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Abstract

The main objective of this research was to study the marine bacteriology of the coast of North Sulawesi. The study was accomplished by calculating the abundance of coliform, heterotrophic, and pathogenic bacteria, and analyzing the coexistence relationship between bacteria and phytoplanktons. This research, which included the sampling and laboratory works, has been carried out on 25 – 28 October, 2000. The results suggested that the abundance of each bacteria was as follows: *coliform* bacteria range between 227-5940 cfu/100 ml with averages 1814.1 cfu/100 ml, found in all stations; *heterotrophic* bacteria range between (1-82) x 10^3 cfu/ml with averages 12.1 x 10^3 cfu/ml, it was high density and has association with phytoplankton *Trichodesmium thieubautii.* It was also found 6 species of pathogen bacteria e.g. *Aeromonas, Citrobacter, Proteus, Pseudomonas, Yersinia* and *Shigella*. The presence of coliform and pathogen bacteria was indicator of low quality of the seawater in the sampling area. Based on bacteriological study, the North Sulawesi Coastal is not suitable for aquaculture and need treatment and controlled for further coastal exploitation.

Keywords: bacteria, distribution, abundance, relationship

1. Introduction

Indonesia is facing dualism in developing natural resources particularly from the marine resources including the coastal resources. Most of the coastal potential located in outside Java and Bali islands, particularly in the eastern part of Indonesia, have not been well developed or never been developed. On the other hand, in the western part of Indonesia, the coastal resources have been intensively exploited, and there is an indication of over-exploitation that in turn causes water pollution, coastal abration, and physical degradation on the coastal habitat such as mangrove, reef and seagrass. A sectoral approach, which has been conducted to solve the problem, did not give good results. Therefore, the implementation of an integrated planning locally and regionally to develop and manage the marine resources becomes very important.

The studies on marine pollution utilizing bacteria have been extensively conducted since bacteria are good pollution indicator to determine the quality of marine environment. In 1977 WHO (World Health Organization) has established a standard parameter to measure water pollution utilizing coliform, pathogenic bacteria and heterotrophic bacteria [1]. Coliform bacteria are found in human intestinum from which they are spread out and contaminate aquatic environment through human excretion (faeces). Furthermore, pathogenic bacteria in the marine or aquatic ecosystem are sourced from the human beings or animals. Temperature and water salinity are also the factors that influence the variation in number of density of the coliform and heterotrophic bacteria within the marine ecosystem [2].

The dynamic of bacteria's growth particularly heterotrophic bacteria within a marine ecosystem relates to the growth of phytoplankton [3], while the growth phytoplankton is influenced by temperature, light and water salinity. Other limitation factor to growth is nutrients including nitrogen and phosphorous. The mutual relationship between heterotrophic bacteria and phytoplankton is an important factor in the energy transfer within the marine food web whereas phytoplanktons, as primary productivity, fix carbon through photosynthesis and making them available for higher trophic levels. Determination of density (number of individuals per volume) of phytoplankton is an adequate

means of getting an approximate measure of primary productivity of marine environment [4]. Phytoplanktons are also used as indicators of environmental conditions within the marine environment because their sensitivity to changes in nutrient levels and water quality conditions.

The objective of this research was to study marine condition by calculating the densities of coliform, heterotrophic, pathogenic bacteria, and phytoplankton. This research, which was part of the oceanographic research project of Puslit-Oseanografi LIPI, has been conducted on 25-28 October, 2000 to the seawater along the coast of North Sulawesi province in Indonesia. Furthermore, the North Sulawesi coastal area has been selected by the government of Indonesia as a Marine Research and Development Areas (KAPPEL) of the Research Center for Oceanography – Indonesian Institute of Science, because this area was assumption become over exploitation by domestic activity.

