

**ISOLATION, CULTURE, SEMI-LARGE PRODUCTION OF SELECTED  
MICRO ALGAL SPECIES FROM FRESHWATER TRIBUTARIES OF  
CARIGARA BAY, LEYTE**

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**ABSTRACT**

Carigara Bay is one of the successful fishing lands in the entire Philippines because of its multi-gear fishery system. Filipinos living around the bay considered its marine life as one of their livelihood. However, the biodiversity and biological importance of microalgae are still unexplored evident by the dearth of published data in scientific journals, thus, this study was undertaken. Sampling was conducted on the five (5) coastal towns surrounding the bay namely Carigara, Capoocan, Barugo, San Miguel and Babatngon, Its freshwater tributaries are Lindog River (Brgy. Uyawan), Bislig River (Brgy. Bislig) situated in Carigara, Himanglos River (Brgy. Hilaba), Canomantag River (Brgy. Canomantag) in Barugo, stream located in Brgy, Libertad, Capoocan, Caraycaray River (Brgy. Caraycaray), Lipasan Falls (Brgy. Pinarigusan) in San Miguel and Tula-an Falls (Brgy. Tula-an), Busay Falls (Brgy. Busay) in Babatngon. Water samples from the bay, stream, rivers and falls were collected using a 2.5-3L Plexi glass sampler, transferred in three (3) 1L cap bottle and brought in the laboratory for phytoplankton identification and micro algal culture. Physico-chemical parameters were also gathered in all sampling sites to correlate with the microalgae diversity. Phyla Bacillariophyta, Chlorophyta and Cyanophyta are 44, 37 and 19% of the total phytoplankton identified. Furthermore, there was a significant positive correlation between water pH ( $r = 0.499$ ;  $p = 0.001$ ), conductivity ( $r=0.519$ ;  $p=0.001$ ) and amount of phosphates ( $r = 0.446$ ;  $p = 0.003$ ). Moreover, six (6) genera namely *Asterococcus*, *Anabaena*, *Nostoc*, *Chlorococcum*, *Synechococcus* and *Oscillatoria* were isolated, cultured and semi-mass produced for optimization procedure.

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12 *Keywords: freshwater tributaries, biodiversity, semi-large scale cultures*

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## 15 **1. INTRODUCTION**

16

17

18 Socio-cultural, economic and scientific evolution in the society nowadays  
19 gives a big difference and distinct impact on the food intake, balance diet and overall lifestyle  
20 of many people. Hence, nutritional content and value and feeding mechanisms play critical  
21 role in many developing and industrialized countries (Anderson and Alford 2014). This is  
22 also in support of the main goal of international agencies like World Health Organization  
23 (WHO) and Food and Agriculture Organization (FAO) which is healthy food for everyone in  
24 all nations (Nicoletti, 2016). Indeed, animal health is one of the struggles of the public today  
25 where synthetic and chemically produced food and feed for human and lower forms of  
26 animals, respectively are being manufactured and commercialized (Meena, 2013). The  
27 reason for this is the fast and growing population of humans where natural resources  
28 diminished proportionally (Ugoala et al., 2012). Consequently, in some instances, the  
29 products are dangerous due to the negative side effects that are being neglected while the  
30 synthetic ones brought positive effects. To address these issues, drugs, pigments and  
31 biochemicals excreted by natural aquatic weeds and grass, higher forms of flora, as well as  
32 macro and microalgae are being established and scrutinized for the development of products  
33 necessary for aquaculture, agriculture, and other industrially important applications for  
34 mankind and lower animals (Kaushik and Chauhan 2008; Voort et al., 2014). Algae have  
35 existing species of about 50,000 and more but only half of the total species are being  
36 experimented and analyzed on the said aspect and only 15 micro algal species are involved  
37 in current commercial production (Richmond 2004; Raja et al., 2008; Ugoala et al., 2012).

38

39 Generally, most of the microalgae have major attributes which are important  
40 for mass cultivation. One of these is the presence of high reproduction and growth rates,  
41 hence, can be cultured in simple ponds and complex bioreactors either indoor or outdoor  
42 (Duong et al., 2012; Chen et al., 2014). In order to grow, develop and reproduce in the said  
43 process, these organisms which are autotrophic in nature, can transform sunlight into  
44 glucose by photosynthesis through fixation of carbon dioxide with high production of dry  
45 matter supplementing its body in comparison to the self-feeder plants (Raja et al., 2008;  
46 Park et al., 2011; Karthikeyan 2012). However, algae can also be heterotrophic organisms  
47 where they use organic compounds as their source of carbon dioxide and light. To compare,  
48 micro algal species' efficiency to do photosynthesis is higher to plants by about 10-20%  
49 (Ferdowshi 2013). They are, indeed, fast growers which means that the species can  
50 duplicate its life for about 3 hours and can reproduce themselves very quickly in simple  
51 medium (Kovac et al., 2013). These microalgae can also tolerate varying concentrations of  
52 light and temperature, pH and oxygen concentration in different aquatic habitats which can  
53 eradicate the presence of pathogenic organisms. Microalgae can also tolerate extreme  
54 conditions of high salinity and large exposure to ultraviolet rays. In all of these, the  
55 microalgae produce high varieties of secondary metabolites with large potency and  
56 effectiveness for biological activities (Ibanez et al., 2012).

