20], tannins, saponins, flavonoids, and steroids are a few of the defensive substances that are often found in seaweed. However, for adaptation purposes, many seaweeds have developed a variety of chemical defense mechanisms by producing bioactive substances to fend off certain herbivores to withstand heavy herbivores grazing[21–23]. They also assimilate a sizable part of carbon dioxide through photosynthesis, making them a natural way to lower greenhouse gas emissions[24]. In the coastal waters of Bangladesh, numerous native seaweeds exist; however, there is a scarcity of research on conducting mineral concentrations from them[13,25]. Several research works were done in Asia to examine the presence of minerals and heavy metals in seaweeds[4,14,26,27] and some works were done on analyzing the bioactive compounds, secondary metabolites, and antioxidant properties of certain seaweeds of the coastal region of Bangladesh[28]. Despite the vast research on seaweed nutrition, the global diversity is extensive, resulting in limited knowledge on nutritional characteristics, bioactive compound use, and the role of lesser-known seaweed species in adaptation and climate change mitigation, creating opportunities to fill knowledge gaps. As a result, five important available seaweed species were collected from North Nuniachara and Rezu Khal estuary, Cox's Bazar district, to evaluate the presence of nutrient contents, heavy metal concentrations, and different bioactive compounds qualitatively and quantitatively and their role in adaptation in stressful environmental conditions and in mitigating climate change.

Results

Phytochemical constituents of seaweed samples

In the present study, all the methanolic extracts of seaweed samples were subjected to phytochemical screening for the presence of tannin, saponin, terpenoid, phenol, flavonoid, and steroid. The largest biochemical compounds were present in the methanolic extract of all seaweeds (Table 1). It was revealed from the results that, *H. musciformis* and *H. spinella* collected from St. Martin's Island had all the phytochemicals except tannin and saponin, while all experimental phytochemicals were present in all seaweed samples of Rezu Khal estuary and North Nuniachara other than saponin.

Nutritive compounds of marine seaweeds

This present investigation showed concentrations of mineral contents that are significantly ($P < 0.05$) different from each other in various locations of Cox's Bazar district (Table 2). The mean highest concentration of sodium (Na) 245.43 ± 13.91 mg/kg was observed in *H. musciformis* which was from St. Martin's Island and the mean lowest concentration 42.08 ± 3.67 mg/kg was recorded in *U. lactuca*, collected from North Nuniachara of Cox's Bazar. The amount of potassium (K) in seaweeds ranged from 69.53 mg/kg to 445.48 mg/kg. The sodium content in the present study was negatively correlated ($r = -0.438$, $P < 0.047$) with the potassium content (Fig. 2). The estimation of heavy metals such as Ni, Cr, Cd, and Pb, showed significant $P < 0.05$ variations among distinct species of seaweed (Fig. 3). However, among all seaweeds, only the samples of St. Martin's Island showed Cd accumulation where the mean concentration of 5.02 ± 0.02 µg/kg and 5.26 ± 0.31 µg/kg in *H. musciformis* and *H. spinella*, respectively. On the contrary, Cd was below the detection level in other species that were collected from the Rezu Khal estuary and North Nuniachara. Nikel was not found only in *H. musciformis*, but all other elements were reported in verified amounts among selected species. From Pearson Correlation Analysis (Fig. 4), Cr showed no relation to other metals, while Pb showed a significant $P < 0.05$ positive relationship with Cd and Ni showed a significantly negative relationship with Cd.

Bioactive compounds of seaweeds

The study showed that the pigment composition was different among seaweed species of various locations (Table 3). The highest amount of chlorophyll-a and b were recorded 0.32 ± 0.003 mg/g and 0.32 ± 0.003 mg/g in *U. lactuca* followed by 0.25 ± 0.01 mg/g and 0.22 ± 0.06 mg/g in *E. intestinalis* in Rezu Khal Estuary and The lowest amounts of chlorophyll-a and b were found 0.11 ± 0.01 mg/g and 0.06 ± 0.008 mg/g in *G. tenuistipitata* followed by 0.11 ± 0.002 mg/g and 0.07 ± 0.008 mg/g in*H. spinella* of St. Martin's Island. The total chlorophyll content was highest (0.45 ± 0.03 mg/g in *E. intestinalis* of North Nuniachara and was lowest (0.17 ± 0.02 mg/g) in *G. tenuistipitata* of Rezu Khal Estuary. There were significant $P < 0.05$ differences among species of distinct locations. On the other hand, carotenoid contents showed significant $P < 0.05$ differences among species with the highest 0.06 ± 0.005 mg/g in *G. tenuistipitata* and lowest in 0.02 ± 0.003 mg/g in *U.lactuca*. However, among the pigments, chlorophyll-a showed a significant $P < 0.05$ positive relationship with chlorophyll-b, but no significant relation existed between carotenoids and total chlorophyll contents (Fig. 4). The percentage of total protein contents in the selected seaweeds of distinct locations showed variations among the distinct species. The mean total protein content ranged from 4.96% to 11.99% among the studied seaweeds where the highest mean total protein contents were recorded in *G. tenuistipitata* (11.99%) and (10.96%) collected from two distinct locations such as Rezu Khal estuary and North Nuniachara of Cox's Bazar. The lowest total protein content was estimated in *U. lactuca* (4.96%) followed by another seaweed *E. intestinalis*

Location	Species	Phytochemical composition					
		Tannin	Saponin	Terpenoid	Phenol	Flavonoid	Steroid
St Martin's Island	*Hypnea musciformis*	-	-	+	+	+	+
	Hypnea spinella	-	-	+	+	+	+
Raju Khal estuary	*Ulva lactuca*	+	-	+	+	+	+
	Gracilaria tenuistipitata	+	-	+	+	+	+
North Nuniachara	*Enteromorpha intestinalis*	+	-	+	+	+	+
	Ulva lactuca	+	-	+	+	+	+
	Gracilaria tenuistipitata	-	+	+	+	+	+

Table 1. Preliminary phytochemical screening of selected five seaweeds from three separate locations of Cox's Bazar of Bangladesh. ** (+) showed present and (-) showed absent.

(8.20%) of North Nuniachara. The total protein contents showed no relation with other bioactive compounds of seaweed (Fig. 5). Alternatively, phenols and flavonoids of selected seaweeds of various locations showed a strong significant relation ($r = 0.670$, $P < 0.001$) between them at 5% significant level (Fig. 5). The maximum mean value (81.48 ± 5.08) was recorded for *G. tenuistipitata* followed by *E. intestinalis* (81.32 ± 3.76) and *U. lactuca* (73.83 ± 6.48) mg GAE/g dry weight collected from North Nuniachara of Cox's Bazar. On the other hand, the minimum mean value (11.20 ± 3.27) mg GAE/g was recorded for *H. spinella* in St. Martin's Island followed by *U. lactuca* (20.21 ± 2.79) mg GAE/g from Rezu Khal estuary and *H. musciformis* (20.49 ± 2.11) mg GAE/g in St. Martin's Island.

