Site-specific Morphological and Nutritional Variability of *Caulerpa lentillifera* in Malaysia: A Chemometric Approach for Tropical Aquaculture

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ABSTRACT

Caulerpa lentillifera is an important tropical green macroalgae cultivated and harvested across Southeast Asia. Nevertheless, its morphological and biochemical responses towards varying environmental conditions remain insufficiently discussed. This study aimed to characterise the site-specific morphological, nutritional, and mineral variability of *C. lentillifera* collected from three Malaysian populations. Samples were harvested from wild populations in Port Dickson (PD) and Semporna (SS), and from

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a cultured pond population from Pulau Langkawi (PL). This study also applied chemometric methods to identify unique compositional patterns relevant to tropical aquaculture. Morphological and proximate analyses found that the PL sample has an assimilator length of up to 94 mm and a moisture content of 96% (wet weight basis), whereas PD samples had higher total nitrogen content (1.36 mg/L), crude fiber (3.45 g/100 g), and rhizoid length (13.8 mm), indicating site-related variability. Principal Component Analysis (PCA), Partial Least Squares Discriminant Analysis (PLS-DA), and hierarchical heatmaps were used to integrate water-quality, morphometric, nutritional, mineral, and heavy-metal datasets and to visualise site-specific clustering patterns. The PCA explained 62.5% of the total variance, and the PLS-DA model showed clear, yet descriptive, group separation (R^2 and $Q^2 > 0.9$; $p = 0.001$). The fibre, calcium, and carbohydrates were identified as the main discriminant factors. Overall, the analyses revealed distinct site-associated compositional trends rather than definitive classifications. These findings provide baseline insights to guide future site selection and cultivation strategies for sustainable tropical aquaculture.

INTRODUCTION

Seaweed has been an integral part of human diets and health for centuries. To date, most seaweed production is concentrated in Asia, accounting for over 81% of the total worldwide production (Ismail et al., 2024). In 2020, the seaweed aquaculture dominated more than half of the total marine aquaculture, with an annual yield reaching more than 35 million tonnes (wet weight) 2020 (Chopin & Tacon, 2021; Stuthmann et al., 2023). Despite growing interest in seaweed cultivation, most exports focus on brown and red seaweed. Less attention had been given to green macroalgae or *Chlorophyta*. This had created a gap in further understanding of their ecological, nutritional values, and aquaculture potential (Moreira et al., 2021).

Among the green seaweeds, *Caulerpa* spp. is the most prominent genus, with more than 75 species reported (Famà et al., 2002; Preez et al., 2020). Within this, *Caulerpa lentillifera* is the most outstanding species due to its rapid growth, palatability, and potential commercial value. In Southeast Asia, including Malaysia, Thailand, Indonesia, and the Philippines, it is known as 'sea grapes or latok', due to its grape-like features. In coastal regions, *C. lentillifera* is often included in main dishes or eaten fresh due to its palatability and nutritional benefits (Chaiklahan et al., 2020; Khandaker et al., 2021). Due to its rising potential as a functional food, this species has attracted growing economic and cultivation interest.

However, *C. lentillifera* exhibits distinct physiological responses under different cultivation and environmental conditions. This helps to address existing gaps in understanding their adaptation and growth performance (Estrada et al., 2020). To date, many studies have focussed on interspecific variation within the genus *Caulerpa*, but comparatively less attention has been given to intraspecific variation. For example, research in Malaysia primarily focuses on differences among *C. racemosa, C. manorensis*, and *C. sertularioides* (Ismail et al., 2020; Yap et al., 2019), while site-specific variability in *C. lentillifera* is relatively underexplored. It is therefore important to understand their site-specific variability within the species and identify the factors that drive intraspecific variation to improve aquaculture cultivation and quality. Although seaweeds exhibit high adaptability, this does not necessarily translate into ecological or cultivation advantages without supporting data.

Thus, this study aims (i) to characterise the morphological, nutritional, and mineral variability across three distinct Malaysian sites; (ii) to identify the key discriminant variables using chemometric approaches; and (iii) to discuss the implications of site-specific variations within the species for tropical aquaculture and resource utilisation. To achieve these objectives, multivariate statistical analyses were employed. Principal Component Analysis (PCA), Partial Least Squares Discriminant Analysis (PLS-DA), and hierarchical clustering were applied to integrate water-quality, morphological, proximate, and mineral data into a comprehensive analysis. The analysis contributes to identifying the key variables that lead to population differentiation within the species. This study also supports Malaysia's National Agrofood Policy 2021-2030 (NAP 2.0), which aims to promote sustainable aquaculture development, improve cultivation, optimise production, and ensure consistency (Ismail et al., 2024).

MATERIALS AND METHODS

Study Area and Sample Collection

Wild samples were harvested from Port Dickson, Negeri Sembilan (PD, Southern Malaysia; Latitude: 2° 24'58.4 N, 101°51' 24.84"E). It is a semi-exposed coastal area close to the coastal development and active tourism. Another wild sample was collected from Semporna, Sabah (SS, East Malaysia; 4° 28'54.4 "N, 118° 36'40.28 "E). This area is closer to a coral reef ecosystem and is less disturbed by urban development. The cultivated samples were collected from a local aquaculture farm in Pulau Langkawi, Kedah, Malaysia (PL, Northern Peninsular Malaysia) (Figure 1). This site was included based on availability and is not environmentally equivalent to the natural site. While the sampling was conducted between January and April, the dates varied across locations due to logistical and weather constraints.

