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The Community Structure of Heterotrophic Bacteria and Microalgae in an Artificial Reef (Tire Modules) in Dalipuga Iligan City (Mindanao, Philippines)

MARIA LUISA W. SASIL
PATRIA ROSALES-APAO

Abstract

The colonization of heterotrophic bacteria and microalgae community structure were assessed from the 7 year old artificial reefs (tire modules) deployed in Barangay Dalipuga, Iligan City at depths of 8 and 24 m. The community structure as used in this study refers to the composition, abundance and biomass. Twenty (20) genera of microalgae belonging to classes Bacillariophyceae, Cyanophyceae and Chrysophyceae with Navicula *as the most abundant were observed. Seventeen (17) bacterial isolates tentatively identified as* Bacillus sp., Pseudomonas sp. *and* Vibrio sp. *With four (4) species possibly related to* Vibrio parahaemolyticus, Vibrio alginolyticus, V. cholerae *and* V. mimicus *were identified. The abundance of these Gt bacteria in the marine environment is an indication of terrestrial contamination. There were similar trends of decreasing microalgal-bacterial interactions.*

Key Words: *colonization, artificial substratum, heterotrophic bacteria, microalgae community structure.*

Introduction

The characteristics of marine organisms as primary colonizers in any surfaces is widely known as in the phenomenon of biofouling (Austin 1988). The initial reactions for attachments between microbial cells and solid surfaces are not clear but are said to depend

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among others upon the nature or electrical changes of the surface and the microbial cells (Marshall, 1976). It is on this bases that various materials are developed into the sea accidentally and intentionally for several purposes additional substrate for rehabilitation of coral reefs, aggregation of fishes, and attachment for other organisms such as invertebrates. The installation of Artificial Reefs took off in the early 80's as one of the country's program on the coastal management but in the later 90's there was a moratorium for the reasons that ARs were not managed properly but became primarily instruments for aggregating fishes to increase fish catch. Several studies had been done on the ARs such as colonization of fishes, macroalgae, and other sessile organisms and the effect of different structural design of Ars on the fish colonization (Bohnsack and Talbot, 1970), however, little is known on the colonization of microorganisms on these substrates.

In any substratum the microalgae are especially important as they serve as a direct source of food for herbivorous fishes (Prince et.al., 1975; Prince and Gotshall, 1976; Prince and Gotshall, 1979) while dissolved organic matter released from these microalgae serves as nutrients for heterotrophic bacteria (Riquelme and Ishida, 1989). Heterotrophic bacteria in an artificial reef recycle nutrients that otherwise would have been lost to bottom sediments, and shorten and modify the food web to increase the reefs net production. They serve as food for smaller organisms such as the meiofauna and various ingesters. A great deal or work has been done on bacteria and microalgae in the water column (Wiebe and Smith, 1977; Ducklow et.al., 1986) but far less in the benthic environment. As part of the Artificial Reef Project of the Mindanao State University-Iligan Institute of Technology, this study was undertaken to determine the community structure of microalgae and heterotrophic bacteria as colonizers in tire modules deployed in 1989 in Barangay Dalipuga, Iligan City.

Materials and Methods

This study area is situated in Sitio Mapalad of Barangay Dalipuga, Iligan City (8 16 North Latitude and 124 15.2 East Longitude). Barangay Dalipuga has an area of 9710.562 km and is situated 12.2 km north of Iligan City bounded by Lugait, Misamis Oriental to the north, to the south by Barangay Kiwalan, and to the west by Iligan Bay (Fig. 1). In this area a total of forty (40) tire reef modules consisted of six tires arranged in four levels or storeys with one tire placed horizontally at the base and three tires positioned vertically at the top of the base. One tire was placed on top of the second level and a last single tire was placed horizontally on top of the third level of tires. All the tires were fixed by nylon ropes (Fig. 20). Of the forty (40) tire modules, two (2) modules one from the shallow (8 m) and one from the deep (24 m) portions were considered as the sampling stations. It was assumed that the two stations would provide a representative sample of all tire modules at the same depth.

