JAESRR

http://publisher.uthm.edu.my/ojs/index.php/jaesrr

e-ISSN : 2821-2819

Journal of Advancement in Environmental Solution and Resource Recovery

Leptospira Serovars Ecological Parameters and Water Quality Analysis at Lata Janggut, Jeli, Kelantan, Malaysia

Siti Nor Aishah Mohd Salleh[1,2*], Mohamad Faiz Mohd Amin[2], Nur Shaylinda Mohd Zin[1], Norshila Abu Bakar[1]

[1]Faculty of Civil Engineering and Built Environment, Universiti Tun Hussein Onn Malaysia, Parit Raja, 86400, MALAYSIA

[2]Faculty of Earth Science, Universiti Malaysia Kelantan, 17700 Ayer Lanas, Kelantan, MALAYSIA

[3]Faculty of Applied Sciences and Technology Universiti Tun Hussein Onn Malaysia, Pagoh Campus, Muar, 84600, MALAYSIA

*Corresponding Author

DOI: https://doi.org/10.30880/jaesrr.2021.01.01.003
Received 09 November 2021; Accepted 01 December 2021; Available online 07 December 2021

Abstract: From 2009 to 2015, the number of Leptospirosis infections and deaths in Kelantan increased, with Jeli District having the greatest number of fatalities. This research focuses on the ecological features of *Leptospira* sp. at Lata Janggut, a natural recreational area. Over the course of six consecutive weeks from July 26 to August 28 of 2016, a total of 18 water samples were obtained individually from the upstream part of the waterfall, from the shaded and direct sun areas. For the *Leptospira* sp. availability examination, water samples were collected for morphological characterisation and staining technique. The physical and chemical properties of water samples were examined both in situ and ex situ. The 16 elements of heavy metal were determined as well. The Water Quality Index (WQI) of the upstream of Lata Janggut was determined to be 64.50 (Class III) in the shade and 77.51 (Class II) in the direct sun areas, respectively, based on the WQI value recommended by Malaysia's Department of Environment (DOE). According to the identification, the research region was free of Leptospirosis throughout the observation period, which was consistent with the ecological criteria of *Leptospira* sp. at Lata Janggut.

Keywords: Leptospirosis, ecological, environment, water, characterisation

1. Introduction

Leptospirosis is a silent killer of humans as the symptoms are hard to detect at an early stage but can be fatal. It typically happens in developing countries. However, due to globalisation and international travel make the developed countries are exposed to it as well [1]. It is a zoonotic disease that originated from animals and can be passed to both animals and people, which can cause morbidity and mortality. Bacteria, parasites, protozoa, fungi and viruses may become the factor of the diseases. As for Leptospirosis, it is originated from pathogenic bacteria, which the animals such as rats and dogs excrete the leptospires both in the active infection and asymptomatic stages [2].

Studies of Leptospirosis in Malaysia have shown that Kelantan has the highest number of cases than other states. The number of Leptospirosis infections and deaths in Kelantan increased between 2009 and 2015, which Jeli District set a new record for fatalities, indirectly creating an uneasy scenario [3]. Due to the big flood that occurred in Kelantan

*Corresponding author: nursha@uthm.edu.my

at the end year of 2014, many places were being degraded, such as the residential area and the agricultural site. The disaster also caused the poor hygiene management of the environment, and the spreading of the disease has skyrocketed to the highest number compared to the previous months. In contrast to the common perception of leptospirosis infected through the flood, contaminated food and drink, the case involving the location of the waterfall began to provoke the question mark.

Lata Janggut is recognised as a natural recreational area by the locals. Therefore, the present study can help the visitors have further knowledge about Leptospirosis disease and raise its awareness to the locals and publics. Testing for pathogenic *Leptospira* in water samples may be beneficial for individuals who come into contact with contaminated water while participating in sports and provide them with an early alert about the detection of *Leptospira.* Necessary precautions should be taken by the authorities to monitor water bodies and to alert the public of contaminated water bodies in view of this.

Leptospires need suitable conditions for them to survive in the environment. By studying its ecological parameters, the appropriate circumstance for pathogenic *Leptospira* serovars can be recognised. This infectious disease also provides a fine example that involves the interaction between the environment, animals and humans [4]. In Malaysia, the majority of leptospirosis cases recorded in were caused by humans being exposed to a pathogenic bacteria-infested environment. In Sabah, Malaysia, for example, a leptospirosis outbreak occurred during the Eco-Challenge in 2000. Eighty out of 189 competitors were diagnosed with leptospirosis, and twenty-nine people were hospitalised, but there were no fatalities [5].

Several factors can influence the continuous existence of pathogenic leptospires in the environment since they cannot multiply outside the host. The factors that shown to affect Leptospirosis epidemiology and provide an appropriate niche for this organism include the physical and chemical parameters for Water Quality Index (WQI) (i.e., pH, temperature, Dissolved Oxygen (DO), Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), ammonia nitrogen (NH_3-N), and Total Suspended Solids (TSS)). Typically, the leptospires are sensitive to heat and dryness, acids and basics disinfectants. Tropical and subtropical countries like Malaysia have high humidity and warm temperatures, making it ideal for *Leptospira* to survive in the environment for long periods.

