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Bacterial indicators reveal water quality status of Rangkui River, Bangka Island, Indonesia

Miratul Maghfiroh^{1*}, Heni Novianti², Lukman¹, Eddy Nurtjahya²

¹Research Centre for Limnology, Indonesian Institute of Sciences (LIPI), Kompleks Cibinong Science Centre, West Java, 16911, Indonesia

²University of Bangka Belitung, Kepulauan Bangka Belitung, 33172, Indonesia

*Corresponding author: miratul@limnologi.lipi.go.id

Abstract. Rangkui River flows across Pangkal Pinang City, the capital of Bangka Belitung Province. Land uses vary and can inevitably be pollution hot-spots for this river. This study aimed to investigate the current status of Rangkui River based on bacterial indicators in association with other water quality parameters. The sample collection was completed in March 2017 within 6 stations. Water quality parameters observed were temperature, TSS, turbidity, pH, DO, salinity, BOD, COD, Nitrate and Phosphate. The results of faecal coliform observation indicated that the stations closer to upstream (station 1, 2, 3) showed lower faecal coliform concentration (530-940 MPN/100 ml) as compared to those closer to downstream (station 4, 5, 6) which had the amount of ~11,000 MPN/100 ml. Phylogenetic study emphasized that isolates of interest were more similar to *Escherichia* group. The water from Rangkui River could be classified from Class I to Class IV according to Indonesia Government Regulation No.82/2001. PCA revealed Station 3 and 6 obviously separated from other stations so that more concerns should be given to improving water quality in these areas.

1. Introduction

Generally, the sources of water pollution differ and originate from point or non-point sources. Runoff of the pollutants occurs from, for instance, livestock and animal wastes, stormwater and sewer. Others may be leaked from septic system or emanated from improper treatment of industrial wastewater [1]. Polluted rivers undergo water quality changes that may include DO (Dissolved Oxygen), BOD (Biochemical Oxygen Demand), COD (Chemical Oxygen Demand), N-NH₃, TP (Total Phosphorus), pH, turbidity [2,3,4]. The value of each parameter tends to increase or decline. DO value may be lower than that of the threshold whereas other parameters such as BOD, or COD demonstrate significant increase in concentration [5,6]. Other than that, the assessment of pollution in river can be carried out by observing the content of organic compounds such as hydrocarbons [7], inorganics such as heavy metal concentration [8], physical and physiological features (colour, taste, odour, turbidity), and biological aspects (faecal coliform, enteric virus) [9,10].

Among the assessments using biological parameters, observation on faecal coliform is the most common water quality parameter to examine since the amount of this bacterial group may relatively describe biological pollution in water systems [11,12]. The occurrence of pathogenic bacteria can lead to the risk to human health through the spread of communicable disease. Pathogenic *Escherichia coli*, *Salmonella* spp, and *Cryptosporidium* spp. that are present in water exceeding government criteria



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would be of community concerns [1]. The contamination of faecal coliforms in water can induce serious problems and is reported to bear negative consequences [13,14].

In Indonesia, the majority of the river has been suffering from multiple pollutions. The deterioration is caused by poor management of land and water resources which includes deforestation and improper agricultural practices [15]. In addition, more than 12% of Indonesians do not have access to sanitation facilities (i.e. toilets) [16], as it is expected that open spaces, e.g. river, are used to discharge biological wastes. This also happens in Rangkui River which has ~7,200 m in length and lies across Pangkal Pinang City, the capital of Bangka Belitung Province. The river comprises approximately 4,300 ha of catchment area. The upstream areas include Bukit Kemiri and Bukit Kelawang whereas at downstream part, Baturusa River which is adjacent to Pangkalbalam Harbour, finally discharges into the sea at the eastern part of Bangka island [17]. Rangkui River flows through three different districts which cover Kecamatan Rangkui, Kecamatan Taman Sari, and Kecamatan Pangkalbalam and along its stream, land uses vary from residential, urban commercial, recreational place, drinking water plant, traditional markets to lead mining sites. These can inevitably be pollution hot-spots for Rangkui River.

This study aimed to investigate the current status of Rangkui River based on bacterial indicators and other water quality parameters. The information also provides insights on (1) how different land use is associated with the dynamics of water quality changes; (2) potential sources of nutrient loads and bacterial emission. Additionally, the information contributes correspondingly to the sustainable management of Rangkui River by the government of Pangkal Pinang.

2. Methods

2.1 Sample collection


We collected the samples in March 2017. Water quality parameters which include temperature, turbidity, pH, salinity, DO were measured *in situ* using water quality checking devices whereas TSS (Total Suspended Solids), BOD, COD, Nitrate (N-NO₃), Phosphate (P-PO₄) were analysed in the laboratory according to standard procedures [18]. Sample collections were carried out within six sites along Rangkui River (Figure 1).



