SPECIES DIVERSITY AND CHLOROPHYLL CONTENT OF GREEN ALGAE IN THE SELECTED INTERTIDAL AREAS OF MALITA, DAVAO OCCIDENTAL, PHILIPPINES

ABSTRACT

The study aimed to determine the species diversity and chlorophyll content of green algae in selected sites in Malita, Davao Occidental, namely: Sitio Alibungog, Sitio Bagumbayan, and Sitio Agdao in Tubalan, Malita, Davao Occidental, Philippines. The three selected sites were designated as three stations. Each site had three transect lines (a total of 9 transect lines). The transect quadrat method was used to establish the study areas, while the chlorophyll determination content of the green algae was done using a spectrophotometric method. There were 7 seven green algae species found in the three selected sites, namely: *Halimeda opuntia, Halimeda discoidea, Codium fragile, Ulva compressa, Caulerpa lentillifera, Caulerpa racemosa*, and *Dictyosphaeria cavernosa.*

The results also showed that Station 2 had the highest Index of diversity. In terms of relative abundance, Caulerpa lentillifera had the highest abundance in Station 1, Halimeda opuntia had the highest abundance in Station 2, and Caulerpa lentillifera had the highest abundance in Station 3. The results showed that the temperature at each of the three stations ranged from 31 to 320 °C, the pH level was 7, and the salinity ranged from 31 to 32 ppt. Regarding chlorophyll determination content, *Dictyosphearia cavernosa, Caulerpa lentillefera,* and *Caulerpa racemosa* contain the highest value in chlorophyll. Then, *Ulva Compressa* contains the lowest value of total chlorophyll content.

KEYWORDS: Biodiversity, Chlorophyll, Green Algae, Photosynthesis, Tubalan Malita

INTRODUCTION

Marine green algae (Chlorophyta) are naturally abundant. It records a high biodiversity in tropical coral reefs and lagoons, often intermixed with associated seagrass habitats. Species diversity is a multidimensional concept that includes species richness, abundance, and evenness[1]. In general, there have been two approaches to measuring species diversity, which incorporate information on the number of species (species richness) and the relative abundances of individuals within each species (species abundance). One method has been to construct mathematical indices broadly known as diversity indices; the other involves comparing observed patterns of species abundance to theoretical species abundance models[2].

Chlorophyll is an essential biochemical component of a molecular apparatus responsible for photosynthesis, the vital process of converting sunlight energy into life-sustaining oxygen and the subsequent energy transduction into high-energy compounds like ATP and NADPH. Chlorophyll enables plants and other chlorophyll-containing organisms to perform photosynthesis[3].

Algal growth can be tracked directly by measuring chlorophyll levels. Surface waters with high chlorophyll levels tend to be high in nutrients, especially phosphorus and nitrogen. The algae can grow or bloom as a result of these nutrients. Dissolved oxygen levels are depleted as algae species bloom, then crash and die in response to changing environmental conditions. High levels of nitrogen and phosphorus can be indicators of pollution from man-made sources, such as septic system leakage, poorly functioning wastewater treatment plants, or fertilizer runoff. As a result, measuring chlorophyll can be used as an indirect indicator of nutrient levels[3].

Measuring chlorophyll content is important in estimating photosynthetic efficiency and detecting light stresses[4]. The methods used for pigment quantification vary in accuracy and cost-effectiveness. Since chlorophyll concentration is easy to analyze, interest was directed to its application as an indicator of productivity[5].

It is essential to explore the extent of applicability of chlorophyll pigments in green algae species. However, little information is available on the chlorophyll assessment in the locality of Malita, Davao Occidental. Hence, in this study, the researchers characterized, determined, and identified the species diversity, abundance, and chlorophyll characterization of pigments of green algae in Tubalan, Malita, Davao Occidental, Philippines.

MATERIALS AND METHODS

Research Locale

This study was conducted in the three (3) selected intertidal sites: Sitio Alibungog, Sitio Bagumbayan, and Sitio Agdao, Tubalan, Malita, and Davao Occidental. It comprises approximately (6°29’35” N 125°34’08” E”) the island of Mindanao. Elevation at these coordinates is estimated at 25.6 meters or 84.0 feet above mean sea level. An estimated number of green algae (Chlorophyta) species were found on the three selected sites of Tubalan, Malita, and Davao Occidental.