Discussion

Seaweed is an essential part of marine ecosystems by reducing human-made effects, CO_2 emissions, and other climate change consequences[29]. It is also a preferable source of bioactive compounds, phytochemicals, polysaccharides, essential fatty acids, and amino acids with all vitamins and mineral components[30]. The present research findings proved the nutritional value and bioactive compounds of five seaweeds growing in three distinct locations of Cox's Bazar, Bangladesh, and their role in adaptation to adverse environmental conditions. Several researchers reported much higher Na and K concentrations of *H. musciformis* (1103 ± 5.0 mg/kg) and (308.0 ± 2.0 mg/kg) collected from the eastern part of St. Martin's Island[25] than the present study. Na content was reported[28] in *G. tenuistipitata* (118.57 ± 6.56 mg/kg) which is about three times higher and in *E. intestinalis* (123.31 ± 5.12 mg/kg) from Cox's Bazar which is about two times higher than present findings. They also found much higher K content in *G. tenuistipitata* and *E. intestinalis* collected from Inani Beach of Cox's Bazar than in the present findings. The variations might have occurred because of environmental modifications, including geographic location, tidal fluctuations, the availability of minerals in salt water, and wave exposure[13]. However, *H. musciformis* and *H. spinella* accumulate more Na than K. Unlike these two species, the ratio of Na/K decreased to 0.097 in *G. tenuistipitata* of Rezu Khal estuary. The decreased amount of Na/K ratio was found such as 0.43 in *G. tenuistipitata*, 0.44 in *E. intestinalis*, and 0.48 in U. lactuca which belonged to North Nuniachara. According to bio-deposited concentrations of elements including Ca, K, Na, Mg, Mn, and P, brown and red algae had higher K and lower Na concentrations. In contrast, green seaweeds had higher Na and lower K concentrations[31]. The key mechanisms for supporting osmoregulation in marine seaweeds are the Na and K pumps that function between saltwater and cell sap, and turgor pressure fluctuations are brought on by the varied ionic composition of vacuoles[32]. In the present investigation, red seaweed (except) *H. musciformis* and *H. spinella* from St. Martin's Island, *G. tenuistipitata* of North Nuniachara and Rezu Khal estuary accumulate more K than Na content. At the same time, green seaweeds *U. lactuca, E. intestinalis* of North Nuniachara and U. lactuca of Rezu Khal estuary also accumulate more K than Na. *H. musciformis* and *H. spinella* collected from St. Martin's Island may defend salinity only by Na inclusion mechanism and other studied *G. tenuistipitata*, *U. lactuca* and *E. intestinalis* from two different locations that accumulate more K than Na may sustain in salinity by operating exclusion mechanism[32]. In the present research, we have found all seaweeds have moderate to lower levels of heavy metals compared with the safe consumption limit according to Food Safety Authority of Ireland[33]. According to[34] *U. lactuca* from the South Adriatic Sea in Italy had Cd 0.20 µg/g and Pb 0.84 µg/g which was much lesser than our study. The concentrations of Pb, Cr, and Cd found in *G. tenuistipitata* were 31 µg/kg, 60 µg/kg and 20 µg/ kg collected from North Nuniachara[12] which were higher than our current investigation. Both *Hypnea* species of St. Martin's Island have all these heavy metals in a larger amount than others and are considered the highest level of heavy metal accumulator among tested seaweeds. *Gracilaria* species have also been found to be good accumulators of heavy metals in agreement with another finding[35]. These two species may act as bioremediating agents for cleaning polluted water from different sources. The chemistry of their cell walls plays a significant role in the species-specific differences in the accumulation of various heavy metals (such as As, Cd, and Pb)[36]. Generally, several environmental factors, such as alterations in salinity, temperature, and pH, variations

Location	Seaweed	Minerals			Heavy metals			
		Na	K	Na/K ratio	Ni	Cr	Cd	Pb
St. Martin Island	*H. musciformis*	245.43 ± 13.91a	71.16 ± 1.26f	3.45	BDL	4.18 ± 0.14e	5.02 ± 0.02a	7.83 ± 0.27d
	H. spinella	110.68 ± 13.91b	69.53 ± 3.47f	1.62	0.97 ± 0.06e	11.08 ± 0.72c	5.26 ± 0.30a	20.42 ± 0.13a
Rezu Khal estuary	*U. lactuca*	93.53 ± 3.68b	111.93 ± 3.33d	0.84	1.71 ± 0.04b	8.13 ± 1.59d	BDL	3.99 ± 0.09e
	G. tenuistipitata	43.31 ± 4.24e	445.48 ± 11.37a	0.09	1.38 ± 0.17c	6.58 ± 0.56d	BDL	3.64 ± 0.03f
North Nuniachara	*E. intestinalis*	67.81 ± 2.19c	155.38 ± 4.73c	0.44	5.61 ± 2.96a	7.43 ± 4.19cde	BDL	8.76 ± 0.15c
	U. lactuca	60.46 ± 3.67d	88.03 ± 3.67e	0.48	0.99 ± 0.008d	14.2 ± 0.48a	BDL	7.46 ± 0.11d
	G. tenuistipitata	42.08 ± 3.67e	139.51 ± 3.65b	0.43	3.85 ± 0.08a	12.8 ± 0.40b	BDL	11.4 ± 0.13b

Table 2. Minerals (mg/kg dw) and heavy metals (µg/kg dw) contents of dry seaweed samples. Values are expressed as mean ± SD (n = 3). Values in a column that do not share the same letter indicate significant differences in their means (P = 0.05). dw = dry weight, BDL = Below Detection Level.

in photoperiods, as well as changes in the availability and concentrations of nutrients, significantly influence the affinity of organisms to absorb heavy metals[37,38]. These might be the reasons for finding various levels of heavy metal content in distinct species along with distinct locations. Seaweed usually forms secondary metabolites to adapt to an inappropriate condition of the environment. They rarely get damaged during their metabolism because of have some protective compounds and mechanisms[19]. Some of the protective compounds commonly found in seaweeds are tannins, saponins, flavonoids, and steroids[20], which were tried to explore in our experiments. The results of the phytochemical analysis revealed the presence of the largest bioactive compounds in methanolic solvents which could act as prime contributors to the antimicrobial and antioxidant potentiality and help to adapt in complex marine habitats. Earlier researchers evaluated the antimicrobial activity of different seaweed extracts, but the present study only revealed the presence or absence of some important secondary metabolites.