Three sampling collection of epibiotic samples were made from November 1996-January 1997 and a total of eighteen (18) samples were collected. In each module the samples were taken randomly from the surface of the tires at the second level as a matter of convenience and with the assumption that the colonization of bacteria and microalgae were similar in all tire surfaces. Epibiotic samples were taken by scraping the tire substrates (Prasad et.al., 1993) using a modified plastic sample, open at both ends. During collection one end was placed on the tire surface while the other was covered with a cellophane bag and the enclosed epibiotic sample was thoroughly scraped using a finger. The scrapings were made sure to be collected inside the properly labeled bag and were sealed after collections before bringing to the surface. The scraped materials were transferred to plastic bottles and were preserved by adding 4% buffered formalin except for the samples that were used for bacterial plate counting. Microalagae were identified up to the genius level only using the taxonomic keys of Yamaji (1982) and Newell and Newell (1963). The abundance of microalgae was microscopically determined using haemacytometer counting chamber. The microalgal count was expressed as units/m2 using the formula of Martinez et.al. (1975). The biomass of microalgae was determined through chlorophyll a analysis following the procedure of Parsons and Strickland (1963). In determining the cell density of bacteria, acridine orange direct count (AODC) by epiflourescence microscopy was employed (Kepner and Pratt, 1964). The biomass of microbial community was determined through protein analysis following the method of Lowry et.al. (1951). The bacterial isolates were subjected to morphological (gram, capsule, spore staining and motility) and physiological tests (oxidase, oxidation/fermentation, nitrate reduction, MR-VP, Arginine dehydrolase/Lysine and Omithine decarboxylase., Influence of 1% NaCl on growth in nutrient broth and Sensitivity to o/129 antibiotic). Tentative identification of the bacterial isolates was based on various authors (Buchanan and Gibbous, 1974; Raymundo and Zamora, 1991; Shewan et.al., 1963).

Results and Discussion

A total of eighteen (18) samples were collected from the two tire modules on the three sampling periods at 8 m and 24 m depth from November-December 1996 and January 1997. Twenty (20) genera of microalgae were recorded belonging to Classes Bacillariophyceae, Cyanophyceae and Chrysophyceae (Table 1). Order Centrales was represented by Coscinodiscus and Rhizosolenia, Bacteriastrum, *Chaetoceros* and Thalassiosira. Order Pennales was represented by Licmophora Cocconeis, Navicula, Diploneis, Mestogloia, Pleurosigma, Nitzchia, and Amphiprora. Two genera belonging to Order Silicoflagellata are Distephanus and Pyrocystis. Only one member belonging to Division Cyanophyta (Cyanobacteria) represented by Anabaena was observed in the tire modules. Navicula was the most abundant (147 units/m^2)followed by Nitzchia (138 units/m^2) at 8 m depth for the three sampling period (Table 2). Similarly at 24 m, Navicula and Nitzchia accounted for the most biomass but he cell density was relatively lower than at 8 m amounting to only 117 units/m^2 and 102 units/m^2, respectively Grammatophora and Licmophora (1.5 units/m^2), Pyrocystis (1.5 untis/m^2), Bacteriastrum (1.5 units/m^2), Amphiprora (1.5 units/m^2) and Licmophora (1.5 units/m^2) had the least total cell density at 8 m and Thalassiotrix (1.5 units/m2), Pleurosigma (1.5 units/m2), Rhabdonema (1.5 units/m2), Anabaena (1.5 units/m2) and Distephanus (1.5 units/m2) at 24 m.

Fragilaria and Licmophora were found at 8 m but were not found at 24 m depth. In contrast, Distephanus was found at 24 m but were not found at 8 m depth. These depth difference may be related to light quantity as microalgae are photosynthetically saturated at different light levels (Ferguson et.al., cited in Vesberg and Versberg, 1981). Other factors such as grazing preferences by microalgal grazers on the specific genus or species of microalgae may have influenced the depth differences.