To avoid bias, samples were harvested around 11:00 a.m. and 3:00 p.m., within a 10-meter radius, and at depths of 0.5 to 1.0 m. This is important to keep preserving site-
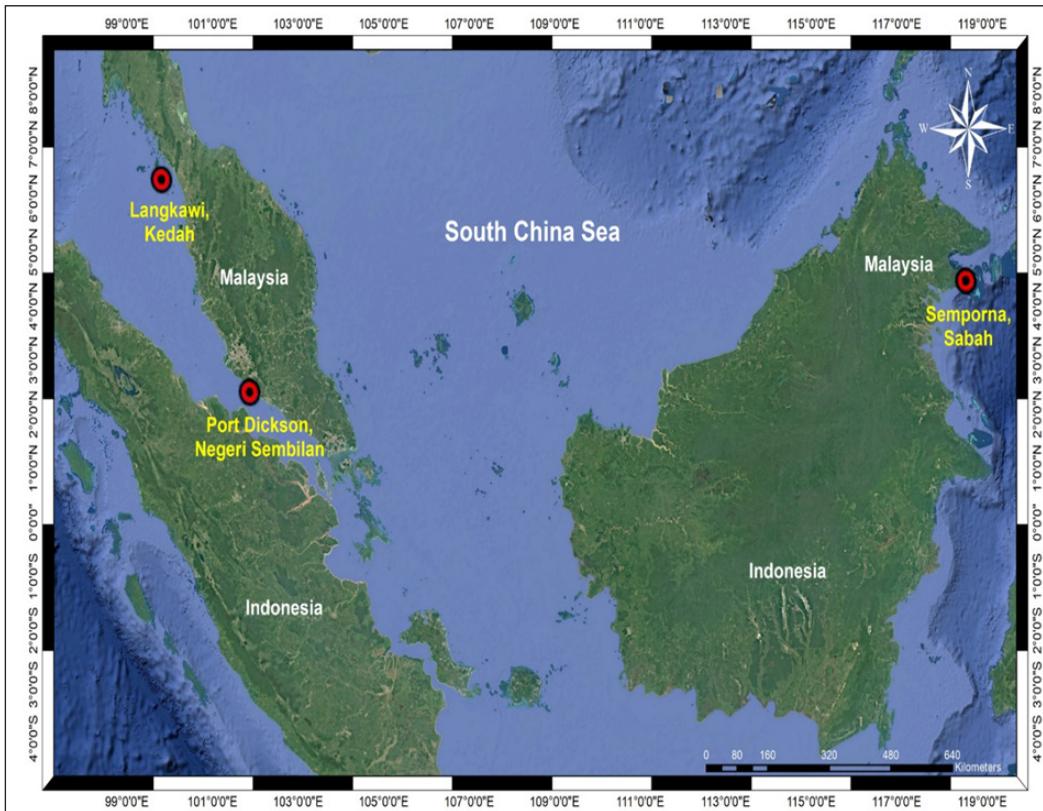
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Figure 1. Location of sampling sites: Port Dickson (PD), Semporna (SS), and Pulau Langkawi (PL)

specific differences. At each site, about 10 individual thalli were randomly collected, kept on ice, and transported to the laboratory within 24 hours. Morphometric identification was conducted on individual thalli, and chemical analysis was performed on a composite sample.

Water Quality Assessment

At each site, water temperature (°C), salinity (ppt), and pH were measured in situ with a calibrated multiparameter probe (HI98194, Hanna Instruments, USA). Independent water samples were aliquoted within a 5-10 m radius using pre-cleaned amber bottles (n=10 per site). Total nitrogen (TN) was determined following the APHA Standard Method (3500 series), while total phosphorus (TP) was quantified after acid digestion using ICP-OES as in USEPA Method 6010D. All results were expressed in mg/L.

Species and Morphometric Identification

Species identification was validated by a phycologist and an aquaculture expert from the International Institute of Aquaculture and Aquatic Sciences (I-AQUAS), Universiti Putra

Malaysia. The identification was recorded based on the seaweed size and structure of the assimilator, branchlet, and rhizoids (Estrada et al., 2020).

Proximate Analysis

Moisture content, ash, crude protein, crude fat, and carbohydrate content of the *C. lentillifera* were determined based on the Association of Official Analytical Chemists (AOAC, 2007) method on the fresh-weight (wet basis). This is to reflect the typical consumption of seaweed (Fisal et al., 2012). The oven-drying method (AOAC 934.01) was used for moisture content, and incineration at 550 °C in a muffle furnace (AOAC 942.05) was used for ash content. The Kjeldahl method with a nitrogen-to-protein conversion factor of 6.25 (AOAC 984.13) was used to determine crude protein, and crude fat was determined by Soxhlet extraction with petroleum ether (AOAC 920.39). Carbohydrate content was determined by difference after subtracting the total percentages of moisture, ash, crude protein, and crude fat, with ten replicates per sample (Abdullah et al., 2021).

Mineral Composition Analysis

Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES; Agilent 5100, USA) was used to quantify the mineral composition of the *C. lentillifera.* The seaweed samples were dried in a muffle furnace at 550 °C, as referred to in AOAC (2005). Next, the samples were digested in 7 mL concentrated hydrochloric acid and diluted to 100 mL. The multi-element calibration standards and reagent blanks were also prepared for quality control measurement (Mohd Naeem et al., 2017).