The presence of tannins, flavonoids, and the absence of saponins, and alkaloids were found[28] in the aqueous extracts of red seaweeds *G. tenuistipitata* and green seaweeds *E. intestinalis* collected from the coast of Cox's Bazar whereas the author also found tannins and saponins in red seaweeds *H. boergesenii* from St. Martin's Island. However, the present study showed the absence of tannins in *G. tenuistipitata* from North Nuniachara while other phytochemicals showed similar results to the earlier findings. However, these variations could be the result of differences in the analytical procedures as well as variations in the solvent used for the study. Seaweed is the most widespread species of photosynthetic organism, with different classes of pigment for light absorption and photo defense. At depths where the light intensity is variable, these pigments oversee absorbing the light required for photosynthesis. Many studies reported that, like green plants, green seaweed had higher concentrations of chlorophyll-a than red seaweeds. Alternatively, carotenoid content fluctuated among seaweed groups with the highest in the red seaweeds and the lowest in the green species. Our present study also showed the same result[39,40]. The highest total chlorophyll content was found[41] in *E. intestinalis*(1.57 mg/g), which was three times greater than that of present findings. The author also reported the lowest carotenoid content in the same species (0.03 mg/g dry weight) is comparable to the present finding. The highest total chlorophyll (0.54 mg/g dry weight) was recorded[42,42] which was a bit higher than the present results. On the contrary, these authors found the lowest amount of carotenoid contents in brown seaweeds than in red seaweeds. It has been known that algae are among the major photosynthetic organisms in the marine environment. According to[43] photosynthetic pigments in marine seaweeds help in cell communications and human health maintenance and have probable antimicrobial activities and promising applications in food and pharmaceutical fields. At the same time, algal carotenoids have antioxidant properties that protect against human diseases brought on by oxidative stress and the growth of cancer cells[44,45]. The present research focused only on the estimation of the soluble essential protein contents of different studied seaweeds from various locations, but most of the researchers estimated the crude protein content of different seaweeds. *U. lactuca* was found in the Persian Gulf of Iran, and[46] analysis revealed that it included 17.11% protein and *E. intestinalis* had 10.51% protein content on a dry weight basis which is greater than the present study. However, differences in protein content may be related to seasonal changes, temperature readings, or the consumption of the protein by seaweed for growth and reproduction. Additionally, it can also be connected to variations in seaweed species, geographic regions, and local environmental circumstances[47,48]. On the contrary, the author[49] found the total phenolic content in *Hypnea* species was 9.84 mg GAE/g which was lesser than the present

Location	Seaweed	Chloro--phyll-a (mg/ g)	Chloro--phyll-b (mg/g)	Total Chlorophyll mg/g	Carotenoid mg/g	Total Protein (%dry wt)	Total Phenolic Content (mg GAE/ g dw)	Total Flavonoid Content (mg QE/ g dw)
St. Martin Island	*H. musciformis*	0.13 ± 0.04de	0.11 ± 0.05bcd	0.26 ± 0.09cd	0.04 ± 0.007bc	9.28bc	20.48 ± 2.11b	19.16 ± 1.83e
	H. spinella	0.11 ± 0.002e	0.07 ± 0.03cd	0.18 ± 0.04d	0.05 ± 0.01ab	8.50c	11.20 ± 3.26c	16.34 ± 0.91f
Razu Khal estuary	*U. lactuca*	0.15 ± 0.008d	0.10 ± 0.006c	0.25 ± 0.01c	0.03 ± 0.001c	8.62c	20.21 ± 2.80b	31.33 ± 1.76c
	G. tenuistipitata	0.11 ± 0.01e	0.06 ± 0.008d	0.17 ± 0.02d	0.04 ± 0.02abcd	11.99a	73.12 ± 5.87a	25.88 ± 2.24d
North Nunia chara	*E. intestinalis*	0.25 ± 0.01b	0.22 ± 0.06b	0.45 ± 0.03a	0.03 ± 0.007cd	4.96d	81.32 ± 3.76a	36.99 ± 1.93b
	U. lactuca	0.32 ± 0.003a	0.32 ± 0.001a	0.40 ± 0.03ab	0.02 ± 0.003d	8.20c	73.83 ± 6.48a	27.64 ± 1.54d
	G. tenuistipitata	0.22 ± 0.01c	0.15 ± 0.01b	0.37 ± 0.02b	0.06 ± 0.005a	10.96b	81.48 ± 5.08a	51.48 ± 1.27a

Table 3. Chlorophyll, carotenoid, and bioactive contents of dry seaweed samples. Values are expressed as mean ± SD (n = 3). Values in a column that do not share the same letter indicate significant differences in their means (P = 0.05). dw= dry weight, GAE = Gallic Acid Equivalent, QE = Quercetin Equivalent

