

Biomass Loss and Carrageenan Content of Longline-Cultivated *Kappaphycus alvarezii* in Ekas Bay, Lombok: A Single-Season Case Study

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Abstract

Seaweed cultivation performance varies with local conditions, changing biomass production and the chemical characteristics of harvested thalli. This study evaluated the growth performance, seawater elemental dynamics, thallus elemental composition, phytochemical profile, and carrageenan content of *Kappaphycus alvarezii* cultivated using the longline method in Ekas Bay, East Lombok, West Nusa Tenggara, Indonesia. The study ran for 45 days, from 21 May to 5 July 2025. We evaluated growth performance from four field replicates, each with three nested subsamples, based on final wet biomass, daily growth rate (DGR), absolute growth, and biomass retention. We measured seawater elemental composition at planting and harvest without independent analytical replication. Thallus elemental composition, qualitative phytochemical profile, and carrageenan content were assessed using three independently analyzed thallus samples. Mean final wet biomass was 19.34 ± 10.62 g from an initial biomass of 100 g, with a DGR of $-4.10 \pm 1.41\%$ day⁻¹, absolute growth of -80.66 ± 10.62 g, and biomass retention of $19.34 \pm 10.62\%$. Potassium and chlorine were the dominant measured elements in the thalli, with concentrations of 10.34 ± 0.50 and 10.09 ± 0.85 mass%, respectively. Seawater concentrations of S, Cl, K, Ca, and Br were higher at harvest than at planting, with increases of 52.74%, 34.84%, 14.35%, 73.73%, and 11.85%, respectively; these observations were descriptive because seawater samples were not independently replicated. Phytochemical screening showed strong positive reactions for flavonoids and alkaloids (+++), weak positive reactions for saponins (+), and variable responses for tannins, terpenoids, phenol/hydroquinones, and steroids. Carrageenan content averaged $40.77 \pm 1.58\%$. The results demonstrate substantial biomass loss during the cultivation period, while the remaining thalli showed measurable elemental, phytochemical, and carrageenan characteristics. The study is limited by the single-site, single-period design, unreplicated seawater elemental sampling, and the use of three independently analyzed thallus samples for chemical characterization, which restricts broader inference regarding seasonal patterns and the causes of biomass loss.

Keywords: biomass loss carrageenan, *Kappaphycus alvarezii*, longline cultivation, Ekas Bay

INTRODUCTION

Kappaphycus alvarezii is an important carrageenan-producing seaweed widely cultivated in Indonesian waters. Its production performance is commonly evaluated through biomass growth, survival or biomass retention, and carrageenan characteristics, which can vary with cultivation method and local environmental conditions. Longline cultivation is widely used in Indonesia because the position of thalli in the water column exposes them to water movement, light, nutrients, and other site-specific conditions. Studies in Indonesian waters have demonstrated that cultivation performance varies among farming systems and locations. For example, longline cultivation of *K. alvarezii* in Southeast Sulawesi produced substantial biomass gains but also showed thallus damage under field conditions (Kasim & Mustafa, 2017). While cultivation studies across Indonesian sites have demonstrated geographic variation in growth and product quality. More recently, Cokrowati *et al.*, (2024; Sulystyaningsih *et al.*, 2022) reported different growth and carrageenan-related characteristics of *K. alvarezii* and *K. striatus* cultivated in Ekas Bay, East Lombok. These findings indicate that cultivation performance should be interpreted within the specific environmental and geographic context of each production site.

A major concern in field cultivation is that biomass gain cannot always be assumed from the initial planting biomass. Biomass loss may occur through thallus breakage, fragmentation, grazing, hydrodynamic disturbance, or unfavorable environmental conditions. Such biomass loss may not necessarily correspond to a proportional change in carrageenan content because biomass

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accumulation and carrageenan biosynthesis represent different biological processes. Previous studies have shown that cultivation method, nutrient availability, seed weight, cultivation duration, and environmental conditions can influence growth and carrageenan-related characteristics (Faisan *et al.*, 2024a; Muyong & Tahiluddin, 2024; Pong-Masak & Sarira, 2018; Rajapaksha *et al.*, 2025; Rasnijal *et al.*, 2024). In Indonesian waters, the relationship between environmental conditions and *K. alvarezii* performance has also been investigated in longline systems, including studies linking water parameters with growth and carrageenan quality (Istiqamah *et al.*, 2025).

