

ORIGINAL RESEARCH ARTICLE

Linking Environmental Factors to Phenolic Concentrations in Seagrasses of Silliman Beach and Olympia Island, Negros Oriental

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ABSTRACT

Seagrass meadows are essential in sustaining marine biodiversity, supporting fisheries, and protecting shorelines. Despite their importance, seagrasses are increasingly exposed to multiple stressors, including sedimentation, nutrient enrichment, pollution, and coastal development. These pressures can trigger biochemical responses such as the accumulation of phenolic compounds. In this study, phenolic concentrations in seagrasses were quantified, and the environmental factors influencing their concentration were identified. Seagrass samples were collected from Silliman Beach (Dumaguete City) and Olympia Island (Bais City), Philippines. Phenolic content was measured using the Folin–Ciocalteu method. Water quality parameters were monitored twice monthly from July 2024 to April 2025, and sediment nutrient analyses were conducted monthly. Results revealed lower phenolic concentrations in the seagrasses at Olympia Island (5.24 to 26.50 mg TAE/g) than at Silliman Beach (6.82 to 50.5 mg TAE/g). *Syringodium isoetifolium* exhibited the highest phenolic concentration at Silliman Beach, followed by *Thalassia hemprichii*, *Cymodocea rotundata*, and *Halodule pinifolia*. *Enhalus acoroides* showed the lowest concentrations in both Silliman Beach and Olympia Island. Canonical correspondence analysis showed that phosphorus primarily influenced phenolic accumulation in *Syringodium isoetifolium*. In other seagrass species, total suspended solids, total dissolved solids, and nutrients correlated with the phenolic concentrations. Increased phenolic production in seagrasses reflects adaptive stress responses and resource allocation toward defense. Therefore, phenolic levels should be incorporated as a biomarker to monitor ecosystem health and guide conservation strategies in Philippine seagrass habitats.

Keywords: Negros Oriental, phenolics, seagrass, stress bioindicator

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INTRODUCTION

The Philippines harbors one of the most diverse seagrass assemblages in the Indo-Pacific, with 18 known species (Fortes, 2018). They are vital components of the Philippine coastal ecosystems, providing essential services such as sediment stabilization, nutrient cycling, carbon sequestration, and nursery habitats for diverse marine species (Unsworth et al., 2019). However, recent assessments indicate a sharp nationwide decline, with an estimated 30–50% of seagrass meadows degraded or lost and an annual loss rate of about 2.6% (Fortes, 2018; Fortes, 2022; Dugong Seagrass Conservation Project, 2024).

The degradation of Philippine seagrass ecosystems results from multiple environmental and anthropogenic stressors, including uncontrolled development in coastal areas (Mascariñas and Otadoy, 2023; Ramos and Caray, 2025). For instance, in Davao Oriental, human-driven pressures such as agricultural runoff, sedimentation, and coastal development degrade water quality and limit light penetration, resulting in lower seagrass species diversity in Taganilao compared to Pujada Island (Sanchez-Delute et al., 2025). Expansion of aquaculture in the

Philippines can also lead to sediment disruption, water pollution, declining water quality, and degradation of the seagrass ecosystems (Tahiluddin et al., 2025). Studies have shown that seagrasses employ adaptive strategies, such as synthesizing secondary metabolites containing phenolic compounds, to withstand stress (Mannino and Micheli, 2020; Kumar et al., 2023).

Despite seagrass' ecological importance, studies in Negros Oriental, Philippines, rarely examine how seagrasses physiologically respond to environmental stress (Cayetano et al., 2025; Oczon and Intoy, 2025). Most focus on species diversity and distribution rather than biochemical defenses such as phenolic production. To address this gap, this study aimed to (1) measure the concentration of phenolics in different seagrass species from Olympia Island and Silliman Beach; (2) compare the phenolic concentrations between the two sites; and (3) correlate water quality parameters, including nitrogen and phosphorus sediment content, with phenolic compound concentrations in seagrass. By examining these relationships, the study provides insights into the ecological interactions and adaptive mechanisms that sustain seagrass resilience and informs effective coastal conservation strategies.

MATERIALS AND METHODS

Description of the study area

The study was conducted in two seagrass ecosystems in Negros Oriental, Philippines. The first study site was Silliman

Beach in Bantayan, Dumaguete City (9°19'51"N, 123°18'35"E) (Figure 1). Silliman Beach is within the city, where the coastline is lined with infrastructure and modified by human activities, such as seawalls, seaports, and ongoing reclamation. In this site, 30 transects were established from the northern to the southern section of Barangay Bantayan.

