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Spatial distribution of coliform bacteria in Batang Arau River, Padang, West Sumatera, Indonesia

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D. Helard, S Indah, and M Wilandari

Department of Environmental Engineering, Faculty of Engineering, Universitas Andalas, Kampus Unand Limau Manis, Padang 25163

Email : dennyhelard@eng.unand.ac.id

Abstract. The objective of this study was to assess spatial distribution of bacterial population in Batang Arau River. The river water was sampled biweekly, during 3 successive months, and analyzed for coliform bacteria (total coliforms, TC, and fecal coliforms, FC). The results showed that the number of TC range 2.61 – 4.89 $\log_{10}$ number/100 mL and FC 2.48 – 4.79 $\log_{10}$ number/100 mL. The concentration of all parameters inspected has increased from upstream to downstream of the river, except for pH and DO. Bacterial coliforms were strongly correlated with some physicochemical parameters (TSS, TDS, EC and pH), with a Spearman correlation coefficient (r) ranged from -0.599 to 0.827. Analysis of the spatial distribution of the one-way ANOVA at 95% confidence level showed that there were significant differences (p<0.05) in the concentration of bacterial coliforms between upstream and downstream sampling stations as a result of differences in land use and human activity. Cluster analysis grouped 8 sampling stations into two clusters, moderate and high polluted, based on similarities of bacterial characteristics. The bacterial data clearly shows that human health is at a very high risk, as WHO guidelines classification for FC or E. coli in water supplies (WHO 1997) or for agriculture use (WHO 2006) and those strategies for improving water quality of Batang Arau River must be expeditiously implemented.

1. Introduction

The microbiological quality of freshwater in urban and densely populated rural areas is frequently endangered by contamination with untreated domestic wastewater. It is caused by the presence of fecal bacteria in the river water and lagoons. The extent of this fecal pollution, in many cases, reaches levels which exceed recommended limits for water to be used for drinking, recreation, or irrigation of crops eaten raw [1]. The use of bacterial indicator bacteria to assess the microbiological quality of surface and ground water has been used for many years [1-7]. The primary objective for using indicator organisms and method commonly related to their examination is to indicate the degree of water contaminated by fecal wasted [2]. Indeed, fecal pollution of surface water is special concern since the most important bacterial gastrointestinal infections in humans are primarily transmitted by water polluted with faeces of infected persons [2, 8]. Nowadays, the most common indicators used are total coliforms, fecal coliforms and enterococci, which are used as surrogates for human pathogens to assess the health risk and quality of the water [6, 9].

Batang Arau River is a medium water course that runs totally in the centre of Padang, West Sumatera, Indonesia, which has an important role for residents with daily activities. In the river catchment area, rural communities are directly dependent on this river for all their water needs

including drinking, bathing, washing, fishing, recreation, livestock watering, irrigation and agriculture. In this area, treatment of wastewaters has not been implemented properly; since most domestic wastewaters are discharged directly to the Batang Arau River, which could potentially degrade water quality in the river water bodies, representing a direct source of microbial contamination to the river [10]. Therefore, the communities in this area have been more prone to water-borne diseases [6]. However, very little are known about the distribution of microbial indicator parameters of Batang Arau River as well as other physicochemical parameters. Hence, an overview of the microbial quality of the Batang Arau River water is a major public health issue. This study is necessary to develop appropriate management strategies to minimize the potential public health risks.

The objective of this study was to determine the bacterial quality (total coliforms and fecal coliform counts) and some physicochemical parameters of the water in the Batang Arau River. Moreover, the spatial distribution of the bacterial population was analyzed. The result of this research will show the effects of the rural population and industries located along the river on the microbial population changes.

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2. Study Area and Methods

2.1. Study Area

Batang Arau River has a length of about 30.6 km. From the upstream to downstream of Batang Arau River is approximately 19.83 km with a circumference of approximately 69.15 km. At the upstream of Batang Arau River, the residential population is relatively rare and the land is used as agricultural area [11]. However, intensive urbanization takes place from midstream to downstream of Batang Arau River and has potentially cause water pollution.

