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Spatial-Temporal Effect on Proximate, Trace Elements, Alginate, and Fucoxanthin Contents, of *Sargassum Polycystum* Brown Seaweed

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Abstract: This article describes the environment’s new role in *Sargassum polycystum* brown seaweed's nutrition and metabolites production, based on locations as well as seasonal variation. These variations enable the discovery of environmental factors with the best influence on this production. In addition, the high precise proximate composition, trace elements, alginates, and fucoxanthin contents were obtained using spectroscopy, gravimetry, Nuclear Magnetic Resonance (^{1}HNMR), and High-Performance Liquid Chromatography (HPLC). For example, this study illustrates the proposed method to optimize the environmental carrying capacity of waters on volcanic islands for high *S. polycystum* metabolite production. The study method allows for an improved understanding of carbohydrates encountering the most macro nutrition contents (36-48%) and manganese as the highest trace element (17.15-103.29 mg/kg). Furthermore, alginate characterization obtained 10.96 22.09%, 27.70-36.57%, 9.47-17.83%, 8.14-8.36, and 284.71-499.10 cPs of yield, ash, moisture, pH, and viscosity, respectively. Meanwhile, the M/G ratio reached 0.35-0.84 and 0.155-0.587 mg/g of fucoxanthin. The metabolites variations between location and season were significantly influenced by nitrate, ammonia, DO, and salinity for the alginate, while fucoxanthin's counterparts were temperature, pH, and copper. Subsequently, the new method's effectiveness was evaluated by statistical calculation of canonical correspondence multivariate analysis. The study results provide improved suggestions on the environment condition's ability to support *S. polycystum* development in small island waters. They are also a fair consideration of *Sargassum* brown seaweed aquaculture development program in Indonesia.

Keywords: water quality, *Sargassum polycystum*, trace elements, alginate, fucoxanthin.

时空对海藻多囊褐藻海藻的含量，微量元素，藻酸盐和岩藻黄质含量的时空影响

摘要：本文根据位置和季节变化来描述环境在海藻多囊藻海藻的营养和代谢产物生产中的新作用。这些变化可以发现对生产有最大影响的环境因素。此外，使用光谱，重量分析，核磁共振和高效液相色谱获得了高精度的近邻成分，痕量元素，藻酸盐和岩藻黄质含量。例如，这项研究说明了为高产多囊藻代谢产物而优化火山岛上水域的环境承载能力的拟议方法。该研究方法可以使人们更好地理解碳水化合物所遇到的大量宏观营养含量（36-48％）和锰作为最高的微量元素（17.15-103.29毫克/公斤）。此外，藻酸盐表征分别获得了10.96 22.09％，27.70-36.57％，9.47-17.83％，8.14-8.36和284.71-

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499.10聚氯乙烯的产率，灰分，水分，酸碱度和粘度。同时，岩藻黄质的中號/G比达到0.35-0.84和0.155-0.587毫克/公克。藻酸盐中硝酸盐，氨，溶解氧和盐度显着影响位置和季节之间的代谢物变化，而岩藻黄质的对应物是温度，酸碱度和铜。随后，通过规范对应多元分析的统计计算来评估该新方法的有效性。研究结果为环境条件支持小岛海域多囊链球菌发展的能力提供了改进的建议。它们也是印度尼西亚海藻褐色海藻水产养殖发展计划的公平考虑因素。

关键词：水质，多囊羊栖菜，微量元素，藻酸盐，岩藻黄质。

1. Introduction

Sargassum is a brown seaweed commonly found in Indonesian coastal waters. This genus dominates the intertidal zone [1] and is characterized by high species diversity and the ability to withstand extreme conditions, including high desiccation, temperature drops, and nutrition limitations [2]. These natural strengths make the plant a potential candidate for producing valuable metabolites, including polysaccharides and fucoxanthin [3].

The compound "Alginate" is an essential economic hydrocolloid, and this property is based on the viscosity character developed through the polymer bond between β-D-mannuronic acid (M) as well as α-L-guluronic acid (G), and the consequent formation of MM, GG, or MG block combinations [4]. This hydrocolloid is used globally in industries as food additives, pharmaceuticals, and the cosmetic industry. More recently, brown seaweed attracted attention as a potential raw material for producing fucoxanthin as a bioactive compound. Fucoxanthin is a carotenoid with unique allenoic bond properties and an essential role in electron transport during photosynthesis [5]. In addition, various studies have reported the carotenoid’s possible application as an anti-inflammatory, anti-obesity, and anti-cancer agent [6].

The *Sargassum polycystum* is one of the most common species in Indonesian coastal waters, especially around small islands. According to [7], brown seaweed has a favorite food and bioactive compound source. The seaweed's growth and metabolite production ability are largely influenced by the surrounding environment's physicochemical conditions. Currently, alginate and fucoxanthin production from brown seaweed is low and has limited application. However, this contrasts with extraction from macroalgae grown in subtropical waters, including *Saccharina japonica*, *Macrocystis pyrifera*, and *Laminaria spp*. [8].