findings, and some studies also found phenolic content for *Hypnea* species was 49.7 mg GAE/ g[50] which was greater compared to our findings from the same genus. The total phenolic content was found[51] 10.25 mg GAE/g in *Gracilaria* species which was almost eight times and seven times lesser than present findings in the same genus. Another author[52] found 30.9 mg GAE/g total phenolic content in *Gracilaria* species. According to[53], seaweed's phenolic compounds can chelate metal ions and stop the generation of free radicals, which enhances the intrinsic coordination of antioxidants. In this way, phenols transfer hydrogen atoms to peroxyl in the cycle of lipid peroxidation that creates aryloxyls thus the peroxidation process is slowed down by the inability of aryloxyls to serve as chain carriers for free radicals. An earlier study found[54] that the total flavonoid content in *Enteromorpha* species was 49.1 mg QE/g. Therefore, in the present study, the total flavonoid content in *Enteromorpha* species was recorded at 36.99 mg QE/g which was supported by the earlier findings. The total flavonoid contents were reported[55] in *U. lactuca* and *H. musciformis* were (36.07 ± 2.57) and (20.02 ± 2.40) mg RU/g from Western Libyan Coast which were comparable to the present findings.Another author[41] reported the total flavonoid contents in *H. musciformis* and *E. intestinalis* were 67 ± 0.0002 mg QE/g and 57 ± 0.0002 mg QE/g respectively, from North Nuniachara which was almost three times and two times greater than present findings. The variation in total flavonoid content between the present study and others might be attributed to environmental factors, species differences, harvesting methods, different solvents used for extraction, and so forth. On the other hand, the total flavonoid contents are subjected to seasonal variation because earlier studies have shown that in the same species, flavonoid content can vary when collected during different seasons[6]. Moreover, in the present study, the total protein showed a significant positive relation with K content and a negative relation with total chlorophyll pigments whereas showed no relation with other variables (Fig 6 Based on the above discussions, we have found that *G. tenuistipitata* from North Nuniachara stood out for its elevated levels of phenols, flavonoids, and protein. So, the present study revealed that five distinct types of seaweeds growing in three different coastal areas have notable mineral concentrations, enabling them to thrive in high-saline environments and might be used as bio-remediators for purifying water contaminated with various sources of heavy metals. However, the levels of these heavy metals stay within permissible limits and are non-toxic, setting up the safety of consuming these seaweeds. Furthermore, their nutritional value positions them as significant food sources for coastal people in Bangladesh. Additionally, these seaweeds have crucial primary and secondary metabolites that can address various human health issues and contribute to technological advancements. Cultivating these seaweeds in Cox's Bazar district has the potential to enhance the blue economy, aligning with the sustainable development goal of reaching Vision 2041. To augment seaweed production and the value of seaweed-derived products, future research should recognize seaweed as an alternative food and source of bioactive compounds. Researchers should also acknowledge the challenges associated with extracting bioactive compounds and explore practical methods for extracting valuable biomolecules, using bioactive chemicals as organic additives across various sectors. Additionally, assessing the carbon sequestration potential of seaweed habitats and deciding the overall carbon impact throughout the seaweed production lifecycle needs a carbon-sequestration-focused life cycle assessment.

Methods

1 Study area

For the present investigation, live and healthy specimens were collected from three locations in the coastal zone of Bangladesh in March 2023 (Fig. 1). Although seaweeds can be found along the entire southern coast, Cox's Bazar is the place where most of the species are discovered to naturally thrive, and an enormous amount of natural seaweed resources are noted on St. Martin's Island. Five Marine seaweeds such as *Hypnea musciformis* (Wulfen) J.V. Lamouroux, *H. spinella* (C. Agardh) Kutzing were collected from St. Martin's Island, *Gracilaria tenuistipitata* var liui Zang and Xia, *Ulva lactuca* L., and *Enteromorpha intestinalis* L. Nees were collected from three different locations, namely North Nuniachhara (21°28'26" N, 91°57'56" E) Rezu Khal estuary (21°17'27" N, 92°03'08" E) and the western coast of St. Martin's Island (20°38'00" N, 92°19'00" E) of Bangladesh. The collection process involved carefully hand-collecting fresh seaweed samples during low tide from the intertidal region attached to the rocky substratum. To ensure the purity of samples, they were carefully washed in flowing seawater to remove any unwanted materials like salt and exterior materials like epiphytes, shells, and sand and then rinsed with fresh water. After this cleaning process, the samples were transported to the laboratory for added processing while being supported in plastic jars filled with local seawater to prevent evaporation.

2 Sample preparation

In the laboratory, the samples were thoroughly washed with deionized distilled water after being meticulously cleaned with tap water many times to get rid of sand and other potential impurities. Following this, samples were dried at room temperature until all moisture was removed from them. They were then at once placed in airtight zip-bags labeled with the names of the species. The shade-dried seaweeds were pulverized using an electronic kitchen mixer to get the course powder, passed through a 60-mesh sieve, and sealed in airtight zip-lock bags at a temperature of -20 ℃ for further analysis[28].

3 Crude Extracts of Seaweed

For preparing seaweed extracts, the specific amount of each sample was weighed. A sample: methanol solvent ratio of 1:10 was employed to prepare the stock solution of the extracts. The extracts were then concentrated by applying lower pressure in a rotating evaporator at around 40 ℃. The dried sample was kept in a refrigerator at 4 °C as a crude sample extract[41].

4 Seaweed Digestion Method

Each seaweed sample was digested for analysis of nutrients and heavy metals by the nitric-perchloric acid digestion method[56] with some modifications. Approximately one gram of each dehydrated through oven-drying at 55 ℃ seaweed samples were taken and kept in a dried 100-ml. glass beaker. The samples were soaked in a solution of HNO_3 for 12 h, and then rinsed with deionized water. Afterward, 12 ml of 65% HNO_3 and 5 ml of 70% $HClO_4$ solution were added to the samples, which were then left at room temperature. Brown-colored fumes of HNO_3 appeared after cooling and were cleared out by evaporating using a hot plate. Once everything had completely evaporated, the residual ash was liquidized in distilled water and filtered using Whatman filter paper. Finally, the samples were diluted with distilled water to make a final volume of 100 ml. used with distilled water to make a final volume of 100 ml.

5 Qualitative phytochemical screening analysis

Topical subheadings are allowed. Authors must ensure that their Methods section includes adequate experimental and characterization data necessary for others in the field to reproduce their work.

5.1 Tannins

For this test, 1 ml of 5% $FeCl_3$ was added to 1 ml of each seaweed extract. A brownish-green precipitate was taken as evidence for the presence of tannins in samples[57].

5.2 Saponins

The stable persistent froth was created by adding 2 ml distilled water into 2 ml of each seaweed extract and shaking vigorously for 15 minutes lengthwise confirming the saponin test[58].

5.3 Terpenoids

For the presence of terpenoids, 2 ml of chloroform and 1 ml concentrated H_2SO_4 were added into 0.5 ml of each seaweed liquid extract. The formation of a reddish-brown color at the interface showed positive for the terpenoids test[59].

5.4 Phenols

A few drops of 10% aqueous $FeCl_3$ solution were added to 1 ml for each seaweed extract. The blue color appearance showed the presence of phenols[60].

5.5 Flavonoids

A dilute ammonium solution of 5 ml was added to 1 ml of each sample and shaken vigorously. A yellowish-green color was considered a positive test for flavonoids[58].

5.6 Steroids

Each sample of 1 ml was dissolved in 2 ml chloroform and an equal volume of concentrated H_2SO_4. The upper layer turned red and H_2SO_4 layer showed yellow with green fluorescence. This was a sign of the presence of steroids[59]

6 Quantitative bioactive compounds analysis

6.1 Estimation of minerals and heavy metals

Minerals (sodium and potassium) were analyzed by a Flame Photometer (PFP7, Jenway and UK). Heavy metals (lead, chromium, cadmium, and nickel) were analyzed by Graphite Furnace Atomic Absorption Spectrometry (GFAAS) (GFA-7000A, Shimadzu and Japan).

