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WATER QUALITY ASSESSMENT AND THE PREVALENCE OF ANTIBIOTIC-RESISTANT BACTERIA FROM A RECREATIONAL RIVER IN KUCHING, SARAWAK, MALAYSIA

KHAIRUNNISA MOHAMMAD HAMDI[1]*, SAMUEL LIHAN[1], NURASHIKIN HAMDAN[2] AND TAY MENG GUAN[2]

[1]Institute of Biodiversity and Environmental Conservation, Universiti Malaysia Sarawak, 94300 Kota Samarahan, Sarawak, Malaysia. [2]Faculty of Resource Science and Technology, Universiti Malaysia Sarawak, 94300 Kota Samarahan, Sarawak, Malaysia.

**Corresponding author: khairunnisahamdi97@gmail.com*
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Abstract: Recreational activities may affect a river's water quality, which may pose health risks to those in direct contact with the water. This study aims to analyse the water quality if Jangoi River in Kuching, Sarawak, by measuring the conventional chemical and biological parameters and to characterise the bacteria isolated from the water based on their antibiotic susceptibility. The determination of the water quality is based on the Department of Environment's Malaysian Water Quality Index (WQI), which was carried out at three sampling stations (upstream, middle stream and downstream) in two different sampling trips. Six water quality parameters were measured: Dissolved oxygen, biological oxygen demand, pH, chemical oxygen demand (COD), ammoniacal nitrogen and total suspended solid. The WQI values of the river ranged from 88% to 92%, classifying the river under Class I. The one-way ANOVA analysis revealed that the parameter values for all the three stations are not significantly different, except for the COD. The higher COD value of the upstream water could be due to the release of wastewater from houses and agricultural activities near Jangoi River. The faecal coliform and total coliform counts ranged from 650 CFU/100 mL to 1,000 CFU/100 mL and 20,050 CFU/100 mL to 23,250 CFU/100 mL, respectively. Nine bacteria were isolated and 16S rRNA PCR and DNA sequencing. The DNA sequencing revealed the presence of *Escherichia coli, Chromobactium violaceum, Lelliota amnigena, Pseudomonas aeruginosa, Achromobacter xylosoxidans, Stenotrophomonas maltophilia, Pseudomonas hibiscicola, Achromobacter mucicolens* and *Bacillus pacificus*. Antibiotic susceptibility tests demonstrated the highest percentage of susceptibility for ciprofloxacin (100%), followed by ampicillin (40%) and chloramphenicol (40%). However, the highest percentage of resistance (60%) was shown by erythromycin. The multiple antibiotic resistant (MAR) index in this study ranged from 0.0 to 0.6. The river's WQI categorisation and microbiological status are inconsistent, indicating the need to modify the WQI formula to include the microbial parameter. Additionally, this study recommends the establishment of water quality and antibiotic resistance pattern monitoring programmes to anticipate the emergence of MAR bacteria in the aquatic environment and to document the continuous water quality state of Sarawak's recreational rivers.

Keywords: River water quality, bacteria, Jangoi River, 16S rRNA sequencing.

Introduction

Rivers are the most vital source of freshwater for all living things, including humans. Human social, economic and political developments during the last several decades have indeed been substantially determined by the availability and distribution of fresh water in riverine systems. Since water is used for a variety of well-being functions, including municipal drinking water, agricultural land irrigation, industrial water, fishing, boating and body-contact enjoyment, there has been an increasing desire for water essentiality.

Today, Sarawak's recreational rivers have developed into one of the state's most prominent natural tourism attractions. The recreational

rivers have gained popularity as one of the most favoured tourist destinations, as river water is crystal pure and the areas are surrounded by lush flora and fauna. There are numerous water-based recreational hotspots in Kuching, including the Adis Buan River, Ranchan Waterfall and Kubah National Park. As Muyibi *et al.* (2008) pointed out, while all water-based leisure activities are beneficial to remain fit, they all come with their own set of risks. The foremost issue that arises in this domain is the public exposure to contaminated water, which may pose health risks. Water pollution occurs when pollutants are dumped into bodies of water without being properly treated.

According to the United States' Centers for Disease Control and Prevention (CDC), numerous pathogenic causative agents are responsible for recreational water illnesses (RWI). Surface water is one of the platforms of disease dissemination caused by pathogens present in the water bodies (Pandey *et al.,* 2014). The significant contributors to pathogens in recreational water are land uses, faecal pollution and anthropogenic activities (Rodrigues & Cunha, 2017). The most common way for germs to spread is through ingestion of, contact or inhalation of mists from the contaminated water (Minnesota Department of Health, n.d.). The CDC has reported a wide range of infections caused by the transmission of germs in recreational water, including wound and neurologic-related infections, gastrointestinal, skin, ear infections and the most well-described RWI is diarrhea.

RWI may be more prevalent in children under 10 due to their proclivity for swallowing contaminated water, hand-mouth exposure, repeated immersion of the head in water, and their act of remaining in water for a long period of time (Wade *et al.,* 2003). Furthermore, bacteria may develop resistance to a variety of antibiotics, altering their responsiveness to medications. Methicillin-resistant *Staphylococcus aureus* (MRSA) is a prominent instance of an antibiotic-resistant bacteria. According to the most recent statistics provided in 2019 by Malaysia's National Surveillance of Antimicrobial Resistance (NSAR), 15% of all *S. aureus* isolated from clinical samples are determined to be MRSA. Nonetheless, there was a downward trend in isolated MRSA (19.8%) from 2018 to 2019. MRSA has a greater rate of antibiotic resistance than *S. aureus* in 2019. This is expected as a result of MRSA's resistant gene cassette showed that it contains genes conferring resistance to drugs other than beta-lactam antibiotics (NSAR, 2019). Sanborn and Takaro (2013) demonstrated the importance of monitoring indicator bacteria levels in recreational water as a means to protect bathers' health.

The drawback of Sarawak's water quality monitoring programme, however, is that the government monitors only a few rivers, which do not include small rivers that are often well known as water-based leisure spots. To our knowledge, only a few studies have evaluated the importance of a continuous water quality monitoring programme for recreational rivers. Jangoi River was chosen for this study because it is one of the several rivers in Kuching that are actively involved in a myriad of leisure activities. Due to the river's placement, all discharges will join the river system, as agricultural and residential areas are located within the river's watershed. As a result, the water quality is impacted. To our knowledge, no prior research has been undertaken on Jangoi River's water quality and microbiological assessment.