57

58 Carigara Bay, the location of the study, is located in the Province of Leyte at  
59 Region 8 or the Eastern Visayas where it is situated in five (5) coastal towns namely  
60 Carigara, Capoocan, Barugo, San Miguel and Babatngon. Freshwater tributaries such as  
61 streams and rivers of the Carigara Bay are present keeping the marine life moist in condition  
62 over many months (Makabenta, 1995). The bay itself and its freshwater resources are  
63 abound with diversity of organisms but poorly studied as shown by dearth of reports. In fact,  
the study on the assessment of water quality and initial identification of zooplankton and  
phytoplankton was solely the existing biological report about Carigara bay (Santos et al.  
1999). Noticeably, too, are current publications stating that most of the micro algal cultivation

64 is sold in the market for animal food and pharmaceutical products (Becker 2004) and the  
65 algal biomass is starting to have a high rate of demand for aquaculture industry including fish  
66 feed providing an initiative revenue for algae industry even microalgae cultivation is only a  
67 few decades old (Anemaet et al., 2010). However, adequate studies on the suitability of  
68 these microalgae as animal feed are limited (Olaizola 2003; Sathasivam et al., 2017). Micro-  
69 algal based bio-resources products necessary in daily living are the new trends of today's  
70 era and scientists dwell more attention on the said matter (Feng et al., 2016). Thus this  
71 study on the identification and assessment of microalgae in Carigara Bay and freshwater  
72 resources in its vicinity, situated at the five (5) towns surrounding the bay would serve as  
73 baseline information for future studies.

74 The objective of this study is to identify and quantify microalgae found in  
75 Carigara bay and its freshwater tributaries; isolate and culture micro algal species;  
76 characterize and identify the micro algal species isolated from the freshwater tributaries of  
77 Carigara bay and screen, select and semi-mass produced micro algal species from the  
78 freshwater tributaries of Carigara bay.

## 80 2. MATERIAL AND METHODS / EXPERIMENTAL DETAILS / METHODOLOGY

81  
82 The island of Leyte where Carigara Bay is sited and connected with freshwater  
83 tributaries, is a sister island of Samar and one of the ten (10) biggest land masses in the  
84 country. Sampling stations in the area were determined in coordination with Dr. Paciente A.  
85 Cordero, Jr. with the supervision of the faculty members and staff of the College of Fisheries  
86 at Eastern Visayas State University, Carigara Campus. Fourteen (14) stations were  
87 established at the upper coastal towns adjoining the Carigara Bay. Station 1 was the  
88 Carigara Bay at Brgy. Libertad (Capoocan); Station 2 was a stream at Brgy. Libertad  
89 (Capoocan); Station 3 Carigara Bay at Brgy. Visoria (Carigara); Station 4 was the Lindog  
90 River at Brgy. Uyawan (Carigara); Station 5 was the Bislig River at Brgy Bislig (Carigara);  
91 Station 6 was the Carigara Bay at Brgy. Duka (Barugo); Station 7 was the Himanglos River  
92 at Brgy. Hilaba (Barugo); Station 8 was the Canomantag River at Brgy. Canomantag  
93 (Barugo); Station 9 was the Carigara Bay at Brgy. Mawod-pawod (San Miguel); Station 10  
94 was the Caraycaray River at Brgy. Caraycaray (San Miguel); Station 11 was the Lipasan  
95 Falls at Brgy. Pinarigusan (San Miguel); Station 12 was the Carigara Bay at Brgy.  
96 Kalangawan Guti (Babatngon); Station 13 was the Tula-an Falls at Brgy. Tula-an  
97 (Babatngon) and Station 14 was the Busay Falls at Brgy. Busay (Babatngon). The water  
98 samples were collected from July 2-8, 2018 which was considered as the 1<sup>st</sup> sampling and  
99 from November 23-26, 2018 which was the 2<sup>nd</sup> sampling. Collection of water samples from  
100 the bay was done by towing 30 meters away from the shoreline. The water samples from the  
101 bay were gathered 30 meters away from the shoreline with a depth of 1-1.5 meters only. The  
102 water samples from the bay were used for phytoplankton quantification analysis only. For the  
103 freshwater tributaries of the bay, the water samples were collected in integrated manner,  
104 thus, from surface, middle and approximately 0.5 meter away from the bottom. Most have  
105 depths ranging from 0.75- 2 meters, therefore, ideal for integrated sampler. Depths were  
106 measured using the plexi glass sampler built in with improvised measurement in meters  
107 and/or the secchi disk. The three (3) L of water samples from the freshwater ecosystems  
108 were divided from which the 1 L was taken and fixed with Lugol's solution to preserve the  
109 cell wall; one (1) L was kept cooled during the transport at University of Santo Tomas,  
110 Manila and immediately stored in freezer or placed in a refrigerated condition for *ex situ*  
111 nutrient determination of the water samples such as nitrate and phosphates and the other  
112 one (1) L was used as a live sample for the isolation of microalgae. Furthermore, prior to  
113 collection physical parameters such as surface water temperature, column depth, light  
114 penetration or transparency, and chemical parameters such as water pH, conductivity and  
115 dissolved oxygen were recorded in situ. All of the parameters were measured using the  
116 Xplorer GLX (PAASCO) water quality sensor except for the light penetration or transparency

117 which was determined using a Secchi disc. In addition analysis of nitrate and phosphorus  
118 were also done through the Hach DR/2010 phosphate data logging spectrophotometer at the  
119 Roque Laboratory, Old Graduate School, University of Santo Tomas.