Elemental and phytochemical characteristics can provide complementary information about the chemical composition of harvested biomass. Elements such as K, Ca, Na, Mg, S, and Cl are commonly detected in *K. alvarezii*, but elemental concentrations measured in dried thalli should be interpreted cautiously because surface salt residues and the polysaccharide matrix may contribute to the measured values. Therefore, elemental composition is considered here as a descriptive chemical characteristic rather than a direct physiological marker. Phytochemical screening provides an additional qualitative description of the harvested biomass because secondary metabolites may vary with environmental and temporal conditions and may be associated with responses to environmental stress (Nunes *et al.*, 2024, 2025; G. E. Schneider *et al.*, 2000). Carrageenan remains a key product-quality parameter, although its measured yield and physicochemical characteristics can also be affected by raw material properties and extraction procedures (Mendes *et al.*, 2024a; Rudke *et al.*, 2022; Sulystyaningsih *et al.*, 2019). Consequently, biomass performance, elemental composition, phytochemical profile, and carrageenan content should be interpreted as complementary characteristics of the harvested biomass rather than as evidence of a single causal physiological pathway.

Despite increasing research on *K. alvarezii* cultivation in Indonesian waters, information remains limited on the outcome of a longline cultivation cycle in Ekas Bay when substantial biomass loss occurs, particularly regarding the chemical characteristics of the biomass remaining at harvest. The 2024 study in Ekas Bay documented growth and carrageenan-related characteristics of *K. alvarezii* in comparison with *K. striatus*, but it did not characterize biomass retention together with seawater elemental composition, thallus elemental composition, phytochemical profile, and carrageenan content during the cultivation cycle (Cokrowati *et al.*, 2024). Other Indonesian studies have focused on comparisons among cultivation systems, locations, cultivars, or water-quality relationships rather than documenting this combination of biomass outcome and chemical characteristics under a single longline cultivation cycle (Hurtado *et al.*, 2001; Istiqamah *et al.*, 2025; Pong-Masak & Sarira, 2018). The present study therefore addresses a site-specific empirical gap by characterizing biomass loss and the associated chemical characteristics of *K. alvarezii* cultivated in Ekas Bay, East Lombok, during a 45-day cultivation period from 21 May to 5 July 2025.

This study aimed to evaluate the cultivation outcome of *K. alvarezii* grown using a longline system in Ekas Bay, East Lombok, Indonesia, over 45 days by quantifying final wet biomass, daily growth rate, absolute biomass change, and biomass retention; characterizing seawater elemental composition at planting and harvest; determining the elemental composition and qualitative phytochemical profile of harvested thalli; and measuring carrageenan content in three independently analyzed thallus samples. Based on the premise that biomass accumulation and carrageenan characteristics may respond differently to field cultivation conditions, we hypothesized that the 45-day cultivation cycle would result in measurable changes in biomass retention and chemical characteristics of the harvested thalli, while carrageenan content would not necessarily change proportionally with biomass loss.

MATERIALS AND METHODS

The study was conducted in Ekas Bay, East Lombok, West Nusa Tenggara, Indonesia, from 21 May to 5 July 2025, covering a 45-day cultivation period. The longline cultivation station was located at approximately 8.864062° S and 116.438514° E. Ekas Bay is an active tropical seaweed farming area where *K. alvarezii* is cultivated commercially. The study examined the growth performance and

biochemical characteristics of *K. alvarezii* under the cultivation conditions observed during the first experimental season.

Healthy *K. alvarezii* propagules were obtained directly from an existing seaweed cultivation area in Ekas Bay, East Lombok, Indonesia. Seedling selection was based on morphological characteristics and visible health conditions. Selected propagules had dense, well-branched thalli, young and actively growing tissue, and no visible symptoms of ice-ice disease. Each experimental unit consisted of a propagule with an initial fresh weight of 100 g. The initial weight was recorded before cultivation and served as the baseline for evaluating changes in biomass throughout the cultivation period. The seedlings were selected to minimize initial differences in thallus condition and to improve comparability among experimental units.

The study employed two cultivation systems, namely the longline and stake methods, to evaluate the growth performance of *K. alvarezii* under different farming arrangements. For the longline system, the cultivation area had an approximate water depth of 30 m. The cultivation rope was positioned approximately 20 cm below the sea surface, and individual seaweed propagules were attached at intervals of 30 cm along the rope. Four experimental groups (A1–A4) were randomly assigned to four separate longline ropes, spaced 30 cm apart. This arrangement maintained spacing between adjacent thalli throughout the cultivation period. Experimental units were randomly positioned within the designated cultivation area to reduce potential placement bias. The initial fresh weight of each propagule was standardized at 100 g, and cultivation continued for 45 days.