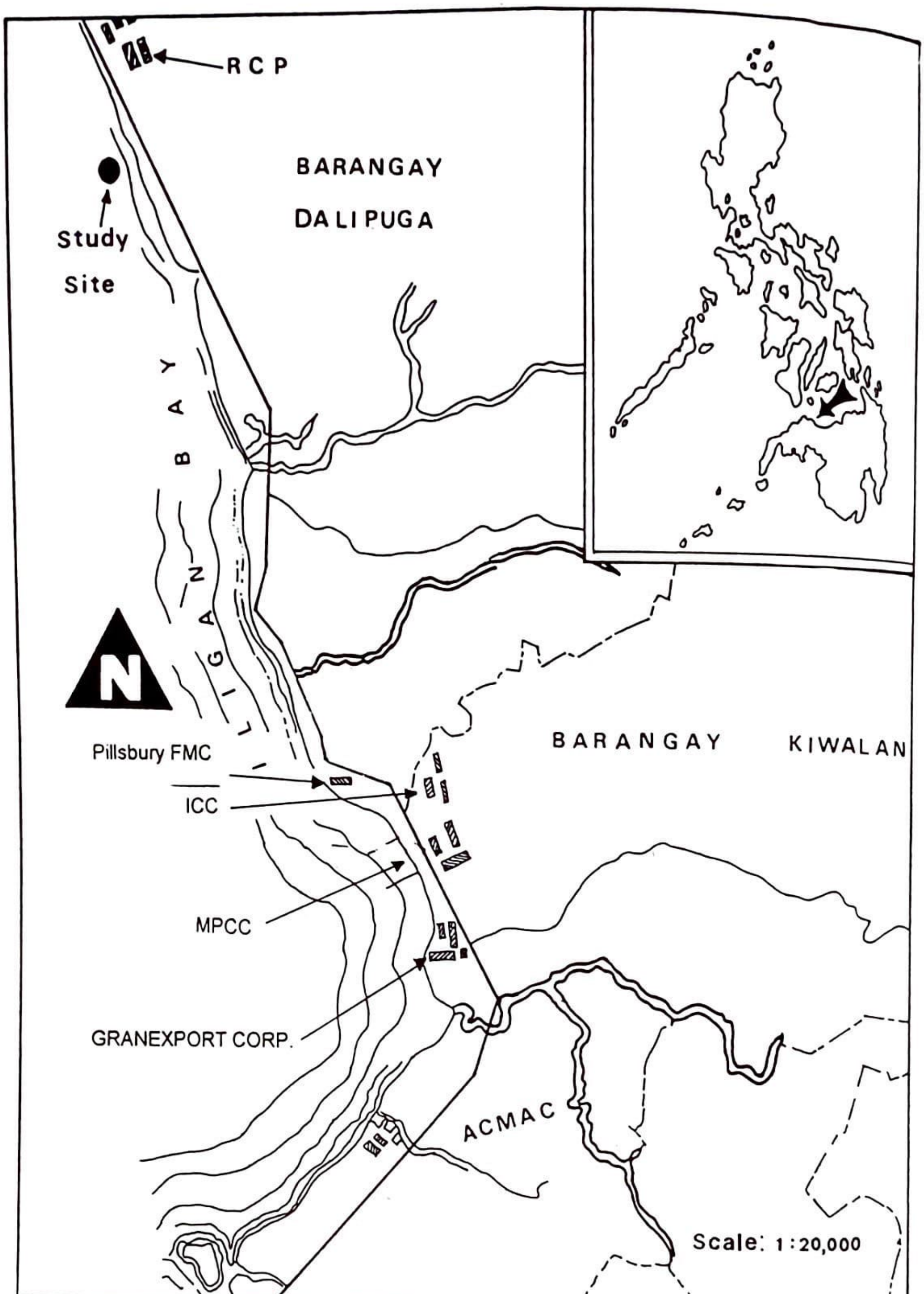

Figure 1. *Location of the study site in Sitio Mapalad, Barangay Dalipuga, Iligan City (Mindanao, Philippines)*