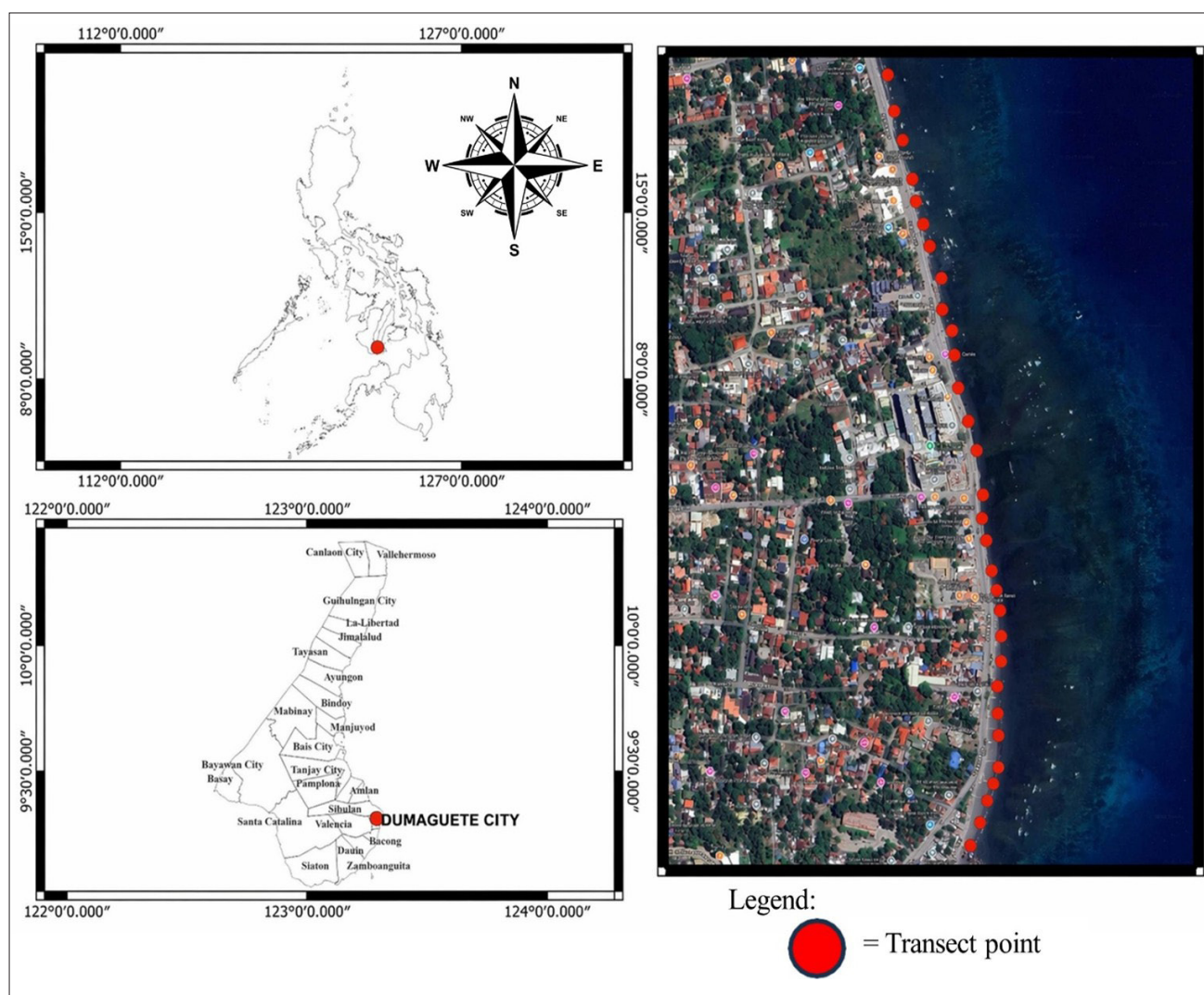


Figure 1. Map of the study site. (A) Map of the Philippines, (B) Map of Negros Oriental, (C) Map of Silliman Beach, Dumaguete City, Negros Oriental, Philippines.

The second study site was Olympia Island within Bais Bay, Bais City, Negros Oriental (9.6164 °N, 123.1461 °E) (Figure 2). Bais Bay is known for its rich fishery resources; however, periodic fish kills have been reported, likely linked to sewage discharge and untreated domestic, agricultural, and industrial effluents that degrade water quality. Thirty transects were established from north to east across the study area.

Measurement of the physico-chemical parameters in the sampling sites

The study involved the collection and analysis of water and sediment samples to assess physicochemical parameters in seagrass beds. Water quality was measured twice monthly from July 2024 to April 2025 at 8:00 AM, 12:00 NN, and 4:00 PM, while sediment samples for nitrogen and phosphorus analyses were collected monthly. Standardized protocols were followed to ensure data collection accuracy and reliability.

Water pH, dissolved oxygen (DO), total dissolved solids (TDS), temperature, and salinity were measured using a calibrated multimeter probe submerged approximately 4 inches below the surface, with stabilized readings recorded (Environmental Management Bureau: Ambient Water Quality Monitoring, 2008). Water clarity was assessed using a Secchi disk (Bruckner, 2007). For total suspended solids (TSS), seawater samples were collected at 0, 50, and 100 meters from the shore to assess sediment load variation caused by wave action, tidal currents, and human activity. Samples were obtained using 1,000 mL HDPE bottles pre-washed with phosphate-free detergent and rinsed with deionized water. Bottles were submerged 8–12 inches below the surface to minimize sediment disturbance, properly labeled, and stored for laboratory analysis. TSS were analyzed by filtering well-mixed water samples through a pre-weighed 45 mm Whatman cellulose filter paper, which was then dried at 102–105 °C until constant weight was achieved. TSS was calculated from the weight difference before and after filtration and expressed in milligrams per liter.