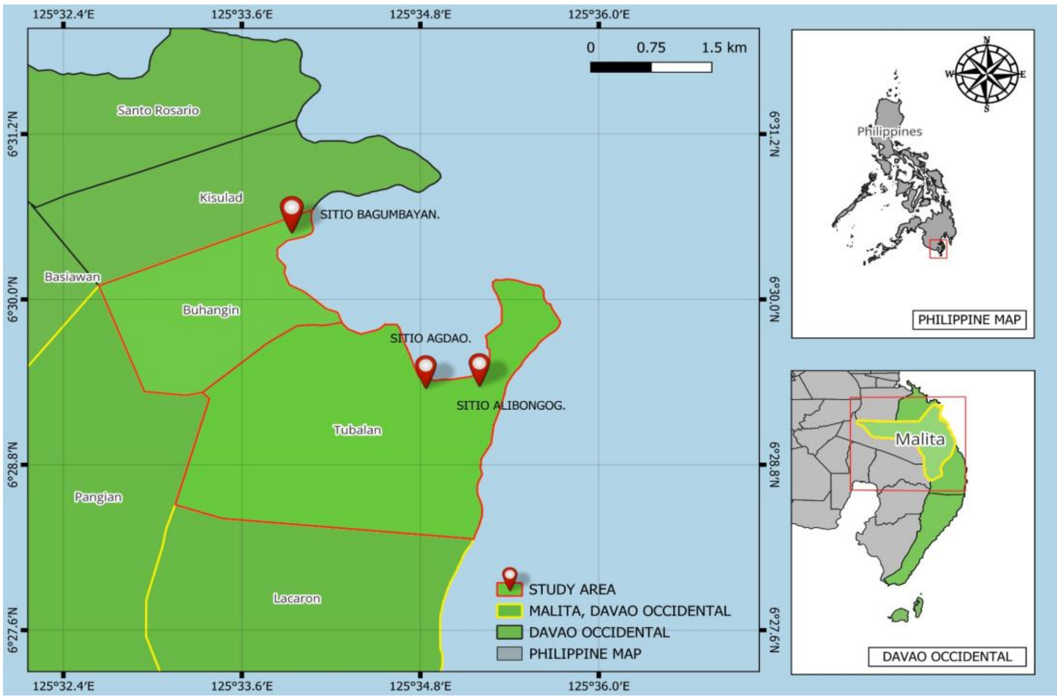

Figure 1: Sampling stations of Tubalan intertidal area

Establishment of the Study Area

The three selected sites were designated as three stations. 50 meter transect lines will assign each station three 50 meters with three replicates. The distance between the quadrat of each transect was ten (10) meters. The quadrat will be used as a dimension of a 1m x 1m quadrat device subdivided into 10 cm sub-squares. Each site had 6 sample replicates, so the three sites will have 18 replicates.

Sample Collection of Green Algae

Aside from the literature review, an actual site visited by the researchers was conducted to determine the presence and most abundant green algae of the three selected sites, namely: Sitio Alibungog, Sitio Bagumbayan, and Sitio Agdao in Tubalan, Malita, Davao Occidental. Random collections of green algae were done from August to December 2021. The green seaweeds were collected by manually picking from the three selected sites, namely: Sitio Alibungog, Sitio Bagumbayan, Sitio Agdao in Tubalan, Malita, Davao Occidental, Philippines ("6 0 30' 50" N, 1250 34' 41" E"). The collected green algae species were identified using pictures and the dichotomous key outlined in the Field Guide to the Philippines' Common Mangroves, Seagrasses and Algae[6].

Extraction Process

Extraction occurred in Research and Laboratory Services Center (RLSC) with low light intensity and a cool room temperature. Green algae species (Chlorophyta) were cut into small pieces and placed in a closed container, followed by 50 mL methanol. The solution was macerated for 3 days, before it turned green and dark red. After the respective maceration periods, the soaked algae mixtures were filtered through white thin cloth, poured into a small cup and stored at -20 ºC until further use. Fifty (50) mL of methanol was poured into the mortar, followed by the remaining plant culture, which was macerated for three minutes before the solution turned green and dark red again. The remainder of the solution was poured into the same flask. For spectrophotometric characterization, 1 mL extract was centrifuged for 5 minutes at the maximum speed to precipitate small particles. Pure extract was put into the micro tube[7].