The water quality status and the pervasiveness of antibiotic-resistant bacteria in Sarawak's recreational rivers have been assessed infrequently, resulting in a dearth of data. As asserted by the Department of Environment (DOE, 2015), the Water Quality Index (WQI) declined from 58% to 52% in 2014 while polluted rivers climbed from 5% to 9%. Regular monitoring of water quality is necessary since Sarawak is well known for its scenic beauty, particularly its rivers, which attract visitors from all over the globe.

The purpose of this study is to determine the quality of the Jangoi River water by measuring conventional chemical and biological parameters

using the DOE's proposed WQI and to describe isolated bacteria based on their antibiotic susceptibility. The findings of this study will be used to initiate the monitoring of the water quality status and antibiotic-resistant patterns of bacteria in recreational waters in Kuching to ensure that the rivers remain healthy and clean for public use. Correspondingly, a database or profile encompassing the assessments of water quality and antibiotic resistance patterns can be generated, which is informative and constructive for future management decisions.

Materials and Methodology

The Collection and Processing of Samples

Sample collection was carried out at a recreational park, namely the Jangoi River Lodge, which is located in Jalan Padawan, Kuching, Sarawak, Malaysian Borneo (N 1°34.994" E 110 °23'3.108"). The locality of the sampling site is shown in Figure 1.

The sample collections were conducted during the high tide (Trip 1) and low tide (Trip 2) and when the weather was good in the morning, when most recreational activities occur (9.00 am to 11.30 am). Water samples were collected using a 500 mL sterile Schott bottle at three points: The upstream (N 01° 38'57" E 110° 20'13"), middle stream (N 01° 13'36" E 110° 23'1") and downstream (N 01° 13'36" E 110° 23'2") (Huys, 2003). The selection of the sampling stations was made based on the classification of land use along the riverside, as shown in Table 1.

Water samples were taken at least 50 cm below the water surface according to Huys (2003). All the collected samples were transported to the laboratory in a cooler box and processed forthwith. The bacteria were isolated from the water samples with the vacuum filtration method using 0.45 µm membranes (Gerba & Pepper, 2004).

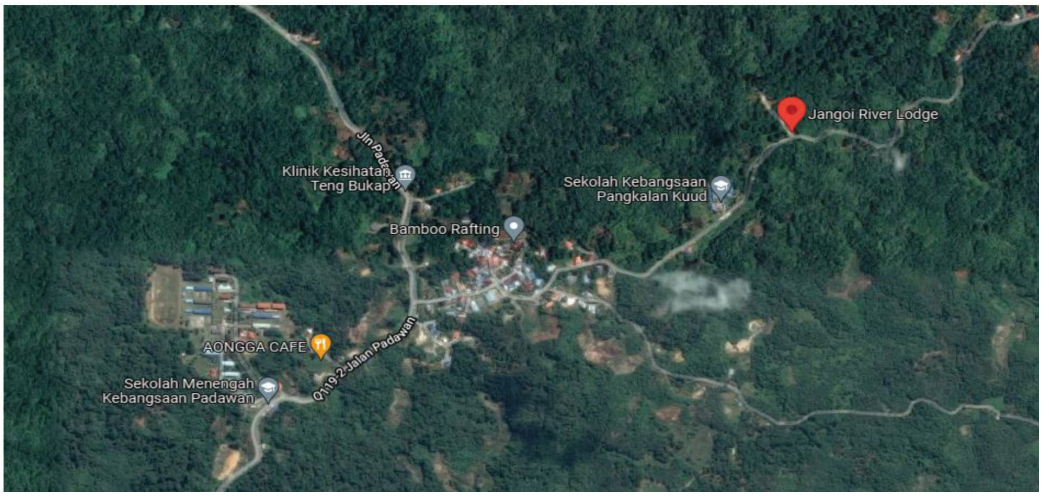

Figure 1: The locality of the Jangoi River Lodge, Padawan (Source: Google Earth maps)

Table 1: The land use at selected sampling stations

Station	Part of River	Land Use/Activity
1	Upstream	Agriculture and farming, residential
2	Middle stream	Recreational, resort (tourism)
3	Downstream	Agriculture and farming, residential

Water Quality

Water quality assessments were conducted according to the American Public Health Association (2017). The water quality parameters, such as pH, temperature, dissolved oxygen (DO) and conductivity were measured *in-situ* and recorded at every station. The YSI Pro Plus Multiparameter Water Quality was used to measure *in-situ* water parameters. The water samples at each station were collected using 1,500 mL plastic bottles at the surface for the analysis of total suspended solids (TSS), chemical oxygen demand (COD) and ammoniacal nitrogen content (NH_3-N). As for biological oxygen demand (BOD_5), 300 mL BOD_5 glass bottles were used to collect water samples at each station and covered with aluminium foil to prevent the production of additional oxygen from photosynthesis (Scholz, 2006). The initial *in-situ* DO read was measured by using YSI Pro Plus Multiparameter Water Quality Meter. The bottles were stored in a dark incubator box for five days, and the final DO values were measured and recorded on the fifth day. The HACH DR/890 portable calorimeter was used to analyse COD and NH_3-N. The WQI for every selected station was calculated by comparing the water quality parameters with their respective regulatory standards. The calculations were done by using the sub-indices values of six water quality parameters: DO, BOD_5, NH_3-N, TSS, COD and pH. The following formula was to calculate the WQI (DOE, 2009).

$$WQI = [0.22 \times SIDO] + [0.19 \times SIBOD_5] + [0.16 \times SICOD] + [0.15 \times SINH_3\text{-}N] + [0.16 \times SISS] + [0.12 \times SIpH] \quad (1)$$

Statistical Analysis

The static statistical analysis was conducted using the SPSS Software. One-way analysis of variance (ANOVA) was performed to determine whether there was a significant difference between all stations for each parameter at a confidence level of p-value = 0.05. The Pearson correlation analyses were also done to analyse the relationship between the parameters.