Environmental water quality was assessed at the cultivation sites to characterize the conditions experienced by *K. alvarezii* during the experimental period. Water quality measurements were conducted at two sampling points: the beginning and the end of the cultivation period. The measured parameters included water temperature, salinity, pH, and dissolved oxygen (DO). Measurements were conducted at the cultivation sites corresponding to the longline and stake systems. The resulting data were used to describe environmental conditions and support interpretation of differences in seaweed growth and biochemical characteristics. Absolute Growth (AG) was calculated as (Coantic & Seguin, 1971):

$$AG = W_t - W_0$$

where (W_t) is the final fresh weight (g) and (W_0) is the initial fresh weight (g).

Daily Growth Rate (DGR) was calculated according to the equation (Coantic & Seguin, 1971):

$$DGR = \left(\frac{\ln W_t - \ln W_0}{t} \right) \times 100$$

where (W_t) is the final fresh weight (g), (W_0) is the initial fresh weight (g), and (t) is the cultivation period (days).

At harvest, *K. alvarezii* thalli were collected from each experimental unit and labelled according to cultivation treatment. Samples were prepared for phytochemical screening, elemental analysis, and carrageenan extraction. Sample preparation procedures, including rinsing, drying, grinding, and storage, were considered important for maintaining sample consistency and analytical reliability (Harborne, 1998) (Rupert *et al.*, 2022). Specific preparation conditions require verification against laboratory records.

Elemental composition of *K. alvarezii* and seawater was examined using X-ray fluorescence spectroscopy. The target elements included Cl, K, Ca, S, P, Si, Fe, Zn, Cu, and Br. The supplied Rigaku Supermini200 manual describes wavelength-dispersive X-ray fluorescence (WD-XRF), including

qualitative, semiquantitative, and quantitative analysis procedures (Rigaku, 2024). Instrumental calibration, sample preparation, and certified reference materials (CRM).

Qualitative phytochemical screening was conducted to identify alkaloids, flavonoids, tannins, saponins, terpenoids, steroids, and phenolic compounds following established phytochemical screening principles (Harborne, 1998). Reagent reactions involved Mayer's, Wagner's, Dragendorff's, FeCl_3 , NaOH , HCl , Mg , and H_2SO_4 , as appropriate. Positive reactions were identified through characteristic color changes, precipitate formation, or persistent foam. Extraction conditions and qualitative scoring criteria (+, ++, +++) require confirmation from laboratory records.

Carrageenan was extracted from harvested *K. alvarezii* through alkaline treatment, separation, and drying. Alkaline extraction is widely applied to recover carrageenan from red seaweeds, although extraction conditions influence recovery and physicochemical characteristics (Rupert *et al.*, 2022). Carrageenan yield was calculated on a dry-weight basis following the general approach described in previous research on *K. alvarezii* (Mendes *et al.*, 2024b) :

$$CY (\%) = \frac{W_c}{W_d} \times 100$$

where W_c is the recovered dry carrageenan weight (g) and W_d is the initial dry seaweed weight (g). KOH concentration, extraction temperature, duration, and sample-to-solution ratio require verification against laboratory record. (Dhewang *et al.*, 2023) described alkaline extraction followed by filtration, KCl-induced precipitation, drying, and gravimetric yield determination. Recent work by Kumalaningrum *et al.* (2025) also evaluated carrageenan from *K. alvarezii* against SNI 8391-1:2017 quality criteria. The laboratory protocol used in this study was aligned with SNI 8391-1:2017, pure carragenan, part 1: Kappa carragenan. It should be noted that BSN subsequently issued SNI 8391-1:2021 as the current revision of the 2017 standard.

Each experimental unit contained 100 g of fresh *K. alvarezii*, serving as the baseline for growth assessment. Longline and stake cultivation systems were compared over a 45-day cultivation period. Previous studies have demonstrated the relevance of cultivation conditions when assessing seaweed growth and carrageenan characteristics (Rupert *et al.*, 2022). No untreated control or baseline biochemical measurements have been confirmed; therefore, biochemical results were interpreted as differences between cultivation treatments rather than changes from initial biochemical conditions.

The experimental design consisted of four separate longline ropes, with one experimental group assigned to each rope. Individual propagules within the same rope were considered subsamples rather than independent line-level replicates. Statistical interpretation accounted for the hierarchical arrangement of samples and was restricted to the study site. Data were summarized using descriptive statistics. Normality was assessed using the Shapiro–Wilk test (Shapiro & Wilk, 1965) , while variance homogeneity was evaluated using Levene's test (Levene, 1960). The Mann–Whitney U test (Mann & Whitney, 1947) was applied to appropriate two-group comparisons. The Kruskal–Wallis test (Kruskal & Wallis, 1952) , followed by Dunn's post-hoc test (Dunn, 1964), was used for comparisons involving more than two groups. Principal component analysis (PCA) was used to examine multivariate elemental variation, following the framework described by (Jolliffe & Cadima, 2016). Heatmaps were used to visualize elemental patterns. Statistical significance was set at $p < 0.05$.