Phytochemical Screening

Dried and fine *C. lentillifera* powder was used in this study to standardise its moisture content (Rasyid, 2017). Aqueous extracts were prepared using a solid-to-solvent ratio of 1:20. Total phenolic content (TPC) was determined using the Folin-Ciocalteau method at 765 nm. Total flavonoid content (TFC) was determined using the calorimetric method at 415 nm. Absorbance was read using a 96-well microplate reader (Model 680, Bio-Rad Laboratories, USA). Calibration curves were prepared using gallic acid (0-250 mg/L) and quercetin (1000-5000 mg/L), with strong linearity (R^2 values = 0.9908 and R^2= 0.9989, respectively) TPC was expressed as mg gallic acid equivalent per gramme dry weight (mg GAE/ g^{-1} DW), while TFC was expressed as mg quercetin equivalents per gram dry weight (mg QE/ g^{-1} DW) (Zheng et al., 2022).

Statistical and Chemometric Analysis

Data are reported as mean ± standard deviation. One-way ANOVA followed by Tukey's post hoc test was calculated using Minitab 21.4.1 software (State College, Pennsylvania,

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United States). Chemometric analyses were performed using MetaboAnalyst 6.0. Variables included in Principal Component Analysis (PCA) and Partial Least Squares Discriminant Analysis (PLS-DA) were selected from complete datasets with no missing values. All variables were mean-centred and auto-scaled to unit variance to account for differences in measurement units. PLS-DA models were validated using k-fold cross-validation, and model performance was assessed using the R^2 and Q^2 values. Variable Importance in Projection (VIP) scores > 1.0 were used to consider important for group discrimination, and clustering patterns were visualised using a heat map.

RESULTS AND DISCUSSION

Water Parameters and Intraspecific Variation

Water temperatures at all sites ranged from 28.67 to 31.5 °C, consistent with standard tropical farming conditions for *Caulerpa* spp. (Zuldin et al., 2019) (Table 1). In the wild environments of PD and SS, elevated temperatures are observed compared to the PL water sample. This difference may reflect local climatic conditions and tidal exposure during in situ sampling, rather than inherent site differences. Importantly, all temperature values remained within the reported tolerance range for *C. lentillifera,* suggesting that temperature variation among sites is unlikely to impose physiological stress during the sampling period (Stuthmann et al., 2023).

There is no significant difference in salinity among sites. This suggests similar patterns of water mixing and tidal influences across sites during sampling (Guo et al., 2015). However, seaweed growth could still be affected by seasonal or short-term freshwater inputs, which can alter salinity. Thus, it is necessary to consider site-specific cultivation practices, especially in an open coastal environment (Mosquera-Murillo, 2016).

Compared to all samples, the highest dissolved oxygen (DO) was observed in PL at 4.31 ± 0.21 mg/L. This range is still comparable to both coastal and semi-aquaculture systems, as reported by Alamsyah, (2016). The variations between samples are likely

Table 1

Comparison of water parameters for C. lentillifera *harvested from different Malaysian sites*

Parameters	PD	SS	PL	*p*-value
Temperature (°C)	31.47±1.27[a]	29.72±1.25[b]	28.67±0.89[b]	0.000
Salinity (ppt)	31.37±0.55[a]	31.39±0.71[a]	31.45±0.72[a]	ns
Dissolved oxygen (mg/L)	4.14±0.09[a]	3.77±0.15[b]	4.31±0.21[a]	0.000
pH	8.05±0.02[b]	8.09±0.02[a]	8. 09±0.01[a]	0.000
Total nitrogen (mg/L)	1.36±0.10[a]	0.93±0.11[b]	1.22±0.18[a]	0.000
Total phosphorus (mg/L)	0.027±0.01[b]	0.006±0.00[c]	1.03±0.02[a]	0.000

Note. Different superscript letters in the row indicate significant differences (Tukey's test, p ≤ .05). Ns (not significant), PD (Port Dickson), SS (Semporna, Sabah), and PL (Pulau Langkawi)

due to the more stable conditions in the pond system rather than biological activity. In addition, the pH values range from 7.5 to 8.5, indicating a suitable range for growth. It is very important to monitor the pH changes, as they might influence pigment synthesis and secondary metabolism (Ly et al., 2021; Nurkolis et al., 2023).

The PL site also demonstrated higher total phosphorus (TP) (1.03±0.02 mg/L) than other sites. Nutrient accumulation under reduced water-exchange conditions in semi-aquaculture pond systems might also contribute to this (Sulistiawati et al., 2020). While essential for the seaweed growth, excessive accumulation can cause eutrophication. Thus, it is very important to monitor the TP, especially in the cultivation system (Fried et al., 2003).

In contrast, PD showed the highest total nitrogen (TN) content, up to 1.36 ± 0.10 mg/L. This indicates higher nitrogen availability than on the other sites. Yet the TN alone does not reflect bioavailable nitrogen, and elevated concentrations may result from tissue accumulation rather than direct uptake. Coastal activities around sites could also contribute to TN concentration. However, without the specific analysis on the source-specific evidence, this interpretation remains speculative (Chen et al., 2019).