2. Materials and Methods

2.1. Samples Collection

Figure 1. Twenty-nine sampling stations designed for this research was located in area of interest along the costline of North Sulawesi, such as at the mouth of Bitung-Newmont (BN) area including Stations 1 to 9, Manado-Amurang-Bolaanguki (MAB) Bays including Stations 10 to 14, Kwandang Bay (KB) including Stations 19 to 32. The water depth is in the range of 3 to 2300 meters. The samples were obtained from the surface of the seawater (photic zone) by using a "water sampler" during the cruise on 25-28 October, 2000. The samples were collected in 200 ml sterilized bottles for further analyses in the BJ.VIII. Laboratory and Microbiology Laboratory in Jakarta.

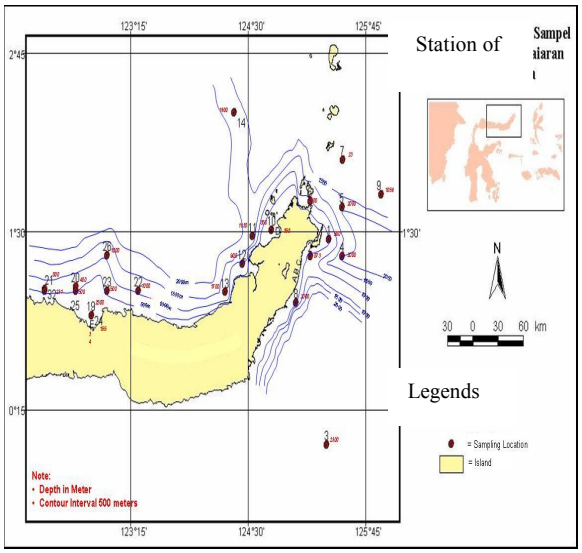

Figure 1. A map showing sampling locations (stations) for microbiology with bathymetry of the North Sulawesi waters. Contour interval is 500 meters. The sea bottom of the Station 19 is showing a very deep that may relate to sub sea growth faulting or other sea-bottom geomorphology

2.2. Isolation, Preparation and Counting Techniques

The Isolation of bacteria was carried out in BJ.VIII laboratory and all of the analyses (purification and identification) were conducted in the Microbiological Laboratory of PUSLIT-Oceanography-LIPI, Jakarta by using Membrane Filter techniques, Total Plate techniques and Selective medium methods. The pathogenic bacteria and phytoplankton were identified up to genus name. Detailed description of each methods and techniques are as follows:

1. A Membrane Filter Technique followed the methods of the American Public Health Association [5] was applied to calculate coliform bacteria within the samples. An MF-Endo agar was used for medium to grow bacteria strains. Bacteria strains were incubated at 35^0C ± 0.5^0C during 24 hours.

2. A Total Plate Count (TPC) Technique was used to calculate the number of heterotrophic bacteria within the seawater samples. Bacteria strains were inoculated by "Pour Plate Method" in a Marine-agar E-2216 (Difco) with the following

procedure: 1 ml of water sample was diluted with buffer phosphate until 10^{-3} in a glass tube, and then was pour onto Marine agar E-2216 (Difco) plates with 3 times replication followed by incubation for 5-7 days at room temperature.

3. The pathogenic bacteria were isolated from the water samples in three steps of isolation as follows: firstly the water samples was cultured in a selective medium of Selenith-broth for growth of pathogenic bacteria and TCBS-agar for growth of *Vibrio* bacteria; then secondly the bacterial strains were transferred in a medium of XLD-agar based on the World Health Organization [1] method; and thirdly the cultures were transferred in a medium of TCBS-agar based on the methods of Barrow & Miller [6].

4. Phytoplanktons were collected from the seawater by using KITAHARA net that the dimension as follows: 31cm in diameter of opening, 120cm of length, and 20µm of mesh. Phytoplanktons were counted based on the number of each individual counting.

