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Species diversity and Chlorophyll content of green algae in the selected intertidal area of Malita, Davao Occidental, Philippines

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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 Philippines. The three selected sites were designated as three stations. Each site had 3 transect lines (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 result also showed that Station 2 gained the highest Index of diversity, while in terms of relative abundance, Caulerpa lentillifera obtained the highest abundance in Station 1, Halimeda opuntia obtained the highest abundance in Station 2, and Caulerpa lentillifera obtained the highest abundance in Station 3. The result showed that the temperature of each of the three stations ranged from 31-32°C, the pH level was 7, and the salinity ranged from 31-32 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 found in large quantities in nature. They exhibit significant biodiversity in tropical coral reefs and lagoons, frequently growing alongside related sea grass environments. Species diversity is a multidimensional concept that includes species richness, abundance, and evenness[1]. Overall, there are two main methods for assessing species diversity, which takes into account both the number of species (species richness) and the distribution of individuals among the various 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].

Assessing chlorophyll concentrations can monitor algae growth. Water surfaces with elevated chlorophyll concentrations usually have high nutrient levels, particularly phosphorus and nitrogen. These nutrients can lead to algae proliferation or blooming. When algal species thrive, the levels of dissolved oxygen decrease as they later die off in response to changing environmental conditions. Elevated nitrogen and phosphorus concentrations may signal pollution from human activities, including leaks from septic systems, inefficient wastewater treatment facilities, or runoff from fertilizers. 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” Northand 125°34’08” East”) of the island of Mindanao. Elevation at these coordinates is estimated at 25.6 meters or 84.0 feet above mean sea level (Figure-1). A preliminary survey of green algae (Chlorophyta) species was conducted on the three selected sites of Tubalan, Malita, and Davao Occidental.

Establishment of the Study Area: The three selected sites were designated as three stations. 50-meter transect lines will assign each station three 50meters 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 (60 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 the 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 a white 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. The 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 was 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].

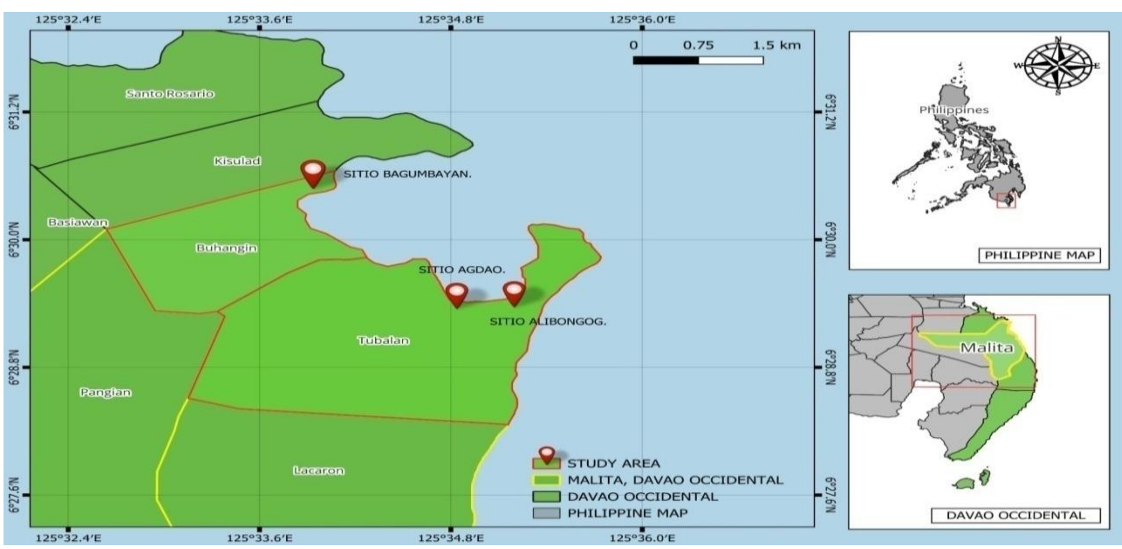

Figure-1: Sampling stations of Tubalan intertidal area.

Data Analysis: The researchers interpret the data gathered using the Kruskal-Wallis test to determine the significance of differences in 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[7-10], and the Field Guide and Atlas of the Seaweed Resources of the Philippines[11] 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:

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

Species Diversity: The 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

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

The diversity index criteria are as follows: $H' \leq 1$ = Low diversity, $1 < H' \leq 3$ = Moderate diversity, $H' \geq 3$ = 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:

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

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 investigate the significance of differences in terms of species, relative abundance, and species distribution among the three selected sites in Tubalan Cove.

Results and Discussion

Table-1 shows the seven (7) green algae species 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, sea grasses, and Algae in the Philippines[6].