RESULT AND DISCUSSION

The growth performance of *K. alvarezii* varied among four longline cultivation groups (A1–A4) over the 45-day cultivation period. All groups exhibited negative daily growth rates (DGR), indicating biomass loss during cultivation. The mean final wet biomass was 19.34 ± 10.62 g, compared with an initial biomass of 100 g, corresponding to a biomass retention of $19.34 \pm 10.62\%$. The mean DGR was $-4.10 \pm 1.41\% \text{ day}^{-1}$, while absolute growth averaged -80.66 ± 10.62 g (Figure 1). The negative DGR

values were observed across all four groups, although the magnitude of biomass loss differed among them. These findings indicate that the cultivated seaweed experienced an overall reduction in fresh biomass rather than positive growth during the experimental period.

The most important result of this study (Figure 1) was the substantial biomass loss of *K. alvarezii* during 45 days of longline cultivation. The mean DGR of $-4.10 \pm 1.41\% \text{ day}^{-1}$ and absolute growth of $-80.66 \pm 10.62 \text{ g}$ demonstrate that the biomass at harvest was considerably lower than its initial weight. Negative DGR indicates that biomass loss exceeded new tissue production during the cultivation period. However, the magnitude of this response varied among field replicates. A3 retained approximately one-third of the initial biomass and showed a DGR of $-2.52\% \text{ day}^{-1}$, whereas A1 retained only 10.13% and showed a DGR of $-5.57\% \text{ day}^{-1}$. This variation indicates that even within one longline farming area, local conditions can produce substantial differences in cultivation performance.

The 45-day cultivation trial in Ekas Bay resulted in substantial biomass loss, with a mean daily growth rate of $-4.10 \pm 1.41\% \text{ day}^{-1}$ and final biomass retention of 19.34%. Negative growth was recorded across all four longline groups, indicating that biomass reduction occurred throughout the experimental arrangement rather than being restricted to a single cultivation line. Comparable negative production was documented by (Pong-Masak *et al.*, 2026), who reported biomass losses across all ten *K. alvarezii* cultivars during the first 45-day cultivation cycle and negative growth among the five cultivars evaluated during the second cycle. Their findings demonstrate that biomass reduction can occur under longline cultivation even when cultivar performance differs substantially.

The mechanisms responsible for biomass loss in Ekas Bay remain uncertain. van Oort *et al.* (2025a) demonstrated that propagule loss, ice-ice disease, epiphyte fouling, temperature, and wind conditions jointly influenced *K. alvarezii* yields in South Sulawesi. However, the present study did not systematically quantify these potential determinants. Therefore, their contribution to the observed biomass decline cannot be established. The findings indicate an unfavorable growth outcome but do not identify a specific biological or environmental cause. Cáceres-Farias *et al.* (2025); Montúfar-Romero *et al.* (2023) reported conditions of approximately 34‰ salinity at a tropical cultivation site and emphasized that temperature, salinity, nutrient availability, and cultivation configuration jointly influence biomass performance. Experimental evidence also shows that changes in salinity modify photosynthetic performance, osmolyte accumulation, antioxidant responses, and ionic balance in *K. alvarezii* (Siddiqui *et al.*, 2022). Therefore, the present biomass loss cannot be explained by salinity alone.