Samples of river water were collected from eight stations along the Batang Arau River at biweekly intervals between August and September 2016. The sampling stations were classified as one baseline station (S1) and seven impact stations (S2 to S8). Baseline station is represented with natural and unpolluted state of river basin and impact stations are used for measuring the quantity of pollutant and extent of pollution due to human interference. Figure 1 shows the locations of the sampling stations while the detailed description of sampling stations is listed in Table 1.

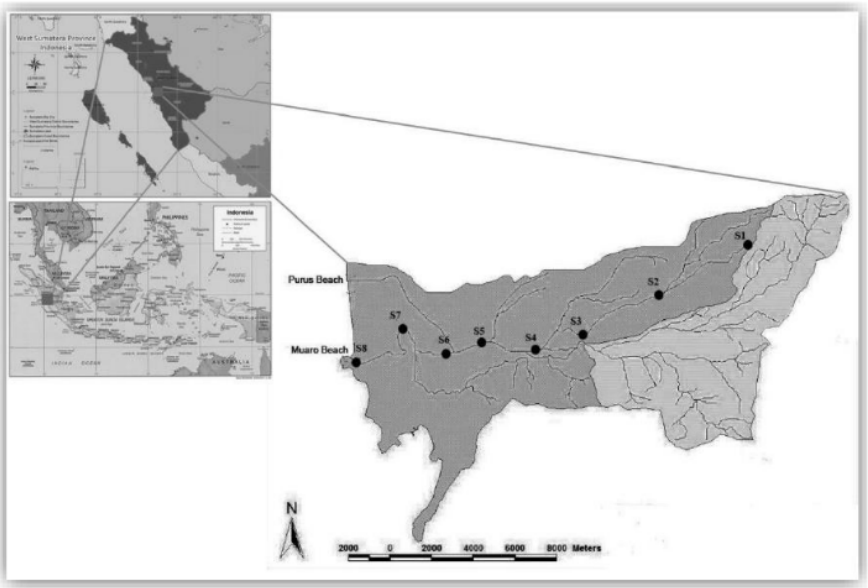


Figure 1. Map of the Batang Arau catchment area indicating the sampling station (Station 1, S1, Lubuk Paraku; S2. Padang Besi; S3. Cengkeh; S4. Lubuk Begalung; S5. Banuaran; S6. Subarang Padang; S7. Palinggam; S8. Muaro).

Table 1. Descriptions of eight sampling stations on the Batang Arau River

Stations	Latitude	Longitude	Elevation, m.a.s.l.* (m)	Distance (from S1), (km)	Description
S1	0°56'49.9"	100° 30'31.5"	229	0	Upstream of the Batang Arau River which is located in a forested area.
S2	0°57'30.4"	100°27'08.0"	124	4.2	Located on a drain which is carrying wastewater from a limestone mill and agricultural activities.
S3	0°57'39.7"	100°25'29.7"	72	10.1	Received wastewater from domestic and commercial activities.
S4	0°57'40.8"	100°24'02.3"	18	13.8	The river has passed through agricultural and industrial areas.
S5	0°57'43.3"	100°22'54.1"	7	16.7	The streams have received wastewater from rubber industry.
S6	0°57'26.8"	100°22'41.1"	6	17.6	Located after the streams couple with the secondary drainage channels, which receive wastewater from the domestic and commercial areas.
S7	0°57'41.4"	100°22'28.4"	3	18.9	The stream couples with the channel of Jati Drain that receives wastewater from domestic and commercial activities.
S8	0°57'44.8"	100°21'51.5"	1	19.9	Downstream of the river; all pollutants along the river accumulated.

*m a.s.l. : meters above sea level

2.2. Sampling and analytical methods

Water samples were collected biweekly from August to September 2016. The samples were collected in sterile sample bottles and transported in cooler boxes at <10°C to the laboratory and analyzed within 3 h. Water quality parameters like EC, pH, DO and T were analyzed on site using portable meter. The other parameters such as total suspended solids (TSS) and total dissolved solids (TDS) were analyzed in laboratory as Standard Methods [12] guidelines.