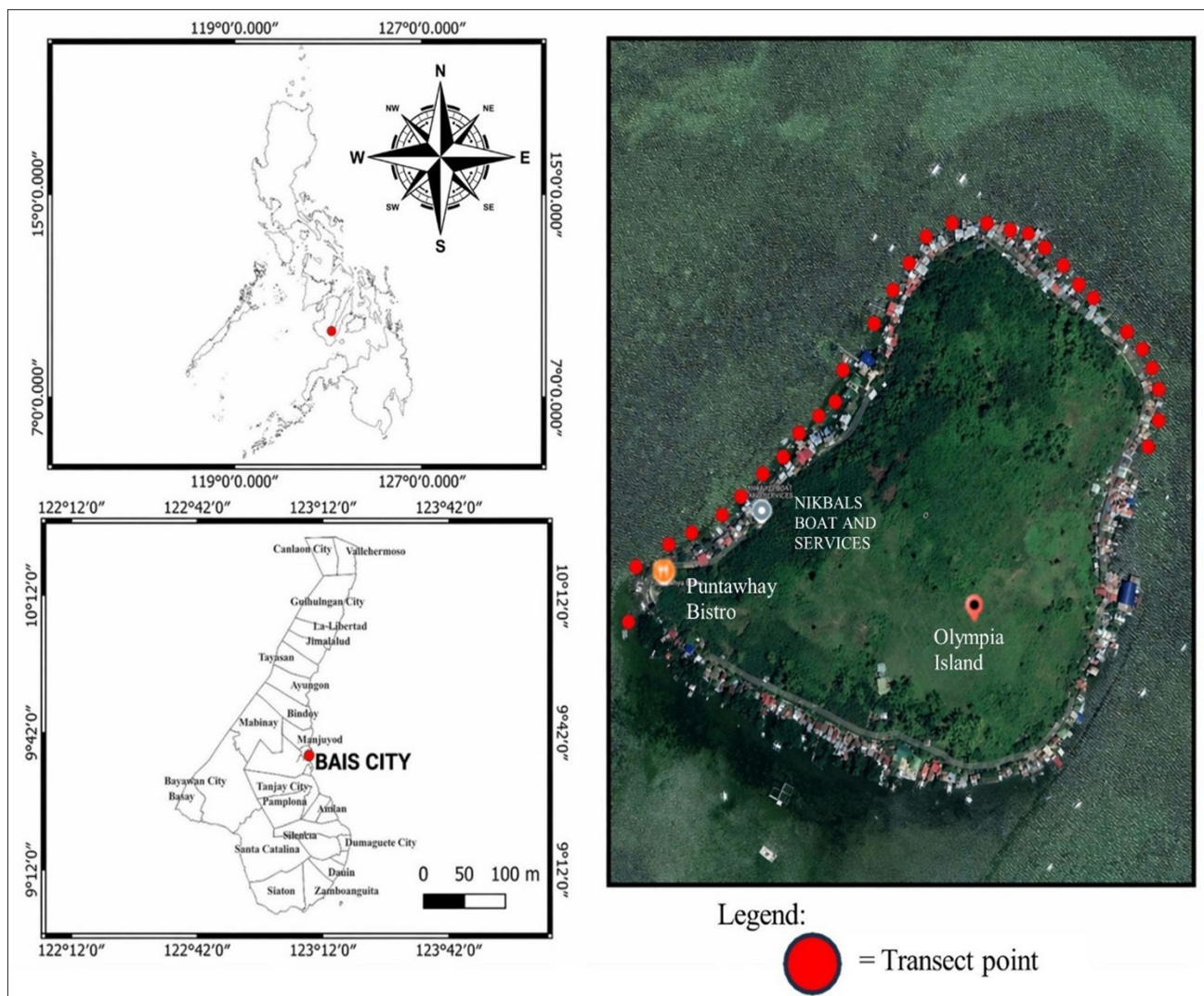


Figure 2. Map of the study site. (A) Map of the Philippines, (B) Map of Negros Oriental, (C) Map of Olympia Island, Bais City, Negros Oriental, Philippines.

Soil nutrient analysis was conducted using a soil test kit provided by the Provincial Agriculturist Office, Soil and Water Management Division. Sediment samples were collected at a depth of 15–20 cm using a corer. Five subsamples per quadrat were composited, air-dried, and sieved through a 2 mm mesh. Approximately 0.5 kg of each composite sample was analyzed according to the kit's standard protocol. Nitrogen and phosphorus levels were determined by adding the Bray No. 1 extractant for nitrogen and Murphy and Riley reagents for phosphorus to the soil sample. After 5–10 minutes of reaction, color development indicated nutrient levels: light to deep red for nitrogen and pale to deep blue for phosphorus. All materials were rinsed with distilled water to prevent contamination, and the used reagents were disposed of in accordance with the provided safety and disposal guidelines.

Collection of seagrass samples and determination of total phenolic concentration

From July 2024 to April 2025, seagrass samples were collected at each station along a 100-meter transect line that was laid perpendicular to the shore, starting where seagrass patches began. Along each transect, 15 quadrats (50 cm x 50 cm) were placed at 5 m intervals, covering the full 100 m range (0-20, 20-40, 40-60, 60-80, and 80-100 m). The intermediate shoots of various seagrass species (*T. hemprichii*, *C. rotundata*,

E. acoroides, *H. pinifolia*, and *S. isoetifolium*) within each quadrat were cut using scissors. Immediately after collection, samples were placed in plastic bags with seawater and stored in a cool box to prevent dehydration and degradation during transport to the laboratory. Upon arrival at the laboratory, seagrass samples were thoroughly washed with tap water to remove sediments and epiphytes, then sorted by species. Samples were air-dried for approximately five days or until constant weight was achieved, then further dried in an oven to ensure complete moisture removal. The dried seagrass was ground to a fine powder using a grinder. For each sample, 500 mg of powdered seagrass was accurately weighed and placed in a flask. The crude extract was prepared by adding 50 mL of 80% methanol to 500 mg of the powdered sample and heating on a hot plate for 30 min. To quantify the total phenolic compound concentration, 500 μ L of the extract was mixed with 500 μ L of 10% Folin-Ciocalteu reagent, 1 mL of 7.5% sodium carbonate solution, and 8 mL of distilled water in a test tube. Tannic acid was used as a standard. The mixtures were incubated at room temperature for 30 min to allow color development. Absorbance was measured at 760 nm using a UV-Vis spectrophotometer (Dynamica, HALO DB-20R). Tannic acid was used as a standard. The phenolic content was calculated from a standard calibration curve and expressed as milligrams of tannic acid equivalents per gram of extract (mg TAE/g) (Balogun et al., 2014).

Data analysis

Canonical correspondence analysis was employed to examine the relationship between physicochemical parameters and total phenolic concentrations in seagrasses. The Mann-Whitney U test was applied to determine whether there was a significant difference in the physicochemical parameters between sampling sites.