Morphometric Traits across Sampling Sites

Figure 2 shows different parts of the *C. lentillifera*, while Table 2 shows the quantitative measurement of it.   A significant difference ($p<0.05$) was observed in both assimilator
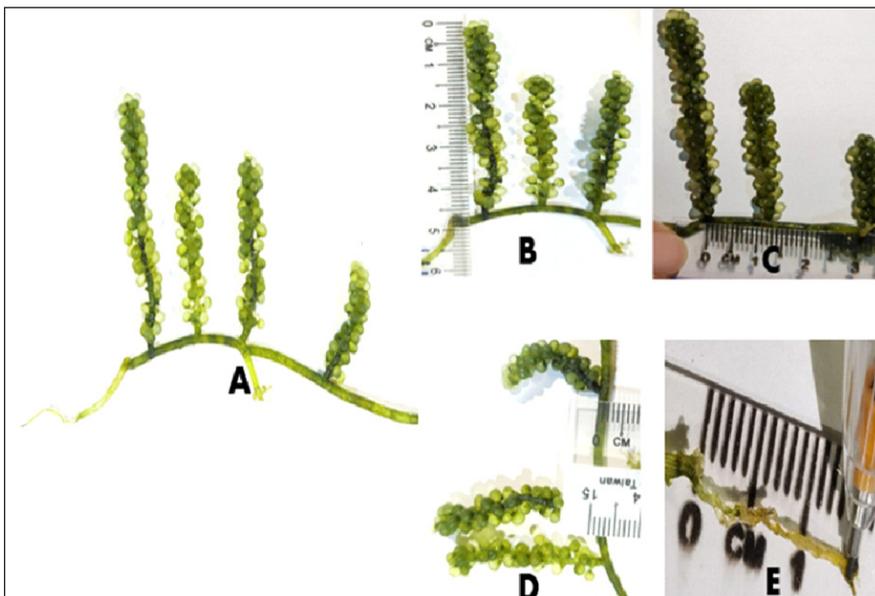

Figure 2. Morphometrics of *Caulerpa lentillifera* based on several parameters. (A) Number of assimilators; (B) Branchlet of the assimilators; (C) Spacing between stolon; (D) Stolon diametre; (E) Rhizoid length

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Table 2

Morphometric parameters of C. lentillifera *from different locations in Malaysia*

Morphometric Parameters/ Location	PD	SS	PL	*p*-value
Assimilators				
Height (mm)	35.30±6.62[b]	40.16±3.82[b]	94.40±7.73[a]	0.000
Width (mm)	1.71±0.51[a]	1.53±0.36[ab]	1.26±0.28[b]	ns
Spacing (mm)	6.00±0.47[b]	7.55±0.78[b]	8.18±1.38[b]	0.000
Number per Stolon	3.15±0.24[a]	3.0±0.00[a]	3.20±0.25[a]	ns
Branchlets				
Ramulus diametre (mm)	1.83±0.23[a]	1.88±0.20[a]	2.00±0.23[a]	ns
Stalk length (mm)	1.81±0.33[a]	1.70±0.63[a]	2.00±0.23[a]	ns
Stalk diametre (mm)	1.00±0.20[a]	1.17±0.27[a]	1.07±0.22[a]	ns
Rhizoids				
Stolon diametre (mm)	1.80±0.24[b]	2.25±0.16[a]	1.08±0.14[c]	0.000
Length (mm)	13.8±3.46[a]	9.2±2.74[b]	6.65±1.70[b]	0.000
Number per rhizoid per stolon	2.80±0.63[a]	2.60±0.52[a]	2.30±0.67[a]	ns

Note. Different superscript letters in the row indicate significant differences (Tukey's test, p ≤ .05). Ns (not significant), PD (Port Dickson), SS (Semporna, Sabah), and PL (Pulau Langkawi)

and rhizoid length. PL samples exhibited higher double-assimilator heights than SS and PD. These differences suggest the PL site provides more stable, favourable growth conditions, water quality, and nutrient availability for *C. lentillifera* (Estrada et al., 2020). In a cultivated pond like PL, a limited and controlled growth environment could also promote the elongation of assimilators. This is further supported by longer rhizoids in both PD (13.8 ± 3.46 mm) and SS (9.2 ± 2.74 mm) compared to PL (6.65±1.70 mm). In wild environmental conditions, the seaweed needs longer rhizoids as an adaptation to secure stronger anchorage to the substrate.

Some part of the morphometric parameters, like branchlet width, assimilator width, the number of branches per stolon, and the number of rhizoids per stolon, showed no significant differences. These parameters are suggested to be more stable and may be influenced by genetic factors rather than adaptation. In short, the morphological plasticity of C. lentillifera is influenced by both genetic and environmental factors. Their adaptation allows them to survive in various hydrodynamic, substrate, and nutrient conditions (Rabia, 2016; Tanduyan et al., 2013).

Proximate Composition on-Site Variation

Table 3 shows the proximate composition of the *C. lentillifera* harvested from different sites. The highest moisture content was detected in the PL sample (96.60±0.81%), aligned with *C. lentillifera* from Indonesia (95.01 ± 0.17%) and Taiwan (94.28 ± 0.24%) (Nofiani

Table 3

The proximate study (wet basis) of C. lentillifera *from different locations in Malaysia*

Parameter/Site	PD	SS	PL	*p*-value
Moisture (%)	94.08±0.45	94.94±1.06[b]	96.60±0.81[a]	0.000
Ash (%)	1.25±0.21[a]	0.45±0.06[b]	0.43±0.06[b]	0.000
Crude lipid	0.06±0.01[b]	0.07±0.00[a]	0.07±0.00[ab]	0.000
Crude protein (g/100 g)	1.0±0.38[a]	0.43±0.13[b]	0.56±0.25[b]	0.000
Carbohydrate	0.16±0.44[b]	3.97±1.00[a]	0.86±0.80[b]	0.000
Crude fibre (g/100 g)	3.45±0.25[a]	0.14±0.05[c]	1.48±0.02[b]	0.000

Note. Different superscript letters in the row indicate significant differences (Tukey's test, $p \leq .05$). Ns (not significant), PD (Port Dickson), SS (Semporna, Sabah), and PL (Pulau Langkawi)

et al., 2018; Ly et al., 2021). This elevated moisture content may be associated with the greater assimilator height observed in PL, as elongated tissues typically retain higher water content to maintain structural integrity and buoyancy.