120 In order to quantify, identify, isolate and separate micro algal species, standard  
121 protocol on washing and plating techniques were done to ensure the isolation of all the  
122 microalgae components from water samples collected (Martinez, 1976; Baldia, 1992; Lee et.  
123 al., 2014). For quantification, an appropriate amount was was loaded in a Neubauer  
124 Counting Chamber for density determination as viewed using the Olympus CH20 compound  
125 light microscope. (Martinez et. al., 1975). The species were identified using several  
126 references on micro algal taxonomy and biodiversity. For the isolation, Binangonan  
127 Research Station Pantastico medium (BRSP) was used for the multiple streaking technique.  
128 After growth colonies and establishment of uni-algal cultures, the isolates were maintained in  
129 each test tubes containing 10 ml of BRSP media at a temperature of  $25 \pm 2^{\circ}$  C, pH of 7.27  
130 and light intensity of 3,000 lx. Six (6) isolates were selected for semi-mass production and  
131 large scale cultivation.

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### 133 3. RESULTS AND DISCUSSION

134

#### 135 Biodiversity of Phytoplankton


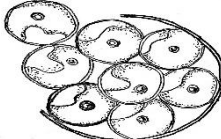
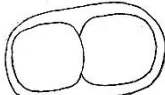
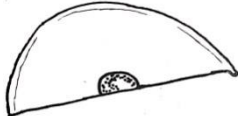
136 The variety of biological organisms is attributed by biotic and abiotic factors obtained  
137 in the study sites. These are the physico-chemical parameters and the type of ecological  
138 features which are bounded in the sampling areas in order to understand and explore the  
139 variety of living organisms present in the said locations. Moreover, studies on phytoplankton  
140 diversity can in turn control the physico-chemical and biological conditions in certain aquatic  
141 ecosystem (Ariyadej et al., 2004; Ali et al., 2010). The species are identified taxonomically,  
142 uni-isolated and mass-cultured for their biological potentials particularly in food industry,  
143 pharmaceutical and biotechnology.


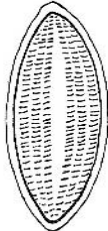
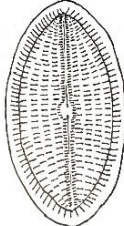
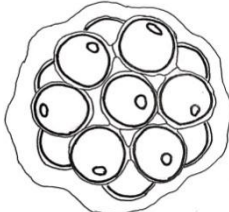
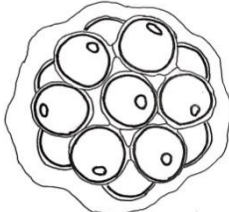
144 The following are the Genus identified and quantified in Carigara Bay and its  
145 freshwater tributaries. For Phylum Bacillariophyta, these are *Cocconeis* (Her.) Grun., 1868;  
146 *Coscinodiscus* Her., 1838 *Fragilaria* Lyng., 1819., emend, Rabenh., 1864; *Synedra*  
147 Ehrenberg, 1830; *Navicula* Bory, 1822 erend, Cl., 1894; *Nitzschia* Hassal, 1845, emend,  
148 Grun., 1880; *Stauroneis* Her., 1843; *Cymbella* Agardh, 1836; *Melosira* Agardh, 1824;  
149 *Diploneis* Ehrenberg, 1894; *Gomphonema* Ehrenberg, 1832 and *Neidium* Pfitzer, 1871. For  
150 Phylum Chlorophyta, these are *Chlorella ellipsoidea* Gerneck 1907 and *Chlorella vulgaris*  
151 Beyerinck 1890; *Staurastrum* Meyen ex Ralfs, 1848; *Stigeoclonium* Kuetzing 1843;  
152 *Schizomeris* Kuetzing 1843; *Sphaerocystis* Chodat 1897; *Ulothrix* Kuetzing, 1833;  
153 *Closteridium* Reinsch, 1888; *Closterium* Brebisson, 1856; *Coelastrum* Naegeli in Kuetzing,  
154 1849 and *Pediastrum* Meyen, 1829. For Phylum Cyanophyta, *Lyngbya* Ag., 1824;  
155 *Oscillatoria* Vaucher, 1803; *Chroococcus* Naeg., 1848; *Merismopedia elegans* A. Braun in  
156 Kuetzing 1849 and *Nostoc* Vaucher, 1803.

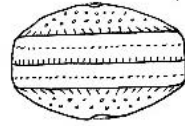
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**Table 1. List of all phytoplankton species with illustrations**

Species	Division	Size	Illustration
<i>Chlorella ellipsoidea</i>	Chlorophyta	Length: 9.2 um Width: 6.9 um	
<i>Chlorella vulgaris</i>	Chlorophyta	Length: 9.2 um Width: 9.2 um	
<i>Chroococcus sp.</i>	Cyanophyta	Length: 6-15 um Width: 9.6 um	
<i>Closteridium sp.</i>	Chlorophyta	Length: 96.6 um Width: 36.8 um	

<i>Closterium sp</i>	Chlorophyta	Length: 48.3 um Width: 9.2 um	
<i>Cocconeis placentula</i>	Bacillariophyta	Length: 32.2 um Width: 13.8 um	
<i>Cocconeis sp.</i> (2)	Bacillariophyta	Length: 46 um Width: 27.6 um	
<i>Coelastrum sp.</i>	Chlorophyta	Length: 18.4 um Width: 11.5 um	
<i>Coscinodiscus sp.</i>	Bacillariophyta	Length: 59.8 um Width: 13.8 um	