RESULTS

Physico-chemical parameter in Silliman Beach and Olympia Island

Figure 3 presents the physico-chemical parameters measured at Silliman Beach and Olympia Island from July 2024 to April 2025. The pH values recorded at Olympia Island ranged from 7.6 to 8.4, while pH values at Silliman Beach ranged from 7.8 to 8.0 (Figure 3A). Total dissolved solids (TDS) values ranged from 23.9 to 25.8 mg/L at Olympia Island and from 23.6 to 24.4

mg/L at Silliman Beach (Figure 3B). Salinity levels ranged from 28.7 to 29.9 ppt at Olympia Island and 28.4 to 28.8 ppt at Silliman Beach (Figure 3C), while water temperature values ranged from 28.9 °C to 30.4 °C and 28.4 °C to 29.7 °C, respectively (Figure 3D). Dissolved oxygen concentrations ranged from 12.8 to 14.7 mg/L at Olympia Island and 9.9 to 14.6 mg/L at Silliman Beach (Figure 3E). Total suspended solids (TSS) values ranged from 7.8 to 23.6 mg/L at Olympia Island and 3 to 16.9 mg/L at Silliman Beach (Figure 3F), while water clarity, measured using Secchi depth, ranged from 2.7 to 6.3 m at Olympia Island and from 3.6 to 5.92 m at Silliman Beach (Figure 3G). Nitrogen concentrations ranged from 0.57 ± 0.12 mg/kg in July to 0.82 ± 0.08 mg/kg in April at Olympia Island and from 0.50 ± 0.00 mg/kg in July and September to 1.65 ± 0.21 mg/kg in August at Silliman Beach (Figure 3H). Phosphorous concentrations ranged from 0.83 ± 0.25 mg/kg in December to 2.54 ± 0.46 mg/kg in March at Olympia Island and from 0.70 ± 0.10 mg/kg in December to 2.40 ± 0.14 mg/kg in August at Silliman Beach (Figure 3I).

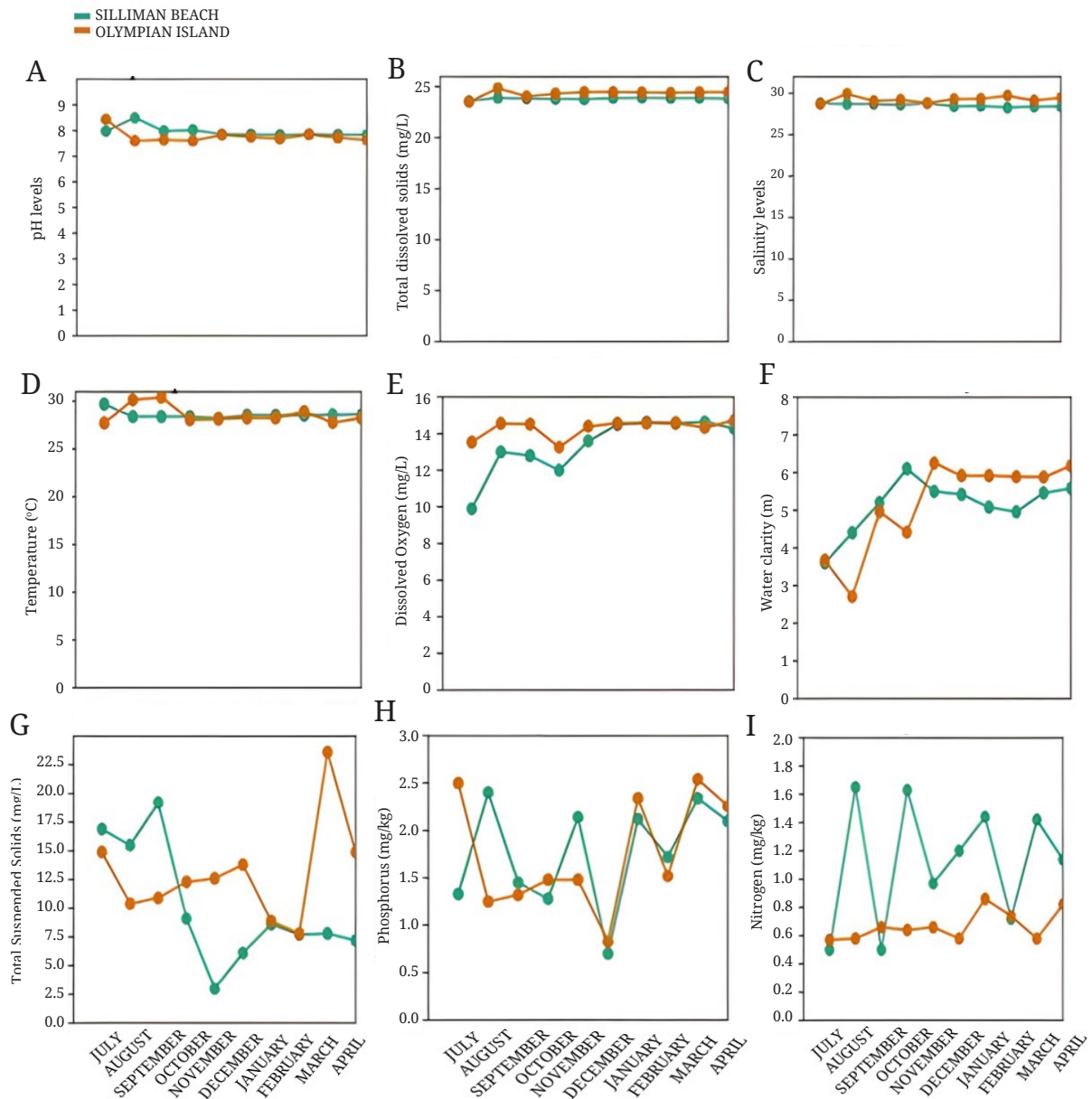


Figure 3. Measurements of the physico-chemical parameters in Olympia Island, Bais City and Silliman Beach, Dumaguete City, from July 2024 – April 2025: (A) pH, (B) Total dissolved solids, (C) Salinity, (D) Temperature, (E) Dissolved Oxygen, (F) Water clarity, (G) Total suspended solids, (H) Phosphorus, (I) Nitrogen.