Higher ash content was detected in PD samples (1.25 ± 0.21%) than in SS and PL samples. Still, the ash content from all samples lies within the expected range for fresh seaweed (<5%) (Nofiani et al., 2018; Syakilla et al., 2022). This might be due to high calcium and zinc levels in the same samples, suggesting that site-specific minerals influenced ash variation (Zhou et al., 2025). The PD sample also showed higher crude protein (1.0±0.38 g/100 g) than the other sites, indicating accumulation of total nitrogen on the site. While this suggests a possible connection between nitrogen and protein accumulation, further analysis is needed.

Importantly, crude fibre, carbohydrate, and protein were identified as the key variables contributing to separation in subsequent PCA and PLS-DA analyses (see Chemometric Analysis section), thereby linking univariate compositional differences to multivariate clustering patterns.

Mineral Profiles on-site Variation

Table 4 shows that the mineral composition in each sample varies among sites. Samples from PD show the highest calcium (104.00 ± 7.54 mg/100 g) and zinc (0.343 ± 0.26 mg/100 g) levels among the sites. This variation may reflect variation in mineral availability and uptake in an open coastal environment, rather than intrinsic differences of the seaweed (Ismail et al., 2014; Preez et al., 2020).

Higher levels of magnesium, potassium, and sodium are found in PL samples. This pattern is likely due to accumulation under relatively stable pond conditions rather than to direct environmental influences or to the seaweed's intrinsic nutritional superiority. Such accumulation is consistent with reduced water exchange and salt retention commonly

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Table 4
Mineral analysis of C. lentillifera *isolated from different locations in Malaysia*

Location/ Mineral (mg/100 g)	PD	SS	PL	*p*-value	RNI/day (mg/day) limit
Calcium, Ca	104.00±7.54[a]	32.00±1.97[b]	26.00±1.63[c]	0.000	1,000.00
Magnesium, Mg	13.24±2.16[b]	13.29±0.95[b]	58.51±1.80[a]	0.000	310-420.00
Potassium, K	2.69±0.10[c]	4.93±0.39[b]	33.60±1.50[a]	0.000	4.70
Sodium, Na	28.21±2.14[c]	333.30±24[b]	550.60±33.8[a]	0.000	1500
Zinc, Zn	0.343±0.26[a]	0.165±0.15[b]	0.116±0.07[c]	0.000	4.40-6.30
Copper, Cu	1.36±1.20[b]	1.52±1.18[a]	1.37±1.31[b]	0.008	900 μg/day

Note. Different superscript letters in the row indicate significant differences (Tukey's test, $p \leq .05$). Ns (not significant), PD (Port Dickson), SS (Semporna, Sabah), and PL (Pulau Langkawi)

associated with pond-based aquaculture systems, which differ fundamentally from open coastal environments (Greer et al., 2020). While these minerals are nutritionally relevant, the present discussion emphasises their roles as indicators of site and system-specific accumulation rather than dietary contribution. Accordingly, comparison with recommended analyses is descriptive and explanatory in nature and is not intended to imply causality that seaweed consumption quantities vary, and that mineral content is reported on a wet-weight basis.

Heavy Metal Content on-site Variation

In the absence of specific regulations on heavy metals in seaweed in Malaysia, the heavy metal content of the *C. lentillifera* was assessed based on the permissible limits established for leafy vegetables (Table 5). This comparison is intended as a general safety reference rather than a direct regulatory equivalence, as seaweeds differ from terrestrial vegetables in mineral accumulation pathways.

Table 5
The Heavy Metal Comparison of C. lentillifera *from different locations in Malaysia*

Location/ Heavy Metal (mg/kg)	PD	SS	PL	*p*-value	Malaysia Food Regulation 1985	FAO/ WHO, 2019 limit
Mercury, Hg	ND	ND	ND	-	0.5	0.004
Antimony, Sb	ND	ND	ND	-	0.5	-
Arsenic, As	3.27±0.15[b]	1.49±0.19[c]	11.23±0.9[a]	0.000	1.00	0.5
Lead, Pb	2.08±0.45[a]	0.95±0.07[b]	1.11±0.09[b]	0.000	2.00	2.0
Cadmium, Cd	ND	1.05±0.07[a]	0.65±0.07[b]	0.000	1.00	1.0

Note. Different superscript letters in the row indicate significant differences (Tukey's test, $p \leq .05$). Ns (not significant), PD (Port Dickson), SS (Semporna, Sabah), and PL (Pulau Langkawi)

The highest total arsenic was recorded in PL (11.2±0.14 mg/kg) and the lowest in SS (1.58±0.02 mg/kg). These values are consistent with a previous study where seasonal and hydrodynamic factors influence arsenic accumulation in *Caulerpa* spp. (Misheer et al., 2006). The higher arsenic content in PL may result from limited water exchange in the pond systems, allowing dissolved elements to accumulate and be absorbed. However, this remains speculative without identifying the arsenic sources. In fact, the arsenic present in this study was reported based on the total value. A previous study reported that most of the arsenic in seaweed exists as arsenosugars, which are less toxic than the inorganic form and can be reduced through washing, soaking, and boiling (Almela et al., 2005; Yu et al., 2024). Yet, arsenic speciation analysis is required to more accurately assess food safety risks, particularly for on-cultivated *C. lentillifera,* and should be prioritised in future studies.