Indonesia is an archipelago country dominated by small islands and characterized by unique habitats between thee islands. Economical brown seaweed production within the archipelago is complicated due to islands' remoteness, inadequate understanding of vital production factors, and a larger-scale production's impact on the coastal ecosystem. This study, therefore, investigates the predominant environmental factors influencing seaweed metabolite production capacity, including nutrition, alginate, and fucoxanthin. In this study, *Sargassum polycystum*’s growth was studied in three islands' coastal waters over two seasons to include potential pressure from the land on waters. The research locations are all situated in Tidung, Sebesi, and Bintan Islands, western Indonesia.

2. Materials and Methods

2.1. Sample Collection and Preparation

Sargassum polycystum was collected from 9 (nine) spots along each island’s coast, during 2 (two) seasons, rainy and dry, represented by March-April and August-September 2019. The seaweed samples were then cleaned and washed gently with seawater to eliminate sand, debris, epiphytes, and other attached excessive matter. Subsequently, the alginate sample was dried, while the remaining material was stored at cold temperature during transportation to the laboratory. Furthermore, seawater samples collected for environmental characterization were treated by HNO_3 addition until a pH of ± 2 was obtained, then stored in dark bottles. Meanwhile, trace elements in seawater samples were preserved in H_2SO_4 solution with an of pH ±2. Figure 1 shows the sampling location map.

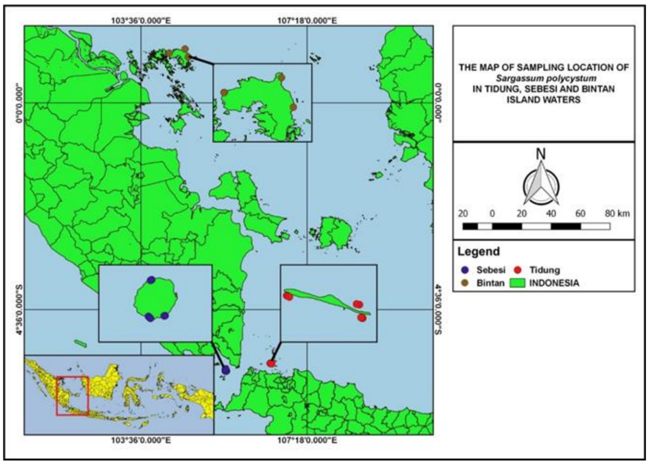

Fig. 1 A map of the sampling locations

2.2. Environmental Parameters Determination

Physio-chemical variables were evaluated in-situ using a multi-parameter quality meter. Meanwhile, Nitrate (NO_3^-), Ortho-phosphate (PO_4^{3-}), and Ammonia (NH_4^+) levels were evaluated with an Ultra Violet-Visible (UV-VIS) Spectrometer. The seawater and seaweed were examined for trace elements, including Barium (Ba), Selenium (Se), Iron (Fe), Manganese (Mn), Copper (Mn), Zinc (Zn), and Molybdenum (Mo). Furthermore, Atomic Absorption Spectrophotometry (AAS) Perkin Elmer PinAAcle 900H involving the Flame (Acetylene-Air) technique was used to determine the quantities, according to the APHA (American Public Health Association) method [52].

2.3. Proximate Composition Analysis

In this analysis, the seaweed's moisture, ash, protein, lipid, fiber, and carbohydrate contents were calculated using the standard AOAC [12] method. Carbohydrate content was determined by weight difference, using moister, ash, protein, lipid, and fiber content data. Meanwhile, crude lipids were extracted from a powdered seaweed sample using the Soxhlet apparatus. Furthermore, methanol and chloroform were used as mixture solvents, while samples were oven-dried overnight. Also, crude lipids were determined gravimetrically, while the Kjeldahl method was used to determine protein content, according to the AOAC method. The seaweed moisture content was determined using a thermoregulated oven at 105°C until a constant sample weight was attained. In comparison, the ash content was obtained by calcination in a furnace at 550°C for 4 hours. Meanwhile, crude fiber content was calculated using an analytical method described by [9].

2.4. Trace Element Content Analysis

Analysis was performed using AAS for trace elements including Barium (Ba), Selenium (Se), Iron (Fe), Manganese (Mn), Copper (Mn), Zinc (Zn), and Molybdenum (Mo).

2.5. Alginate Extraction and Characterization

The alginate was extracted using a method described by [9]. A sample was soaked with 4% HCl solution and 2% Na_2CO_3 as a solvent, as well as 10% $NaHCO_3$, to convert the acid into sodium alginate. Subsequently, the solution was mixed with 99% isopropyl alcohol (IPA), as required in the deposition process. Meanwhile, the M/G block ratio was determined using a Jeol ECS 400 MHz Nuclear Magnetic Resonance (^{1}HNMR) spectrometer, according to [10] and [11]. The yield was valued in flour form from the total dry sample, while the alginate's water and ash content were evaluated based on the AOAC method during pH and viscosity analysis [12].

2.6. Fucoxanthin Content Analysis

This analysis utilized an Ultrasound-Assisted Extraction (UAE) based on [13] and required the use of a water bath Ultrasonic LC60H on a 35 kHz frequency within 120 minutes. Meanwhile, 90% acetone acquired from Mallinckrodt chemical, USA (Pro analyst) was used as the solvent at a 1: 6 weight/volume ratio. Subsequently, the fucoxanthin content was analyzed using a Shimadzu LC-20A High-Performance Liquid chromatography (HPLC) equipped with a UV-Vis detector and a C18 column (20 mm x 250 mm) during separation (Luna Phenomenex). In addition, the fucoxanthin quantity was calculated in mg/g by converting the active sample fraction area (regarding the standard fucoxanthin curve developed by Sigma-Aldrich, Germany).