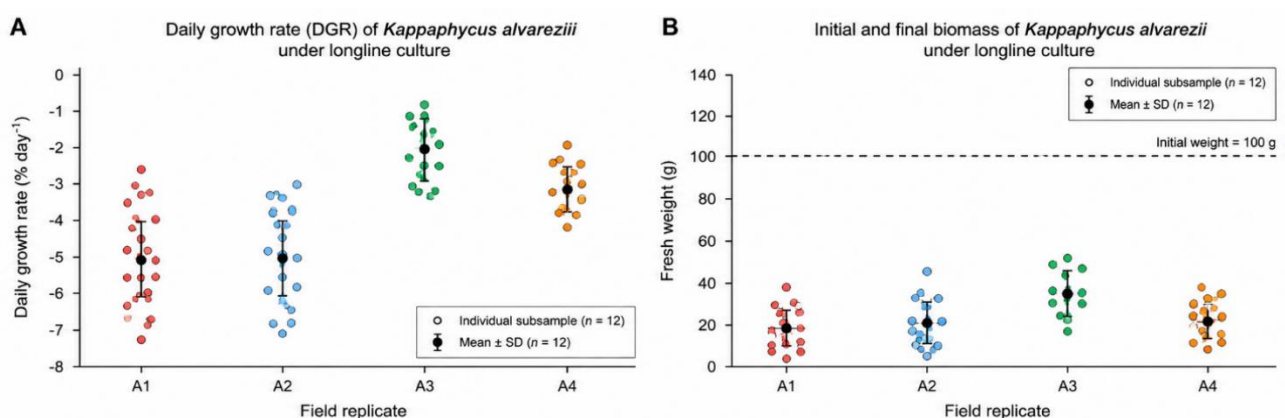


Figure 1. Growth performance of *K. alvarezii* cultivated using the longline method during the first cultivation season (45 days). (A) Daily growth rate (DGR, % day⁻¹) showing individual subsample values (n= 12 per field replicate) and mean ± SD for each replicate. (B) Final fresh weight (g) of individual subsampels (n= 12 per field replicate) compared with the initial fresh weight (100g; dashed line), with mean ± SD for each replicate

The environmental dataset also requires cautious interpretation. The recorded salinity of 34 ppt was inconsistent with the substantially lower Cl and Na concentrations reported in Table 1, suggesting a need to verify analytical procedures, measurement units, and sample preparation. Similarly, the recorded pH of 6.7 was unusually low for seawater and requires confirmation against instrument calibration records. Because these measurements were not independently validated and environmental monitoring was limited to the beginning and end of cultivation, neither salinity nor pH can be reliably linked to the observed biomass decline. Future studies should integrate repeated water-quality measurements with systematic assessments of propagule loss, disease incidence, epiphyte fouling, and hydrodynamic conditions throughout the cultivation cycle. Van Oort *et al.* (2025b) similarly emphasized that limited environmental sampling may be insufficient to identify meaningful relationships between individual water-quality variables and seaweed yield. Future cultivation trials in Ekas should therefore record pH, temperature, salinity, dissolved oxygen, nutrients, current velocity, and weather conditions repeatedly throughout each cultivation cycle.

The reported seawater elemental concentrations were higher at harvest than at planting for S, Cl, K, Ca, and Br (Table 1). The largest calculated percentage difference was observed for Ca (+73.73%), followed by S (+52.74%) and Cl (+34.84%). However, these results were obtained from only two sampling times without independent analytical replication. Moreover, the reported Cl and Na concentrations are inconsistent with the recorded seawater salinity of 34 ppt, indicating that the original analytical units, dilution factors, and sample preparation procedures require verification. Therefore, these values are retained as provisional descriptive results and cannot be used to infer validated temporal changes in seawater elemental composition or to explain the observed biomass loss.

Seawater elemental concentrations were (Table 1) higher at harvest than at planting for S, Cl, K, Ca, and Br. The strongest proportional increase occurred for Ca (+73.73%), followed by S (+52.74%) and Cl (+34.84%). Nevertheless, these differences should be regarded as temporal observations rather than direct evidence of nutrient enrichment, depletion, or release by *K. alvarezii*. The water samples represent two points in time and lack independent replication. Changes could result from tidal exchange, evaporation, freshwater input, suspended material, sediment interaction, or other hydrographic processes.

The thallus composition provides stronger evidence that *K. alvarezii* maintained a distinct mineral profile despite poor biomass performance. Potassium and chlorine were the dominant measured elements at 10.34 ± 0.50 and 10.09 ± 0.85 mass%, respectively. High K abundance is consistent with the known mineral-rich composition of *K. alvarezii*. Rakhasiya *et al.* (2023) reported potassium as the dominant macro-element in products derived from *K. alvarezii*, supporting the strong K signal observed in the present thalli. Recent compositional characterization of cultivated *K. alvarezii* further confirms that the chemical quality of the harvested biomass reflects a complex combination of mineral and biochemical constituents, supporting the use of compositional analysis alongside conventional growth measurement (Nunes *et al.*, 2024)

Ionic regulation may be important for maintaining cellular function under variable marine conditions. Siddiqui *et al.* (2022) demonstrated that *K. alvarezii* adjusts Na^+ and K^+ accumulation, osmolytes, photosynthetic activity, and antioxidant responses when exposed to different salinities. The relatively consistent K concentrations among L1, L2, and L3 may therefore indicate maintenance of an important ionic component even when total biomass declined. However, the present study did not measure intracellular ion transport or physiological markers, so this interpretation should remain mechanistic rather than causal.

The differences observed for Ca, Mg, Si, Al, and Fe among thalli indicate microscale variability in elemental accumulation. Such variability can arise from differences in exposure to water movement, suspended particles, surface deposition, or physiological status. The finding supports the use of elemental profiling together with conventional growth parameters because biomass measurements alone cannot describe changes in tissue composition.