Figure 1. Location of sample collections (numbers represent the sampling stations).

Each station has specific features to represent different land use variation (table 1). Water samples were taken ~1 L each and kept in sterile bottles. The temperature was maintained at ~4°C during transportation to the laboratory for further analysis.

Table 1. Site description.

	Coordinate	Attributed land use	Images
Station 1	2°13.109 S; 106 ° 06.372 E	Plantation and drinking water plant are identified in this area - upstream point in Kecamatan Pangkalan Baru	
Station 2	2°12.473 S; 106° 06.047 E	Intensive plantation site in Kecamatan Pangkalan Baru	
Station 3	2 ° 10.994 S; 106 ° 05.280 E	Metal mining spots (~50 m in length); (located in Kecamatan Pangkalan Baru)	
Station 4	2 ° 07.729 S; 106 ° 06.711 E	Urban commercial and residential of Kecamatan Rangkui	
Station 5	2 ° 07.577 S; 106 ° 07.023 E	Traditional market in Kecamatan Taman Sari	
Station 6	2 ° 05.978 S; 106 ° 08.344 E	Downstream part (Baturusa watershed) in Kecamatan Pangkalbalam	

2.2 Enumeration of faecal coliform and identification of isolate of interest

The determination of faecal coliform was done using MPN (Most Probable Number) with multiple tube method [18]. Lactose broth (LB) and Brilliant Green Lactose Bile broth (GLBB) were used respectively for presumptive/isolation and confirmatory test. Incubation was 24-48 hours at 35-37°C. Triplicate was applied to provide more representative data. Isolates of interest were obtained by the observation morphological and Gram characteristics [19]. Sequential single colony isolations (SCIs) were intended to produce pure colony. EMB Agar (EMBA) was chosen for SCI routine and Nutrient Agar (NA) was

the bacterial medium for culturing purpose. DNA (deoxyribonucleic acid) from isolate of interests were extracted using DNA – GF-1 Bacterial DNA Extraction Kit (Vivantis) followed by amplification of 16S rRNA gene sequence. We used 2X Taq Pol Master Mix (Vivantis) and universal primer 9F(5'-GAGTTTGATCCTGGCTCAG) and 1492R(5'-GGCTACCTTGTTACGACTT). The setting of PCR (Polymerase Chain Reaction) as follows: 95°C for 2 mins, 95°C for 30 secs, 55°C for 30 secs, 75°C for 1 min, 75°C for 5 mins, 20°C for 1 min which cycled 35 time. Prior to sequencing, amplicons were purified (PCR Clean-up kit, Vivantis). Sequences were matched with those deposited in GenBank. Phylogenetic tree was constructed and analysed with MEGA 6.0 [20].

2.3 Data analysis

Data accumulated from water quality parameters and faecal coliform were then compared with the guideline published by the Government of Indonesia through Government Regulation (PP) No. 82/2001 entitled Management of Water Quality and Water Contamination Control (table 2). Thus, the status of Rangkui River is based on the criteria set by national government regulation. The abundance of faecal coliform is explained descriptively. PCA (Principal Component Analysis) was served by R programming language [21] coupled with R commander [22] and FactoMineR packages [23].

Table 2. Indonesia Government Regulation No. 82/2001 regarding water classification.

Unit	Class I	Class II	Class III	Class IV
TSS	50	50	400	400
pH	6 – 9	6 – 9	6 - 9	5 – 9
BOD	2	3	6	12
COD	10	25	50	100
DO	6	4	3	0
Phosphate	0.2	0.2	1	5
Nitrate	10	10	20	20
Faecal Coliform	100	1000	2000	2000

Notes:

Class I: water suitable for drinking water after further treatment

Class II: water for recreational, aquaculture, animal husbandry, farming, agriculture

Class III: water for aquaculture, animal husbandry, farming, agriculture

Class IV: water for farming, agriculture

3. Results and discussion

Rangkui River caters local people with numerous ecosystem services. This river supplies water for drinking water, recreational, animal husbandry and agriculture. Furthermore, Rangkui River is the haven for groups of fish and other aquatic organisms. We studied the status of Rangkui River to present information as the baseline data, thereby, the current condition of Rangkui River can properly be described and better comprehended.