The Most Probable Number (MPN) of coliforms bacteria were determined by multiple fermentation tube technique [13]. Each of separate sets of 10 tubes of Lactose broth (LB) was prepared. Then, each of the 5 tubes of 10 ml double strength LB with 1 ml of the undiluted water sample was inoculated. These LB tubes along with inverted Durham tubes were incubated at 35 ± 0.5 °C for 48 hours and at 44 ± 0.5 °C for 24 hours for TC and FC, respectively. Tubes were tested for gas production and turbidity of the medium at the end of 24/48 hours incubation. Positive tubes with gas formation and turbidity of the medium were sub-cultured for confirmation into Brilliant Green Lactose Bile (BGLB) broth and E.coli (EC) medium for TC and FC, respectively, with inverted Durham tubes. BGLB tubes were incubated for 48 hours at 35 ± 0.5 °C and for 24 hours at 44 ± 0.5 °C for EC tubes. Tubes were examined for gas production. Estimated counts of total coliform and fecal coliform were obtained from MPN tables and expressed in log10 number per 100 ml.

Data were presented in the form of matrix plot. Spearman's R correlation analyses were performed to determine whether significant relationships exist among bacterial measure and water quality [14]. Statistical significance was set at p values <0.05. River water quality variable data were normalized by log10 transformations and then subjected to Kurskall-Wallis non-parametric one-way ANOVA to examine the spatial variations [7]. Cluster analysis (CA) are used to identify sources of water quality inputs and to group sampling sites into homogeneous zones (z-transformation of the input data, Euclidean distance as similarity index and Ward's method of linkage [15] using data collected from Batang Arau River. CA using the Ward's method is regarded as a very efficient method and was

applied to the standardized data considering previous reports [16-18]. All descriptive statistics and graphs were carried out using PAST (Paleontological Statistics) software V3.04 [19]. The software is available from: http://folk.uio.no/ohammer/past/.

3. Results and Discussion

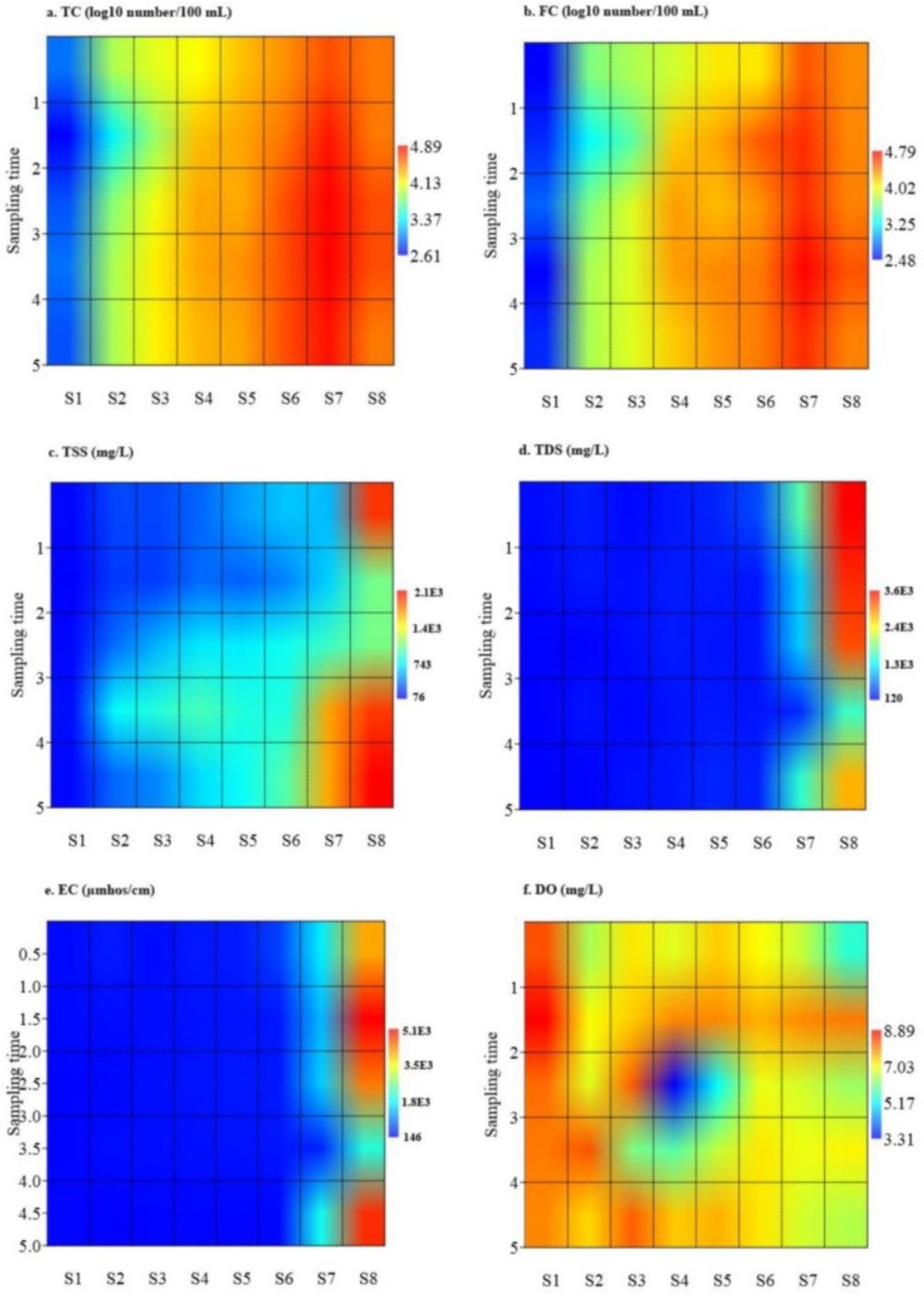
A summary of bacterial counts (TC and FC) and physicochemical water quality (TSS, TDS, EC, DO, pH and T) including ranges, mean and standard deviation for each sampling station along the Batang Arau River for the period August to September 2016 is provided in table 2.