Table 1. Elemental concentrations of seawater at planting and harvest

Element	Planting (mg L ⁻¹)	Harvest (mg L ⁻¹)	Change (%)
S	37.630	57.477	+52.74
Cl	890.400	1200.600	+34.84
K	39.644	45.333	+14.35
Ca	16.324	28.359	+73.73
Br	3.763	4.209	+11.85
Na	NR	345.690	NR

Note: NR = not reported at the corresponding sampling time. Percentage changes were calculated only for elements measured at both planting and harvest. Seawater samples were not independently replicated at either sampling time. The reported Cl and Na concentrations require verification against the original laboratory records because they are inconsistent with the recorded salinity of 34 ppt. Accordingly, the elemental concentrations and percentage differences are presented descriptively and should not be interpreted as validated changes in seawater chemistry, followed by S (+52.74%) and Cl (+34.84%). Nevertheless, these differences should be regarded as temporal observations rather than direct evidence of nutrient enrichment, depletion, or release by

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X-ray fluorescence (XRF) analysis identified potassium (K) and chlorine (Cl) as the predominant measured elements in the dried thalli of *K. alvarezii*. On a dry-weight basis, potassium accounted for 10.34 ± 0.50 mass%, followed by chlorine (10.09 ± 0.85 mass%), sulfur (2.37 ± 0.08 mass%), calcium (1.25 ± 0.35 mass%), and sodium (1.01 ± 0.09 mass%) (Table 2). These concentrations represent the reported elemental mass fractions in dried samples rather than the complete chemical composition of the seaweed. Light elements, particularly carbon (C), hydrogen (H), oxygen (O), and nitrogen (N), were not quantified using the applied XRF method. Accordingly, the measured elemental percentages should not be interpreted as accounting for the total dry biomass. Elemental concentrations were expressed as mass percentages relative to the dry sample mass.

Table 2. Elemental composition of dried *K. alvarezii* thalli determined by X-ray fluorescence (XRF)

Element	L1 (mass%)	L2 (mass%)	L3 (mass%)	Mean $\pm$ SD (mass%)
Na	1.110	0.939	0.984	1.011 $\pm$ 0.089
Mg	0.225	0.294	0.230	0.250 $\pm$ 0.039
Al	0.132	0.085	0.113	0.110 $\pm$ 0.024
Si	0.387	0.338	0.443	0.389 $\pm$ 0.053
P	0.152	0.137	0.151	0.147 $\pm$ 0.008
S	2.360	2.300	2.460	2.373 $\pm$ 0.081
Cl	9.950	11.000	9.320	10.090 $\pm$ 0.849
K	10.200	10.900	9.920	10.340 $\pm$ 0.505
Ca	1.470	0.841	1.440	1.250 $\pm$ 0.355
Fe	0.229	0.181	0.182	0.197 $\pm$ 0.027
Br	0.101	0.108	0.103	0.104 $\pm$ 0.004
Sr	0.0083	0.0080	0.0093	0.0085 $\pm$ 0.0007
Cu	0.0072	ND	ND	NC

Note: Elemental concentrations are expressed as mass percentages (mass%) on a dry-weight basis. Values are presented as mean $\pm$ standard deviation (SD) calculated across three independently analyzed thallus samples (L1, L2, and L3; n = 3). The SD represents variation among the analyzed samples and should not be interpreted as instrumental measurement uncertainty. The reported elements do not represent the complete chemical composition of the biomass because C, H, O, and N were not quantified.

Seawater elemental concentrations were recorded as higher at the time of harvest compared to the time of planting for the elements S, Cl, K, Ca, and Br. The largest proportional increases occurred in Ca (+73.73%), followed by S (+52.74%) and Cl (+34.84%). These water samples represent two time points and lack independent replication. The observed changes could be attributed to tidal exchange, evaporation, freshwater input, suspended matter, sediment interactions, or other hydrographic processes. Thallus composition provides stronger evidence that *K. alvarezii* maintains a distinctive mineral profile despite relatively low biomass performance. Potassium and chlorine were the dominant elements measured, accounting for $10.34 \pm 0.50\%$ and $10.09 \pm 0.85\%$ of the total mass, respectively. The high abundance of K aligns with the known mineral-rich nature of *K. alvarezii*. Rakhasiya *et al.* (2023) reported potassium as the dominant macro-element in *K. alvarezii* derivatives, supporting the finding of high K levels in the thallus in this study. Characterization of the cultivated material confirms that the chemical quality of the harvested biomass reflects a complex combination of mineral and biochemical constituents, thereby supporting the use of compositional analysis alongside conventional growth measurements (Nunes *et al.*, 2024).

Ionic regulation likely plays a crucial role in maintaining cellular function amidst fluctuating marine conditions. Siddiqui *et al.* (2022) demonstrated that *K. alvarezii* adjusts Na^+ and K^+ accumulation, osmolyte levels, photosynthetic activity, and antioxidant responses when exposed to varying salinity levels. Therefore, the relatively consistent K concentrations across groups L1, L2, and L3 may indicate the maintenance of essential ionic components, despite a decline in total biomass.