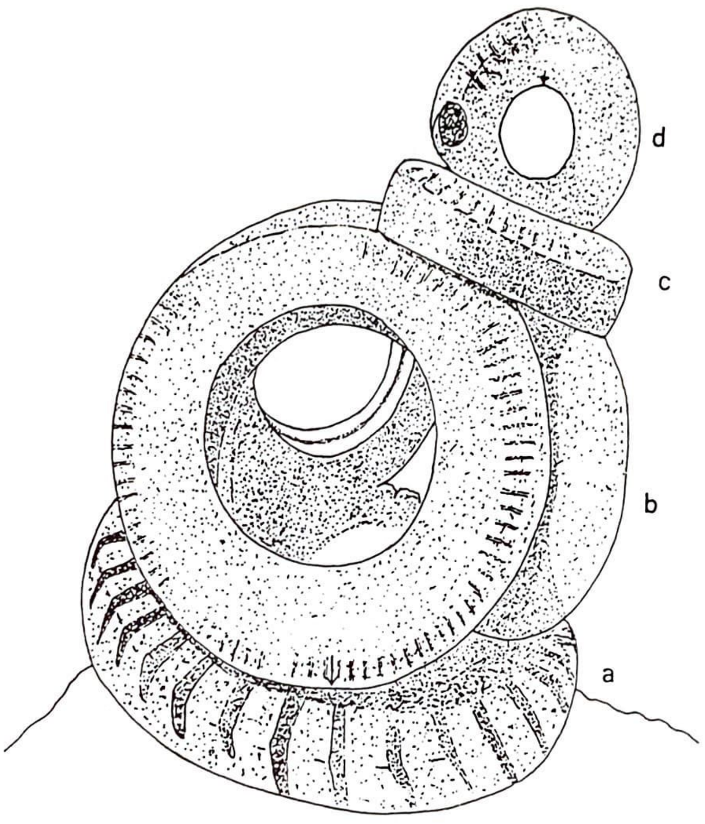

Figure 2. *Illustration of a tire module with dimensions of the tires a) base tire 45 x 50 cm b) second level 24.13 x 20.32 cm c) third level 17.78 x 19.05 cm d) topmost level 13.97 x 13.97 cm.*

Table 1. *Classifcation of microalgae found in the tire reefs at Sitio Mapalad, Barangay Dalipuga, Iligan City at 8 m and 24 m depth during the entire sampling period.*

CLASS	ORDER	SUBORDER	FAMILY	GENUS
Bacillariophyceae	Centrales		Coscinodiscaceae	*Coscinodiscus*
		Rhizosoliniineae	Rhizosoleniaceae	*Rhizosolenia*
			Bacteriastraceae	*Bacteriastrum*
		Biddulphiineae	Chaetoceraceae	*Chaetoceros*
			Thalassiosiraceae	*Thalassiosira*
	Pennales	Biraphidineae	Naviculaceae	*Navicula*
				Diploneis
				Mestogleia
				Amphiprora
				Cocconeis
				Pleurosigma
			Nitzchiaceae	*Nitzschia*
		Araphidineae	Fragilariaceae	*Fragilaria*
				Thalassiothrix
			Tabellariaceae	*Rhabdonema*
				Grammatophora
				Licmophora
			Siliicoflagillidae	*Distephanus*
Chrysophyceae	Silicoflagellata			*Pyrocystis*
Cyanophyceae	Oscillatoriales		Nostocaceae	*Anabaena*

Table 2. *The total cell density (units/m²;10⁶) of microalgae (genus only) observed at the tire modules at 8 and 24m depth for the entire sampling period (November 1996 – January 1997).*

Genus	8 m Total cell density Units/m2 (x10⁶)	24 m Total cell density units/m2 (x 10⁶)
1. *Navicula*	147	117
2. *Nitaschia*	138	102
3. *Chaetoceros*	51	17
4. *Cocconeis*	20	4.5
5. *Mestogloia*	14	1.23
6. *Thalassiothrix*	6.6	1.5
7. *Coscinodiscus*	12	6.6
8. *Diploneis*	14	8.7
9. *Pleurosigma*	19	1.5
10. *Thalassiosira*	8.7	5.1
11. *Rhabdonema*	8.7	1.5
12. *Anabaena*	7.2	1.5
13. *Grammatophora*	1.5	4.5
14. *Rhizosolenia*	3.0	3.0
15. *Pyrocystis*	1.5	4.5
16. *Bacteriastrum*	1.5	5.1
17. *Distephanus*	0	1.5
18. *Amphiprora*	1.5	3.0
19. *Fragilaria*	3.6	0
20. *Licmophora*	1.5	0
TOTAL	460.3	300.8