Isolation of Bacteria

The homogenisation of water samples was done before performing the two-fold serial dilution according to Huys (2003). A total of 1 mL of homogenised water was diluted with 9 mL of 0.85 (w/v) saline solution. A total of 100 µL of water samples were plated on Hicrome Coliform Agar and incubated at 37°C for 12 to 24 hours (Poznyak *et al.,* 2019). A few colonies were randomly isolated and streaked onto Hicrome Coliform Agar until pure isolates were obtained. Slanted agar was used to keep the isolates as working stock at 4°C and in 15% glycerol stock for long-term preservation at -20°C (Addgene, 2012).

Coliform Count

The number of bacterial colonies present on Hicrome Coliform Agar was enumerated in CFU/100 mL by using the formula (Kumar, 2011) shown below:

$$\text{CFU/Ml} = \text{no. of colonies x dilution factors/ volume of culture plate} \quad (2)$$

Bacterial DNA Extraction

The extraction of bacterial DNA was done using the boiling centrifugation method (Kathleen *et al.,* 2011) with few modifications. The nutrient broth was used as the enrichment broth. The bacteria were picked up using a sterilised loop, dipped into the prepared broth and grown overnight. The broth culture was transferred into a sterile 2 mL centrifuge tube and centrifuged at 10,000 rpm for 5 minutes. Next, the supernatant was discarded, and the pellet was resuspended in 500 μL of ddH_2O and vortexed. The suspension was then heated at 100°C for 10 minutes using a heat block and then cooled for 5 minutes. The suspension was centrifuged at 10,000 rpm for 10 minutes. A 100 µL supernatant was then transferred to a sterile tube and stored at -20°C for PCR testing later (Al-Griw *et al.,* 2017).

16S rRNA Sequencing

Nine bacterial isolates were successfully identified using 16S rRNA sequencing. The

amplification by PCR was performed according to Kathleen *et al.* (2014) in a standard 30 µL reaction mixture, which consists of 15 µL of exTEN PCR Mastermix (1st Base, SG), 1.2 µL 27F and 519R primers, 9 µL sterile distilled water a with 3.6 µL of total genomic DNA. The amplification begins with an initial denaturation at 95°C (10 minutes), denaturation at 94°C (30 seconds), followed by 26 cycles of annealing, extension at 55°C (1 minute) and 72°C (1.5 minutes), respectively. The final extension was carried out at 72°C for 1 minute. A total of 30 µl of the PCR product were electrophoresed on 1.0% (w/v) of agarose gel. The agarose gel was pre-stained with 1 ***µ***L of 10 mg/mL ethidium bromide (Promega, USA) in 1X Tris-Borate -EDTA (TBE) buffer for 30 minutes at 90 V. The agarose gel was visualised under a UV transilluminator (Maestrogen, TW).

DNA Purification

The protocol of electrophoresis was carried out according to Kathleen *et al.* (2014) with modification. The amplified products were purified by using a QIAquick PCR purification kit (Qiagen, Germany). The DNA product from extraction, amplification and purification was run through 1.0% (w/v) agarose gel. A total of 1 µL of 10 mg/mL of EtBr (Promega, USA) was mixed with the agarose gel during the preparation. In order to confirm the presence of purified DNA, the PCR products were electrophoresed in 1 X TBE buffer for 30 minutes under 80 V, followed by the visualisation of the stained gel under a UV transilluminator. Next, the purified DNA products were sent to Apical Scientific Sdn Bhd for DNA sequencing. After obtaining the DNA sequences, the comparison of 16S rRNA nucleotide sequences with the available 16S rRNA nucleotide sequences in the database from GenBank was made using the BLAST server by the National Centre for Biotechnology Information (NCBI).

Antibiotic Susceptibility Test (AST)

The disc diffusion method was implemented to identify the antibiotic susceptibility according to the Clinical and Laboratory Institute (CLSI, 2016). The inoculation of bacteria was done by swabbing the bacterial culture onto Mueller Hinton Agar (MHA) with a sterile cotton swab. Following that, the antibiotic-containing disc was placed on the agar medium and the plate was incubated at 30°C for 18 to 24 hours. This study employed commercial antibiotics, such as erythromycin (15 g), ampicillin (10 g), chloramphenicol (30 g), ciprofloxacin (5 g) and tetracycline (30 g). The zone of inhibition that formed after 24 hours was measured with a standardised ruler. Using the usual interpretive table, the results were examined and classified as susceptible (S), intermediate (I) and resistant (R). The multiple antibiotic resistance index (MARI) was calculated as the ratio of the number of antibiotics to which the isolate is resistant against: (a) the total number of antibiotics to which the isolates were tested, (b) i.e., MARI = a/b. *E. coli* ATCC 25922 and *S. aureus* ATCC 29213 were used as positive controls. In this study, only *A. xylosoxidans, S. maltophilia, P. hibiscicola, A. mucicolens* and *B. Pacificus* were tested for their antibiotic susceptibility.

Results and Discussion

pH

The pH is proportional to the hydrogen ion concentration in the water. Natural changes typically occur as a result of the water's interactions with the underlying rock, particularly carbonate forms and precipitation, particularly acid rain. Additionally, the pH can fluctuate due to wastewater or mining wastes entering the water (EPA, 2012). Per the results obtained, the water from Jangoi River has a pH range of 7.51 to 7.97 and is therefore classified as Class I (good quality). According to Lentech (2013), the natural pH values for freshwater lakes, ponds, and streams range between 6 and 8, contingent on the soil and bedrock in the area. The bulk of living things can survive at a pH range of 6.5 to 9.0, including fish and mussels (Fondriest Environmental, 2019). The pH level of the water is affected by many factors, both natural and manufactured. Acid rain, elevated

CO_2 levels, drainage or mining discharges can affect the pH value (EPA, 2012). pH monitoring is pivotal because changes in pH can have a detrimental effect on a variety of chemical and biological processes occurring in the water. Water bodies with an abnormally high or low pH value may become unfavourable to the survival of the majority of aquatic life, as their preferred pH range is between 6.5 and 9.0 as stated before, even though some can survive in water with a pH value outside of this range (Fondriest Environmental, 2019). In this study, the one-way ANOVA analysis revealed that the mean pH values between all three stations were not significantly different from each other.

Dissolved Oxygen (DO)

The DO of Jangoi River is classified as Class II, as it ranged between 5.53 mg/L and 6.25 mg/L, which is deemed good. According to the ANOVA analysis output, the p-values obtained for Trip 1 were $p = 0.850$ and for Trip 2 were $p = 0.090$, indicating that there is no significant difference in DO for all three stations. The DO denotes the concentration of free oxygen molecules in water. Its concentration will fluctuate according to season, location, and water depth. Aquatic growth requires a minimum of 4 mg/L DO. Below that level, aquatic species may perish. The DO concentrations over than 6 mg/L indicate a healthy ecosystem with enough water flow in a river (Ibrahim & Kutty, 2013).