Table 2. Overall range, means and standard deviations of coliform bacteria and physicochemical water quality at the eight sampling points in the Batang Arau River

Variable	Station							
	S1	S2	S3	S4	S5	S6	S7	S8
TC (Log_{10} number/100 mL)	2.61 - 2.95[a]	3.34 - 3.88	3.83 - 4.18	4.13 - 4.40	4.32 - 4.46	4.46 - 4.66	4.66 - 4.89	4.53 - 4.66
	2.84±0.14[b]	3.75±0.23	4.06±0.14	4.32±0.11	4.39±0.05	4.60±0.09	4.82±0.09	4.58±0.08
FC (Log_{10} number/100 mL)	2.48 - 2.78	3.26 - 3.77	3.52 - 3.92	3.88 - 4.32	4.09 - 4.38	4.09 - 4.53	4.54 - 4.79	4.37 - 4.54
	2.59±0.12	3.61±0.21	3.81±0.18	4.18±0.18	4.26±0.12	4.36±0.17	4.66±0.09	4.42±0.07
TSS (mg/L)	76 - 112	224 - 756	228 - 840	342 - 938	326 - 824	418 - 966	568 - 1654	1052 - 2078
	94±13	392±212	462±250	597±252	622±208	716±213	1077±535	1611±510
TDS (mg/L)	124 - 168	120 - 246	146 - 188)	200 - 238	204 - 288	204 - 446	300 - 1658	1518 - 3560
	139±20	184±58	169±20	220±16	244±35	262±105	1106±522	2884±820
EC (µmhos/cm)	172 - 206	146 - 308	182 - 234	182 - 298	183 - 312	183 - 559	369 - 1910	2018 - 5097
	189±17	216±60	216±21	249±43	262±49	316±142	1362±594	4051±1212
DO (mg/L)	7.9 - 8.9	6.4 - 8.3	6.1 - 8.2	3.3 - 7.9	5.2 - 7.9	6.9 - 7.6	6.6 - 7.9	5.5 - 8.0
	8.2±0.4	7.2±0.7	7.4±0.9	6.2±1.8	6.9±1.1	7.2±0.3	7.0±0.5	6.7±0.9
pH	8.0 - 8.9	8.1 - 9.1	7.8 - 9.6	7.4 - 8.5	7.1 - 8.2	7.2 - 8.1	7.2 - 8.2	6.9 - 8.1
	8.3±0.4	8.7±0.4	8.7±0.9	7.9±0.4	7.7±0.5	7.6±0.4	7.6±0.4	7.5±0.6
T (^{0}C)	24.2 - 26.2	26.4 - 31.2	28.1 - 34.7	29.3 - 33.4	29.2 - 33.7	29.6 - 33.6	29.7 - 32.3	29.5 - 32.4
	24.9±0.8	28.6±1.8	30.7±2.5	30.6±1.7	30.5±1.8	30.5±1.7	30.4±1.1	30.4±1.1