Spectrophotometer determination of Chlorophyll

The pigment determination of macroalgae extract was done using a spectrophotometric process. For non-polar pigment determination, the spectrophotometer was calibrated using methanol. The centrifuged sample extracts were applied by solute until the limit marker on the cuvette reached and homogenized with a micropipette. The spectrophotometer was set to 300 nm, and on a Schimadzu UV-1800 spectrometer, the absorbance, transmission, and concentration of the blank and sample extract in cuvettes were measured and modified until 0. From 300 nm to 750 nm, the process was repeated for wavelength intervals of 25 nm. Blank was used to calibrate each new wavelength. Based on the highest wavelength produced in each sample extract, the absorption results were transformed into graphics for analysis[7]. The pigment amount was calculated according to the formulas of Lichtentaler and Wellburn 1985[8].

Data Analysis

The researchers interpret the data gathered using the Kruskal-Wallis test to determine the significance of differences of variables, the presence of species, species abundance, chlorophyll content, and physicochemical parameters.

Identification of green algae species

The collected green algae species were identified using the taxonomic Key to the Green Algae [7,8,9,10], and the Field Guide and Atlas of the Seaweed Resources of the Philippines[11] (Gavino and Field Guide to common mangroves, Sea grasses, and Algae of the Philippines)[6].

Relative Abundance

The relative abundance of each species of green algae was based on the formula:

$$RA=\frac{\textit{Total number of species}}{\textit{Total number of all species}} \text{x } 100$$

Species Diversity

Diversity index (H') mathematically states the circumstances of the organism's population to analyze the number of individuals in each growth step or genus in a habitat community. The most commonly used diversity index is the Shannon-Weiner index [12].

Where:

H'=Shannon-Weiner Index of Diversity

n_i= number of individuals per species

N= total number of individual species

$$H' = -\sum_{i=1}^{S} Pi \ln Pi$$

The diversity index criteria are as follows:

H' ≤ 1 = Low diversity

1 < H'≤ 3 = Moderate diversity

H '≥ 3 = high diversity

Physico-chemical Parameters

The prevailing level of the physicochemical parameters was determined per station during sampling. Temperature, pH, and salinity were analyzed using a Multiparameter tester following the supplier's specifications.

Quantification of Pigments

To calculate the corrected chlorophyll a concentration by inserting the corrected absorbance values in the following equation:

$$\text{Chlorophyll a } (\mu g/l) = \frac{\mathbf{26.73(618\boldsymbol{b}-666\boldsymbol{a})\boldsymbol{E}}}{\boldsymbol{V}}$$

Where:

E= the volume of methanol used for extraction (ml)

V= the volume of water filtered

666_a=the turbidity corrected Abs at 666 nm after acidification

618_b= the turbidity corrected Abs at 618 nm before acidification

Statistical Analysis

The data was analyzed using a specific tool to identify the results based on the data being gathered. Analysis of variance (One-way ANOVA) was performed to analyse the significance of the difference in terms of species, relative abundance and species distribution in the three selected sites of Tubalan Cove.

RESULTS AND DISCUSSION

The table one(1) showed the Seven (7) green algae species were found in the study area. The following species were identified: *Halimeda opuntia, Halimeda discoidea, Codium fragile, Ulva compressa, Caulerpa lentillifera, Caulerpa racemosa*, and *Dictyosphaeria cavernosa* using pictures and the dichotomous key outlined in the Field Guide to the Common Mangroves, Seagrasses, and Algae in the Philippines[6].

Table 1. List of Green Algae found in the study area

SPECIES NAME	STATION 1			STATION 2			STATION 3		
	T1	T2	T3	T1	T2	T3	T1	T2	T3
H. opuntia	+	-	+	+	+	+	+	-	-
H. discoidea	+	+	-	+	-	-	+	-	-
C. fragile	+	+	+	+	+	+		+	+
U. compressa	-	-	-	+	-	-	-	-	-
C. lentillifera	+	+	+	+	+	+	+	+	+
C. racemosa	-	-	-	+	+	-	-	-	-
D. cavernosa	-	-	-	-	+	-	-	-	-

Table 2 showed the relative abundance of the species of green algae in the study area. Result revealed that in Station 1, *Caulerpa lentillifera* obtained the highest abundance with 28.26%, followed by *Codium fragile* with 26.08%, *Halimeda discoidea* with 19.57%, and *Halimeda opuntia* were the least abundant with 13.04%. The result revealed that *Caulerpa lentillifera* is more abundant than the other species present in station 1.

In Station 2, *Halimeda opuntia* obtained the highest abundance with 21.28%, followed by *Caulerpa lentillifera* with 19.15%, *Codium fragile* with 14.89%, *Halimeda discoidea* and *Caulerpa racemosa* with 10.64%*, Ulva compressa* with 6.38% and *Dictyosphaeria cavernosa* were least abundant with 2.13%. The result revealed that *Halimeda opuntia* is more abundant than the other species present in station 2.