Biological Oxygen Demand (BOD_5)

The BOD_5 for all stations was categorised under Class II, except for station 2, during Trip 1. It was found to be the highest (3.41 mg/L) and was classified as class III. The water sample from station 2 was taken near the riverfront due to the middle section being inaccessible due to its depth. Considering the minimal water flow, organic matter in the water accumulated along the river's banks. Water samples from sections 1 and 3 were taken in the middle section with fast water flow. Owing to the fact that moving water dilutes and decomposes contaminants more rapidly than standing water, the BOD_5 level at station 1 was lower than the BOD_5 level at station 2. The BOD_5 level was higher during Trip 1 than it was during Trip 2 due to the tidal variation. During the wet season, more nutrients will run off into the river, intensifying the impacts. According to the analysis, the BOD_5 concentration in Jangoi River was between 2.68 mg/L and 3.41 mg/L. Generally, uncontaminated water has a BOD_5 concentration of less than 2 mg/L. Therefore, Jangoi River has traces of organic material.

Nitrate and phosphate emission may result in an increase in BOD_5. In the case of the Jangoi River, one of the identified causes of the BOD_5 elevation is the picnic activities held in the middle stream. Dishwasher use, as well as beverages and food additives, are all possible sources of phosphate release. Additional nitrate and phosphate sources include manure runoff, fertiliser use and sewage effluent from residential/ tourism sites. Excessive concentrations of these chemicals will result in the increase of organic matter in the water. This will eventually increase the bacteria present to breakdown them. As a result, the BOD_5 level will be elevated (CIESE, 2019). The increase in BOD_5 levels will result in algal blooms and the death of aquatic organisms such as fish. Degradation of water quality will have a significant impact on the public's ability to use rivers for recreational purposes, lowering the value of the country's tourism attractions and properties. Therefore, the authorities should improve the nutrient discharge and sewage treatment management to limit the amount of excessive nutrient being released into the river.

Chemical Oxygen Demand (COD)

The COD concentrations of Jangoi River ranged between 6.67 mg/L and 11.7 mg/L. During Trip 1, water samples taken at stations 1 and 2 reported higher COD values, classified as Class II, while the remainder are classified as Class I. The ANOVA analysis revealed that the mean COD between all three stations were not significantly different, with $p > 0.05$ ($p = 0.438$) for Trip 1 and $p > 0.05$ ($p = 0.854$) for Trip 2. The higher COD concentration of the upstream water indicates the discharge of wastewater

from dwellings or agricultural locations next to Jangoi River's upper stream. Animal manure, fertilisers, herbicides and pesticides, as well as food and beverage waste from adjacent residential and agricultural areas, may all contribute to the increase in COD levels in the water.

COD and BOD_5 values appeared to be different, with COD values typically greater than BOD, although both represent the decomposition of organic waste. The COD test employs potassium dichromate (in a 50% sulfuric acid solution) that "oxidises" both inorganic and organic compounds in a water sample, resulting in a significant COD reading than the BOD_5. The BOD_5 test, on the other hand, solely determines the biodegradable portion of an organic waste process (USEPA, 2012).

In some circumstances, the BOD_5 value may be greater than the COD value, that is as measured by dichromate oxidation. As explained by Kani (2014) and Anderson (2017), the most frequent instance is a sample that contains a huge amount of decomposable organic matter and a significant concentration of ammonium and organic nitrogen, such as effluents from the processing of fish. Despite the fact that chromate does not convert ammonium to nitrate, the BOD_5test inoculum may contain nitrifying bacteria that use oxygen in the process. To prevent this, certain BOD_5 methods use a nitrification inhibitor. A refinery's effluent may include sulfur or organic nitrogen that can be oxidised biologically, but not by chromic acid in the COD process.

In the case of Jangoi River, one of the contributing factors of the BOD_5 elevation is the picnic activities held in the middle stream. Dishwasher use, as well as beverages and food additives, are all potential sources of phosphate release. Additional nitrate and phosphate sources include manure runoff, fertiliser use, and sewage effluent from the residential or tourism sites. Excessive concentrations of these chemicals will increase the presence of organic matter in the water, which will increase the presence of bacteria to break them down.

As a result, the BOD_5 level will be elevated (CIESE, 2019), thus leading to the occurrence of algal blooms and the death of aquatic creatures, such as fish. The degradation of water quality will have a significant impact on the public's ability to use rivers for recreational purposes, lowering the value of the country's tourism attractions and properties. As a result, the authorities should improve nutrient discharge and sewage treatment management to limit the amount of nutrients entering the river.

Ammoniacal Nitrogen (NH_3-N)

In this study, NH_3-N was not detected because the chemical is present outside the method's detection limit. The test range for NH_3-N detection using the Hach method 8155 on the HACH DR/890 calorimeter is 0.01- 0.50 mg/L.

NH_3 is a very harmful pollutant that can affect the aquatic environment (Oram, n.d.) and is typically found in water as a result of fertiliser runoff. Accordingly, NH_3-N levels in unpolluted water should not exceed 0.1 mg/L. Otherwise, it is considered polluted.

According to Mohammad Razi *et al.* (2020), NH_3-N is one of the leading pollutants along the Kuching, Betong and Samarahan shorelines. Following their findings, it is proposed that the contaminants originated from extensive agricultural and aquaculture activities, commercial establishments and coastal towns. Pantai Damai in Kuching had the highest NH_3–N content in their investigations, at 0.41 mg/L, when compared with other coastal waters in 2016.

Majit (2008) in his study, paid particular attention to assessing the water quality of several rivers in Sarawak used for recreational activities. The recreational sites studied were Ranchan Waterfall, Giam Waterfall and Rayang River. The rivers' NH_3-N levels were found to be between 0.031 to 0.054 mg/L, which was considered to be unpolluted.

Accordingly, Giam Waterfall and Sungai Rayang did not attract many people due to the secluded river's limited access (Majit, 2008).