3. Results and Discussion

3.1. Coliform bacteria

Twenty nine water samples taken from near the surface of seawater have been studied for coliform bacteria's density. The coliform bacteria's density ranges widely from 2 – 59 cfu / 100 ml with the average of 19 cfu/ 100ml. Figure 2 shows that the maximum number of coliform bacteria was found at the station-24 (Kwandang Bay), while the minimum number of coliform bacteria was found at the station-22.

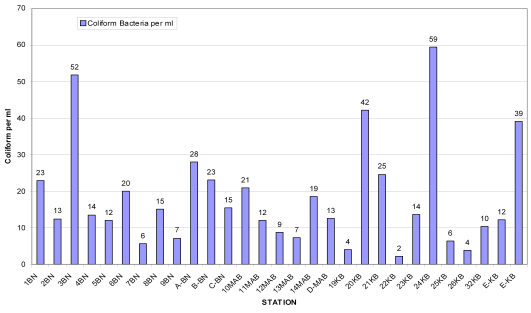

Figure 2. Number of Coliform bacteria's colonies per 100 ml sample in each station. The average density of coliform bacteria is greater in BN and MAB areas that may reflect greater contamination in this area. BN = Bitung-Newmont Area; MAB = Manado-Amurang-Bolaanguki bay; KB = Kwandang Bay

In general, the density of coliform bacteria in the three areas (Kwandang Bay, BN and MAB) showed a significant differentiation in terms of distribution and density. In the BN and MAB bay Areas, coliform bacteria are widely distributed with high average number of 12 colonies per ml. The lowest density was found in the station 7 and 9, while the higher density, which is approximately 52 colonies per ml, was found in the station 3. This result is very interesting because the sampling location of the Station-3 is approximately 60 Km from the shoreline. Sea-bottom morphology together with current direction may have swept the bacteria from nearshore area to the open marine area. In addition, the BN area is known as an industrial area that can be a potential source of coliform bacteria. In the Kwandang Bay, the average density of coliform bacteria is 8 colonies per ml. The density of coliform bacteria in this area was widely varies from the range of 2 to 59 colonies per ml. The greater number of coliform was found in the nearshore where this area is very close to inhabitant's area as a main source of coliform bacteria.

The presence of coliform bacteria within this area may relate with bad sanitation "management" from the surrounding area. The other sources of coliform bacteria is domestic and non-domestic (industry) sewages as seen in several stations near by Bitung, Manado Bay, and Kwandang Bay. Moreover, the growth of coliform bacteria in these areas is also determined by the availability of nutrient that may come from sewage effluent.

Mehlman [7] and Thayib [8] has defined that bad quality aquatic environment is characterized by high density of bacteria such as *Coliform, Fecal coli* and *Fecal streptococcus*. In addition, the U.S. Environmental Protection Agency [9] and APHA [5] recommends that the density of coliform bacteria in the water for public used should not exceed 200

colonies/ml, while Indonesian Standard of Marine Environment Quality (Standard Baku Mutu Lingkungan Laut, 1988) has established a standard that the density of coliform bacteria for the hatcheries or other aqua cultures should not exceed 10 colonies/ml. Therefore, the marine along the coast of North Sulawesi is still moderately good except for hatcheries and aqua culture.

3.2. Heterotrophic Bacteria

Twenty nine samples have been studied for heterotrophic bacteria's density. The results show that heterotrophic bacteria occur in a wide range of 4,000 – 888,000 cfu / ml. As seen in Figure 3, there is a significant different in average density between the BN area and the MAB and KB areas.

In the BN area, the average density of heterotrophic bacteria is 269,000 colonies per ml sample, while in other areas it is 110,000 colonies per ml sample. In Stations C, D, E, and 24, heterotrophic bacteria occur in very low density (4,000 to 16,000 colonies per ml), while in the Station 1, they occur in very high density (888,000 colonies per ml).