In Station 3, *Caulerpa lentillifera* obtained the highest abundance, 23.53%, followed by Codium fragile, 14.71%, Halimeda opuntia, 11.67%, and Halimeda discoidea, the least abundant species, 5.88%. The result revealed that Caulerpa lentillifera is more abundant than the other species in Station 3.

This indicates that *Caulerpa lentillifera* was relatively abundant compared to other species in the study area. Hence *Caulerpa lentillifera* has shown potential ability to remove basic dyes from waste streams[13], heavy metals from industrial wastewater[14,15] and nutrients from aquaculture effluents[16].

Table 2: Relative abundance of green algae in the selected intertidal area of Tubalan

SPECIES NAME	STATION 1	STATION 2	STATION 3	SITE (MEAN)
H. opuntia	15%	25%	21.05%	20.20%
H. discoidea	22.5%	12.5%	10.53%	16.16%
C. fragile	30%	17.5%	26.32%	24.24%
U. compressa	0%	7.5%	0%	03.03%
C. lentillifera	32.5%	22.5%	42.11%	30.30%
C. racemosa	0%	12.5%	0%	05.05%
D. cavernosa	0%	2.5%	0%	1.01%

Table three (3) shows the species diversity, Station 2 gained the highest value of 1.79, followed by Station 1 with a value of 1.35, and lastly, Station 3 gained the smallest value of 1.28. The species diversity of the study ranges from 128 to 1.79. The results indicate that the green algae around the three sites was categorized as moderate diversity.

Species diversity in a community can be shown by the diversity index (H') value; the higher the H' value, the higher the diversity and stability of the community. H' is categorized as low (H'<1), moderate (1≤ H'≥2), and high (H'>2)[17]**.** The results showed that the green algae around the three sites was categorized as moderate diversity (1≤ H'≥2). This showed that the number of species in the three stations was still diverse and stable. This implies that increasing species diversity can influence ecosystem functions such as productivity by increasing the likelihood that species will use complementary resources and that a particularly productive or efficient species is present in the community[18].

Table 3. Species Diversity of Green Algae study area

STATION	SPECIES DIVERSITY
1	1.35
2	1.79
3	1.28

Table four (4) shows the prevailing levels of the following physico-parameters in the study area, such as temperature, pH, and salinity, were determined per station and observed during sampling. The temperature of the study area ranges from 31-32^0 C. Station 2 obtained the highest temperature reading of 320 C, followed by station 1, which had a reading of 310 C, and station 3, which had a reading of 31.5^0 C. In terms of salinity, Station 1 has 31 parts per thousand (ppt), Station 2 has 32 parts per thousand (ppt), and Station 3 has 32 parts per thousand (ppt). However, the hydrogen ion concentration of the pH level on each sampling station was 7 ppm.

Table 4. Level of the physico-chemical parameter in the study area

STATION	TEMPERATURE	PH	SALINITY
1	31^0 C	7 ppm	31 ppt
2	32^0 C	7 ppm	32 ppt
3	31.5^0 C	7 ppm	32 ppt

Table 5 showed the chlorophyll determination content. Each sample of green algae species: *Halimeda opuntia, Halimeda discoidea, Codium fragile, Ulva compressa, Caulerpa lentillifera, Caulerpa racemosa and Dictyosphaeria cavernosa* was measured absorbance at wavelengths of 618 and 666 nm. The results obtained in the form of a chlorophyll absorption spectrum. The result reveals that *Dictyosphearia cavernosa, Caulerpa lentillefera* and *Caulerpa racemosa* contain the highest value of 155.034 µg/L in chlorophyll a. Then, Ulva Compressa contains the lowest value of 38.7585 µg/L in chlorophyll a.

The result showed that the chlorophyll content of green algae species was characterized by chlorophyll a as the major photosynthetic pigment. Dictyosphearia cavernosa, Caulerpa lentillefera, and *Caulerpa racem*osa obtained the highest concentration.