2.7. Data Analysis

The environmental parameters, the proximate composition, trace elements, alginate, and fucoxanthin contents' mean values were analyzed by analysis of variance (ANOVA), followed by Tukey's post hoc comparison test, to check for differences between data, and statistical significance was determined at the 5% level ($p<0.05$). Furthermore, IBM SPSS statistic 24 was employed for ANOVA and post hoc tests. Also, the significant water quality characteristics between locations and seasons were analyzed using multivariate Principal Component Analysis (PCA), while the relationship between water quality characteristics, alginate, and fucoxanthin content was determined using canonical correspondence analysis (CCA). In addition, multivariate statistical analysis was performed using the Past Statistics Software V4.02 [14].

3. Results

3.1. The Environmental Conditions of Research Areas

A total of 8 environmental parameters were determined, including the water's nutrient content (nitrate, ammonia, and phosphate) and physicochemical properties. All variables were also present in all locations, albeit in variable quantities over the year. Table 1 shows the seasonal variations, where nitrate concentration was higher in dry seasons, especially within Bintan, while phosphate concentration dropped significantly from 0.058 mg/L in the rainy season to 0.008 mg/L in dry seasons. However, in Sebesi, phosphate concentration was significantly higher in the dry seasons.

Table 1 Physio-chemical variation of the seawater (mean ± SD; n=9)

Parameters	**Tidung**		**Sebesi**		**Bintan**	
	Rainy	**Dry**	**Rainy**	**Dry**	**Rainy**	**Dry**
Nitrate (mg/L)	0.23±0.1	0.24±0.2	0.27±0.1	0.26±0.1	0.77±0.3	1.00±0.7
Phosphate (mg/L)	0.047±0.01	0.022±0.004	0.004±0.003	0.027±0.003	0.058±0.009	0.008±0.005
Ammonia (mg/L)	0.09±0.003	0.84±0.05	0.27±0.04	0.06±0.01	2.35±0.3	0.81±0.1
DO (mg/L)	5.61±0.1	6.53±0,8	5.58±0.3	7.53±1.4	5.88±0.5	7.77±0.6
pH	7.40±0.2	7.35±0.3	7.55±0.2	7.26±0.3	7.68±0.1	7.43±0.4
Temperature (℃)	31.6±0.4	27.53±0.1	30.8±1.6	28.67±0.7	30.47±0.9	29.8±3.8
Salinity (‰)	34.67±0.6	35.53±0.4	33.93±1.6	34.33±1.2	32.2±0.9	34.33±1.15
Brightness (%)	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	93.3±11.6

Meanwhile, ammonia concentration varied with location and season, decreasing considerably in Bintan and to a lesser degree in Sebesi, from the rainy to the dry season, but increasing within Tidung. Similarly, the physicochemical parameter results also varied, depending on the location and season. The DO and salinity concentration increased from rainy to dry season in all locations, while the reverse was the case for pH and temperature. Also, the brightness levels were only lower values in Bintan during the dry season, with a value of 93%, compared to 100% in the rainy season.

Macroalgae growth depends on seawater quality in terms of nutrients, and micro minerals or trace elements, including selenium, are essential components for macroalgae photosynthesis. In the seawater samples, trace elements (TE) detected varied with location and season. Table 2 shows a total of two TEs were detected in all locations and seasons. These are zinc, dominant in all waters in at a range of 0.0077 ± 0.002 - 0.019 ± 0.004 mg/L, and selenium, present in lower concentrations of 0.001 ± 0.00006 - 0.0015 ± 0.0004 mg/L. The highest TE accumulation was observed in Sebesi Island's waters, especially during the rainy season. Figure 2 shows the principal component analysis used to determine spatial and temporal factors influencing seawater quality. Based on the analysis, principal components 1 (PC 1) and 2 (PC 2), with variations representing 84.294% of the total water physicochemical and TE data, were obtained. Also, nitrate, ammonia, DO, and Fe were identified as the first and second components' dominant parameters. Nitrate and ammonia content variations were significant in Bintan Island. Meanwhile, DO, and Fe results were quite identical in Tidung and Sebesi Island's coastal waters.