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***Cymbella sp.***

**Bacillariophyta**

Length:  
69 um  
Width:  
25.3 um



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***Diploneis sp.*  
(1)**

**Bacillariophyta**

Length:  
39.1 um  
Width:  
16.1 um

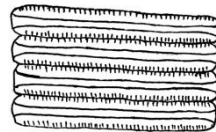


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***Fragilaria sp.*(1)**

**Bacillariophyta**

Length:  
41.4 um  
Width:  
4.6 um

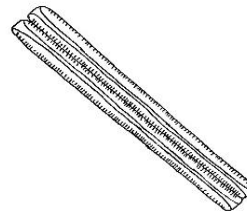


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***Fragilaria sp.*  
(2)**

**Bacillariophyta**

Length:  
59.9 um  
Width:  
25.4 um



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***Fragilaria sp.(3)***

**Bacillariophyta**

Length:  
52.2 um  
Width:  
18.4 um



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***Fragilaria sp.*  
(4)**

**Bacillariophyta**

Length:  
211.6  
um  
Width:  
9.2 um



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***Fragilaria sp.*  
(5)**

**Bacillariophyta**

Length:  
39.4 um  
Width:  
3.9 um

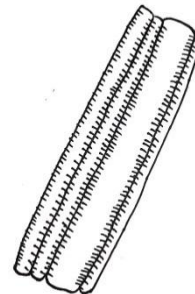


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***Fragilaria sp.*  
(6)**


**Bacillariophyta**

Length:  
52.9 um  
Width:  
11.5 um






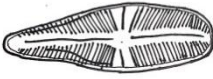
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<b><i>Gomphonema</i> sp. (1)</b>	<b>Bacillariophyta</b>	Length: 36.8 um Width: 11.5 um	
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<b><i>Gomphonema</i> sp. (2)</b>	<b>Bacillariophyta</b>	Length: 36.8 um Width: 11.5 um	
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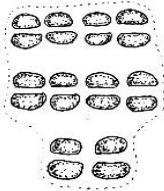
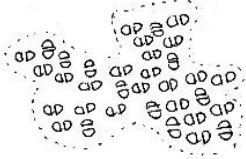

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<b><i>Gomphonema</i> sp. (3)</b>	<b>Bacillariophyta</b>	Length: 41.4 um Width: 13.8 um	
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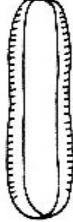
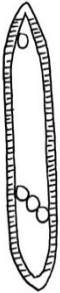

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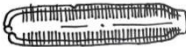


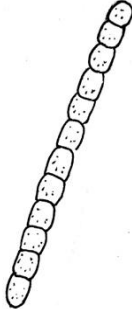
<b><i>Lyngbya</i> sp.(1)</b>	<b>Cyanophyta</b>	Length: 32.2 um Width: 9.2 um	
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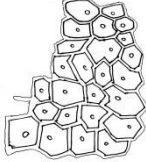

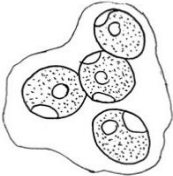
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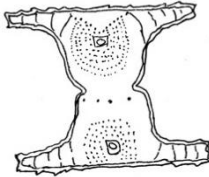




<i>Lyngbya sp.(2)</i>	Cyanophyta	Length: 87.4 um Width: 9.2 um	
<i>Melosira sp.</i>	Bacillariophyta	Length: 18.4 um Width: 9.2 um	
<i>Merismopedia sp.(1)</i>	Cyanophyta	Length: 103.5 um Width: 11.5	
<i>Merismopedia sp.(2)</i>	Cyanophyta	Length: 69 um Width: 13.8 um	
<i>Navicula sp.(1)</i>	Bacillariophyta	Length: 55.2 um Width: 23 um	





<i>Navicula sp.(2)</i>	Bacillariophyta	Length: 23 um Width: 18.4 um	
<i>Navicula sp.(3)</i>	Bacillariophyta	Length: 46 um Width: 9.2 um	
<i>Navicula sp.(4)</i>	Bacillariophyta	Length: 39.1 um Width: 11.5 um	
<i>Navicula sp.(5)</i>	Bacillariophyta	Length: 55.2 um Width: 16.1 um	
<i>Neidium sp.</i>	Bacillariophyta	Length: 46.0 um Width: 13.8 um	

<i>Nitzschia sp.(1)</i>	Bacillariophyta	Length: 105.8 um Width: 9.2 um	
<i>Nitzschia sp.(2)</i>	Bacillariophyta	Length: 78.2 um Width: 20.7 um	
<i>Nitzschia sp. (3)</i>	Bacillariophyta	Length: 78.2 um Width: 13.8 um	
<i>Nitzschia sp. (4)</i>	Bacillariophyta	Length: 59.8 um Width: 13.8 um	

<i>Nitzschia</i> sp. (5)	Bacillariophyta	Length: 46 $\mu$ m Width: 18.4 $\mu$ m	
<i>Nitzschia</i> sp. (6)	Bacillariophyta	Length: 59.8 $\mu$ m Width: 13.8 $\mu$ m	
<i>Nitzschia</i> sp. (7)	Bacillariophyta	Length: 115 $\mu$ m Width: 13.8 $\mu$ m	
<i>Nostoc</i> sp.	Cyanophyta	Length: 8 $\mu$ m Width: 6.7 $\mu$ m	
<i>Oscillatoria</i> sp.(1)	Cyanophyta	Length: 41.4 $\mu$ m Width: 2.3 $\mu$ m	