The results showed that Navicuala was the most dominant genus of microalgae in the tire reefs. Similarly, in a study on a concrete reef module installed in 1996 at Brgy. Samburon and Brgy. Magoong (Linamon, Lanao del Norte) *Navicula* was observed to be the most abundant colonizer (Mangorsi, 1997; Apao and Sasil, 1996, Table 3). From the data in Table 3, one can infer that during the early and late stage of microalgal colonization on different substrates in an artificial reef, the genera *Navicula* and *Nitzchia* predominate. In view of the small size of these genera they are known to outnumber other microalgae and they contribute to most of the algal biomass (Reise cited in John et.al., 1992) The abundance of these genera may possibly be attributed to some inhibitory substances produced by these microalgae thus preventing the faster colonization and growth of other microalgal species (Ferguson et.al. cited in Vermberg and Vermberg, 1981).

The microalgal biomass and cell counts in the tire reefs were highest at 8 m than at 24 m depth throughout the sampling period. The decrease in depth coincided with the decrease in bacterial counts as microalgae are also dependent on bacteria through photophagotrophy (Kimura and Ishida cited in Riquelme and Ishida, 1989, Bird and Kalff, 1986), bacterial products such as vitamins (Furuki et.al., 1985) siderophores (Murphy et.al., 1976_ and other trace elements. The concentration of chli a coincided with the total microalgal counts indicating similar trends for the biomass and cell counts (Fig. 3) The bacterial cell number as well as the total epibiotic biomass as measured by protein concentration was highest at 8 m than 24 m depth (Fig. 4 and 5)

Table 3. *A comparison of the microalgal community structure showing the number of genera, total cell density (cells or units/m²) and the dominant genus during different colonization period in two types of artificial reefs in the coastal waters of Iligan Bay.*

Location	Type of AR	Colonization Period	No. of Genera	Total Density (cells/m²)	Dominant Genera	Source
Magoong Lanao del Norte	Concrete	7 days (early)	12	3.3×10^{11} 2.4×10^{11}	*Navicula* *Nitzchia*	Rosales Apao and Sasil (1996)
Samburon Mangorsi Lanao del Norte	Concrete	7 days (early)	6	6.9×10^{13} 6.7×10^{13}	*Navicula* *Nitzchia*	(1997)
Mapalad, Dalipuga, Iligan City	Used tire	7 years (late)	20	2.64×10^{8} 2.40×10^{8}	*Navicula* *Nitzchia*	Sasil and Rosales-Apao this study

The depth-differences of the bacterial cell densities and biomass may be attributed to the exudates of extra cellular organic compound released by microalgae present on the tire surfaces which can be utilized by the bacteria as an energy and carbon source (Williams and Yentsch, 1976; Larson and Hangstrom, 1982; Wolter, 1982; Coveney, 1982; Cole et.al., 1982; Lancelot, 1984; Brock and clyne, 1984; Sondergaard et al., 1985). The contribution of dissolved organic carbon released by microalgae to bacterial production varied from 20 to 90% (Coveneym 1982; Larsson and Hangstrom, 1982' Sondergaard et.al., 1985). All of those studies have demonstrated that extracellular organic carbon is important for supporting heterotrophic bacterial growth in natural aquatic environments. As illustrated in Fig 6, the decreasing trend in the bacterial cell density with depth coincided with that of the microalgal counts. This means that as the abundance of microalgae increases the cell density of the bacteria also increases and vice versa.