Table 2 Trace element content in the seawater (mg/L) based on locations and seasons (1= rainy, 2= dry; mean ± SD; n=3). Decimal mean ± SD was reduced to get better displayed. Symbol of < is under the detectable limit of AAS

Parameters	**Tidung**		**Sebesi**		**Bintan**	
	1	**2**	**1**	**2**	**1**	**2**
Barium	0	0	0.01±0.001	0.003±0.01	0	0.01±0.0
Selenium	0.0012±0.0001	0.001±0.00006	0.0013±0.0001	0.0012±0.0001	0.0021±0.0007	0.0015±0.0004
Iron	0	0.22±0.007	0.45±0.08	0.159±0.01	0.117±0.06	0.024±0.04
Manganese	0	0	0	0	0	0.0057±0.0006
Copper	0.0097±0.0001	0.0083±0.0006	0.0113±0.002	0	0.005±0.001	0.005±0.001
Zink	0.018±0.002	0.014±0.003	0.019±0.004	0.015±0.005	0.0077±0.002	0.01±0.002
Molybdenum	0	0	0	0	0	0

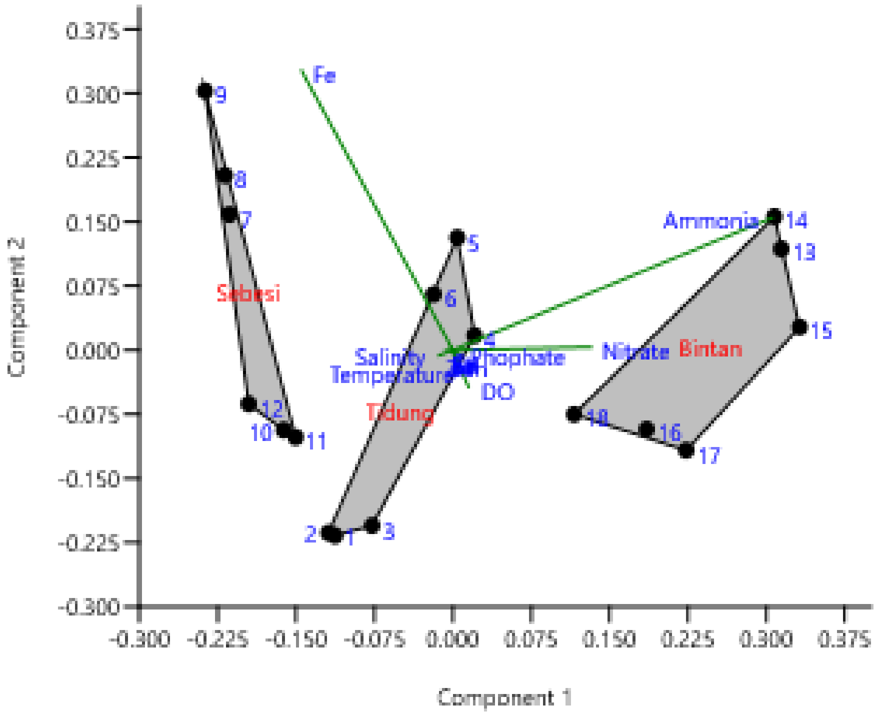

Fig. 2 PCA water quality characters in the research location

3.2. Proximate Composition and Trace Element Contents

The sample's nutritional aspect was examined through proximate groups and micromineral content. Table 3 shows the proximate composition based on habitat diversity and season. According to the analysis, carbohydrate (36-48%) and crude lipid (0.2-0.6%) contents were the major and minor components, respectively, in all locations and seasons. This study's crude lipid values are relatively low compared to other studies. A subsequent one-way ANOVA identified significant differences in ash and carbohydrate contents ($p<0.05$) between the research sites. Meanwhile, a Tukey post-test disclosed that Bintan has significantly different ash and carbohydrate contents than the other study locations. The proximate content prevalence was found to follow the pattern of carbohydrate > ash > moisture > crude fiber > protein > lipid in all locations. Meanwhile, seasonal variations showed changing patterns in all locations and a specific trend, where the dry season was characterized by high carbohydrate and moisture content. In contrast, high ash crude lipid, protein, and crude fiber contents were prevalent during the wet counterpart.

Table 3 Proximate composition (%) of dried S. polycystum as raw material for alginate (1 = rainy season; 2 = dry season). Different superscript letters are significantly different (p<0.05). All data presented are in SD (n=6)

Location		Moisture	Ash	Crude Lipid	Crude Protein	Crude Fibre	Carbohydrate
Tidung	1	9.63 ±0.05a	31.61±0.23a	0.61±0.02a	4.08±0.05a	5.37±0.11a	48.69±0.24a
	2	12.19±0.0a	30,78±0.31a	0.32±0.01a	4.92±0.24a	3.80±0.09a	47.99±0.14a
Sebesi	1	12.07±0.01a	33.51±0.24a	0.52±0.03a	6.28±0.01a	5.64±0.32a	41.99±0.59a
	2	12.81±004a	27.56±0.43a	0.29±0.05a	4.28±0.22a	6.82±0.05a	48.24±0.21a
Bintan	1	10.11±0.35a	40.94±0.37b	0.46±0.02a	4.93±0.02a	7.29±0.08a	36.27±0.17b
	2	10.93±0.24 a	41.56±0.62b	0.22±0.03a	4.06±0.04a	4.21±0.12a	39.02±0.57b

Seaweed is a source of many essential minerals, with benefits for humanity in food and health. Based on the mineral content analysis, manganese was the primary element (17-103 mg/kg), while copper showed the lowest values (1.1-4.99 mg/kg) in all locations and seasons, and most of the remaining element concentrations showed no significant variation. However, 3 (three) elements, barium, selenium, and manganese, differed significantly with seasons ($p<0.05$). This was observed for barium in Tidung and Bintan, selenium in Sebesi and Bintan, and manganese, in Bintan. Table 4 shows *S. polycystum*'s trace elements contents.