On the other hand, the PD sample shows a higher lead level (2.08 ± 0.45 mg/kg) than the SS and PL samples. Although higher, all values remained within tolerable intake limits reported for both adults and children (Sharma & Rhyu, 2014; Nag & Cummins, 2022). Mercury (Hg) and antimony (Sb) were detected in any of the samples, further supporting its safety for human consumption. Overall, while the heavy metals were below international and guideline limits, it is very important to continuously monitor these locations to ensure long-term food safety.

Phytochemical Analysis on *C. lentillifera* across Sites

In Table 6, no significant difference ($p > 0.05$) was found in the total phenolic content (TPC) of the aqueous extract from all samples. The uniformly low TPC values are consistent with previous reports, indicating that aqueous extraction (0.15-2.04 mg GAE/g DW) of *C. lentillifera* yields relatively low concentrations of polar phenolic compounds (Yap et al., 2019; Syakilla et al., 2022). This suggests that phenolic abundance is not a dominant driver of site-specific variation under the extraction conditions applied in this study.

Nevertheless, a significant difference ($p < 0.05$) in TFC values was observed across all locations, with the values ranging narrowly from 4.57 to 1.63 QE/g DW. The differences might be contributed to by cultivation practices including controlled growth conditions, reduced physical stress, and post-harvest handling. These factors may play a more significant role than environmental exposure alone. In addition, flavonoid content has been shown to vary with extraction parameters, drying method, genetic diversity, and fluctuations due to biological, environmental, seasonal, and yearly variations (Kumar & Roy, 2018; Aryal et al., 2019). Given that PL samples originate from an aquaculture system, these differences should be interpreted with caution, as cultivation practices confound comparisons with wild populations.

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Table 6

The total phenolic (TPC) and flavonoid content (TFC) of C. lentillifera *were isolated from different locations in Malaysia*

Test/Sample	PD	SS	PL	*p*-value
TPC, mg GAE/g DW	0.44±0.09[a]	0.42±0.08[a]	0.51±0.06[a]	ns
TFC, mg QE/g DW	1.63±0.8[b]	1.72±1.46[b]	4.57±3.23[a]	0.000

Note. Different superscript letters in the row indicate significant differences (Tukey's test, $p \leq .05$). Ns (not significant), PD (Port Dickson), SS (Semporna, Sabah), and PL (Pulau Langkawi)

Chemometric Analysis of Intraspecific Variations in *Caulerpa lentillifera*

Unsupervised Principal Component Analysis (PCA) was adopted to visualise overall patterns of intraspecific variation in *C. lentillifera* variation, followed by supervised Partial Least Squares-Discriminant Analysis (PLS-DA). These analyses are descriptive and explanatory in nature and are not intended to imply causality between environmental parameters and biological traits. This framework is consistent with previous studies that reported that growth-related parameters and biochemical traits may respond independently to environmental conditions rather than exhibiting a direct linear relationship (Wichachucherd, 2019).

The PCA biplot (Figure 3) accounted for 62.5% of the total variance, with PC1 and PC2 accounting for 39.7% and 22.8%, respectively. The positive axis of PC2 is dominated by PD samples and is associated with high calcium (Ca), crude fiber, zinc, and protein content. This clustering indicates that PD samples are enriched in mineral and proximate compositions. However, the PCA alone could not determine whether this is due to the nutrient enrichment or enhanced growth. While Pb had accumulated in the same region, it is not the only factor driving species separation. This is due to the variation in seaweed being driven more by microminerals and nutrient components rather than trace contaminants (Kreissig et al., 2021).

On the other hand, the negative PC1 side is dominated by PL samples. Assimilator height, moisture, phosphate, and total phenolic content were identified as the driving factors. Stable water depth reduced hydrodynamic exposure, and maintenance cultivation could contribute to these. Previously, pond-based studies also suggested that phenolic and antioxidant properties were influenced more by environmental and management factors than by growth (Wichachucherd, 2019). Yet, without direct measurements, this observation is likely descriptive rather than mechanistic.

As for the SS samples, the clustering was found to be distant from both groups. It also shows a strong association between carbohydrate and copper content. This suggests a different metabolic orientation in the samples and reflects energy storage and site-specific
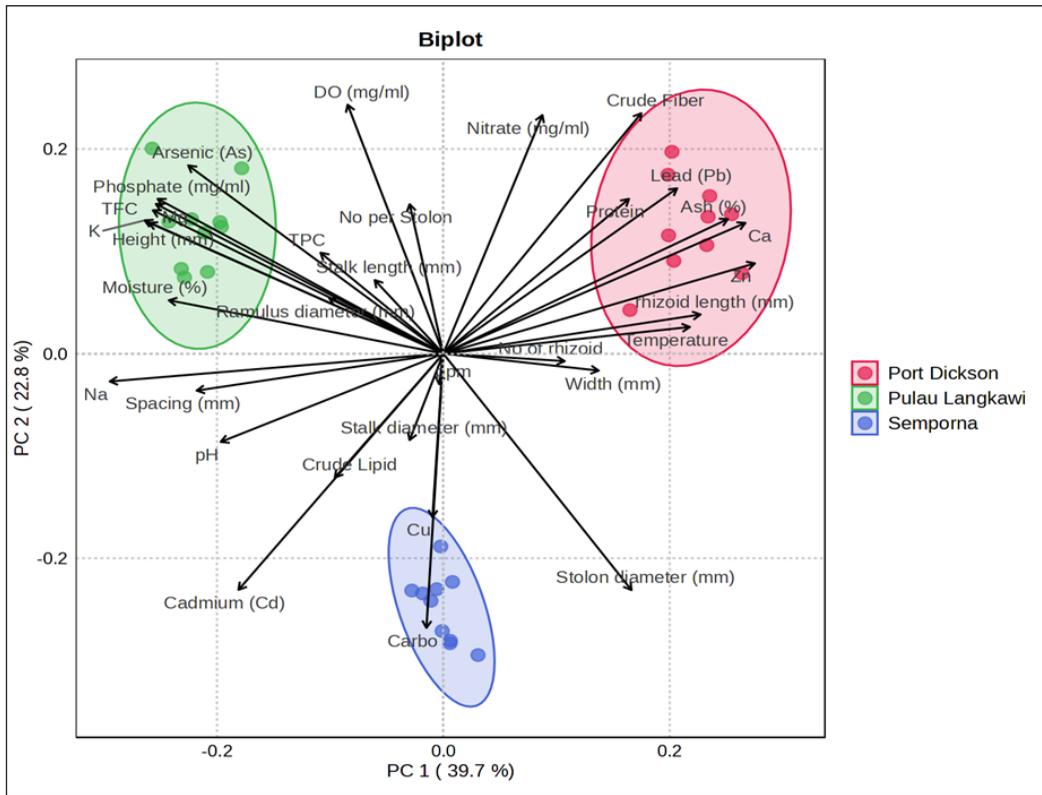
Figure 3. The biplot of C. lentillifera harvested from Port Dickson (PD), Pulau Langkawi (PL), and Semporna (SS)