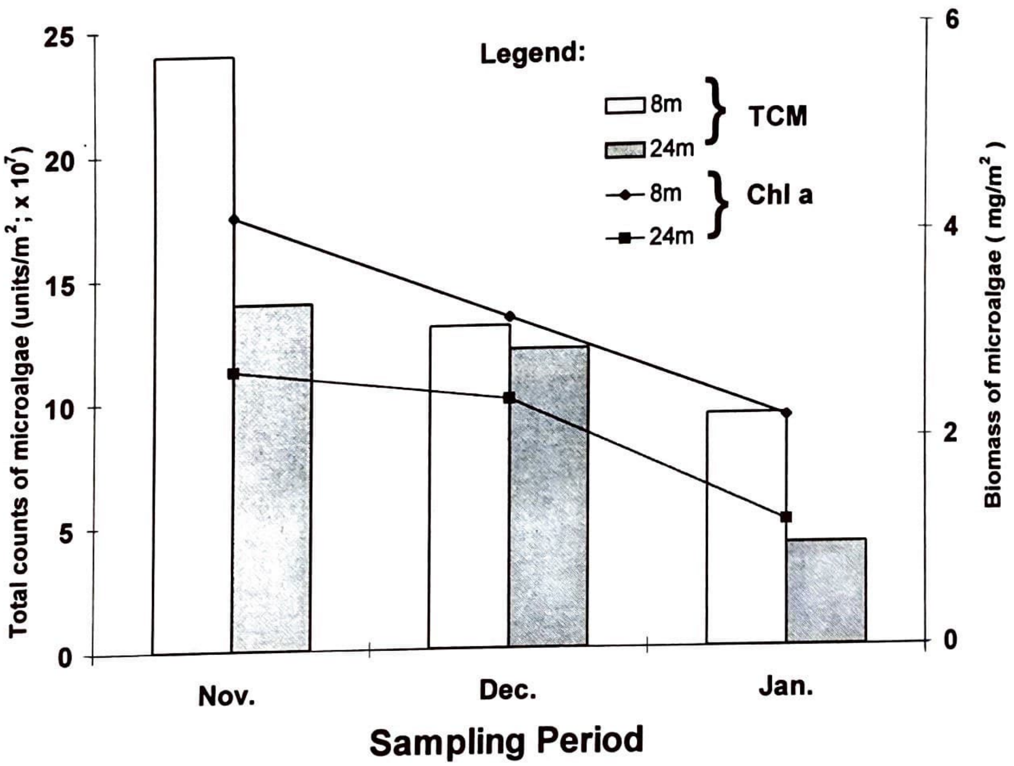

Figure 3. *Relationship between the total counts of microalgae (TCM, units/m²) and the biomass as determined by chlorophyll concentration (mg/m²) in the tire reefs collected at 8 m and 24 m depth for three sampling periods (Nov. 1996 – Jan. 1997) at Sitio Mapalad, Barangay Dalipuga, Iligan City.*

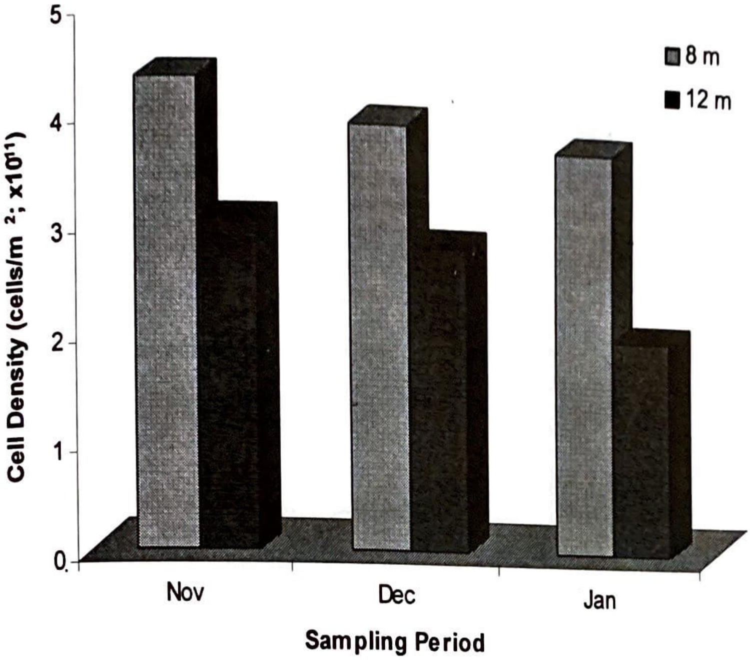

Figure 4. Cell density (cells/m²) of epibiotic bacteria on tire reefs collected at 8 m and 24 m depth for three sampling periods (Nov. 1996 – Jan. 1997) at Sitio Mapalad, Barangay Dalipuga, Iligan City.