The observed differences in Ca, Mg, Si, Al, and Fe levels within the thallus suggest micro-scale variability in elemental accumulation. This arises from differences in exposure to water movement, suspended particles, surface deposition, or physiological status. These findings support the use of elemental profiling alongside conventional growth parameters, as biomass measurements alone cannot characterize changes in tissue composition.

Qualitative phytochemical screening revealed the presence of several metabolite groups in the three longline samples. Flavonoids and alkaloids showed consistently strong positive reactions (+++) in L1M1, L2M1, and L3M1. Saponins showed weak positive reactions (+) in all samples. Greater variation was observed for tannins, terpenoids, phenol/hydroquinones, and steroids (Table 3).

Table 3. Qualitative phytochemical profile of *K. alvarezii* cultivated using the longline method.

Phytochemical group	L1M1	L2M1	L3M1
Flavonoids	+++	+++	+++
Alkaloids	+++	+++	+++
Saponins	+	+	+
Tannins	+	+	++
Terpenoids	++	+	++
Phenol/hydroquinones	+	+	++
Steroids	+	–	+

Note: – = not detected; + = weak positive reaction; ++ = moderate positive reaction; +++ = strong positive reaction.

The phytochemical profiles revealed patterns (Table 3) that differed from the biomass growth trends. Flavonoids and alkaloids remained strongly detectable (+++) across all three thallus samples, whereas saponins consistently showed a weak positive reaction. The levels of tannins, terpenoids, phenols/hydroquinones, and steroids varied among the samples. This pattern indicates that the presence of major phytochemical groups was maintained despite a significant reduction in biomass.

K. alvarezii contains a chemically diverse bioactive fraction. Nunes *et al.* (2024) documented several phenolic compounds in *K. alvarezii* and demonstrated that processing conditions can influence the recovery and stability of bioactive constituents. Sasonal environmental variability may further contribute to these biochemical differences. Schneider *et al.* (2026) demonstrated seasonal variation in the biochemical composition of cultivated *K. alvarezii*, indicating that metabolite profiles can respond differently across cultivation periods. Recent studies also identify phenolics and flavonoids as important constituents of *K. alvarezii* biomass.

However, the variations observed among L1M1, L2M1, and L3M1 reflect the presence and relative reaction intensities of specific compound classes rather than providing direct concentration data. These results serve primarily as complementary evidence, demonstrating that the biochemical profile remains diverse under the given cultivation conditions. Qualitative phytochemical tests indicate the relative presence of compound classes but do not provide direct concentration data. *K. alvarezii* contains a chemically diverse bioactive fraction. Nunes *et al.* (2024) documented several phenolic compounds in *K. alvarezii* and demonstrated that processing conditions can influence the recovery and stability of bioactive constituents. Recent studies also identify phenolics and flavonoids as important constituents of *K. alvarezii* biomass.

Carrageenan extraction from the harvested *K. alvarezii* thalli produced a mean yield of 40.77 ± 1.58% across the three analyzed samples (L1M1, L2M1, and L3M1) (Figure 2). The limited variation among these samples indicates relatively consistent extraction yields at harvest, despite the substantial reduction in fresh biomass during the 45-day cultivation period. However, the reported percentage represents carrageenan recovered relative to the dry biomass subjected to extraction and does not indicate that carrageenan content remained unchanged during cultivation.

The mean carrageenan extraction yield of 40.77 ± 1.58% indicates that carrageenan-containing material remained recoverable from the harvested *K. alvarezii* thalli despite substantial biomass loss. Nevertheless, extraction yield expressed as a percentage of dry biomass should not be equated with total carrageenan production. The marked reduction in harvested biomass suggests that carrageenan output per cultivation unit may also have declined, even though the extraction percentages showed limited variation among the analyzed samples.

Based on the final dry biomass of 2.53 g per cultivation unit cited in the reviewer's calculation, a carrageenan extraction yield of 40.77% would correspond to approximately 1.03 g of recovered