Table 4 The trace elements content of the seaweed (mg/kg). Different superscript letters are significantly different (p<0.05). Capital letters indicate significant differences between locations, and lower-case letters - between seasons. Average ± SD; n = 3

Site/Season		Barium	Selenium	Iron	Manganese	Copper	Zinc
Tidung	1	21.86±1.7 aA	0.25±0.02aA	3.86±1.8 aA	17.15±12.6 aA	3.39±1.5 aA	18.47±8.8 aA
	2	14.19±1.7 bA	0.22±0.02aA	4,12±0,05 aA	25.46±21.0 aA	1.97±0.29 av	23.42±8.8 aA
Sebesi	1	20.55±1.1 aA	0.30±0.01 aA	1.70±2.9 aA	98.48±57.3 aA	3.54±0.7 aA	3.54±0.7 aA
	2	17.36±5.5 aA	0.18±0.002 bA	4,84±0,02 aA	18.76±9.9 aA	2.79±0.9 aA	10.09±1.4 aA
Bintan	1	10.65±1.3aA	0.31±0.02 aA	34.20±27.7 aA	103.29±15.3 aA	1.13±0.9 aA	12.64±4.2 aA
	2	22.02±3.7bA	0.18±0.02 bA	9,15±0,02 aA	33.89±6.8 bA	4.99±2.3 aA	34.82±16.8 aA

3.3. Alginate Contents

Table 5 shows the *S. polycystum* alginate characteristics. Based on the results showed the yield varied between 10.96% - 22.09%, with the lowest and highest values observed in Tidung and Sebesi Islands, during rainy and dry seasons, respectively. Meanwhile, moisture and ash contents varied across islands and seasons, with the least and most significant values observed in Sebesi (9.47 ± 1.6 and 27.70 ± 1.6%) and Bintan islands (17.83 ± 2.3 and 36.57 ± 1.5), respectively. However, the pH variations were limited, ranging from 8.14 - 8.36. Conversely, the viscosity showed highly different results, although discrepancies were not significant between locations and seasons. The highest and lowest values were obtained from the same locations on Sebesi Island (499.58 ± 0.4 and 284.71 ± 158.6 cPs, respectively). Statistical analysis showed the alginate yield differed significantly between Sebesi and Tidung, and the yield difference between Sebesi and Binan was influenced by season (*p*<0.05). Also, seasonal variation influenced the alginate's viscosity, especially alginates from Sebesi and Bintan Islands; however, locations had no influence.

Table 5 Alginate characteristics. Different superscript letters are significantly different (p <0.05). Capital letters indicate significant differences between locations, and lower-case letters - between seasons. Average ± SD; n = 3

Location/season		Yield (%)	Ash (%)	Moisture (%)	pH	Viscosity (cPs)
Tidung	1	10.96 ± 1.1aB	29.28±2.6aA	14.97±0.9aA	8.14 ± 0.22aA	393.31 ± 111.01aA
	2	13.97 ± 5.0aB	29.34±2.5aA	10.80±1.9aA	8.21 ± 0.06aA	499.1 ± 0.97aA
Sebesi	1	14.92 ± 1.5bA	27.70±1.6bA	17.83±2.3aA	8.27± 0.1bA	284.71 ± 158.6bA
	2	22.09 ± 2.8aA	29.82±3.4bA	9.47±1.6aA	8.27± 0.07aA	499.58 ± 0.4aA
Bintan	1	12.29 ± 1.2bB	31.89±1.8cB	13.98±0.4aA	8.36± 0.02aA	436.77 ± 109.5bA
	2	16.04 ± 1.3aB	36.57±1.5cB	10.73±1.04aA	8,27± 0.08aA	498.83 ± 2.02aA

Alginate characteristics were assessed in the form of M (β-d-mannuronate) and G (α-l-guluronate) block ratio as essential factors in gel-formation (gel performing). This property is exhibited through the development of copolymer glycosidic bonds between both blocks. Table 4 shows the M/G ratio recorded in this study, with significant differences observed regarding location and season (*p*<0.05). Generally, the values ranged from 0.57 - 0.99, indicating the G fraction was more significant compared to the M. Table 6 shows the M/G ratio based on single and double-bonds.

Table 6 Characteristics of single and double fractions of M and G alginate blocks. Different superscript letters are significantly different (p <0.05). Capital letters indicate significant differences between locations, and lower-case letters - between seasons. Average ± SD; n = 3

Location/season		G	M	FGG	FMM	FGM	FMG	M/G Ratio
Tidung	1	0.69	0.31	0.46	0.23	0.08	0.23	0.47 ± 0.2aA
	2	0.6	0.4	0.38	0.22	0.18	0.22	0.7 ± 0.3aA
Sebesi	1	0.56	0.44	0.5	0.06	0.39	0.06	0.81 ± 0.2aA
	2	0.64	0.36	0.4	0.24	0.13	0.24	0.58 ± 0.2bA
Bintan	1	0.55	0.45	0.37	0.18	0.28	0.18	0.84 ± 0.1aA
	2	0.92	0.29	0.23	0.69	-0.4	0.69	0.35 0.2bA

3.4. Fucoxanthin Contents

In this study, fucoxanthin was extracted from *S. polycystum* through an ultrasonic-assisted extraction method using acetone as a solvent, while quantification was performed using HPLC. The highest and least contents were obtained from the sample collected at Sebesi during the dry (0.587 mg/g) and wet (0.155 mg/g) seasons. That implies season has a serious impact on the quantity of fucoxanthin produced by *S. polycystum*. According to quantification results, seasonal differences were statistically significant (*p*<0.05), but the location counterparts were not. Table

7 shows the differences in the samples' fucoxanthin contents based on location and season.