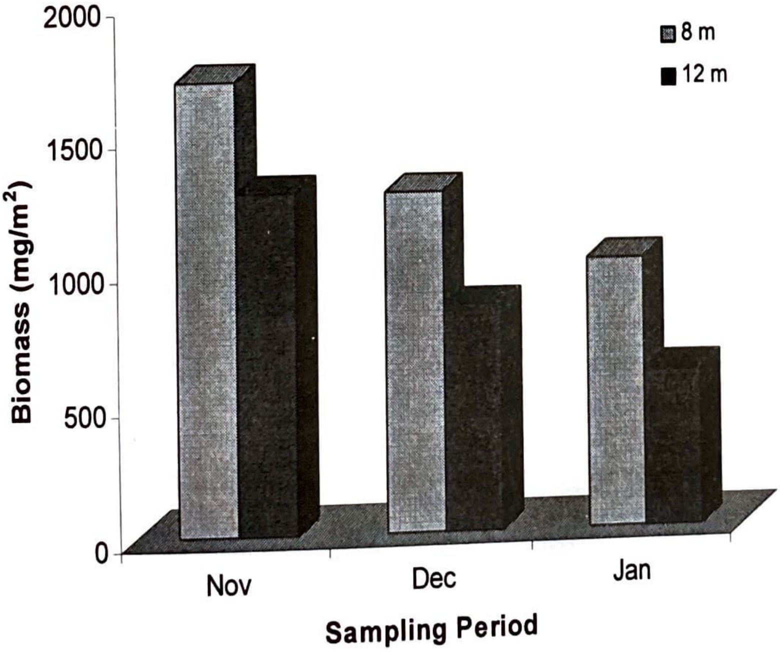

Figure 5. Biomass (mg/m²) of epibiotic bacteria on tire reefs collected at 8 m and 24 m depth for three sampling periods (Nov. 1996 – Jan. 1997) at Sitio Mapalad, Barangay Dalipuga, Iligan City.

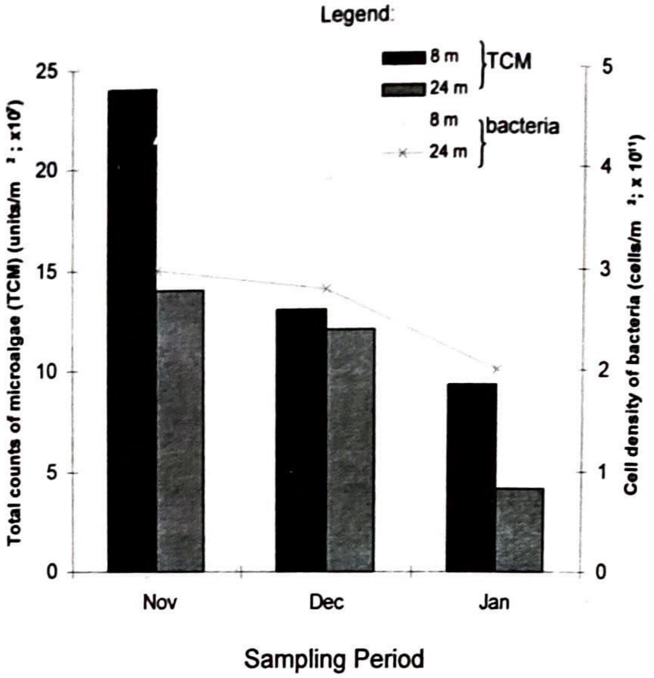

Figure 6. Relationship between the total counts of microalgae (TCM, units/m^2) and the cell density of bacteria (bacteria, cells/m^2) in the tire reefs collected at 8 m and 24 m depth for three sampling periods (Nov. 1996 – Jan. 1997) at Sitio Mapalad, Barangay Dalipuga, Iligan City.