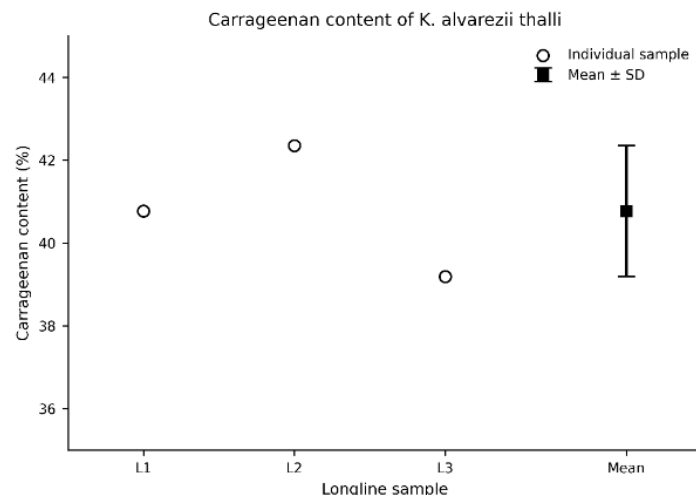


Figure 2. Carrageenan extraction yield (%) from three analysed harvested thallus samples (L1M1–L3M1), shown with mean $\pm$ SD ($n = 3$)

material per unit. This calculation illustrates the distinction between percentage extraction yield and absolute carrageenan output. However, without measured initial dry biomass and baseline carrageenan content, the magnitude of the reduction in carrageenan production cannot be established.

The relatively high extraction percentage in the remaining biomass may reflect differences in the composition of retained thallus tissues. For example, preferential loss of younger or more fragile tissues could alter the relative proportion of carrageenan-containing material in the harvested biomass. This explanation remains hypothetical because tissue-specific biomass loss and carrageenan distribution were not measured. Furthermore, residual salts and other co-extracted substances may influence gravimetric extraction yields if purification is incomplete.

Faisan *et al.* (2024a) compared growth performance and carrageenan characteristics between tissue-cultured and farm-sourced *K. alvarezii* seedlings. Although their findings provide useful context regarding variation in growth and carrageenan characteristics, their experimental conditions do not directly represent severe biomass loss during cultivation. Therefore, the present findings cannot be interpreted as evidence of a physiological decoupling between biomass growth and carrageenan production.

The absence of baseline carrageenan measurements, the limited number of analyzed samples, and the lack of carrageenan quality assessments, including gel strength, viscosity, sulfate content, and ash content, restrict the interpretation of these results. Further investigation is required to determine whether biomass loss affects carrageenan quantity, purity, and functional properties.

Cultivation duration and farming strategy can nevertheless influence both growth and carrageenan yield. Rajapaksha *et al.* (2025) showed that cultivation method and harvest period affected the growth and carrageenan performance of *K. alvarezii*, confirming that biomass productivity and carrageenan production need to be evaluated together rather than used interchangeably. Likewise, Muyong & Tahluddin (2024) demonstrated that nutrient enrichment and farming method can modify growth, carrageenan yield, gel strength, and disease prevalence.

The carrageenan extraction yield of $40.77 \pm 1.58\%$ indicates that carrageenan-containing material remained recoverable from the harvested thalli despite substantial biomass loss. However, this result represents the extraction yield at harvest rather than evidence that carrageenan content

was maintained throughout cultivation. Although previous studies have reported differences in growth performance and carrageenan characteristics among cultivation treatments and cultivars (Faisan *et al.*, 2024b; Pong-Masak *et al.*, 2026; Rajapaksha *et al.*, 2025). these comparisons do not establish that biomass loss and carrageenan production are independent in the present study. The absence of baseline measurements and carrageenan quality assessments limits further interpretation.

CONCLUSION

The first cultivation season in Ekas Bay revealed four principal findings. First, *K. alvarezii* cultivated using the longline method experienced substantial biomass loss over 45 days, with a mean daily growth rate of $-4.10 \pm 1.41\% \text{ day}^{-1}$, absolute growth of $-80.66 \pm 10.62 \text{ g}$, and final biomass retention of $19.34 \pm 10.62\%$. Negative growth was observed across all four cultivation groups. Second, elemental analysis of the harvested dried thalli identified potassium and chlorine as the predominant measured elements, while qualitative phytochemical screening showed strong responses for flavonoids and alkaloids. These findings describe the elemental and phytochemical profiles of the harvested biomass but do not establish changes during cultivation. Third, the harvested thalli produced a mean carrageenan extraction yield of $40.77 \pm 1.58\%$. However, the absence of baseline carrageenan measurements and quality assessments prevents conclusions regarding the stability of carrageenan content, total production, or functional properties. Fourth, the study documents substantial biomass loss alongside measurable elemental, phytochemical, and carrageenan extraction profiles at harvest. Nevertheless, the single-site design, limited environmental monitoring, and absence of baseline biochemical measurements prevent identification of the causes of biomass loss or determination of how biochemical characteristics changed during cultivation. Future studies should incorporate independent cultivation sites, replicated cultivation lines, repeated measurements of water quality and biomass, and paired initial-final assessments of carrageenan yield, purity, and functional properties. Direct observations of propagule loss, disease incidence, and epiphyte fouling are also needed to identify the mechanisms contributing to biomass reduction.

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