The river was mostly used by locals from surrounding dwellings along the riverbed for daily activities, such as laundry. Because of the enormous increase in the human population and socioeconomic development, the preliminary data are out of date (Soo *et al.,* 2017). Therefore, the recreational rivers may have been used for a variety of additional purposes, and the rivers' tourism status may have evolved. In line with previous studies, this study also addresses the requisite for continuous and frequent monitoring of the water quality status of Sarawak's recreational rivers.

Total Suspended Solids (TSS)

The TSS of Jangoi river was recorded at 1 mg/L for all sampling stations and is classified as Class I.

Temperature

Water temperature is influenced and affected by sunlight radiation. Water samplings were carried out at 9.00 am to 11.00 am, starting from the upstream to the downstream. The temperature of Jangoi River ranged between 24.8°C to 25.1°C. There was an increasing trend in the recordings, and the highest temperature recorded was at downstream part of the river, which was the last station. The difference in the temperature was due to the difference in timing when the water sampling as conducted and taken in situ for water temperature. Water temperature in the morning is low, therefore, the temperature at the upstream was the lowest since it was taken first before the other stations.

Coliform Count

Faecal coliform test estimation ranged from 650 CFU/100 mL to 1000 CFU/100 mL. Meanwhile, the total coliform estimation ranged from 20,050 CFU/100 mL to 23,250 CFU/100 mL. The infiltration of faecal coliform bacteria into waterways is expected to come from direct waste discharged from animals or birds, the runoff caused by storms, untreated human sewage and release of faecal material from the residential areas. Another possibility is that untreated human waste is spilled into nearby rivers as a result of individual homes' septic tanks overflowing during the rainy season. Additionally, as Buckley (2004) indicated, swimming activities may contribute to the coliform count, as their study reported that *E. coli* concentrations rose during recreational activities. When the concentration of faecal coliforms is significant, pathogenic organisms are more likely to be present. Furthermore, swimming in waters with high faecal coliform levels raises the risk of having a fever, nausea or stomach cramps as a result of swallowing disease-causing organisms or pathogens that enter the body through cuts in the skin, mouth, ears and nose, especially in young children. Additionally, the elderly and individuals with compromised immune systems are more vulnerable and are at risk (Oram, n.d.). In recreational water bodies, some organisms, such as *Campylobacter* and *E. coli*, may cause waterborne illnesses. Lihan *et al.* (2017) also documented the presence of Enterobacteriaceae pathogenic members in a recreational river. The increase was driven by either physical input introduced by swimmers or bacteria resuspended in the streambed sediments. According to the DOE's water quality guideline, the maximum allowable faecal count in recreational water is 400 counts/100 mL of water.

Water Quality Index

The results of each water quality parameter were tabulated as shown in Appendix I. The WQI was calculated using the Malaysian water quality index formula as shown in Appendix III. The classification of water parameters for sampling Trips 1 and 2 are shown in Table 2. The DOE Water Quality Classification based on WQI is shown in Appendix IV and Appendix V.

An indexing system, WQI was implemented to aid in the organisation of the massive amount of data collected in accordance with the Interim National Water Quality Standards. The indices were created to be an easily understood method for evaluating water quality (Bordalo *et al.,* 2006). It is composed of sub-index values assigned to each pre-identified parameter through

Table 2: Classification of water parameters based on the DOE-WQI classification for Trips 1 and 2

Sampling Stations	Parameters	Value	Trip 1 Class	Value	Trip 2 Class
Station 1	pH	7.90	I	7.86	I
	DO (mg/L)	5.76	II	5.98	II
	BOD_5 (mg/L)	2.86	II	2.81	II
	COD (mg/L)	11.7	II	6.70	I
	NH_3-N (mg/L)	0.00	I	0.00	I
	TSS (mg/L)	1.00	I	1.00	I
	WQI	**88**	**I**	**91**	**I**
Station 2	pH	7.97	I	7.64	I
	DO (mg/L)	5.92	II	6.14	II
	BOD_5 (mg/L)	3.40	III	2.79	II
	COD (mg/L)	10.3	II	7.30	I
	NH_3-N (mg/L)	0.00	I	0.00	I
	TSS (mg/L)	1.00	I	1.00	I
	WQI	**89**	**I**	**91**	**I**
Station 3	pH	7.76	I	7.51	I
	DO (mg/L)	5.91	II	6.25	II
	BOD_5 (mg/L)	2.89	II	2.71	II
	COD (mg/L)	8.00	I	7.00	I
	NH_3-N (mg/L)	0.00	I	0.00	I
	TSS (mg/L)	1.00	I	1.00	I
	WQI	**90**	**I**	**92**	**I**

comparison to a parameter-specific rating curve, dynamically weighted and integrated into the final index. However, the existing WQI formula has a few drawbacks in terms of accurately portraying the water's actual qualities.

This paper has asserted the constraint. As stated in Table 2, the WQI for all stations is between 88 and 92; thus, they are classified as Class I. Nonetheless, this study unequivocally verifies the WQI formula shortcomings and

ineffectiveness as a method for assessing water quality. The physio-chemical indices provided excellent assessment marks to the water quality, which do not correspond to the actual qualities of the river water. According to the data acquired, all stations are optimally classed as Class I, with an excellent WQI, but failed in its microbial evaluation.

As Zainudin (2010) pointed out, most indices are constructed using a predefined set of water quality elements. A site may have an excellent WQI score, but still having a degraded water quality owing to a few significant elements that are not included in the index.

The impracticality has been embedded in Malaysia's current WQI, where the six constituents of WQI are primarily physicochemical in purpose, with no consideration for coliform indications (*E. coli*). The existing formula is flawed due to the lack of microbiological parameters, as *E. coli* is associated with skin contact (recreation) and even drinkable water supplies.

Sim and Tai (2018), in their study, also revealed deteriorated water quality of the tested river as a result of microbiological contamination. Thus, the WQI formula should incorporate the microbiological component, as it is a significant determinant for the WQI. A similar conclusion was also reached by Naubi *et al.* (2016), where their findings demonstrated the ineffectiveness of the DOE's WQI formula since it omitted numerous critical elements, such as nutrients, heavy metals, and faecal coliform.

A favourable WQI at a specific station does not always imply that the water quality was favourable. The issue that occurs in reality is that the WQI provides a positive value, when on-site conditions indicate otherwise (Zainudin, 2010). Apart from the omission of microbiological indicators, Mamun *et al.* (2007) found contradictions between the WQI and the Environmental Quality Act's effluent discharge regulations in Malaysia. For example, whereas NH_3-N is identified as a major contaminant contributing to the pollution of many rivers, there is no limit on this parameter in the effluent discharge standards.