The box plot distributions of water quality parameters at Silliman Beach and Olympia Island reveal significant differences between the two sites (Figure 4). The pH values at Silliman Beach were significantly lower compared to Olympia Island (Figure 4A; $p < 0.001$), indicating a slightly more acidic aquatic environment (Figure 4A). TDS and salinity were also significantly lower at Silliman Beach than in Olympia Island (Figure 4B & C; $p < 0.001$). The seawater temperature was significantly lower at Silliman Beach than in Olympia Island (Figure 4D; $p < 0.001$). DO levels at

Silliman Beach were slightly lower, compared to Olympia Island (Figure 4E; $p = 0.626$). Water clarity was significantly poorer at Silliman Beach (Figure 4F; $p = 0.315$), likely due to increased turbidity. This is further supported by the significantly higher concentration of TSS at Silliman Beach (Figure 4G; $p = 0.043$). Regarding nitrogen and phosphorous concentrations, Silliman Beach had higher level than in Olympia Island (Figure 4H; $p = 0.354$, Figure 4I; $p = 0.124$).

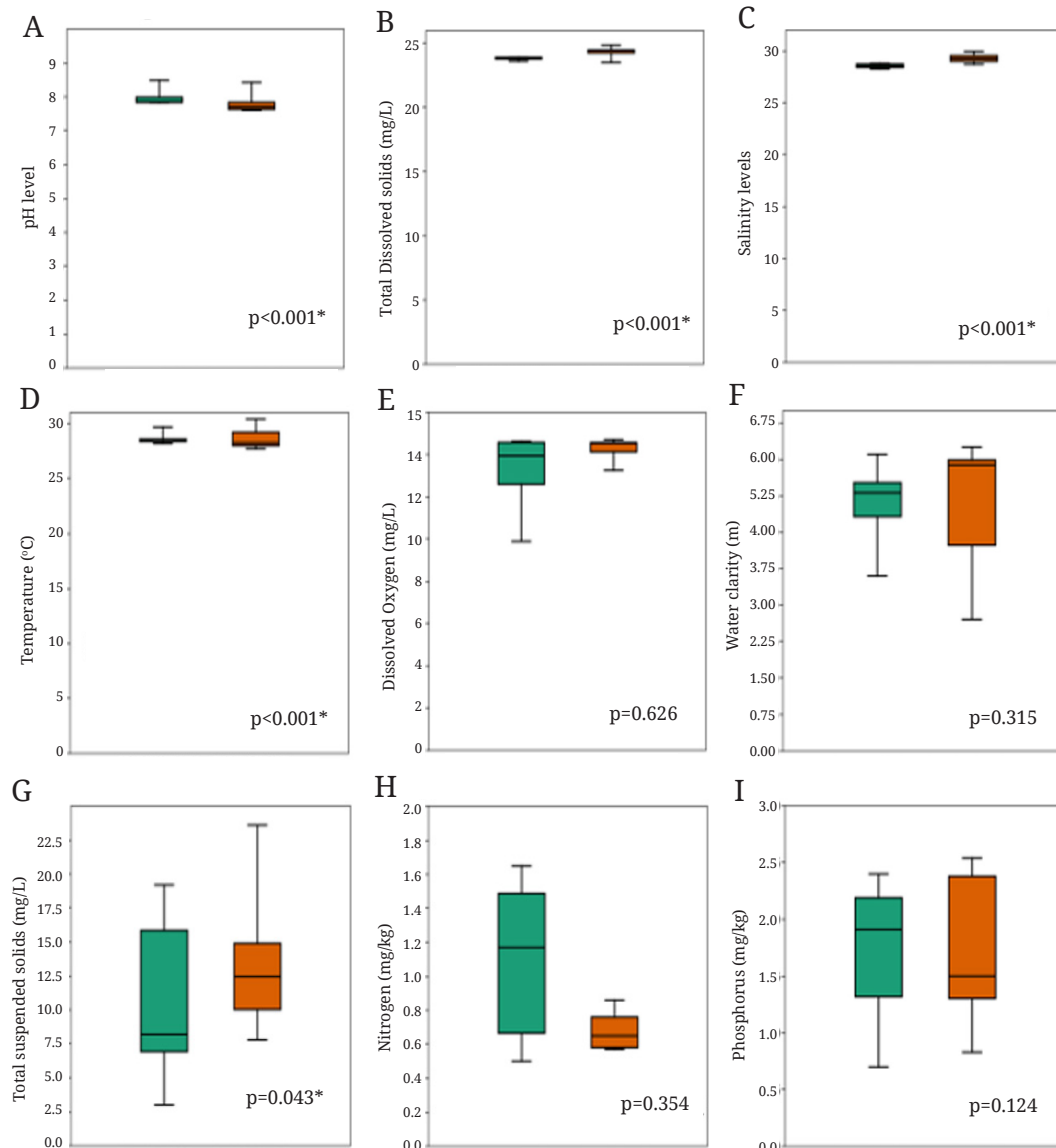


Figure 4. Comparison of the physico-chemical parameters between Olympia Island, Bais City and Silliman Beach, Dumaguete City, from July 2024 – April 2025. *statistically significant

Phenolic concentration in seagrasses

Standard calibration curve

The standard calibration curve for total phenolic content illustrates the relationship between the concentration of the

standard (Tannic acid) and the corrected absorbance at 760 nm (Figure 5). The high coefficient of determination ($R^2 = 0.99$) indicates a strong linear relationship between concentration and absorbance, demonstrating the reliability of this calibration for subsequent sample analysis.