From these results it may be inferred that an interrelationship between the microalgae and bacteria may occur in the tire reefs as algal-bacterial interactions are common in aquatic environments (Sieburth, 1968; Kogure et.al. 1979) and that this relationship may be considered symbiotic. Substances produced by bacteria such as vitamins which cannot be synthesized by microalgae are utilized by the latter and microalgal exudates are taken up by bacteria (Rheinheimer, 1984).A total of seventeen (17) bacterial isolates from the tire substratum of which three (3) genera were tentatively identified as *Bacillus* sp., *Pseudomonas* sp., *Vibrio* sp., and four (4) species tentatively related to *Vibrio parahaemolyticus*, *Vibrio alginolyticus*, *Vibrio cholerae* and *Vibrio mimicus* (Table 4). Although the presence of *Vibrio* species were observed in the tire reef module this does not necessarily imply that the coastal waters of Mapalad are unsafe especially for bathers since 99.9% of the marine bacteria are harmless and are not pathogens (Rheinheimer, 1992). Majority of the bacteria obtained were gram-positive and were identified to be *Bacillus* sp., in contrast to the general observation of the dominace of gram-negative bacteria in marine environments (Rheinheimer, 1992). The major occurrence of gram-positive bacteria maybe brought about by terrestrial contamination probably due to the extensive dredging and reclamation activity at the neighboring coastal barangay as these bacteria are not typically marine but sporeformers of terrestrial origin.The tire substratum offers a good surface for the colonization of fouling organisms which are naturally found in the water column. The biofilm layer produced by the first colonizers (Wahl, 1989) afford a suitable substrate for the subsequent attachment of other bacteria and microalgae through our results showed the presence of colonizers which may be dominant during the early microalgae and heterotrophic bacteria as shown by the similar trends of biomass and counts between the two groups. Positive interactions as well as negative interactions could be assumed between these organisms resulting to the specific composition and abundance in such a small ecosystem. Natural biota of the marine waters were not the only colonizers in the tire substrate but terrestrial vibrios as well illustrating contamination of the waters. Future studies on monitoring of the terrestrial vibrio content in any material submerged underwater will be of use in determining the extent of contamination from terrestrial origin.

Table 4. Morphological and physiological characteristic of seventeen (17) bacterial isolates in Plate Count (PCA) agar. Samples were taken from the tire reefs at Sitio Mapalad, Barangay Dalipuga, Iligan City

Characteristics	BACTERIAL ISOLATES																
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Morphological																	
a. motility	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
b. gram reaction	-	+	+	+	+	+	-	-	+	-	+	+	+	-	-	-	-
c. size (µm)	3.0	4.5	4.5	4.5	6.0	3.0	1.5	3.0	4.5	1.5	4.5	3.0	3.0	3.0	3.0	6.0	3.0
d. capsule	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
e. spore	-	+	+	+	+	+	-	-	+	-	+	+	+	-	-	-	-
f. arrangement	s	s-c	s-c	s-c	s-c	s	s	s-c	s	s-p	s-c	s-c	s-c	s	s	s	s
g. shape	rod	rod	Rod	rod	rod	rod	rod	rod	rod	Rod	rod	rod	rod	rod	Rod	rod	rod
Physiological																	
a. Cytochrome oxidase	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
b. Hugh and Leifson Medium																	
oxidative	+	-	-	-	-	+	-	-	+	-	-	-	-	-	-	-	-
fermentative	-	+ acid	+ acid	+ acid	+ acid	-	+ acid	+ acid	-	+ acid	+ acid	+ acid	+ acid	+ acid	+ acid	+ acid	+ acid
c. MR-VP Medium																	
MR														+	+	+	+
VP														-	+	+	-
d. Nitrate Reduction														+	+	+	+
e. Growth in Nutrient Broth with																	
0% NaCl														-	-	+	+
1% NaCl														+	+	+	+
f. Sensitivity to 0/129 (vibriostatic agent) 150 µg																+	
Tentative Identification	Ps	Ba	Ba	Ba	Ba	Ba	Ps	Vib	Ba	Ps	Ba	Ba	Ba	V pa	V al	V ch	V mi

Legend:

+ positive/presence
- negative/absence
V al - *V. alginolyticus*
s-c - single, chain
s-p - single, pair
V ch - *V. cholerae*
Ps - *Pseudomonas*
Ba - *Bacillus*
V mi - *V. mimicus*
Vib - *Vibrio*
V pa - *V. parahaemolyticus*

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