6.2 Estimation of seaweed pigments

Seaweed's pigments chlorophyll-a, b, and total chlorophyll content were estimated by using 80% acetone extraction method[61]. Carotenoid content was estimated by using the same extracts according to[62] method.

6.3 Estimation of total protein content

Buffer extracts (0.1 M tris HCl buffer, pH 7.5) of dry seaweed materials were used to estimate total protein content[63] spectrophotometrically at 750 nm. Bovine serum albumin (BSA) was used as standard which is expressed by the following equation, and results were expressed as a percentage of algal dry weight.

$$Y = 0.0005X - 0.0446; \quad R^2 = 0.992 \tag{1}$$

6.4 Estimation of total phenol content

This was estimated with a Folin-Ciocaltue assay followed by[64] with slight modification. For this purpose, 1 ml of methanolic dry extract of seaweed sample (1 mg/ml) and 5 ml 10% Folin-Ciocalteau was added into 5 ml 7.5% sodium carbonate solution. The mixtures were incubated for 20 minutes at 25º C, and the solution's absorbance was quantified at 760 nm using a spectrophotometer (SHIMADZU UV-Spectrophotometer) against blank. The calibration curve was constructed using gallic acid as standard which is expressed by the following equation.

$$Y = 0.0039X + 0.0162; \quad R^2 = 0.998 \tag{2}$$

6.5 Estimation of total flavonoid content

This was determined by the $AlCl_3$ colorimetric method[65] with little modification. 1 ml of methanolic dry extract of seaweed sample (1 mg/ ml), 3 ml of methanol, 0.2 ml of 10% Aluminum chloride solution, and 0.2 ml Sodium acetate were mixed with 5 ml distilled water. The mixture was incubated at room temperature for 30 minutes and the absorbance of this mixture was measured at 420 nm using a UV-Spectrophotometer (SHIMADZU UV-Spectrophotometer) against a blank. Quercetin is used as the standard for the construction of the calibration curve which is expressed by the following equation.

$$Y = 0.011X + 0.017; \quad R^2 = 0.973 \tag{3}$$

7 Statistical analysis

The data were presented as the mean ± standard deviation from three replicated determinations. To assess potential variations among the studied seaweed parameters, a one-way ANOVA, followed by Tukey HSD post hoc tests (with a significance level of $P < 0.05$), was employed. This analysis aimed to find significant differences between the various seaweed parameters. Furthermore, Pearson correlation analysis was performed using Minitab software (version 21) to investigate the associations between nutritive compounds and bioactive compounds.

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Author contributions statement

Liza Kamal carried out sampling and designed the experiment, performed the experiments, analysed the data, conceived the study, and drafted the manuscript; Mohammad Ashraful Alam carried out sampling and helped in data analysis, and manuscript preparation; Asma Rahman conducted heavy metals analysis; MD. Faruque Hossain edited the manuscript. Ashfaque Ahmed carried out sampling, arranged funding, designed the study, supervised experimentation, proofread, and approved the final version of the manuscript.

Additional information

To include, in this order: **Accession codes** (where applicable); **No potential conflict of interest was reported by the authors** (mandatory statement).

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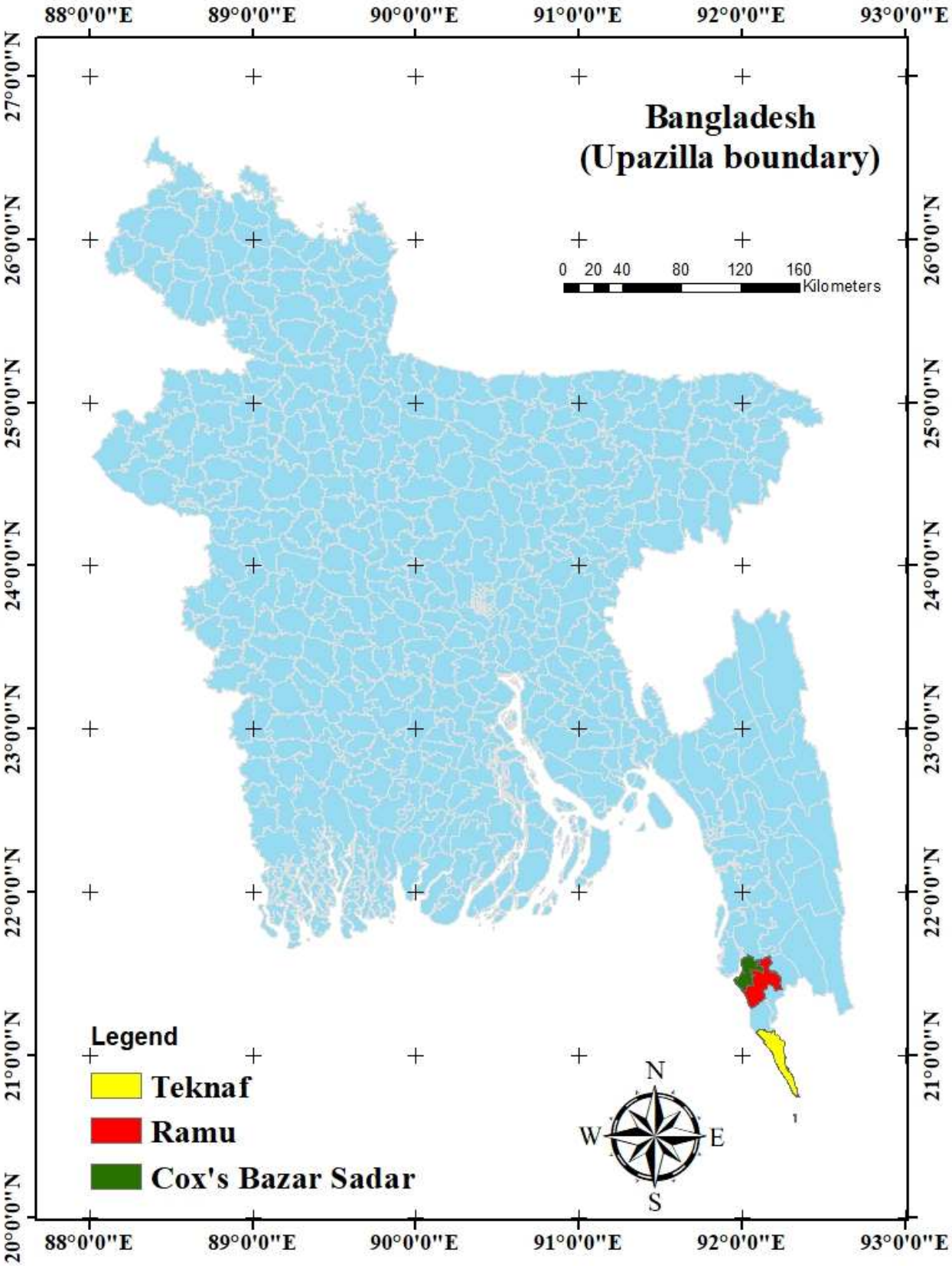

Figure 1. Map showing the three sampling locations of coastal regions of Cox's Bazar District, Bangladesh. A yellow color shows the Western Coast of St. Martin's Island; a green color shows North Nuniachhara area; and a red color shows Rezu Canal Estuary sites for seaweed collection.