<b><i>Oscillatoria</i> sp.(2)</b>	<b>Cyanophyta</b>	Length: 64.4 um Width: 2.3 um	
<b><i>Oscillatoria</i> sp.(3)</b>	<b>Cyanophyta</b>	Length: 92 um Width: 2.3 um	
<b><i>Pediastrum</i> sp.</b>	<b>Chlorophyta</b>	Length: 29.9 um Width: 16.1 um	
<b><i>Schizomeris</i> sp.</b>	<b>Chlorophyta</b>	Length: 9.2 um Width: 9.2 um	
<b><i>Sphaerocystis</i> sp.</b>	<b>Chlorophyta</b>	Length: 9.2 um Width: 9.2 um	

<b><i>Staurastrum</i></b> <b><i>sp.</i></b>	<b>Chlorophyta</b>	Length: 27.6 um Width: 13.8 um	
<b><i>Stauroneis</i></b> <b><i>sp.(1)</i></b>	<b>Bacillariophyta</b>	Length: 27.6 um Width: 13.8 um	
<b><i>Stauroneis</i></b> <b><i>sp.(2)</i></b>	<b>Bacillariophyta</b>	Length: 29.9 um Width: 13.8 um	
<b><i>Stigeoclonium</i></b> <b><i>sp.(1)</i></b>	<b>Chlorophyta</b>	Length: 18.4 um Width: 4.6 um	
<b><i>Stigeoclonium</i></b> <b><i>sp.(2)</i></b>	<b>Chlorophyta</b>	Length: 23 um Width: 9.2 um	

<b><i>Stigeoclonium</i> sp. (3)</b>	<b>Chlorophyta</b>	Length: 18.4 um Width: 9.2 um	
<b><i>Stigeoclonium</i> sp. (4)</b>	<b>Chlorophyta</b>	Length: 35.1 um Width: 2.9 um	
<b><i>Synedra</i> sp.(1)</b>	<b>Bacillariophyta</b>	Length: 285.2 um Width: 2.3 um	
<b><i>Synedra</i> sp.(2)</b>	<b>Bacillariophyta</b>	Length: 62.1 um Width: 11.5 um	



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***Ulothrix sp.***

**Chlorophyta**

Length:  
18.4  $\mu\text{m}$   
Width:  
9.2  $\mu\text{m}$



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These number of micro algal cells found in Carigara bay and its freshwater resources was further correlated on the physico-chemical parameters gathered in the area as shown in Table 2.

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**Table 2. Correlation Analysis on the Mean of the Physico-chemical Parameters and the Total Number of Micro algal Cells found in Carigara Bay and its Freshwater Resources**

Physico-chemical Parameters	Mean	Standard Deviation	Correlation Analysis (N=42)	Number of Micro algal cells (M=16.88; SD=15.86)
Surface Water Temperature, °C	29.10	3.20	Pearson correlation	0.136
			Sig (2-tailed)	0.390
Column Depth, meters	0.9464	0.50	Pearson correlation	-0.047
			Sig (2-tailed)	0.770
Light Penetration, meters	0.65	0.25	Pearson correlation	-0.091
			Sig (2-tailed)	0.566
Water pH	8.20	0.40	Pearson correlation	0.499**
			Sig (2-tailed)	0.001
Conductivity, Siemens per meter	264.14	295.75	Pearson correlation	0.519**
			Sig (2-tailed)	0.000
Dissolved Oxygen, milligrams per liter	7.76	1.82	Pearson correlation	-0.351*
			Sig (2-tailed)	0.023
Nitrates, milligrams per liter	3.22	2.83	Pearson correlation	0.244
			Sig (2-tailed)	0.119
Phosphates, milligrams per liter	5.22	3.83	Pearson correlation	0.446**
			Sig (2-tailed)	0.003

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**\*\*Correlation is significant at the 0.01 level (2-tailed)**

**\*Correlation is significant at the 0.05 level (2-tailed)**

The above table shows that there was a significant positive correlation between water pH (Mean = 8.20; SD = 0.40) and the total number of micro algal cells found in the water sample (M = 16.88; SD = 15.86),  $r = 0.499$ ;  $p = 0.001$ . Same results were obtained between conductivity (Mean= 264.14; SD=295.75) and total number of micro algal cells,  $r=0.519$ ;  $p=0.001$ . It was also found that there exists a significant positive correlation between the amount of phosphates (Mean= 5.22; SD= 3.83) and the total number of micro algal cells found in the water sample,  $r = 0.446$ ;  $p = 0.003$ . This means that increasing the water pH, conductivity and amount of phosphates in the water sample will lead to an increase in the number of micro algal cells found in the water sample. On the other hand, there exists a negative relationship between the amount of dissolved oxygen (Mean= 7.76; SD= 1.82) and total number of micro algal cells,  $r=-0.351$ ;  $p=0.023$ . This means that a higher amount of dissolved oxygen in water will result to a corresponding decrease in the total number of micro algal cells in the water sample. Finally, no significant relationships were observed between the total number of micro algal cells and the following parameters: water temperature (Mean= 29.10; SD= 3.20), column depth (Mean= 0.9464; SD= 0.50), light penetration (Mean= 0.65; SD= 0.25), and nitrate content (Mean= 3.22; SD= 2.83). This means that changes in these parameters do not affect the total amount of micro algal cells in the water samples.