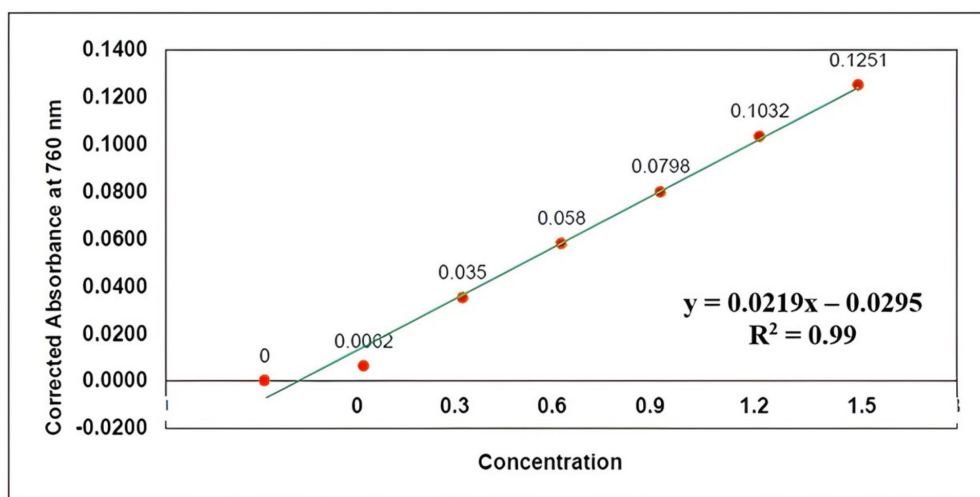


Figure 5. Standard curve for total phenolic concentration using tannic acid as standard.

Total phenolics in seagrass species

At Silliman Beach, analysis of five seagrass species, *E. acoroides*, *H. pinifolia*, *C. rotundata*, *T. hemprichii* and *S. isoetifolium* revealed notable species-specific and temporal variation in phenolic content. *Enhalus acoroides* had the lowest phenolic concentration, ranging from 6.48 to 25.36 mg TAE/g, with the lowest in October 2024 and the highest in December 2024. *Halodule pinifolia* exhibited phenolic concentrations

ranging from 7.63 mg TAE/g in December 2024 to 13.43 mg TAE/g in March 2025. The phenolic concentration of *C. rotundata* was highest in August 2024 at 27.39 mg TAE/g and decreased to 7.53 mg TAE/g in January 2025. *Thalassia hemprichii* showed a decrease in phenolic content from July to October 2024 and an increase from January to April 2025, reaching its highest value in April (34.46–36.85 mg TAE/g). *Syringodium isoetifolium* exhibited the highest phenolic concentrations, peaking at 50.50 mg TAE/g in July 2024 (Table 1).

Table 1. The concentration range of the total phenolic content (mg TAE/g) in the seagrass of Silliman Beach from July 2024 to April 2025.

Species	Concentration range of phenolics in silliman beach (mg TAE/g)									
	July	August	September	October	November	December	January	February	March	April
<i>Halodule pinifolia</i>	12.27-12.51	10.70-11.41	11.67-12.17	11.29-12.37	11.35-12.46	7.63-8.89	12.04-12.56	11.67-12.34	12.73-13.43	11.82-12.46
<i>Syringodium isoetifolium</i>	48.35-50.50	40.65-48.78	*ND	*ND	29.63-30.72	18.50-20.39	18.53-19.69	47.81-49.81	40.52-42.21	24.91-27.79
<i>Cymodocea rotundata</i>	26.60-27.36	25.00-27.39	10.96-11.47	10.33-11.30	9.62-10.08	12.57-13.28	7.53-7.81	21.78-24.66	10.86-11.41	11.22-11.61
<i>Enhalus acoroides</i>	11.37-12.45	10.63-10.70	*ND	6.48-7.18	11.04-12.06	23.49-25.36	18.51-19.92	11.71-12.19	11.94-12.50	11.64-12.21
<i>Thalassia hemprichii</i>	21.32-21.53	20.42-21.50	10.40-10.50	10.40-10.50	*ND	25.05-27.04	31.33-32.86	23.49-25.38	31.53-33.58	34.46-36.85

Note: * undetected

At Olympia Island, total phenolic concentrations in *C. rotundata*, *E. acoroides*, and *T. hemprichii* showed clear species-specific and temporal variation from July 2024 to April 2025. *Cymodocea rotundata* exhibited a decline in phenolic from

July 2024 to January 2025. The phenolic level of *E. acoroides* and *T. hemprichii* was higher from July to December 2024 than in January to April 2025. Among the three seagrass species, *E. acoroides* had the lowest phenolic concentration (Table 2).

Table 2. The concentration range of the total phenolic content (mg TAE/g) in the seagrass was recorded Olympia Island from July 2024 to April 2025.