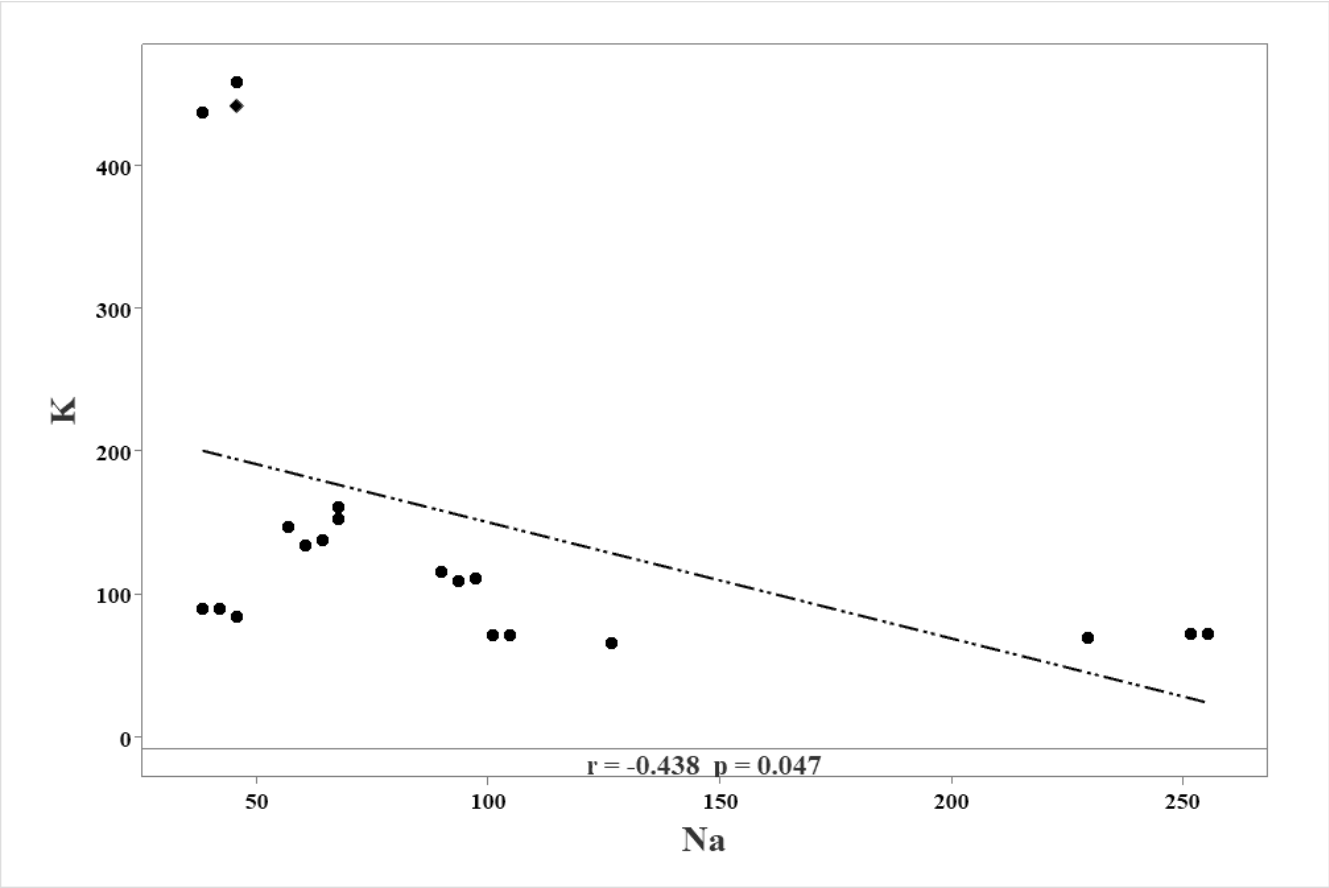

Figure 2. Simple linear co-relation matrix (r) between Na and K content of the studied seaweeds.