### Isolation of Phytoplankton

After quantification of phytoplankton, the water samples were subjected for the isolation, purification and identification of micro algal species. There were 18 isolates that were purified and grown in BRSP liquid medium but only few were chosen and at the same time screened for their suitability as microalgae for large-scale production. The following are the species of microalgae isolated and identified. These were arranged according to phylum regardless of the stations where these microalgae were found. Phylum Chlorophyta has six (6) genus; Phylum Cyanophyta has five (5) genus and Phylum Bacillariophyta has two (2)

201 genus. For Phylum Chlorophyta, these are *Genus Asterococcus Scherffel 1908*; *Genus*  
 202 *Chlorella Beyerinck 1890*; *Genus Stigeoclonium Kuetzing 1843*; *Genus Chlorococcum Fries,*  
 203 *1825 (incl. Cystococcus Naeg.)*; *Genus Hormidium Kuetzing 1843* and *Genus Gloeotheca*  
 204 *Naegeli 1849*. For Phylum Cyanophyta, *Genus Anabaena Bory, 1822*; *Genus Nostoc*  
 205 *Vaucher, 1803*; *Genus Chroococcus Naeg., 1848*; *Genus Synechococcus Naegeli 1849* and  
 206 *Genus Oscillatoria Vaucher 1803* were isolated. For Phylum Bacillariophyta, we have *Genus*  
 207 *Fragilaria Lyng., 1819., emend, Rabenh., 1864* and *Genus Navicula Bory, 1822 erend, Cl.,*  
 208 *1894.*

209 Furthermore, the isolated microalgae were tabulated in terms of their quantitative  
 210 and qualitative forms. Table 2 and 3 summarize the qualitative and quantitative data of the  
 211 18 microalgae that were isolated. The microalgae were arranged according to stations. We  
 212 can also deduce from these tables the list of microalgae that were found in various  
 213 freshwater stations. There were nine (9) freshwater ecosystems that served as sampling  
 214 stations. These freshwater stations are Stations 2, 4, 5, 7, 8, 10, 11, 13 and 14.

215 Table 2 presents the microalgae isolated from each station as well as the  
 216 measurements of each of the cells of the various species of microalgae. The sizes of the  
 217 micro algal cells are in average form.  
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**Table 3. Quantitative analysis on the microalgae isolated from the freshwater stations**

Station No.	Name of the Freshwater Ecosystem	Name of Microalgae	Quantitative	
			Size (Length)	Size (Width/diameter)
2	Stream, Brgy Libertad, Capoocan	<i>Asterococcus limneticus</i> G.M. Smith	10.2 um	22.3 um
		<i>Nostoc</i> sp.	4.6 um	4.1 um
		<i>Anabaena</i> sp.	6.9 um	4.6 um
		<i>Anabaena azollae</i> Strasburger	6.1 um	4.6 um
4	Lindog River, Brgy. Uyawan, Carigara	<i>Chlorococcum humicola</i> (Nageli) Rabenhorst	11.5 um	6.9 um
		<i>Stigeoclonium attenuatum</i> (Hazen) Collins	22.3 um	5.7 um
		<i>Synechococcus</i> sp.	8.2 um	5.6 um
5	Bislig River, Brgy. Bislig, Carigara	<i>Chlorella ellipsoidea</i> Gerneck	8.1 um	6.3 um
		<i>Chlorococcum humicola</i> (Nageli) Rabenhorst	10.3 um	6.5 um
		<i>Nostoc</i> sp.	4.6 um	4.1 um
		<i>Chroococcus limneticus</i> Lemmermann	8.3 um	6.1 um
7	Himanglos River, Brgy. Hilaba, Barugo	<i>Chlorella vulgaris</i> Beyerinck	7.2 um	5.9 um
		<i>Asterococcus limneticus</i> G.M.	10.2 um	22.3 um

		Smith		
		<i>Synechococcus</i> sp.	8.2 um	5.6 um
8	Canomantag River, Brgy. Canomantag, Barugo	<i>Chlorella vulgaris</i> Beyerinck	7.2 um	5.9 um
		<i>Anabaena azollae</i>	6.1 um	4.6 um
		<i>Synechococcus</i> sp.	8.2 um	5.6 um
10	Caray-caray River, Brgy. Caray-caray, San Miguel	<i>Gloeocystis ampla</i> Kutzing	10.1 um	9.2 um
		<i>Chlorococcum humicola</i> (Nageli) Rabenhorst	10.3 um	6.5 um
		<i>Oscillatoria</i> sp.	25.4 um	3.4 um
11	Lipasan Falls, Brgy. Pinarigusan, San Miguel	<i>Chroococcus dispersus</i> (Keissler) Lemmermann	4.3 um	3.5 um
		<i>Fragilaria brevisstrata</i> Grun	41.4 um	4.6 um
		<i>Navicula</i> sp.	10.3 um	6.5 um
13	Tula-an Falls, Brgy. Tula-an, Babatngon	<i>Gloeocystis ampla</i> (Kuetz) Lagerheim	10.1 um	9.2 um
		<i>Chlorococcum infusionum</i> (Schrank) Meneghini	12.8 um	6.8 um
		<i>Navicula cincta</i> (Ehrenberg) Ralfs	9.8 um	7.1 um
14	Busay Falls, Brgy. Busay, Babatngon	<i>Chlorococcum humicola</i> (Nageli) Rabenhorst	10.3 um	6.5 um
		<i>Fragilaria brevisstrata</i> Grun	41.4 um	4.6 um
		<i>Hormidium klebsii</i> G.M Smith	9.2 um	23 um
		<i>Oscillatoria</i> sp.	25.4 um	3.4 um