mineral accumulation. This is due to the carbohydrate accumulation and mineral uptake, ate depend on the environmental and growth stage of the seaweed (Kreissig et al., 2021; Syakilla et al., 2022). Nevertheless, without correlation or regression analysis, the observed association should be interpreted with caution.

The PLS-DA models in Figure 4a showed a strong explanatory power with R^2 and Q^2 exceeding 0.90. Permutation testing confirmed the group separation was not due to random chance, as $p = 0.001$ (Figure 4b). Variables with VIP scores greater than 1.3 indicate that crude fibre, calcium, and carbohydrates are key differentiators that support PCA clustering. These variables were consistent with those identified in the univariate proximate and mineral analyses, without implying a causal relationship. Thus, the analytical approaches mirror earlier reports where multivariate separation reflected differential weighting of growth-related biochemical variables rather than a single dominant driver (Ismail et al., 2014; Syakilla et al., 2022).

The hierarchical heatmap was developed to observe trait differences among sites and their clustering within groups (Figure 5). The well-defined clusters on the dendrogram
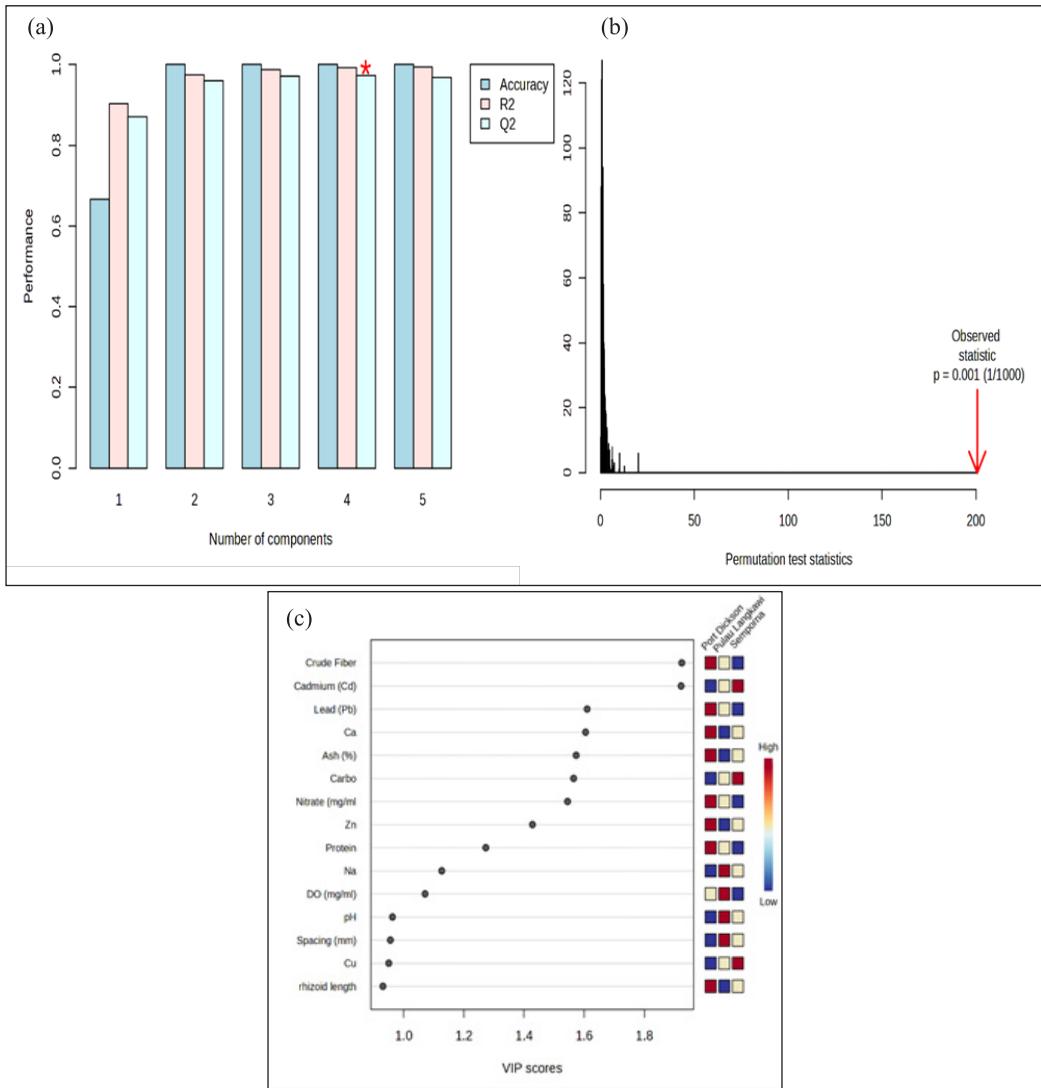
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Figure 4. Partial Least Squares Discriminant Analysis (PLS-DA) model performance and validation. (a) PLS-DA scores plot; (b) permutation test; and (c) model evaluation accuracy