According to previous studies, it is conceivable that most of Malaysia's rivers were found to have a high level of microbiological pollution. Mamun and Zainudin (2013) reported that the elevated coliform levels were caused by poultry wastes and residential wastes. Moreover, Malaysia's environment is ideal for pathogen growth. As a result, there may be suggestions to integrate the coliform count (*E. coli*) as one of the parameters in the formula used to represent the actual quality of the water.

Correlation Analysis

The Pearson's correlation coefficient value of the water quality parameters of pH, temperature, DO, BOD_5, COD, NH_3-N, TSS for all the three selected sampling stations during Trips 1 are shown in Appendix II. For Trip 1, the analysis shows that all seven parameters are not correlated to each other. For Trip 2, most of the parameters are not correlated to each other except for pH, which is positively and significantly correlated ($p < 0.05$) with BOD_5 (0.692), and DO, which is positively and significantly correlated ($p < 0.05$) with BOD (0.680). NH_3-N and TSS cannot be computed for both trips because the values of both parameters were below the detection limit.

Identification of Bacteria and Antibiotic Susceptibility Test

Nine bacterial isolates were identified using the 16S rRNA PCR analysis and DNA sequencing. The percentage similarity is $\geq$ 90% for all isolates. The list of identified bacteria and the mean diameter of the halozone formed by each isolate are shown in Tables 3 and 4, respectively.

Referring to Table 4, the mean diameter of the halozone generated by all bacterial isolates ranged between 0.0 to 34.0 mm. Ciprofloxacin exhibited the highest percentage of susceptibility (100%) against all bacterial isolates, followed by ampicillin and chloramphenicol (40%). Conversely, erythromycin had the highest rate of resistance against all bacterial isolates examined (60%).

Table 3: The identified bacteria after 16S rRNA sequencing

No.	Bacteria
1	*Escherichia coli*
2	*Chromobacterium violaceum*
3	*Lelliota amnigena*
4	*Pseudomonas aeruginosa*
5	*Achromobacter xylosoxidans*
6	*Stenotrophomonas maltophilia*
7	*Pseudomonas hibiscicola*
8	*Achromobacter mucicolens*
9	*Bacillus pacificus*

Table 4: The mean halozone formed by each isolate

Bacteria Isolates	Mean Halozone Diameter (mm)					MAR Index
	AMP	TE	CIP	E	C	
Achromobacter xylosidans	0 (R)	16.0 (I)	21.7 (S)	15.7 (I)	16.7 (I)	0.2
Stenotrophomonas maltophilia	23.3 (S)	18.3 (I)	34.0 (S)	10.3 (R)	13.7 (R)	0.4
Pseudomonas hibiscicola	24.7 (S)	13.0 (R)	30.3 (S)	11.7 (R)	12.7 (R)	0.6
Achromobacter mucicolens	15.0 (I)	24.0 (S)	20.3 (S)	10.7 (R)	24.7 (S)	0.2
Bacillus pacificus	19.3 (I)	16.3 (I)	21.6 (S)	20.0 (S)	25.7 (S)	0.0

[R = Resistant, I = Intermediate, S = Susceptible]

The occupancy of *E. coli* in the water environment implies that the river has been polluted by animal and human faeces. As in this case, its prevalence has been attributed to direct faecal emission from livestock and wildlife in the adjacent waterways of the studied river. Particularly, the poultry farms observed along the riverbank may have served as reservoirs for antibiotic-resistant *E. coli.*

Additionally, it is hypothesised that the presence of *E. coli* in the water is a result of the undisclosed volume of poorly treated wastewater being discharged into the river, opening up the possibility of isolating *E. coli* with antibiotic-resistant characteristics. Residents also introduce a variety of fish into the upper course of the river. Nonetheless, the fish were not confined but were left free in the river. With that being said, all faeces, waste and uneaten feed may contribute to the growth of coliform bacteria, degrading the water quality.

The isolation of *C. violaceum* is inevitable, as it is a ubiquitous soil and water inhabitant. But even so, the abundance of this bacterium is relatively harmful to consumer health. In Malaysia, there have been multiple incidences of infection and mortality caused by *C. violaceum.* Skin lesions and localised abscesses were the most common manifestations. Correspondingly, there have been cases of *C. violaceum*-related diarrhoea and a fatal case of *C. violaceum* infection inextricably linked to ruptured appendicitis, with ingestion of contaminated water being the most likely route of infection.

The organism was found to be relatively susceptible to carbenicillin, cefoxitin and ticarcillin, which are utilised to resist hydrolysis.

There is not much information available on the species *Lelliottia amnigena*. This name became validly published in the same year it appeared on Validation List No. 154 (Namesforlife, 2018). The name *amnigena* means "born in a river", which is intended to imply that it originated from water, thus clarifying why it was discovered and isolated from the Jangoi River water.

Following an investigation of the land usage, it is hypothesised that the *P. aeruginosa* came from animal feeds and faeces, as well as untreated and contaminated water near the riverbank. However, these bacteria are found naturally in soil and water, which justifies their existence in the river water. Furthermore, Pseudomonas bacteria have been shown to be extremely well adapted to survive in a wide variety of conditions, despite being disinfected. Yet, additional data on the occurrence of ntibiotic-resistant bacteria (ARB) in aquatic environments should be analysed to monitor this organism's antibiotic resistance pattern.

An interesting criterion of *A. xylosidans* will be its resistance to a variety of antibiotics. In this study, *A. xylosidans* was shown to be susceptible to ciprofloxacin (CIP) but resistant against ampicillin. Achromobacter species are known to be commonly resistant when tested with ampicillin. The findings of this study corroborate Weitkamp *et al.* (2000). According to Amoureux *et al.* (2013), *A. xylosoxidans* is an uncommon environmental bacterium that is seldomly identified or discovered in most clinical tests. However, if there is detection in the clinical samples, it would most probably be *P. aeruginosa.* This bacterial species was found to be ampicillin-resistant in this study. The respective prior study by Jakobsen *et al.* (2013) explored the mechanism of resistance of *A. xylosoxidans* strain NH44784-1996 towards metals, such as mercury, chromium and zinc, also similar *to P. aeruginosa*. Traglia *et al.* (2012), on other hand, had published well-documented research indicating that *A. xylosoxidans* clinical isolates have a rich heterogeneity of genetic elements, which are frequently related to resistance genes and their dissemination. The assertion is predicated on the premise that *A. xylosoxidans* has developed into a reservoir for horizontal genetic transfer factors linked to the spread of antibiotic resistance. Amoureux *et al.* (2013) showed that this bacterium's active efflux pump possesses the ability to extrude multidrugs per pump type, including AxyABm, which extrudes ciprofloxacin and chloramphenicol, and Axy XY-Opr3, which extrudes tetracycline.