a = range
b = mean±stdev.

Spatial distribution of coliform bacteria as well as physicochemical parameters in Batang Arau River over the sampling period is presented in figure 2a-h.


a. TC (log10 number/100 mL)
Sampling time
1
2
3
4
5
S1 S2 S3 S4 S5 S6 S7 S8
4.89
4.13
3.37
2.61
b. FC (log10 number/100 mL)
Sampling time
1
2
3
4
5
S1 S2 S3 S4 S5 S6 S7 S8
4.79
4.02
3.25
2.48
c. TSS (mg/L)
Sampling time
1
2
3
4
5
S1 S2 S3 S4 S5 S6 S7 S8
2.1E3
1.4E3
743
76
d. TDS (mg/L)
Sampling time
1
2
3
4
5
S1 S2 S3 S4 S5 S6 S7 S8
3.6E3
2.4E3
1.3E3
120
e. EC (µmhos/cm)
Sampling time
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
S1 S2 S3 S4 S5 S6 S7 S8
5.1E3
3.5E3
1.8E3
146
f. DO (mg/L)
Sampling time
1
2
3
4
5
S1 S2 S3 S4 S5 S6 S7 S8
8.89
7.03
5.17
3.31

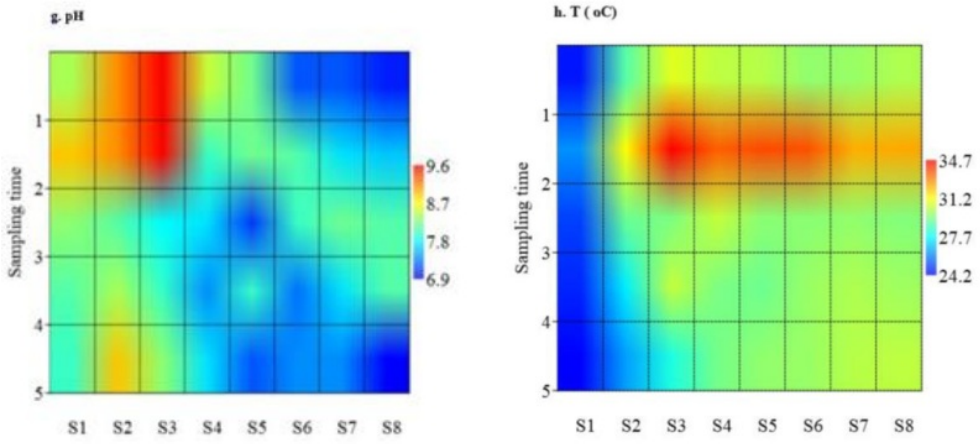


Figure 2a-h. Spatial distribution of bacterial and physicochemical parameters along Batang Arau River: a. TC; b. FC; c. TSS; d. TDS; e. EC; f. DO; g. pH; h. T.
(*Note: Sampling date: 1. 01-Aug-16; 2. 15-Aug-16; 3. 29-Aug-16; 4. 12-Sep-16; 5. 26-Sep-16*)

Among the sampling stations, TC counts of all stations were highly variable with Station 1 and Station 7 constantly recording the lowest and highest mean counts, respectively, during the sampling period. These concentrations and wide variations are similar to findings elsewhere [6, 7, 20, 21]. The upstream of the river, S1, is less affected by anthropogenic, therefore the lowest coliform counts was obtained. The bacterial contamination of the river occured to reflect human activities around, with mean total coliform counts ranking in the following spatial order; S7 > S8 > S6 > S5 > S4 > S3 > S2 > S1. The bacterial counts indicate sewage waste disposal in the rural Batang Arau watershed, implying a lack of treatment infrastructure. The significant differences between S1 and other all stations were clarified by one-way ANOVA ($p < 0.05$) from the mean counts analyzed at the other seven sampling stations (p value varies from 0.0104 to 0.0117).