Table 6 Fucoxanthin content of *S. polycystum* based on different location and seasonal variations (Different superscript letters are significantly different ($p<0.05$). Capital letters indicate significant differences between locations, and lower-case letters - between seasons. Average ± SD; n = 9)

Parameter/season		**Tidung**	**Sebesi**	**Bintan**
Fucoxanthin (mg/g)	Dry	0.447 ± 0.30aA	0.587 ± 0.16aA	0.404 ± 0.16aA
	Rainy	0.166 ± 0.01bA	0.155 ± 0.02bA	0.1570.01bA

3.5. The Relationship between Water Quality and Metabolites Production

The statistical CCA showed significant data from axis 1 and 2 (49.9 and 29.89%), respectively, amounting to 79.79%. That indicates a strong correlation between several parameters related to the water quality group, alginate characteristics, and fucoxanthin contents. Tidung Island showed high alginate moisture content, which correlates to temperature, pH, phosphate, Cu, and Zn compositions. Meanwhile, Sebesi Island data indicated a positive association between high alginate yield, viscosity, ratio, and ammonia, DO, salinity, as well as iron contents. Conversely, Bintan Island was characterized by substantial nitrate, salinity, barium, and manganese contents, and these were collectively implicated in a high M/G ratio and yield. Thus, fucoxanthin content is assumed to be closely related to variations in ammonia, temperature, pH, and copper composition observed within Tidung and Sebesi Islands. Figure 3 shows the canonical correspondence analysis results.

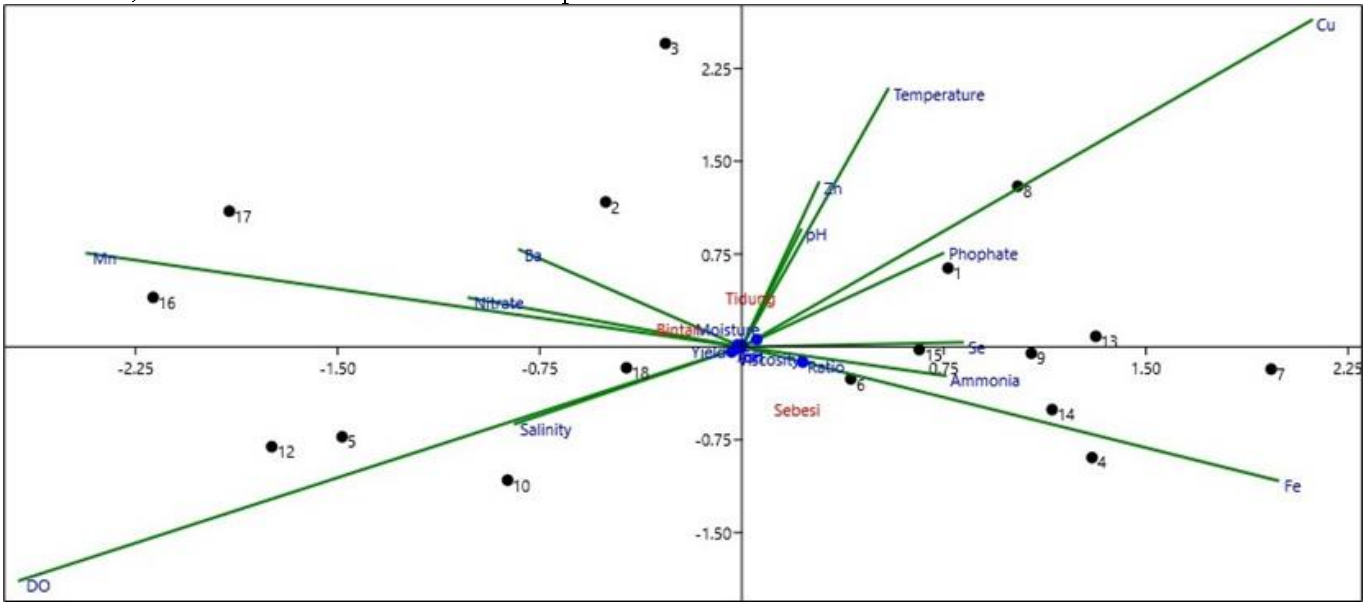

Fig. 3 The relationship between spatial-temporal water quality with the characters of alginate and fucoxanthin content. Axis 1 is indicating the X-axis, and axis 2 is the Y-axis

4. Discussion

Generally, an area's environmental conditions are related to land activity and hydro-oceanographic processes within the waters. The water's environmental quality around three different islands was analyzed during two distinct seasons in this study. The parameters analyzed were all found to be able to support macroalgae growth (Table 1). Macroalgae require sufficient nutrient level for adequate growth, including a combination of nitrate and phosphate, to attain maximum biomass [15]. These two inorganic substances are bound to exhibit fluctuating levels, depending on human activities (anthropogenic), and possibly lead to increased dissolved inorganic material levels in the water. This situation tends to influence macroalgae growth in general [16]. According to [17], anthropogenic stress in the form of freshwater runoff or chemical fertilizer applications triggers variations in the water's nutritional composition, and the direct impact of these variations is related to physiology, diversity, and seaweed beds' growth pattern [18], [19], [20], [21].