Heterotrophic bacteria in a marine ecosystem determine the productivity of the ecosystem in which the primary productivity by phytoplankton is dependent upon the number of dissolved nutrient contents. In addition, as decomposer and balancing agents for all aquatic life [10], heterotrophic bacteria are dependent upon the concentration of raw organic materials in the environment. These organic materials sourced from the terrestrial origin were brought by a river to a marine ecosystem, and will be decomposed into nutrients required by phytoplankton and other higher trophic level. Furthermore, heterotrophic bacteria in a marine ecosystem develop a

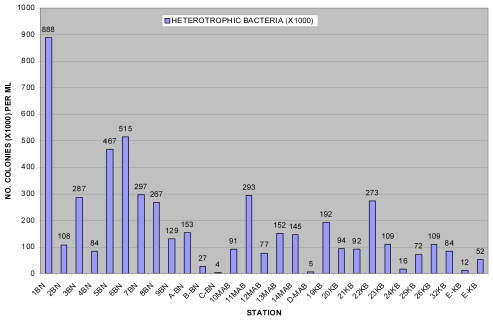

Figure 3. Number of Heterotrophic bacteria's colonies per ml of sample. The average density of heterotrophic bacteria in the Bitung-Newmont area is greater than that in other area, which may reflects the nutrient richness of the Bitung-Newmont area. BN = Bitung-Newmont Area; MAB = Manado-Amurang-Bolaanguki bay; KB = Kwandang Bay

symbiotic association with phytoplankton called as algaecidal bacteria [2]. As a result, the higher density of heterotrophic bacteria as seen in the BN area may be an indicative of nutrient richness. 3.3**.** Phytoplankton.

Fourteen samples from 14 stations have been studied for phytoplankton study. Phytoplanktons were identified up to the family group only, such as Dinoflegellatae and Diatomae. The number of dinoflagellate, diatome and other phytoplankton is listed in table-3.

The density of phytoplankton within the study area was found vary widely within the range of 330 – 32,737 organisms/ml (Figure 4). The phytoplankton density graphic in Figure-4 reveals that the lower density of phytoplankton with 330 – 1,300 organisms/ml was found in the nearshore of BN area, while the higher density of phytoplankton with 5,000 – 32,000 organism/ml was found in MAB and KB areas and in stations 5,6 and 8 in the BN area.

Phytoplanktons are the foundation of the marine food chain, and are important factor in primary productivity. Phytoplankton is primary producers that have ability to fix carbon through photosynthesis, and making them available as a food source for higher trophic levels.

Figure 4 shows a comparison between phytoplankton and heterotrophic bacteria populations, and Phytoplankton-Heterotrophic Bacteria Ratio (Ph/HB Ratio) in the study area. The Ph/HB ratio is utilized in this study to determine the quality and productivity of marine ecosystem by measuring the ratio of phytoplankton and heterotrophic bacteria population in a certain period of time. In normal condition, heterotrophic bacteria in a marine ecosystem

develop a symbiotic association with phytoplankton [2]. In addition, heterotrophic bacteria produce nitrogenase that can be utilized as a food source by phytoplankton [11] and other higher trophic level. Accordingly, the higher number of heterotrophic population in a certain environment will indicate the nutrient rich environment. Heterotrophic bacteria utilize organic materials in the environment and covert them into nutrients that can be an important food source for phytoplankton and other higher trophic level organism. However, the results show that Ph/HB ratio in the BN area is much lower than the ratio in the MAB and KB areas.

There are several possibilities including organic materials supply from terrestrial dumped into the sea, intensity of light penetrating the marine, and the presence of pollutant that may cause phytoplankton can not grow well in the BN area.

Diatomae and Dinoflagellate are two important phytoplanktons in the study area. This phytoplankton will compete each other to obtain nutrient [12].