strongly support site-level structuring as observed in both PCA and PLS-DA. Furthermore, the colour patterns serve as a signature for each sample and location. For instance, PD samples are high in nitrate, protein, and crude fibre; PL indicates elevated phenolic content; and SS has a high carbohydrate content. Collectively, the chemometric analyses reveal site-specific compositional fingerprints rather than performance rankings, in agreement with previous chemometric studies that emphasise origin-dependent compositional signatures in seaweed (Kreissig et al., 2021). In short, the PCA, PLS-DA, and hierarchical clustering offered complementary insights into intraspecific variability in *C. lentillifera.*

This is important for supporting a basis site selection, origin classification, and cultivation strategies. However, the results do not indicate causal relationships and are influenced by cultivation conditions.
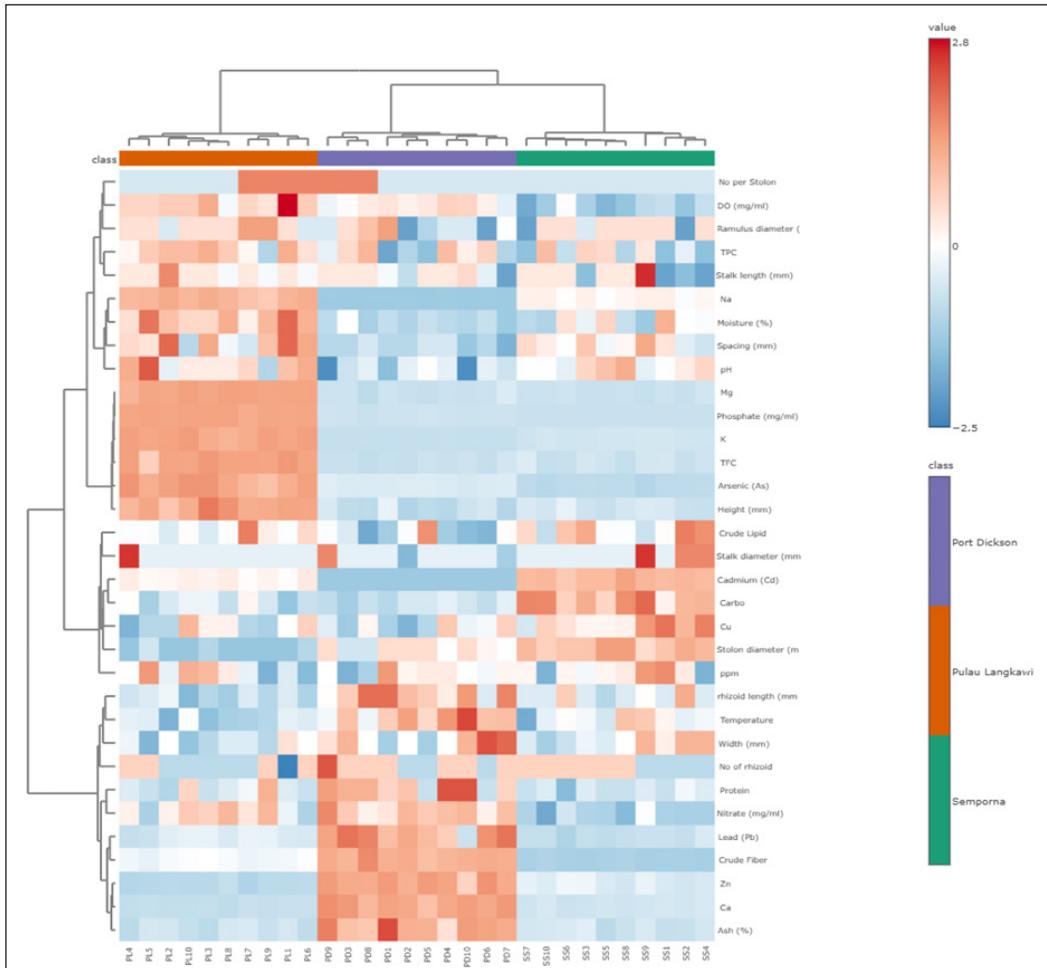

Figure 5. Hierarchical heatmap of nutritional, mineral, and morphometric traits of C. lentillifera from three sites in Malaysia

CONCLUSION

These findings showed site-related differences in morphological, mineral, phytochemical, and elemental composition of *C. lentillifera* harvested across Malaysia. These differences are primarily associated with environmental conditions and cultivation practices rather than intraspecific variation. Chemometric analysis identified crude fibre, calcium, carbohydrate, protein, and phenolic content as the key contributors to site differentiation. PD samples generally had higher protein and calcium levels, while SS samples showed stronger

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carbohydrate-related traits. PL samples showed higher phenolic content and variation in morphometric characteristics. Although heavy metals are detected at low levels, continuous monitoring is needed.

ACKNOWLEDGEMENT

FUNDINGS

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