High levels of ammonia in seawater have been observed to inhibit photosynthesis and seaweed growth [22]. Furthermore, [23] discovered macroalgae can absorb and release dissolved and particulate matter in the habitat, leading to variations in seawater nutrient content. This phenomenon was observed with regard to tidal fluctuations and hydrodynamic processes [24], and these natural factors also significantly influence several heavy metals (Cu, Fe, and Mn) spatial distributions in China's Yangtze waters [25].

Microelements are essential for macroalgae growth, as clearly observed about selenium, positively influencing photosynthesis [26]. However, adverse outcomes have been reported in areas characterized by excess copper [27]. In addition, nitrate and phosphate naturally support seaweed growth [16], [17]. The compounds' distributions are also a form of interaction between biotic and abiotic factors, especially in the coastal intertidal zone [28].

Variations in nutrient content and physio-chemical parameters are generally influenced by water hydrodynamics [22]. Generally, microminerals accumulated in seawater come from river flows, tend to vary in levels, according to tidal induce and geochemical reactions [24]. Coastal water conditions are also strongly correlated with land activities, as anthropogenic factors significantly affect N and P contents [29], leading to increased dissolved inorganic materials in river flows.

Table 3 shows this study's overall proximate results. They hardly differ, compared to previous studies by [30], in terms of moisture (9.95 ± 0.55), ash (42.40 ± 0.41), lipid (0.29 ± 0.01), and protein content (5.40 ± 0.07), of *S. polycystum* grown in Malaysian waters. However, a slight difference was observed in the report by [31] on samples obtained from Tamil Nadu waters in India, with 29.0% ash, 7.6% lipid, 14.8% protein, 21.3% crude fiber. This specific macroalgae’s proximate characteristics differ, compared to other macroalgae, including *S. oligocystum*, with 21.27-22.54% ash, 3.51-5.66% lipid, 7,12-9 protein, 26%, and 7.35-12.63% crude fiber content [9], as well as *S. muticum*, with 13.2-30.5% ash, 1.6-3.2% lipid and 7-11% protein [32].

The different outcomes for several proximate compositions are possibly due to diversity in the analytical methods used. This study utilized relatively straightforward equipment compared to previous studies, and this probably caused differences in results. Also, diversity in Sargassum's proximate composition is generally influenced by species, geographical locations, and seasons [9], [32] or correlated with changes in environmental conditions, including temperature [33].

Based on this study’s findings, *S. polycystum* is a potential source of trace elements. These trace elements vary with location and season. However, the accumulation levels were below the ecological risk index (RI), according to Liu et al. [25]. Macroalgae can absorb large portions of minerals due to the flat filamentous and sheet-like leaf [34]. The presence of minerals in seaweed comes from uptake and assimilation, meaning minerals from seawater can enter cells by complex diffusion through the cell membrane [35]. This study discovered manganese was the most prominent trace element accumulated and was dominant in all locations and seasons. An extensive depletion occurred in Bintan Islands from the rainy to dry season, possibly due to high anthropogenic stress on the water/land and comparatively more rivers flowing into the ocean than other study locations. From this finding, mineral accumulation in *S. polycystum* within Bintan probably offers a critical example for biomonitoring the marine coastal environment's health.

In addition, the percentage of ash and water contents meet commercial standards, at 18-27% and 12%, respectively, while the pH was above the recommended value of 6.1-7.8 [12]. The viscosity values reported were of commercial medium quality (340-680 cPs) and relatively higher than other studies, as observed in *S. duplicatum* and *S. hystrix, at* 14,333 23,333 cPs and 75.37-126.00 cPs, respectively [36], [37]. However, [3], [38] reported that Sargassum's viscosity was generally lower than other brown seaweed, *Laminaria* sp, *Macrocystis pyrifera*, and *Ascophyllum nodosum* as the primary alginate source.

Alginate yields were found to differ significantly with locations and seasons, possibly due to distinct environmental characteristics, but were comparable to the previous studies' results, at 17.12% - 27.64%; 15.85% -20.00%; and 11.22% -29.54% [11], [39], [40]. Also, the environment plays a prominent role in alginate as part of a carbohydrate produced in seaweed tissue. That is supported by [41], [7], stating that alginate content depends on the season and geographic location and variations in nutrition absorbed by the seaweed.

A study conducted by [42] showed that the correct combination of solvent amount, temperature, bleach, and time in the sodium alginate extraction method was crucial for obtaining the optimum alginate quality. These findings were congruent with [43]’s report, stating environmental conditions significantly influenced macroalgae growth, nutrition, and characteristics of the alginate produced. Similarly, [44] acknowledged the impact of water characteristic changes, including pH, on alginate properties of Sargassum Vulgare species grown in the waters of Ischia island, Italy. The impact of seasonality and coastal location in Tamil Nadu, India, was also observed in the yield from *S. polycystum* [39].