Stenotrophomonas maltophilia is a naturally present bacterium that thrives in an aquatic and humid environment. Previously, *Bacterium bookeri* was isolated in 1943 and was later renamed *Pseudomonas maltophilia* and finally *Xanthomonas maltophilia* (Hugh & Leifson, 1963). However, there is a raging debate over the terminology. Additionally, the clinical strains of *S. maltophilia* are often non-susceptible to a variety of antibiotics, but the causes of resistance are poorly understood (Zhang *et al.,* 2000). In this study, *S. maltophilia* is responsive to ampicillin and ciprofloxacin, but resistant against erythromycin and chloramphenicol. Our findings were in accordance with Adegoke and Okoh (2014).

According to Crossman *et al.* (2008), numerous antibiotic-resistance genes, which encode for multidrug efflux pumps, β-lactamase and aminoglycoside-modifying enzymes, were present after the DNA sequencing of *S. maltophilia* K279a strain. As has been previously reported in the literature by Nicodemo and Paez (2007), the intrinsic resistance properties of this bacterial species are determined by the multidrug efflux pumps and the outer membrane's low permeability. This condition may seize resistance genes previously identified on integrons, plasmids and transposons. According to Svensson-Stadler *et al.* (2012), isolating *S. maltophilia* has proven difficult due to its association with polymicrobial illnesses. Furthermore, the difficulties are due to several previously proposed species being

recognised to be allied to *S. maltophilia* and it might be referred to as the "*S. maltophilia* complex", including *Pseudomonas geniculata*, *Stenotrophomonas pavanii*, *P. hibiscicola*, *Stenotrophomonas africana* and *Pseudomonas beteli*. In this study, *P. hibiscicola* was found to be resistant against tetracycline, erythromycin, and chloramphenicol. However, there is insufficient data on this bacterial species' antibiotic resistance activity. In this study, *P. hibiscicola* was also identified and is classified as a member of the genus Stenotrophomonas.

As for *Bacillus pacificus*, this bacterium is classified under *Bacillus cereus,* a facultatively anaerobic bacterium that is broadly found in soil. Due to the scarcity of data on this bacterium species, the antibiotic susceptibility profile of *B. pacificus* is not well described in the literature. Additionally, Bello *et al.* (2012) documented that *B. cereus* has been discovered as a potentially damaging enterotoxin producer, and their presence in recreational water may pose a threat to human health, particularly when ingested with contaminated water. In this study, the MAR index ranged from 0.0 to 0.6.

A MAR index value that is larger than 0.2, on the other hand, suggests a high-risk source of contamination. The identified isolates are discovered from areas where antibiotics are often used. Thus, it is imperative to regularly monitor antibiotic-resistant patterns to forecast the emergence of MAR bacteria and prevent their spread. While antibiotic use is frequently implicated in the incidence of MAR, the outcomes of this study proposed that land use may also play a role in driving the MAR event. Several examples of land use that may actually add to the prevalence of MAR include the use of animal dung as fertiliser to improve the soil's fertility for agricultural use. Manure use in agriculture may contribute to the expansion of antibiotic resistance reservoirs by ARB and antibiotic resistance genes into the soil. Rainy seasons may culminate in the runoff of manured soil into rivers, causing an influx of water. The extensive use of antibiotics in livestock farming leads in their discharge into the surrounding environment, making it one of the significant contributors to this prevalence. This was supported by a study by Heuer and Smalla (2007).

Conclusion

To conclude, it is shown that most of the water parameters recorded at every station had similar values to each other, supported by the one-way ANOVA analysis. The WQI of Jangoi River was determined, and it was established that the river's water is categorised as Class I (clean water). Nevertheless, the faecal and total coliform counts were significantly higher in Jangoi River, which was classified as Class III. Thus, the outcome of this study illustrates the ineffectiveness of the present WQI, as it excludes critical criteria (in this case, faecal coliform count; *E. coli)*. The WQI of Jangoi River does not represent the actual conditions or properties of the water, where it is assigned to an excellent class under WQI but is contaminated with coliform bacteria. On that note, this research proposes that the present WQI formula should be modified to incorporate several significant and influential characteristics to increase the formula's effectiveness and accuracy.

The faecal pollution likely came from surrounding residences. Therefore, tourists should take precaution while engaging in recreational activities. Of the five antibiotics tested, erythromycin showed the highest rate of resistance (60%), followed by chloramphenicol (40%). Additionally, ciprofloxacin recorded the highest percentage of susceptibility (100%) against all isolates, whereas tetracycline and erythromycin had the lowest percentages of susceptibility against all isolates at 50% and 20%, respectively There is at least one isolate with a MAR index of 0.0 in this study, which is *B. pacificus.* However, the MAR index ranged between 0.0 and 0.6 in this study, which is concerning because a MAR index value larger than 0.2 suggests a high-risk source of contamination.

Previously, the water quality and antibiotic resistance pattern of bacteria in Sarawak recreational rivers were only analysed to a

limited extent owing to the unavailability of a continuous monitoring programme. Therefore, the findings of this study may initiate efforts for an ongoing monitoring programme of water quality status and antibiotic resistance patterns of bacteria in Sarawak's rivers. Additionally, this study recommends the need to alter the existing WQI formula to obtain a more precise measurement of the WQI, therefore assuring the excellent quality of river water as a means to suit the government's mission to improve and increase water-based recreational activities and aqua-tourism in the country. Lastly, it is intended that this research should be continued in the future, especially on the antibiotic resistance patterns to assist future management decisions by the environmental and health authorities.