The concentration changes of the FC were very similar to the spatial distribution trends of the TC. One-way ANOVA demonstrated a significant difference in FC levels between S1 and other stations ($p < 0.05$; in the range of 0.0107 to 0.0117), similar to the spatial variability trends of TC counts. The fecal component of the bacteria counts indicates that domestic sewage forms a significant portion of the pollutants in the river. The high coliform counts in water sample at S2 could be as a result of the discharge of untreated wastewater and solid waste from residential areas around the station. Similar studies by Amisah (2014) observed that discharges from human settlements along catchment area contribute to the pollution of the river [7]. Moreover, the lack of suitable permanent disposal sites for both liquid and solid wastes in catchment has conduced in the use of river as a waste dumping ground. Inefficient sewage treatment systems has resulted in insanitary practices, such as urinating or defecating into open spaces, which may be released into the river to increase the microbial load of the river. The mean FC counts ranked in the following spatial order; S7 > S8 > S6 > S5 > S4 > S3 > S2 > S1 during the study. Overall, significant spatial variations in the microbial loads of the water over the sampling period were observed which involves the different land-use activities by the communities around this area.

Mean TC and FC counts increased downstream with respect to locations, except S8. These microbial counts decreasing, from S7, may be due to a physical effect, i.e. osmotic pressure [22] since the concentration of EC in this estuary area increase significantly. With high osmotic pressure, such as concentrated salt brine, water inside the microbial cell moves out through the membrane and into the brine, causing a partial dehydration of the cell. This slows metabolic processes and interferes with multiplication of the microorganisms. Bacterial metabolism is sensitive to salt, because salt exhibits specific ionic and water binding properties [23]. The latter effect is of greatest importance because the

addition of salt to the fermentation liquor leads to a decrease in water activity, aw. Decreases in aw below the optimum values for growth often result in a linear decrease of the growth rate [24].

Between bacterial counts and some physicochemical parameters, a strong correlation was demonstrated (i.e.: TSS: $r = 0.807$ and 0.805; TDS: $r = 0.733$ and 0.723; EC: $r = 0.709$ and 0.680; pH: $r = -0.599$ and -0.606; for TC and FC respectively). Details of the various correlation coefficients are presented in table 3. Young and Thackston (1999) found that fecal bacteria counts in urban tributaries were much higher in sewered basins than in nonsewered basins and in general were related to housing density, population, development, percent impervious area, and domestic animal density [25]. Mallin et al. (2000) found that fecal coliform densities were strongly correlated with turbidity (positively) and salinity (negatively) [26].

Table 3. Spearman rank correlation coefficient of coliform bacteria and physicochemical water quality in the Batang Arau River

Spearman R	TC	FC	TSS	TDS	EC	pH	DO	T
TC	1							
FC	**0.9779**	1						
TSS	**0.8069**	**0.8052**	1					
TDS	**0.7333**	**0.7225**	**0.640**	1				
EC	**0.7091**	**0.680**	**0.6064**	**0.8267**	1			
pH	**-0.5987**	**-0.6061**	**-0.6643**	-0.4942	-0.3919	1		
DO	-0.4290	-0.4001	-0.5288	-0.3335	-0.4662	0.3751	1	
T	0.4271	0.4419	0.2438	0.4131	0.4813	-0.1309	-0.2156	1

To obtain similarity groups between the sampling sites cluster analysis (CA) was applied. The dataset was treated (after data scaling by z-transformation) by the Ward's method of linkage with squared Euclidean distance as measure of similarity [17]. Figure 3 presents the dendrogram obtained from CA that reveals that eight sampling stations on the stream are grouped into two statistically significant clusters. Cluster 1 consists of station S1; while cluster 2 consists of stations S2 to S8. The cluster classifications change with significant level because the stations in the groups have similar characteristic features that are influenced by similar sources. Compared to the average bacterial indicator data and information in clusters with drinking water guidelines [27-28], it could be resumed that clusters 1 and 2 conformed to relatively moderate and high polluted regions, respectively.