The distribution of faecal coliform at six different sites is revealed in table 3. Faecal coliform observed in Station 1, 2, and 3 met Class II criterion of water classification Government Regulation (PP) No. 82/2001. This type of water supply is suitable for recreational, aquaculture, animal husbandry, farming, agriculture. However, faecal coliform in the rest of the stations showed more abundant numbers. Coliform contamination seems apparent for these sites (Station 4, 5, and downstream). The significant number of faecal coliforms might have been discharged to the river through waste from livestock or untreated human sewage. This finding also confirmed another result that faecal indicator

organisms were more abundant at densely populated sites [24]. To eliminate more striking coliform contamination in the future, alternatively, conventional management of sewage system should be updated or replaced [25].

Table 3. Faecal coliform distribution.

Station	MPN/100 mL (avg.)	Attributed land use
Station 1	530	Plantation and drinking water plant are identified around this area (upstream point)
Station 2	740	Intensive plantation site
Station 3	940	Metal mining spots
Station 4	>11000	Urban commercial and residential
Station 5	>11000	Traditional market
Station 6	>11000	Downstream part (Baturusa watershed)

We also tried to observe other bacterial pathogens (presumptive) and found that *Salmonella* spp., *Vibrio* spp., *Aeromonas* sp., and *Shigella* sp. could be recovered from water sample of Station 6 (data not shown). We identified occasional colonies of bacterial pathogens (one or two bacterial groups) in other water samples apart from Station 6. This is suggested that bacterial pathogens are more predominant at downstream area.

Sequencing method allowed us to characterize our isolates of interest. Here, we selected four isolates each from designated Station 1, 3, 4 and 5. The determination is based on morphological and Gram staining. Additionally, these four isolates also demonstrated notable behaviour when challenged with antibiotic at various concentration (data not shown). UPGMA algorithm was run to generate tree construction as shown in Figure 2. Four isolates clustered within *Escherichia* clade. Isolate ET1 and ET4 were more similar to *Escherichia coli*.

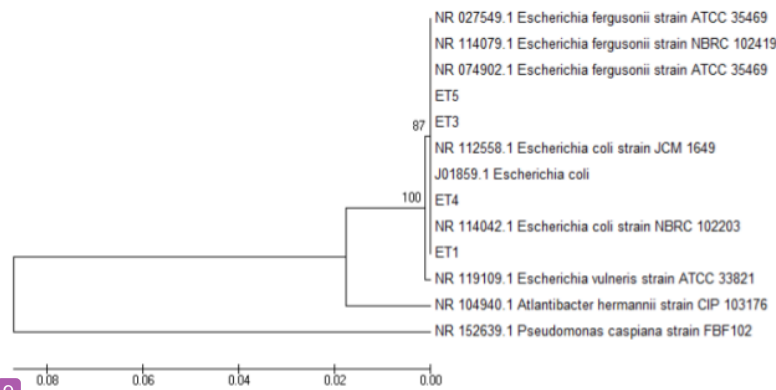


Figure 2. Phylogenetic tree based on 16S rRNA gene sequences across 1050 bp.

We also tried to describe the dynamics of water quality parameters in six stations along Rangkui River. According to table 4, The highest turbidity (also TSS) was noticed at Station 3 where the mining spot is at proximity. The lowest turbidity (and TSS) was around the upper stream area. pH was slightly below pH 7 but one pH tends to be a bit more acidic around mining area [26]. Salinity gradient got

greater by the downstream part. The lowest DO was found at Station 3. Greatest concentration of BOD and COD was recognized at downstream part. This suggests that organic compounds were predominantly accumulating at downstream. Nitrates may be associated with agriculture practices. Nitrates from fertilizer can be carried through rain and contaminate the water [27]. Nitrates from agriculture contribute to the elevated level of nitrate at upstream site. Phosphates at high level can trigger the bloom of algae. At Station 6, the amount of phosphates should be of concern since it was observed abundantly.

Table 4. Observations of water quality parameters.