Species	Concentration range of phenolics in silliman beach (mg TAE/g)									
	July	August	September	October	November	December	January	February	March	April
<i>Cymodocea rotundata</i>	25.89-26.85	20.7-20.79	20.46-20.50	10.4-10.48	15.03-16.11	7.84-8.10	7.80-8.28	13.8-14.78	10.42-11.00	10.66-11.15
<i>Enhalus acoroides</i>	12.05-12.57	18.23-19.07	15.68-16.15	12.15-12.23	13.68-14.18	13.77-14.58	5.72-6.03	4.64-5.67	9.23-9.97	8.16-8.30
<i>Thalassia hemprichii</i>	21.33-21.54	16.7-16.96	*ND	17.22-17.43	20.12-20.40	15.81-16.42	14.25-14.33	11.21-12.16	10.55-10.92	10.61-10.89

Note: * undetected

Correlation between the physico-chemical parameters of the water and the phenolic concentration in the seagrasses. Figure 6 presents the Canonical Correspondence Analysis (CCA) for Silliman Beach, illustrating the influence of the key environmental variables: TDS, TSS, water clarity, pH, phosphorus, and nitrogen on the phenolic concentration of the five seagrass species. The biplot's axes (CCA1 and CCA2) show that TSS strongly influenced the phenolic increase in *H. pinifolia* and *C. rotundata*. On the other hand, phosphorus and pH were positively correlated with phenolic concentration in *S. isoetifolium*, whereas water clarity was associated with higher phenolic concentration in *T. hemprichii*. TDS and nitrogen were correlated with the phenolic concentration in *T. hemprichii*

and *E. acoroides*. Figure 7 presents the Canonical Correspondence Analysis (CCA) for Olympia Island, demonstrating the correlation of TSS, salinity, TDS, nitrogen, phosphorus, DO, and temperature to the phenolic concentrations in three seagrass species. The CCA1 and CCA2 axes capture gradients of these environmental factors. TSS, salinity, and TDS show strong, overlapping correlations, indicating that increased suspended solids, salinity, and dissolved solids resulted in a higher phenolic concentration in *T. hemprichii*. DO and temperature are associated with *C. rotundata* and *E. acoroides*. Overall, the CCA highlights that environmental parameters have distinct and sometimes overlapping effects on phenolic concentrations, with species-specific responses evident.

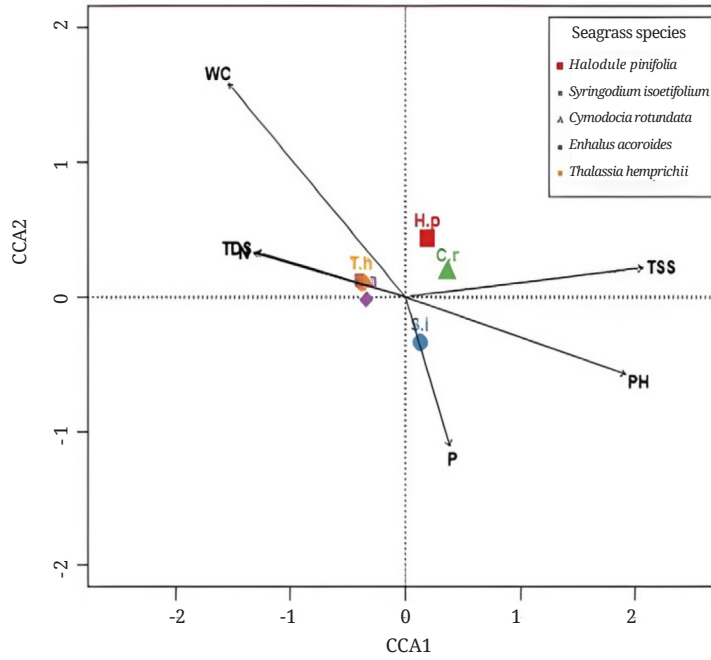


Figure 6. Canonical Correspondence Analysis (CCA) of phenolic concentrations in seagrass species and physicochemical parameters at Silliman Beach.

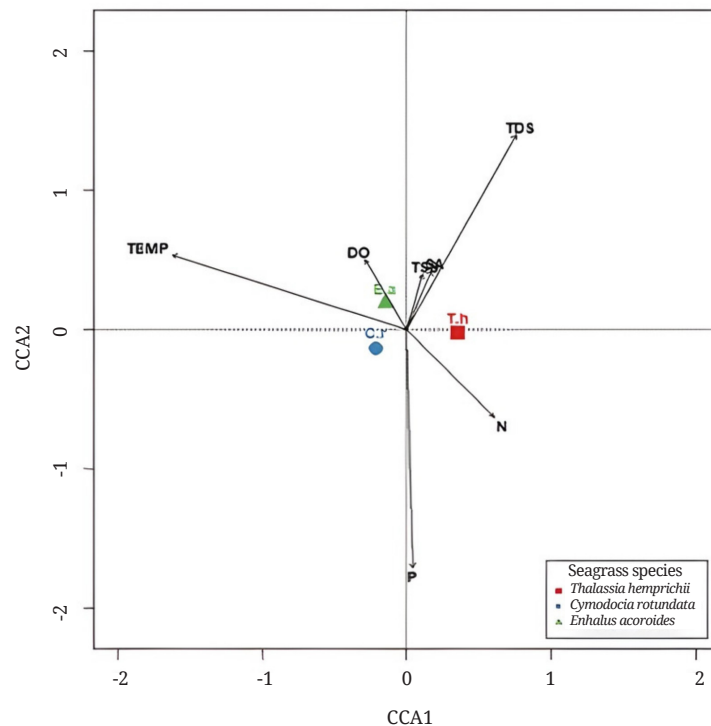


Figure 7. Canonical Correspondence Analysis (CCA) of phenolic concentrations in seagrass species and physicochemical parameters at Olympia Island.