Cluster 1 is located at the upstream area (S1) of the Batang Arau River, in forested area and there is no influence of human activities on water quality, representing the natural background concentration of microbial indicator. However, since the TC and FC counts at this station were exceeded the WHO guidelines, the origin of the microbial indicators could be from wild animal manure, soil and submerged wood [29]. Next to S1, at stations S2 to S8, the increasing of bacterial indicator counts were obtained, reflecting the discharge of pollutants from human activities. These stations are included in this Cluster 2 and located towards to the downstream of the Batang Arau River. At the downstream, all pollutants from all human activities along the river is accumulated, so that the counts of microbial indicator were higher than the previous stations. Therefore, cluster 2 is corresponded to relatively high pollution stations. The results may be a direct reflection of the human population distribution along the river. The higher the concentration of indicator bacteria in water, the higher the risk of illness especially to domestic users [6].

The result of CA suggests that there is no significant difference in the microbial quality at the station in the same cluster, so that for monitoring the microbial quality, sampling at one of stations in the same cluster is possible. The result points that this approach may offer the possible design of a future spatial sampling strategy in an optimal way and provides a reliable classification of surface waters in the whole region. The number of the sampling site could be optimized, for rapid quality assessment studies, not all monitoring sites can be used, but only representative sites from each

cluster. This, in turn, will reduce the number of analysis and the cost of the risk assessment procedure [17].

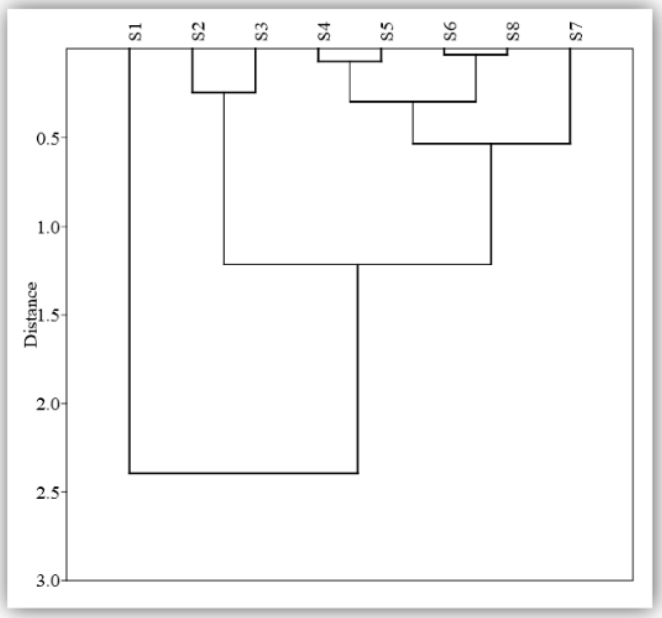


Figure 3. Dendogram showing different clusters of sampling station located in Batang Arau catchment area according to coliform bacteria parameters.

4. Conclusions

The results indicated that, in general, all the coliform bacteria have similar spatial distribution pattern, with an increasing trend in concentration from upstream to downstream of the Batang Arau River, indicating the influences of natural and anthropogenic sources along the river. This study denotes that the coliform bacteria contamination of the Batang Arau River waters is the result of contributions from upstream inputs, tributaries, and several non-point pollution sources (domestic wastewater and agriculture)Spatial variation of coliform bacteria in Batang Arau River, confirmed using one-way ANOVA and CA, classified all the sampling stations into two main groups of spatial similarities. Cluster 1, correspond to S1, was located in upstream area with a moderate pollution region, while cluster 2, from the middle course to the downstream of the river (S2 to S8) were in a region of relatively high pollution. The results may offer the optimal design of a future spatial sampling strategy that will reduce the number of analysis and the cost of the risk assessment procedure.

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