	Unit	Station					
		1	2	3	4	5	6
Temp.	$^{\circ}\text{C}$	25,17 \pm 0,29	27,20 \pm 0,29	31,17 \pm 0,29	28,00 \pm 1,23	29,50 \pm 0,25	30,00 \pm 1,48
Turbidity	NTU	1,55 \pm 0,16	1,19 \pm 0,17	408,33 \pm 10,5	33,44 \pm 21,27	86,00 \pm 4,00	88,00 \pm 5,20
TSS	mg/L	0,06 \pm 0,01 ^a	0,04 \pm 0,02 ^a	15,93 \pm 1,84 ^a	1,31 \pm 0,47 ^a	3,44 \pm 0,95 ^a	3,52 \pm 0,24 ^a
pH	-	6,54 \pm 0,03 ^a	6,63 \pm 0,06 ^a	5,51 \pm 0,17 ^d	6,71 \pm 1,03 ^a	6,45 \pm 0,49 ^a	6,86 \pm 0,02 ^a
Salinity	Ppt	2,00 \pm 1,09	1,50 \pm 0,43	1,50 \pm 0,25	2,00 \pm 0,75	2,50 \pm 0,43	12,00 \pm 1,09
DO	mg/L	5,39 \pm 0,83 ^b	4,88 \pm 0,4 ^b	2,91 \pm 0,12 ^d	3,63 \pm 0,59 ^c	3,06 \pm 0,11 ^c	3,01 \pm 1,34 ^c
BOD	mg/L	1,20 \pm 0,08 ^a	1,47 \pm 0,23 ^b	2,14 \pm 0,47 ^a	1,05 \pm 0,39 ^a	3,41 \pm 0,91 ^b	3,80 \pm 0,60 ^b
COD	mg/L	2,16 \pm 0,05 ^a	7,21 \pm 2,46 ^a	9,41 \pm 3,62 ^a	4,94 \pm 0,31 ^a	8,38 \pm 0,96 ^a	12,90 \pm 1,49 ^b
Nitrate	mg/L	1,90 \pm 0,36 ^a	1,89 \pm 0,12 ^a	1,30 \pm 0,15 ^a	1,70 \pm 0,74 ^a	1,90 \pm 0,79 ^a	1,70 \pm 1,04 ^a
Phosphate	mg/L	0,07 \pm 0,05 ^a	0,05 \pm 0,01 ^a	0,17 \pm 0,02 ^a	0,08 \pm 0,01 ^a	0,09 \pm 0,01 ^a	0,89 \pm 0,03 ^c

Notes: ^aperscripted letters denote that the value meets criterion of water classification (PP No.82/2001) from: (a) Class I (b) Class II (c) Class III (d) Class IV

Water classification according to PP No. 82/2001 comprises of four Classes which represent the suitable purposes of water to be used. Water quality parameters at Station 1 and 2 demonstrated that the waters are classified to Class I or Class II. At Station 4, 5, and 6, the waters might be classified to Class III if we consider the low level of DO and the high content of phosphates at Station 6. The water at Station 3 displayed poor quality of DO and pH, thus, the water seems to be suitable for farming and agriculture (Class IV). To mitigate worst water quality in the future, improvement should be addressed. The improvement of water quality can be done by water planning and better management via public participation [28].

PCA, as defined in Figure 3, revealed distinct clustering among stations. Station 3 and 6 obviously separated from other stations. At Station 6 (downstream), it is apparent that associated water qualities were the abundance of faecal coliform, the concentration of phosphates, BOD, COD, and salinity. In Station 3, it was clearly associated with great concentration of TSS and turbidity. Since these two stations relatively had poor water quality performance, more concerns should be given to improving these areas.

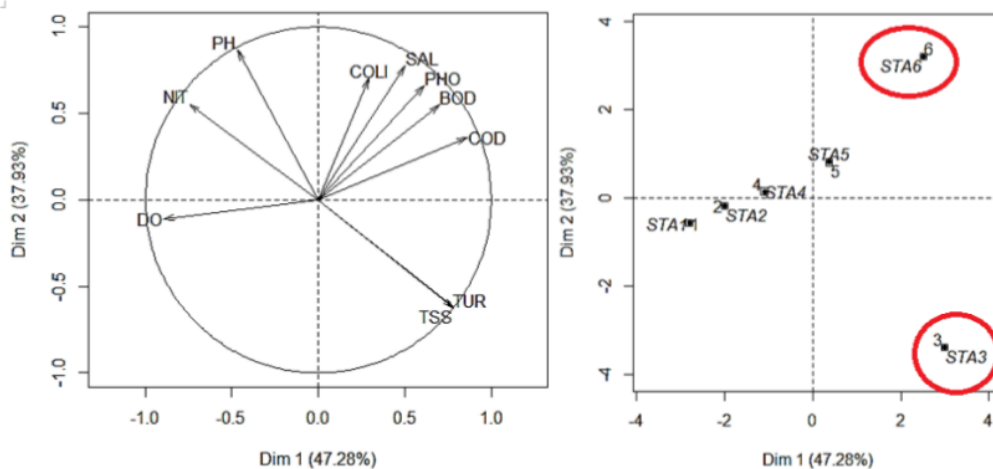


Figure 3. PCA built from water quality variables and stations as factors.

4. Conclusion

This paper identified current water quality status of Rangkui River with special reference to faecal coliform as bacterial indicators in association with other physical and chemical parameters of the river. Faecal coliform, BOD, COD, turbidity, TSS, Phosphates were a set of limiting parameters that distinguished the areas with poor water quality performance. We recommend the improvement of water quality by which water planning and better management can be implemented via public participation. Long term monitoring of water quality is indispensable to ensure the progress of its improvement.

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