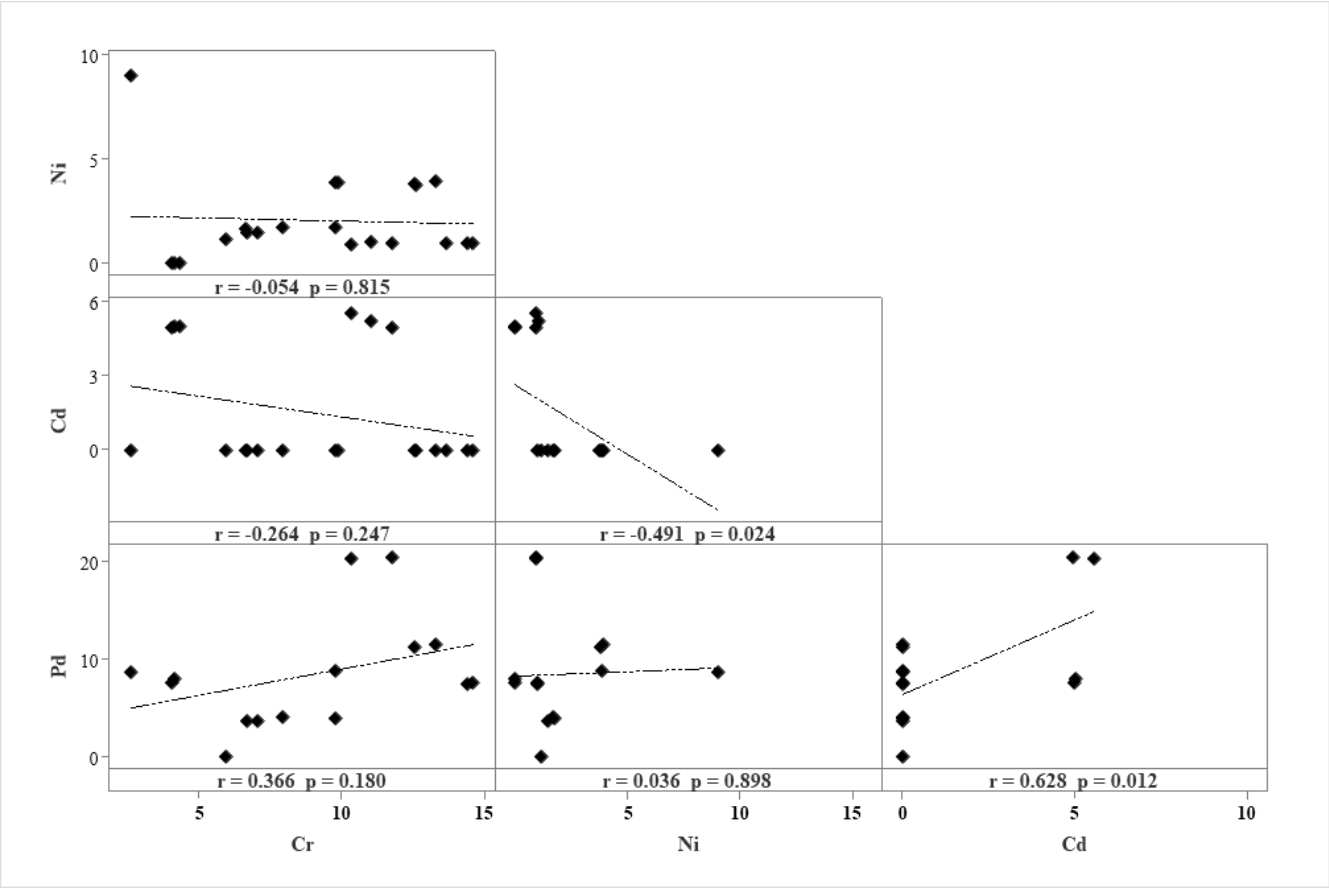

Figure 3. Simple linear co-relation matrix between heavy metals (Cr, Ni, Pb, and Cd) of the studied seaweeds.

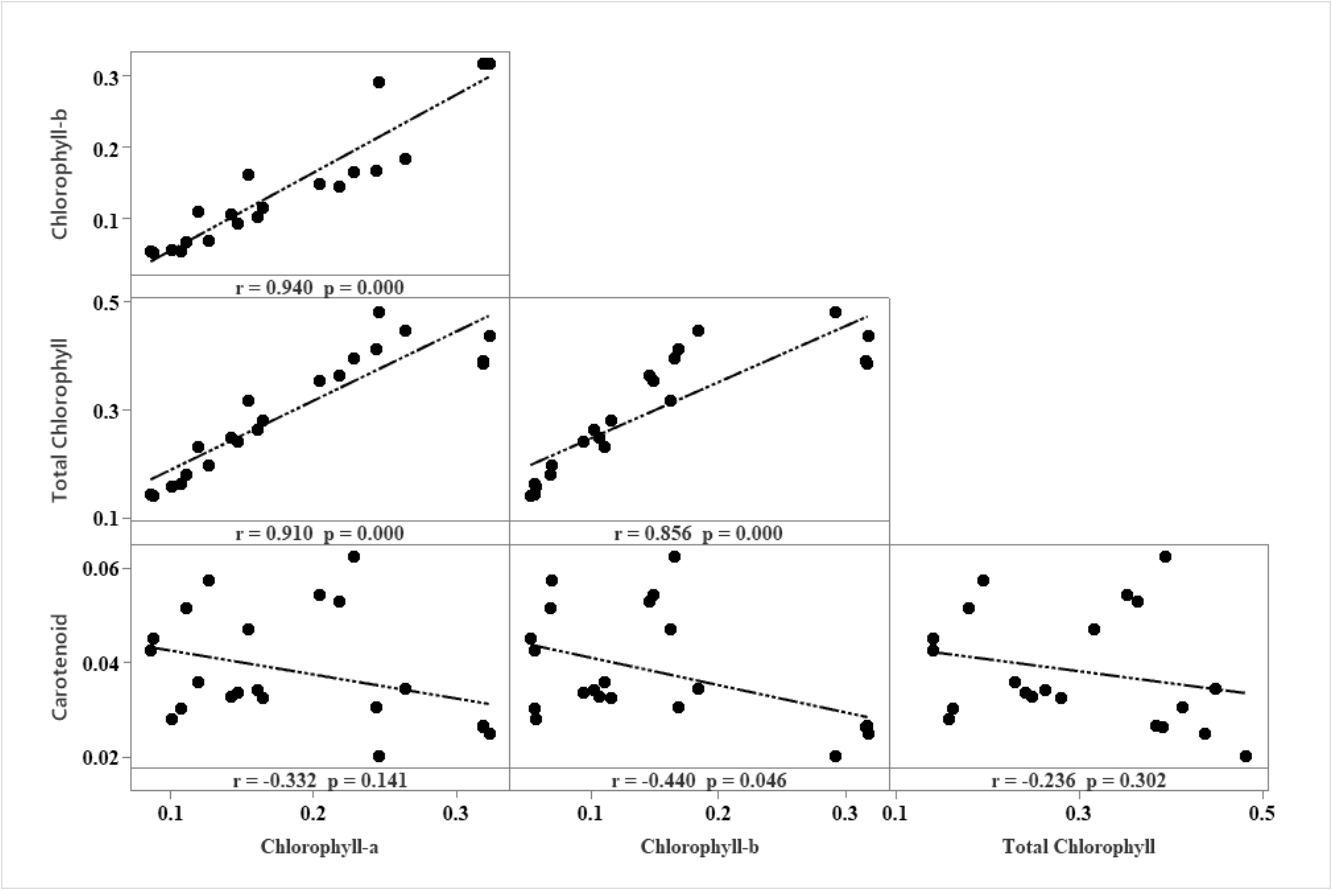

Figure 4. Simple linear correlation (r) matrix between different pigments (Chlorophyll-a, b, Total Chlorophyll, and Carotenoid) of the studied seaweeds.