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Appendix

Appendix I: The results for the water parameters for Trips 1 and 2

Parameters	Station 1		Station 2		Station 3	
	Trip 1	**Trip 2**	**Trip 1**	**Trip 2**	**Trip 1**	**Trip 2**
pH	7.90 ± 0.2	7.86 ± 0.2	7.97 ± 0.2	7.64 ± 0.2	7.76 ± 0.2	7.51 ± 0.2
Dissolved Oxygen (mg/L)	5.53 ± 0.7	5.98 ± 0.14	5.9 ± 0.10	6.14 ± 0.11	5.90 ± 0.05	6.25 ± 0.15
Ammoniacal Nitrogen (mg/L)	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected
Biochemical Oxygen Demand (mg/L)	2.86 ± 0.01	2.81 ± 0.01	3.40 ± 0.01	2.79 ± 0.01	2.89 ± 0.01	2.71 ± 0.01
Chemical Oxygen Demand (mg/L)	11.7 ± 2.1	6.7 ± 3.2	10.3 ± 4.9	7.3 ± 2.3	8.0 ± 2.0	7.0 ± 3.0
Total Suspended Solids (mg/L)	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0
Temperature (^{o}C)	24.8 ± 0.0	25.0 ± 0.0	24.9 ± 0.0	25.1 ± 0.0	25.0 ± 0.0	25.1 ± 0.0

Appendix II: The correlation between the water quality parameters for Trip 1

		pH	DO	BOD	COD	AN	TSS	TEMP
pH	Pearson Correlation	1						
	Sig. (2-tailed)	9						
	N							
DO	Pearson Correlation	.502	1					
	Sig. (2-tailed)	.169						
	N	9	9					
BOD	Pearson Correlation	.364	.126	1				
	Sig. (2-tailed)	.335	.747					
	N	9	9	9				
COD	Pearson Correlation	.184	-.282	.242	1			
	Sig. (2-tailed)	.636	.462	.531				
	N	9	9	9	9			
AN	Pearson Correlation	.a	.a	.a	.a	.a		
	Sig. (2-tailed)	.	.	.	.	.		
	N	9	9	9	9	9		
TSS	Pearson Correlation	.a	.a	.a	.a	.a	.a	
	Sig. (2-tailed)	.	.	.	.	.	.	
	N	9	9	9	9	9	9	
TEMP	Pearson Correlation	-.324	.642	-.643	.0	.a	.a	1
	Sig. (2-tailed)	.394	.063	.062	1.0	.	.	
	N	9	9	9	9	9	9	9

The correlation between the water quality parameters for Trip 2

		pH	DO	BOD	COD	AN	TSS	TEMP
pH	Pearson Correlation	1						
	Sig. (2-tailed)	9						
	N							
DO	Pearson Correlation	-.563	1					
	Sig. (2-tailed)	.115						
	N	9	9					
BOD	Pearson Correlation	.692*	.680*	1				
	Sig. (2-tailed)	.039	.044					
	N	9	9	9				
COD	Pearson Correlation	.297	.124	.066	1			
	Sig. (2-tailed)	.437	.750	.865				
	N	9	9	9	9			
AN	Pearson Correlation	.a	.a	.a	.a	.a		
	Sig. (2-tailed)	.	.	.	.	.		
	N	9	9	9	9	9		
TSS	Pearson Correlation	.a	.a	.a	.a	.a	.a	
	Sig. (2-tailed)	.	.	.	.	.	.	
	N	9	9	9	9	9	9	
TEMP	Pearson Correlation	-.640	.192	.048	-.484	.a	.a	1
	Sig. (2-tailed)	.063	.620	.902	.187	.	.	
	N	9	9	9	8	9	9	9

Appendix III: Formula for WQI

DOE – WQI =

(0.22×SIDO) + (0.19×SIBOD) + (0.16×SICOD) + (0.15×SIAN) + (0.16×SISS) +(0.12×SIPH)

Subindex for DO (in % saturation): SIDO

SIDO = 0 for $x \leq 8\%$

= 100 for $x \geq 92\%$

= $-0.395 + 0.030\ x^2 - 0.00020\ x^3$ for $8\% < x < 92\%$

Subindex for BOD: SIBOD

SIBOD = $100.4 - 4.23x$ for $x \leq 5$

= $108e^{-0.055x} - 0.1x$ for $x > 5$

Subindex for COD: SICOD

SICOD = $-1.33x + 99.1$ for $x \leq 20$

= $103e^{-0.0157x} - 0.04x$ for $x > 20$

Subindex for AN: SIAN

SIAN = $100.5 - 105x$ for $x \leq 0.3$

= $94e^{-0.573x} - 5\ |x - 2|$ for $0.3 < x < 4$

= 0 for $x \geq 4$

Subindex for SS: SISS

SISS = $97.5e^{-0.00676x} + 0.05x$ for $x \leq 100$

= $71e^{-0.0016x} - 0.015x$ for $100 < x < 1000$

= 0 for $x \geq 1000$

Subindex for pH: SIPH

SIPH = $17.2 - 17.2x + 5.02x^2$ for $x < 5.5$

= $-242 + 95.5x - 6.67x^2$ for $5.5 \leq x < 7$

= $-181 + 82.4x - 6.05x^2$ for $7 \leq x < 8.75$

= $536 - 77.0x + 2.76x^2$ for $x \geq 8.75$

Appendix IV: The DOE-WQI classification (Source: Benchmarking River Water Quality in Malaysia, 2010)

Parameters	Unit	Classes				
		I	II	III	IV	V
AN	mg/L	< 0.1	0.1-0.3	0.3-0.9	0.9-2.7	> 2.7
BOD	mg/L	< 1	1-3	3-6	6-12	> 12
COD	mg/L	< 10	10-25	25-50	50-100	> 100
DO	mg/L	> 7	5-7	3-5	1-3	< 1
pH	-	> 7	6-7	5-6	< 5	> 5
TSS	mg/L	< 25	25-50	50-150	150-300	> 300
WQI	mg/L	> 92.7	76.5–92.7	51.9-76.5	31.0-51.9	< 31.0

Appendix V: The DOE's water quality classification based on WQI (Source: Benchmarking River Water Quality in Malaysia, 2010)

Parameters	Index Range		
	Clean	Slightly Polluted	Polluted
SIBOD	91 – 100	80 – 90	0 – 79
SIAN	92 – 100	71 – 91	0 – 70
SISS	76 – 100	70 – 75	0 – 69
WQI	81 – 100	60 – 80	0 – 59