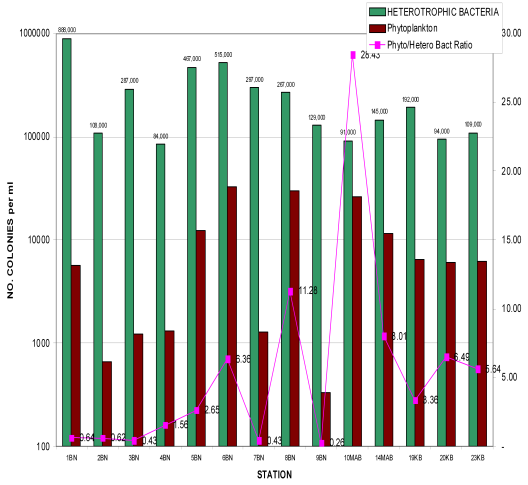

Figure 4. Comparison between the number of heterotrophic bacteria population and phytoplankton in the study area. This graphic also shows the Ph/HB Ratio that may indicate the nutrient availability within the environment. Heterotrophic Bacteria are decomposer bacteria that provide nutrient for other living organism within an aquatic environment

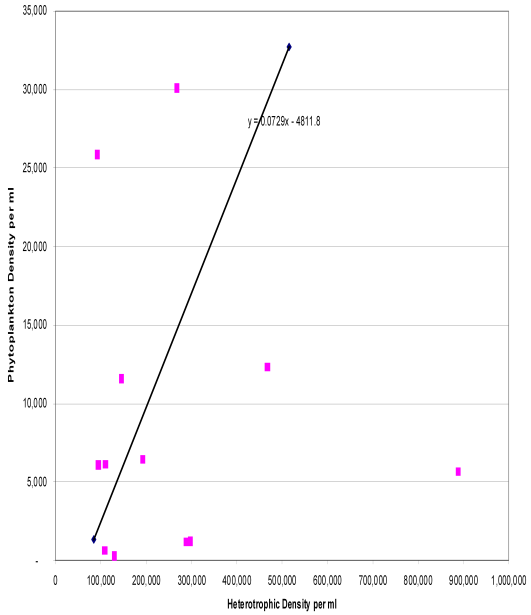

Figure 5. Heterotrophic bacteria–phytoplankton cross plot. Regression line on the chart is best fit line that shows the mutual relationship between heterotrophic bacteria and phytoplankton in the study area

Dinoflagellate associated with bacteria can cause a single species blooming as seen in red tide phenomenon [13]. However, Density of Diatomae in the study area is greater than desnsity of Dinoflagellate; therefore, this phenomenon is not the important issue in the study area.

A simple statistical analysis has been conducted to the data from the study area. Although the result is inconclusive as seen in Figure 5, there might be significant relationship between heterotrophic bacteria and phytoplankton where phytoplankton will utilize the nutrient decomposed from organic materials for their growth. Accordingly, it is very important to conduct further study the relationship between phytoplankton and heterotrophic bacteria within the study area.

3.3.1. *Trichodesmium*

Trichodesmium is an important cyanobacterium in the study area. It is approximately 90% of the total phytoplankton is dominated by the presence of *Trichodesmium thieubautii*. *Trichodesmium* is a marine planktonic cyanobacterium that is commonly found in the study areas. *Trichodesmium* colonies provide a unique pelagic habitat that supports a complex assemblage of consortial organisms. These colonies often represent a large fraction of the plant biomass in tropical, oligotrophic waters and contribute substantially to primary production. This phytoplankton is biogemically very important in a marine ecosystem to fix nitrogen under fully aerobic conditions while photosynthetically evolving oxygen [14] as a major source of nitrogen cycle to the marine environment. Subramaniam et *al*. [15] stated that *Trichodesmium* are noxious, and at least one species contains a neurotoxin and has been reported to be toxic and cause ecosystem damage, such as fish kills. It is also known to induce respiratory difficulties, such as *Trichodesmium* fever, in humans. Identification of these blooms and monitoring their presence would provide vital information to alleviate the impact of these deleterious events.