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For Station 2 which is the stream located at Brgy. Libertad, Capoocan, there were 4 microalgae isolated namely *Asterococcus limneticus* with length of 10.2 um and width of 22.3 um, *Nostoc* sp. with length of 4.6 um and 4.1 um and two (2) species of Genus *Anabaena* with length ranged from 6.1-6.9 um and both width of 4.6 um. With regards to Station 4, new species of microalgae were observed and isolated. These are *Chlorococcum humicola* with length and width of 11.5 and 6.9 um; *Stigeoclonium attenuatum* with length and width of 22.3 and 5.7 um and lastly *Synechococcus* sp. with length and width of 8.2 and 5.6 um. In Station 5, there are four (4) micro algal species where two (2) of them are new isolates in the said station namely *Chlorella ellipsoidea* (length=8.1; width= 6.3um) and *Chroococcus limneticus* (length=8.3; width=6.1um) and two (2) were already found at Station 2 and 4 namely *Nostoc* sp. and *Chlorococcum humicola* respectively. Furthermore, at Station 7, three (3) microalgae were examined such as *Chlorella vulgaris* (length=7.2; width=5.9 um) which is first time to be isolated in the said station, *Asterococcus limneticus*

234 and *Synechococcus* sp. *A. limneticus* and *Synechococcus* sp. were already isolated at  
 235 Station 2 and 4. Moreover, no new isolates were observed at Station 8. The three (3)  
 236 isolates found were the following: *Chlorella vulgaris*; *Anabaena azollae* and *Synechococcus*  
 237 sp. In addition at Station 10, three (3) isolates were surveyed, two (2) of them are new  
 238 isolates namely *Gloeocystis ampla* (length=10.1; width=9.2  $\mu$ m) and *Oscillatoria* sp.  
 239 (length=25.4; width= 3.4  $\mu$ m) and one (1) microalgae, *Chlorococcum humicola* was already  
 240 isolated at Stations 4 and 5. Uniquely, in Station 11, the three (3) microalgae observed were  
 241 new isolates namely *Chroococcus disperses* (length=3.5; width=4.3  $\mu$ m), *Fragilaria*  
 242 *brevistrata* (length=41.1; width=4.6  $\mu$ m) and *Navicula* sp. (length=10.3; width=6.5  $\mu$ m). In  
 243 another scenario, three (3) species of phytoplankton were also isolated at Station 13 where  
 244 in two (2) of them were first time to be studies and one (1) microalgae, *Gloeocystis ampla*  
 245 was already observed at the previous stations discussed. The two (2) microalgae are  
 246 *Chlorococcum infusionum* (length=12.8; width=6.8  $\mu$ m) and *Navicula cincta* (length=9.1;  
 247 width=7.1  $\mu$ m). Lastly, Station 14 has four (4) isolates but three (3) of the microalgae were  
 248 already examined from the previous stations and one (1) is a new isolate namely, *Hormidium*  
 249 *klebsii* (length=25.4; width=3.4  $\mu$ m). From the above discussion, most of the stations have  
 250 one (1) new micro algal isolates scrutinized. The most observed phytoplankton were  
 251 *Chlorococcum humicola* and *Synechococcus* sp. found in 3-4 stations. These microalgae  
 252 belong to Phylum Chlorophyta and Cyanophyta respectively. Species of Phylum  
 253 Bacillariophyta were also noted but most of them are found in only one (1) station.

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**Table 4. Qualitative Analysis on the Microalgae isolated from the freshwater stations.**

Station No.	Name of the Freshwater Ecosystem	Name of Microalgae	Qualitative			
			Color/Pigmentation	Shape	Arrangement	Unique Trait/s
2	Stream, Brgy Libertad, Capocan	<i>Asterococcus limneticus</i> G.M. Smith	Green	spherical	colonial	contains pyrenoids
		<i>Nostoc</i> sp.	blue-green	cells are globose	trichomes are chain-like	more akinetes
		<i>Anabaena</i> sp.	blue-green	cells are ellipsoid	trichomes are coiled	contents are granular
4	Lindog River, Brgy. Uyawan, Carigara	<i>Anabaena azollae</i> Strasburger	blue-green	cells are subglobose	trichomes are coiled	contents are granular
		<i>Chlorococcum humicola</i> (Nageli) Rabenhorst	Green	spherical	solitary	lateral notch and single pyrenoid
		<i>Stigeoclonium attenuatum</i> (Hazen) Collins	Green	cells are cylindrical	filaments are branched and elongated	prostrate portion of thallus little developed
		<i>Synechococcus</i> sp.	blue-green	oblong	solitary	no sheath