Table-1: Species composition of green algae in the study areas.

Species name	Station 1			Station 2			Station 3		
	T1	T2	T3	T1	T2	T3	T1	T2	T3
Halimeda opuntia	+	-	+	+	+	+	+	-	-
Halimeda discoidea	+	+	-	+	-	-	+	-	-
Codium fragile	+	+	+	+	+	+	-	+	+
Ulva compressa	-	-	-	+	-	-	-	-	-
Caulerpa lentillifera	+	+	+	+	+	+	+	+	+
Caulerpa racemosa	-	-	-	+	+	-	-	-	-
Dictyosphaeria cavernosa	-	-	-	-	+	-	-	-	-

Table-2 shows the relative abundance of the species of green algae in the study area. Results revealed that in Station 1, Caulerpa lentillifera obtained the highest abundance at 28.26%, followed by Codium fragile at 26.08%, Halimeda discoidea at 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.

Table-2: Relative abundance of green algae in the study areas.

Species name	Station 1(%)	Station 2(%)	Station 3(%)
Halimeda opuntia	15	25	21.05
Halimeda discoidea	22.5	12.5	10.53
Codium fragile	30	17.5	26.32
Ulva compressa	0	7.5	0
Caulerpa lentillifera	32.5	22.5	42.11
Caulerpa racemosa	0	12.5	0
Dictyosphaeria cavernosa	0	2.5	0

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-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.

The diversity index (H') can illustrate the species diversity within a community; a higher H' value indicates greater diversity and stability of the community. H' values are classified as low (H'<1), moderate (1≤ H'≥2), and high (H'>2)17. The findings revealed that the green algae at the three locations were classified as having moderate diversity (1≤ H'≥2). This indicates that the number of species at the three stations remains diverse and stable. This suggests that increasing species diversity can impact ecosystem functions such as productivity by enhancing the chances that species will utilize complementary resources and ensuring the presence of particularly productive or efficient species within the community[18].

Table-3: Diversity of green algae in the study area.

Station	Diversity Index
1	1.35
2	1.79
3	1.28

Table-4 shows the prevailing levels of the following physical parameters in the study area, such as temperature, pH, and salinity, which 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 32^{0}C, followed by Station 1, which had a reading of 31^{0}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: Physico-chemical properties of water sample in the study areas.

Station	Temperature (^{O}C)	pH	Salinity (ppt)
1	31	7	31
2	32	7	32
3	31.5	7	32

Table-5 shows 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 were 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 155.034µg/L in chlorophyll a. Then, Ulva Compressa contains the lowest value of 38.7585 µg/L in chlorophyll a.

Table-5: Chlorophyll Content of Green Algae from the Intertidal area of Tubalan, Malita.

Species name	Absorbance (666 nm)	Absorbance (618 nm)	Chlorophyl content(µg/L)
Halimeda opuntia	1.968	2.026	77.517
Halimeda discoidea	1.884	1.942	77.517
Codium fragile	2.447	2.54	66.825
Ulva compressa	1.812	2.476	38.758
Caulerpa lentillifera	1.812	1.928	155.034
Caulerpa racemosa	2.193	2.309	155.034
Dictyosphaeria cavernosa	2.16	2.276	155.034

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

Hence, the result indicates that the higher the value of chlorophyll-a, the higher the 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. Oxygen levels in water decline when algae species proliferate, subsequently die, and decompose due to fluctuating environmental conditions. Elevated nitrogen and phosphorus concentrations may signal contamination from human activities like septic system failures, ineffective wastewater treatment facilities, or runoff from fertilizers. Consequently, assessing chlorophyll can serve as an indirect measure of nutrient concentrations[3].

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-7 shows 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-6: The statistical difference of species diversity in the three-sampling stations.

Station	Diversity	F-value	P-value	Interpretation
1	1.35	4.576	0.062	Not significant
2	1.79			
3	1.28			

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

Species	Relative abundance (%)	F-value	P-value	Interpretation
Halimeda opuntia	20.35	11.293	0	Significant
Halimeda discoidea	15.17			
Codium fragile	24.6			
Ulva compressa	2.5			
Caulerpa lentillifera	32.37			
Caulerpa racemosa	4.16			
Dictyosphaeria cavernosa	0.83			

Conclusion

The surveyed sites are highly suited for green algae, as evidenced by the sighting of the seven (7) green algae species in the area, 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 lentilliferais highly evidenced by its abundance. The study areas also have a moderate diversity of green algae. 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, which implies the suitability of the areas for green algae species. The moderate diversity could be attributed to the slight anthropogenic activities in the area, such as the presence of fish cages and some recreational activities.

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.

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

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