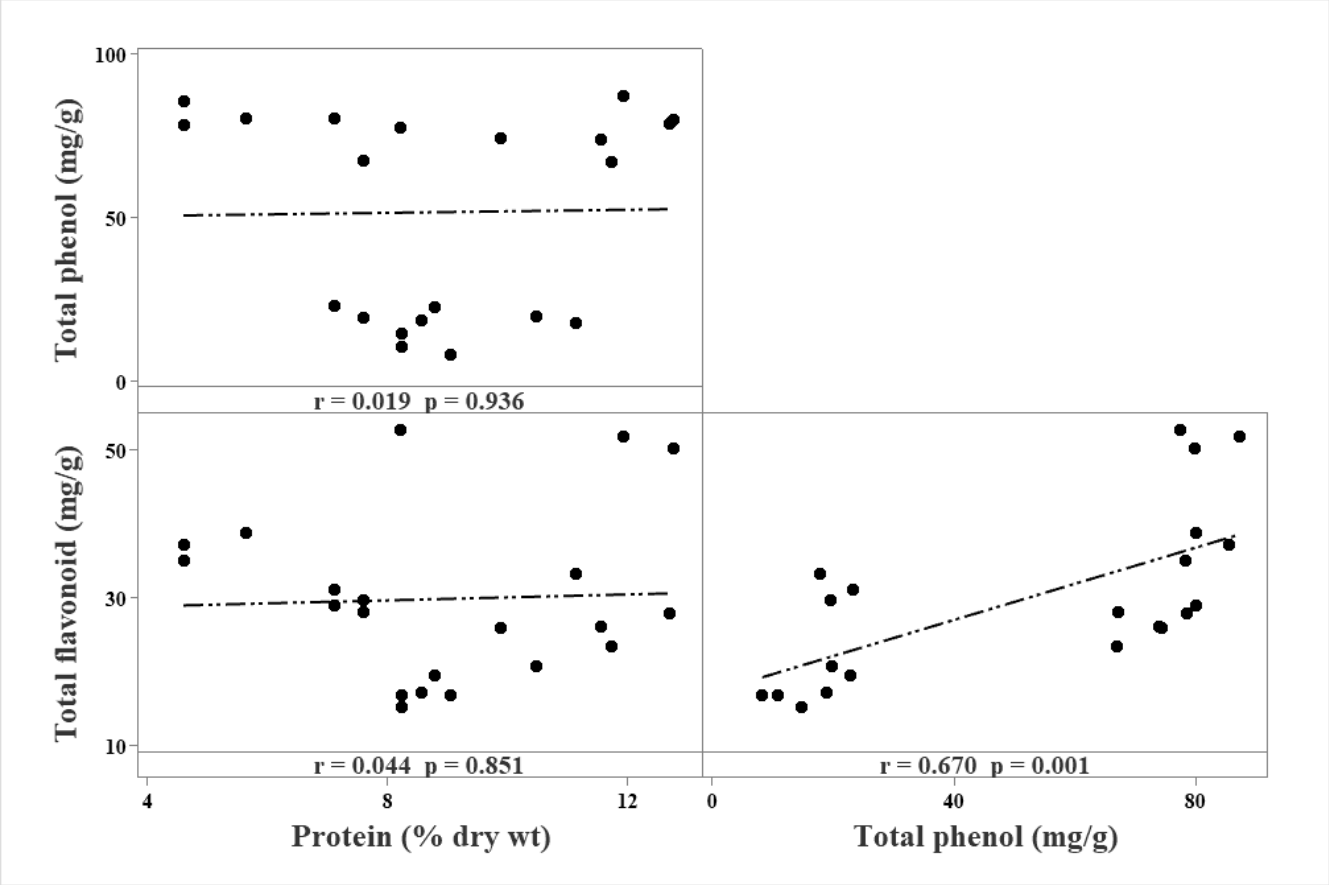

Figure 5. Simple linear correlation co-efficient matrix (r) between bioactive compounds (Total Phenol, Total Flavonoid and Total Protein) of the studied seaweeds.

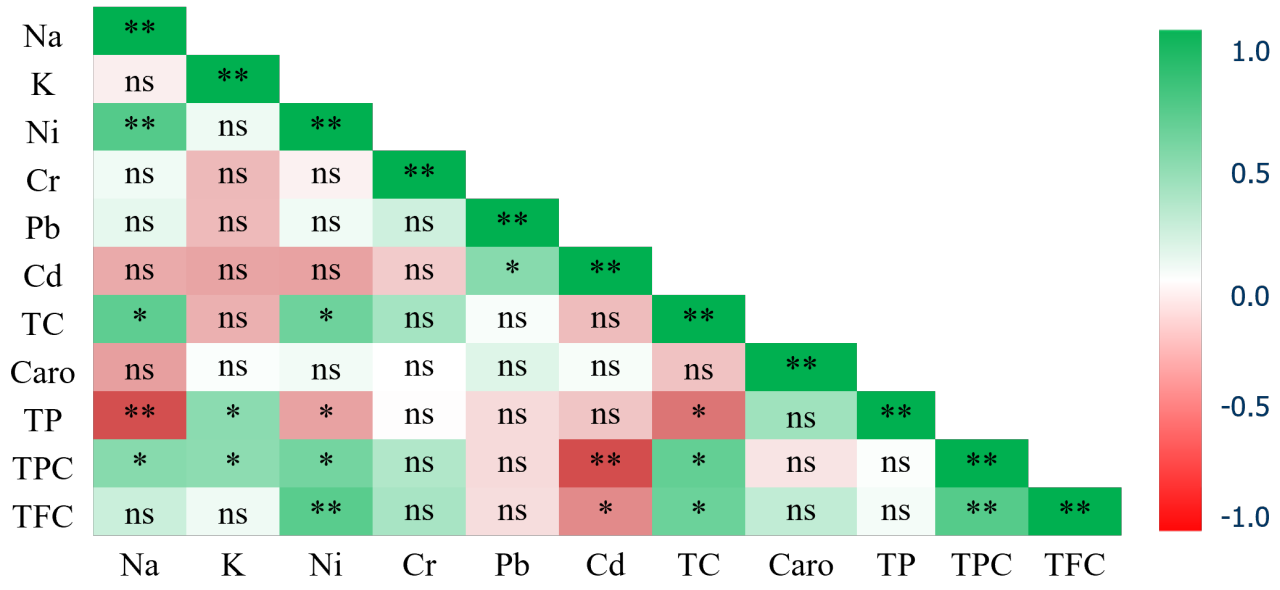

Figure 6. Pearson Correlation matrix (r) between overall variables of the studied seaweeds (** denotes significant at 1% level, where P < 0.01; * denotes significant at 5% level, where P < 0.05 and ns denotes not significant). Na = Sodium, K = Potassium, Ni = Nickel, Cr = Chromium, Pb = Lead, Cd = Cadmium, TC =Total Chlorophyll, Caro = Carotenoid, TP = total Protein, TPC = Total Protein Content, TFC = Total Flavonoid Content.

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