5	Bislig River, Brgy. Bislig, Carigara	<i>Chlorella ellipsoidea</i> Gerneck	Green	ellipsoidal	solitary	chloroplast folded over the cells
		<i>Chlorococcum humicola</i> (Nageli) Rabenhorst	Green	spherical	solitary	lateral notch and single pyrenoid
		<i>Nostoc</i> sp.	blue-green	cells are globose	trichomes are chain-like	more akinetes
		<i>Chroococcus limneticus</i> Lemmermann	Green	ovate	colonial	with mucilaginous envelopes
7	Himanglos River, Brgy. Hilaba, Barugo	<i>Chlorella vulgaris</i> Beyerinck	Green	spherical	solitary	chloroplast like a parietal cup
		<i>Asterococcus limneticus</i> G.M. Smith	Green	spherical	colonial	contains pyrenoids
		<i>Synechococcus</i> sp.	blue-green	oblong	solitary	no sheath
8	Canomantag River, Brgy. Canomantag, Barugo	<i>Chlorella vulgaris</i> Beyerinck	Green	spherical	solitary	chloroplast like a parietal cup
		<i>Anabaena azollae</i>	blue-green	cells are subglobose	trichomes are coiled	contents are granular
		<i>Synechococcus</i> sp.	blue-green	oblong	solitary	no sheath
10	Caray-caray River, Brgy. Caray-caray, San Miguel	<i>Gloeocystis ampla</i> Kutzing	Green	ovoid	colonial	embedded in unlamellated gelatinous envelopes
		<i>Chlorococcum humicola</i> (Nageli) Rabenhorst	Green	spherical	solitary	single pyrenoid
		<i>Oscillatoria</i> sp.	blue-green	elongated	filamentous	distinct sheath-like called calyptra
11	Lipasan Falls, Brgy. Pinarigusan, San Miguel	<i>Chroococcus dispersus</i> (Keissler) Lemmermann	Green	ovate	colonial	with mucilaginous envelopes
		<i>Fragilaria brevivtrata</i>	brown	linear	solitary	striae are short

		Grun				
		<i>Navicula</i> sp.	Brown	linear to elliptic	solitary	striations are transverse
13	Tula-an Falls, Brgy. Tula-an, Babatngon	<i>Gleocystis ampla</i> (Kuetz) Lagerheim	Green	ovoid	colonial	embedded in unlamellated gelatinous envelopes
		<i>Chlorococcum infusionum</i> (Schrank) Meneghini	Green	spherical	colonial	notch on one side and single pyrenoid
		<i>Navicula cincta</i> (Ehrenberg) Ralfs	Brown	linear	solitary	striations are transverse
14	Busay Falls, Brgy. Busay, Babatngon	<i>Chlorococcum humicola</i> (Nageli) Rabenhorst	Green	spherical	solitary	single pyrenoid
		<i>Fragilaria brevisstrata</i> Grun	Brown	linear	solitary	striae are short
		<i>Hormidium klebsii</i> G.M. Smith	Green	cells are cylindrical	unbranched filaments	Chloroplast a parietal plate
		<i>Oscillatoria</i> sp.	blue-green	elongated	filamentous	distinct sheath-like called calyptra

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For Table 3, a thorough discussion on the morphological characteristics of the 18 micro algal isolates that were studied in the 9 freshwater tributaries of Carigara bay. Most of the isolates are chlorophytes (green) and cyanophytes (blue-green) and few are bacillariophytes (brown algae). The brown algae are the following: *Fragilaria brevisstrata*, *Navicula cincta* and *Navicula* sp. These microalgae are linear to oblong, solitary in nature and have striations which are short and transverse. Furthermore, the blue-green algae are *Nostoc* sp., *Synechococcus* sp., *Oscillatoria* sp. and two (2) species of Genus *Anabaena*. The *Nostoc* sp. and *Anabaena* spp. are ellipsoidal to globose, with trichomes which are chain-like and coiled and with akinetes. On the other hand, *Synechococcus* sp. are solitary and oblong and *Oscillatoria* sp. are filamentous and elongated in nature. The difference of this 2 species is the absence and presence of sheath respectively. Lastly, the green algae has the highest in number in which seven (7) genera were isolated and studied. These are *Asterococcus limneticus*, *Chlorococcum* spp. and *Chlorella* spp. which are all spherical and the first species is colonial and the two (2) latter species are solitary in nature. In addition, *Stigeoclonium attenuatum* and *Hormidium klebsii* are also observed in which their cells are cylindrical and filaments are elongated but differ in terms of the unbranching and branching of their filaments respectively. Finally, *Chroococcus* spp. and *Gleocystis ampla* which are ovate and colonial but different in the contents of their envelopes which are mucilaginous

275 envelopes for the foremost species and lammellated gelatinous envelope for the latter  
276 species.

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#### 279 4. CONCLUSION

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281 Carigara Bay and its freshwater resources are bounded with different micro algal  
282 species. Phyla Bacillariophyta, Chlorophyta and Cyanophyta are 44, 37 and 19% of the total  
283 phytoplankton identified. Furthermore, there was a significant positive correlation between  
284 water pH ( $r = 0.499$ ;  $p = 0.001$ ), conductivity ( $r=0.519$ ;  $p=0.001$ ) and amount of phosphates  
285 ( $r = 0.446$ ;  $p = 0.003$ ) and a negative relationship between the amount of dissolved oxygen  
286 (Mean= 7.76; SD= 1.82) and total number of micro algal cells,  $r=-0.351$ ;  $p=0.023$ . Moreover,  
287 six (6) genera namely *Asterococcus*, *Anabaena*, *Nostoc*, *Chlorococccum*, *Synechococcus*  
288 and *Oscillatoria* were isolated, cultured and semi-mass produced for optimization procedure.

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