Hence, the result indicates that the higher value of chlorophyll-a, the higher efficacy of photosynthetic activity. They can effectively measure trophic status and potentially indicate maximum photosynthetic rate[19]. Algal growth can be tracked directly by measuring chlorophyll levels. Surface waters with high chlorophyll levels tend to be high in nutrients, especially phosphorus and nitrogen. The algae can grow or bloom as a result of these nutrients. Dissolved oxygen levels are depleted as algae species bloom, then crash and die in response to changing environmental conditions. High levels of nitrogen and phosphorus can be indicators of pollution from man-made sources, such as septic system leakage, poorly functioning wastewater treatment plants, or fertilizer runoff. As a result, measuring chlorophyll can be used as an indirect indicator of nutrient levels[3].

Table 5. Chlorophyll Content of Green Algae

SPECIES	ABSORBANCE (666)	ABSORBANCE (618)	CHLOROPHYLL VALUE
H. opuntia	1.968	2.026	77.517 µg/L
H. discoidea	1.884	1.942	77.517 µg/L
C. fragile	2.49	2.54	66.825 µg/L
U. Compressa	2.447	2.476	38.7585 µg/L
C. lentillefera	1.812	1.928	155.034 µg/L
C. racemosa	2.193	2.309	155.034 µg/L
D. cavernosa	2.16	2.276	155.034 µg/L

Based on the statistical analysis of the significant difference in green algae in the three sampling stations, the results revealed no significant difference in species diversity in the three stations considered for the study, as shown in Table 6. Since the p-value is greater than 0.05, the significance level implies that the difference is insignificant among the three stations.

Table 6: The statistical difference of species diversity in the three-sampling stations

STATION	INDEX OF DIVERSITY	F VALUE	P-VALUE	INTERPRETATION
1	1.35	4.576	0.062	Not significant
2	1.79			
3	1.28			

Table 7 showed the result of relative abundance, which revealed a statistically significant difference in the mean difference of each species. It reveals that *Halimeda opuntia* is more abundant than *Ulva compressa and Dictyosphaeria cavernosa.* Then, *Codium fragile* is more abundant than *Ulva compressa*, *Caulerpa racemosa* and *Dictyosphaeria cavernosa.* And *Caulerpa lentillifera,* is more abundant than *Caulerpa racemosa Caulerpa racemosa and Dictyosphaeria cavernosa.* Since the p-value is less than the 0.05 significance level, implying that the abundance difference is significant among the species. Hence, there is a significant difference in species abundance in the study area.

Table 7: The statistical difference of relative abundance in the study area

GREEN ALGAE SPECIES	ABUNDANCE	F VALUE	P-VALUE	INTERPRETATION
H. opuntia	20.20%	11.293	0.000	Significant
H. discoidea	16.16%			
C. fragile	24.24%			
U. compressa	03.03%			
C. lentillifera	30.30%			
C. racemosa	05.05%			
D. cavernosa	1.01%			

CONCLUSION

This study aims to determine the species diversity, abundance, and chlorophyll determination content of green algae in the selected sites, Tubalan, Malita, Davao Occidental. Based on the results, the main findings can be summarized as follows:

There were (7) seven green algae species (Chlorophyta) namely: *Halimeda opuntia, Halimeda discoidea, Codium fragile, Ulva compressa, Caulerpa lentillifera, Caulerpa racemosa and Dictyosphaeria cavernosa* found in the three selected sites of Tubalan cove.

Caulerpa lentillifera had the highest relative abundance, and Dictyosphaeria cavernosa had the lowest. In terms of population density, Caulerpa lentillifera had the highest population density, and Dictyosphaeria cavernosa exhibited the lowest density. Regarding species diversity, the results showed that each of the three stations ranged from 1.28 to 1.79.

The results for the physicochemical parameters showed that each station's temperature ranged from 31-32 °C, the pH level ranged from 7 ppm, and the salinity ranged from 31-32 ppt.

In terms of chlorophyll determination content, Dictyosphearia cavernosa, Caulerpa lentillefera, and Caulerpa racemosa contain the highest value of chlorophyll, and *Ulva Compressa* contains the lowest value of chlorophyll.

There is no significant difference in species diversity among the 3 stations of the study. However, relative abundance revealed a statistically significant difference in the mean difference of each species' chlorophyll value.

RECOMMENDATION

The study examined the species diversity, abundance, and chlorophyll determination content of green algae in selected Tubalan, Malita, and Davao Occidental sites. The researchers made the following recommendations.

1. A monitoring study of the different seasons, monitoring the species diversity and chlorophyll content.
2. Further studies are needed to compare the species richness of green algae during high tides and low tides in Tubalan, Malita, and Davao Occidental.
3. Further study may be conducted on the characterization of chlorophyll pigments, including different types of chlorophyll.

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