DISCUSSION

Phenolic compounds are generally synthesized in high concentrations as a response to environmental stress (Mannino and Micheli, 2020). Environmental stress elevates reactive oxygen species (ROS), leading to oxidative stress. To mitigate oxidative damage, both enzymatic and non-enzymatic antioxidant defenses, including phenolic compounds, were synthesized. Phenolic compounds neutralize free radicals, chelate biometal ions such as Fe (II) and Cu(I) to limit the formation of lipid alkoxyl radicals involved in lipid peroxidation, and rapidly scavenge reactive species through hydrogen atom donation or electron transfer. In seagrasses, phenolic compounds are synthesized via the shikimic acid pathway in specialized tannin cells (Kumar et al., 2023). The present study found clear species-specific and spatial variation in seagrass phenolic concentrations. At Silliman Beach, *S. isoetifolium* showed the highest levels, followed by *T. hemprichii*, *C. rotundata*, and *H. pinifolia*, while *E. acoroides* had the lowest. Previous work from Palk Bay, India, also reported higher phenolics in *S. isoetifolium* than in *T. hemprichii*, though lower than in *C. serrulata* and *E. acoroides* in the Gulf of Mannar (Baby et al., 2017). The observed variation in phenolic concentrations among seagrass species highlights their differing responses to environmental stressors.

Canonical correspondence analysis showed that phosphorus strongly correlated with the phenolic concentration in *S. isoetifolium*. Phosphorus is a key limiting nutrient for the growth of *Syringodium isoetifolium*, and enrichment has been shown to enhance its biomass and productivity significantly (Short et al., 1990). However, the high phosphate levels recorded in this study may promote excessive growth of macroalgae, epiphytes, and phytoplankton, which can outcompete seagrasses for light and ultimately hinder their growth and survival. In response, seagrasses may produce phenolic compounds to cope with environmental stressors and protect themselves from biological pressures (Kumar et al., 2023). As observed in *Posidonia oceanica*, the presence of competitors such as *Caulerpa taxifolia* increased tannin cell development, where phenolic compounds are produced (Dumay et al., 2004; Mannino and Micheli, 2020). In the Gulf of Mannar, India, *C. serrulata* exhibited high tannin content as a defense against epiphytes (Baby et al., 2017). In this study, the high phenolic concentrations in *S. isoetifolium* likely reflect a stress response under high phosphate conditions, functioning as a protective mechanism against environmental pressures. This supports the idea that, under stress, seagrasses prioritize defense over growth by allocating more resources to secondary metabolite production, including phenolics (Mannino and Micheli, 2020).

Total suspended solids, total dissolved solids, and nitrogen significantly affected phenolic concentrations in *C. rotundata*, *T. hemprichii*, *H. pinifolia* and *E. acoroides*. Total suspended solids (TSS) are closely linked to light availability: higher TSS levels mean more particles suspended in the water, increasing turbidity and limiting the amount of light reaching seagrasses. In this study, the TSS level is comparable to the TSS level of Vanuatu, South Pacific, which reported turbid and poor water quality due to a combination of coastal hydrodynamic circulation patterns that limit flushing in the sea, and high nutrient and TSS inputs from the surrounding area (Lincoln et al., 2021). High phenolic concentrations in *C. rotundata*, *T. hemprichii*, *H. pinifolia*, and *E. acoroides* could indicate increased photoprotective phenolic synthesis in response to low-light stress induced by high TSS and TDS.

Among the five seagrass species examined, *E. acoroides* showed the lowest phenolic content, suggesting a greater tolerance to environmental stress. On the coast of Tagbilaran, Bohol, Philippines, *E. acoroides* has demonstrated robustness to disturbance

and resilience to light reduction and enhanced sedimentation (Mascariñas and Otadoy, 2023). *Enhalus acoroides* develops comparatively longer and tougher blades than other species, typically reaching 30–150 cm, enabling it to extend higher in the water column and access available light even under turbid conditions. It also possesses thick, sturdy rhizomes and strong roots that enhance resistance to siltation and burial in muddy environments. In contrast to faster-growing, light-dependent species, *E. acoroides* maintains substantial energy reserves, allowing it to withstand prolonged periods of environmental stress (Touchette et al., 2000; Erfreimeijer and Lewis, 2006; Lincoln et al., 2021).

CONCLUSION

This study demonstrated that seagrass phenolic compounds are strongly influenced by species identity and environmental conditions. Significant variation in phenolic concentrations was observed among species, with *Syringodium isoetifolium* exhibiting the highest levels at Silliman Beach, while *Enhalus acoroides* showed the lowest, highlighting species-specific adaptive strategies. Canonical correspondence analysis further revealed that phosphorus was the primary driver of phenolic accumulation in *S. isoetifolium*. In contrast, total suspended solids, total dissolved solids, and nitrogen were key factors influencing phenolic levels in *Cymodocea rotundata*, *Thalassia hemprichii*, *Halodule pinifolia*, and *E. acoroides*. The results supported the idea that increased phenolic production in seagrasses reflects adaptive defense mechanisms under varying environmental stressors. Therefore, phenolic compounds can serve as reliable biomarkers of environmental stress in seagrasses and as measurable indicators of ecosystem condition. Future studies should determine stress thresholds and recovery patterns in seagrass phenolic responses and incorporate molecular and physiological approaches into phenolic analyses further to establish phenolic compounds as robust biomarkers of environmental stress.

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AUTHOR CONTRIBUTIONS

L. C. M: Data collection, visualization, data analysis, writing and editing of the manuscript. T. G. A: Data collection, visualization, data analysis, writing and editing of the manuscript. R. D. B: data collection, visualization, data analysis, writing and editing of the manuscript. V. B. K: conceptualization, research design, supervision, critical review and editing of the manuscript

DECLARATIONS

Informed consent statement

The study was carried out in accordance with applicable guidelines and regulations. Seagrass samples were collected

of Bais and Dumaguete City. All sampling procedures were conducted carefully to minimize disturbance to the natural habitat and to support the sustainability of local seagrasses. No endangered or protected species were deliberately collected, and all methods were designed to prevent any long-term ecological impact.

Conflict of interest


The authors of this research affirm that they have no conflicts of interest.

AI Disclosure

The authors declare that no Artificial Intelligence (AI) or AI-assisted technologies were used in the preparation of this manuscript.

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