In this study, the M/G block ratio varied between locations, and significant differences in water quality were found in Sebesi and Bintan over the seasons. Similarly, the variation in alginate characters was significant. Based on the ratio obtained, the results were comparable to the report on *S. polycystum* grown in Teluk Kemang, Malaysia (0.733) [11], Ujung Kulon Binuanguen waters, Banten, Indonesia at 0.59. and 1.22, respectively [40]. According to [45], the real M/G ratio value is specific to locations and seasons. These variations are possibly related to the measurement processes following adaptation to environmental conditions and seasons. The results showed dry season significantly impacted the decline in G blocks and during the rains. This finding is in line with Tidung Island reports, where lower ratios were obtained in the dry season; however, the reverse was experienced in the coastal waters of Sebesi and the Bintan Islands. All findings were consistent with a report by [41], where alginate content and M/G block ratio significantly depended on the organism type and age, environmental conditions, and habitat.

Seasonal variation was also significantly influenced the fucoxanthin content. Table 7 shows the highest and lowest contents in Sebesi and Tidung waters during the dry (0.587 ± 0.16 mg/g) and rainy seasons (0.166 ± 0.01 mg/g), respectively. Meanwhile, increasing fucoxanthin concentrations were observed from the rainy to dry season in all study locations. These variations are probably attributed to the impact of habitat or development stage. Carotenoid production is significantly dependent on the species, reproduction stage, location, and thallus part [46] since the main function of seaweed is transporting electrons for photosynthesis and antioxidative property, reducing light exposure impacts [5]. That is in accordance with seaweed samples harvested in a different period and followed the minimum tide level. Thus, this condition possibly led to the maximum light and UV irradiation during sampling, especially during the dry season.

In addition, differences were observed in the fucoxanthin concentration increase pattern obtained during this study. That is evidenced by a higher concentration-effect observed during the dry season, and this is possibly influenced by the period of sunlight, UV irradiation level, and water surface temperatures at the sampling time. The finding is supported by [47], stating geographic characteristics related to seawater temperature and depth impacted the fucoxanthin content of various macroalgae types worldwide.

During this study, fucoxanthin measurements were suggested to be obtained during the dry season and preferably in an area with similar characteristics, compared to the Sebesi waters. Therefore, environmental variations are key determinant factors that influence numerous bioactive materials' productions. A study by [48] reported a close relationship between bio-functional substance accumulation and environmental factors (temperature, light intensity, seawater nutrient profile, and water depth). Meanwhile, [49] acknowledged season has a significant impact on fucoxanthin content. A maximum concentration of 4.49 mg/gr was obtained from S. horneri species, in January or during winter. However, a value of 10.80 mg/g is attainable after a combined treatment of temperature, light, and depth control.

Environmental conditions are the primary driving mechanism for seaweed to produce alginate and fucoxanthin quite specifically. According to CCA's statistical analysis for defining the relationship between water quality and metabolites production, every location has a specific character as an environmental response. The water's fertility as determined by nitrate, phosphate, and ammonia tends to differ with location and season and largely determines alginate as well as fucoxanthin production. Particularly, in Bintan, nitrate played a significant role in alginate yield and ratio, while phosphate supported the high alginate moisture content in Tidung, and ammonia impacted alginate viscosity in Sebesi. Conversely, coastal waters of different islands form special habitat features to encourage specific responses in macroalgae.

In addition, oceanographic conditions, including wave motion and tidal current, can change nutrition and promote macroalgae spatial distribution [50]. The hydrodynamic seawater promotes micronutrient dissolved diversity and influences accumulation in seaweed tissue [51]. Thus, the coastal area's hydrodynamic processes, anthropogenic pressures, and geographical features are bound to promote trace element content variation. That is evident in the distinct correlation between these minerals and alginate formation and fucoxanthin within all study locations. Trace elements and heavy metals can encourage stress in macroalgae, leading to bioaccumulation, and consequently, lesser biomolecule formation [25].

In summary, habitat characteristics formed on volcanic island types, coral-formed islands, and monadnock islands and seasonal variations have a significant effect on *S. polycystum* nutrition and metabolites content. That is relevant to the previous studies' results; however, different island type variables are currently the new variables studied. Also, this study's results confirm the environment's carrying capacity plays an important role in wild seaweed development, thus, enabling the use as a reference for improved management, for instance, cultivation development. However, to achieve ideal conditions, laboratory-scale research is needed to produce the most optimal environmental variable precision in supporting the development of this sargassum cultivation type.

5. Conclusions

The three islands in Indonesia's western part have different water condition characteristics, especially for variations in nitrate, ammonia, DO, and Fe. These variations affect *S. polycystum* seaweed’s alginate and fucoxanthin content, as the seaweed’s growth is based on location and season. Thus, this study shows seaweed from the Sebesi waters produced in the dry season had the highest alginate and fucoxanthin content. However, environmental impact studies are required to support large-scale cultivation efforts.

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