3.4. Pathogenic bacteria

There are 6 pathogenic bacteria (Table-1) were isolated from the North Sulawesi seawater included *Aeromonas, Citrobacter, Proteus, Pseudomonas, Yersinia* – which is commonly found in the samples - *and Shigella-which is rarely found in the samples*. Unlike *Salmonella* and *Vibrio* which can cause gastroenteritis and *Proteus* and *Citrobacter* can cause diarrhea [10,16], *Aeromonas, Citrobacter, Proteus, Pseudomonas, Yersinia* bacteria are not categorized as dangerous pathogenic bacteria. In addition, *Pseudomonas* bacterium is highly resistant to the temperature fluctuation. This *Pseudomonas* bacterium will infect human through the weak part of the body such as eyes, ear or wounded body [1]. There are 2 species of *Yersinia* in the world include *Yersinia pseudotuberculosis* and *Yersinia enterocolitica*, and only the latest bacterium that can cause arthritis and enteritis to human being.

Most of those bacteria live at the surface of the seawater where temperature, salinity and nutrient are the more suitable for their growths.

4. Conclusion

1. Coliform bacteria are found in all stations within the study area. The density of coliform bacteria varies and it was dependent upon the proximity to the shoreline. The average density of coliform bacteria is approximately is 10 colonies per ml. However, in some stations such as Stations 3, 20, 24, and E the density of coliform bacteria was greater than 38 colonies per ml.
2. The presence of coliform bacteria within this area may relate with bad sanitation “management” from the surrounding area. The other source of coliform bacteria is domestic and non-domestic (industry) sewages as seen in several stations near by Bitung, Manado Bay, and Kwandang Bay.
3. The BN area was characterized by the higher density of heterotrophic bacteria; however, the phytoplankton density was low. It revealed that phytoplankton was not well grown within this area due to several reasons that required further studies. In this study area, *Trichodesmium thieubautii* was a dominant species of phytoplankton.
4. There were six species of pathogenic bacteria found in the study area. Accordingly, the North Sulawesi coastal is not suitable for aqua culture. These bacteria are required to be eliminated and controlled for further coastal exploitation.

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Appendix.

Table 1.
Pathogenic bacteria isolated from North Sulawesi waters, On October - November 2000

No. St	Position		Water Sample					
	Latitude (N)	Longitude (E)	*Aero-monas*	*Citro-bacter*	*Pseudo-monas*	*Proteus*	*Shigella*	*Yersinia*
1	01º 26’ 966”	125º 21’765”		+	+			
2	01º 19‘ 792”	125º 09‘747”				+		
3	00º 00‘ 236”	125º 20‘137”	+	+		+		
4	01º 19’ 786”	125º 30’217”			+		+	
5	01º 40’ 191”	125º 30’024”		+		+		
6	01º 42‘786”	125º09’689”	+				+	
7	02º 00’ 139”	125º 30’339”				+		+
8	01º 00’168”	125º00’522”	+	+				
9	01º 45’523”	125º 55’074”						
10	01º 30’ 695”	124º45’011”		+				
D	T. Manado	T. Manado	+					
11	01º 28‘ 065”	124º 32’863”				+	+	
12	01º 16’ 341”	124º 26’291”		+				
13	01º 04‘ 563”	124º 15’301”				+		
14	02º 20’ 038”	124º 20’954”	+				+	
19	00º 54’ 880”	122º 49’933”				+	+	
20	01º 07’ 024”	122º 39’988”	+	+				
21	01º 05’ 978”	122º 19’977”			+			

22	01º 05’ 00”	123º 20’ 00”					+	
23	01º 05’ 00”	123º 00’ 00”					+	
24	00º 54’ 880”	122º 49’933”	+					
E	T Kwandang	T. Kwandang						+
E	TKwandang	T. Kwandang						+
25	01º 05’ 00”	122º 40’ 00”		+		+		
26	01º 20’ 00”	123º 00’ 00”				+		
32	01º 05’ 00”	122º 20’ 00”						