Leptospires lack a waterproof membrane, thus they must stay submerged in water to exist. If their habitat dries up, they will die quickly unless they are freeze-dried, which is intentionally prepared. In the natural environment, the inability to thrive outside of water is a key control aspect, as it ensures they cannot spread illness over dry surfaces. Because pathogenic leptospires are particularly sensitive to a wide range of substances, they are relatively straightforward to eradicate. Detergents, acids, and heavy metals may all kill pathogenic bacteria at extremely low concentrations, making it impossible for them to live in heavily polluted water [6]. Besides, natural disasters also can accelerate the widespread of the disease to worst due to global climate change. The disasters including floods, earthquakes, landslides, tsunamis and typhoons that occur all around the world. The main reason for this outbreak due to the lacking of sanitation facilities and clean drinking water amid the inconvenience [7].

This study of leptospirosis was done at the shaded and direct sun areas of Lata Janggut's upstream, Jeli Kelantan, Malaysia. A thorough and conscientious study about the ecological parameters of *Leptospira* sp. and the water quality analysis were carried out as waterfalls become one of the potential breeding sites for *Leptospira* when the area is rocky and has a water reservoir causing bacteria to spread.

2. Materials and Methods

2.1 Study Area and Water Sampling

This study was conducted at Lata Janggut, which is located 12 kilometres southwest of Jeli, approximately seven kilometres from the Jeli town and about 15 kilometres from University Malaysia Kelantan (UMK) Jeli Campus. On the map, the coordinate is N5 40'05", E101°46'17". Fig. 1 shows the study area of Lata Janggut. The recorded physical-chemical parameters in this study were served as an indication of the water quality status of the different sections (shaded and direct sun areas) of Lata Janggut's upstream. Fig. 2 shows specifically the shaded sun and direct sun areas at upstream of Lata Janggut. A total of 18 water samples were collected individually for six consecutive weeks from July 26 until August 28 of 2016. The samples were taken thrice of selected days in a week to represent the whole week. Collected samples were preserved in the cool box during sampling and transported to the laboratory for analysis following standard procedure in accordance with Standard Method of Examination of Water and Wastewater [8]. The in-situ parameters (i.e., pH, temperature, DO, salinity, conductivity) were measured during sampling using a portable multiparameter (YSI, USA).

Fig. 1 - (a) The upstream of Lata Janggut; (b) the downstream of Lata Janggut

Fig. 2 - (a) Shaded sun area; (b) direct sun area

2.3 Laboratory Testing

Morphological identification and determination: The techniques used to determine leptospires were media culture preparation, serial dilution and bacteria isolation, slide preparation and observation, and simple gram staining.

Physical and chemical properties of water samples: Collected samples were analysed in a laboratory to determine physical and chemical properties of water from Lata Janggut. The properties could help to assess the quality concern of the water body. In the present study, the concentration of BOD, COD, NH_3-N, and TSS were analysed with respective HACH standard methods, i.e., Method 8043 for BOD, Method 8000 for COD, Method 8155 for NH_3-N, and Method 630 for TSS [9].

Heavy metal analysis: The water samples from the study area were collected in glass bottles. In this research, the water samples were tested for all 16 elements of heavy metals, which were silver (Ag), aluminium (Al), arsenic (As), calcium (Ca), cadmium (Cd), chromium (Cr), copper (Cu), iron (Fe), mercury (Hg), potassium (K), manganese (Mn), magnesium (Mg), sodium (Na), nickel (Ni), lead (Pb) and zinc (Zn) for its initial characterisation by using atomic absorption spectroscopy (AAS) (PinAAcle 900F, PerkinElmer, USA). There were two procedures used: filtration and dilution method. The analysis was done with triple repetitions for each element.

Water Quality Index (WQI): Water quality status classification was determined by using the WQI. WQI value for the upstream of Lata Janggut was calculated by entering the six water quality parameters mean values of DO, BOD, COD, NH_3N, TSS and pH. First of all, the values were converted to Sub-Indices according to the equation guided by the Department of Environment (DOE). Then, the WQI was derived from the equation (1) as follows:

$$WQI = (0.22*SIDO) + (0.19*SIBOD) + (0.16*SICOD) + (0.15*SINH_3N) + (0.16*SITSS) + (0.12*pH) \quad (1)$$

Ecological parameters analysis: The gathered data were merged as the ecological parameters of Lata Janggut. All the parameters, i.e., the physical and the chemical parameters, were taken into considerations.

3. Results and Discussion

3.1 Morphological Identification of *Leptospira* sp. from Gram Staining Technique

Leptospires specifically have corkscrew-shaped at its end and a Gram-negative-like cell envelope consisting of a cytoplasmic and outer membrane [10]. Based on the results and observations with its morphological characteristics as the references, supported by gram staining technique with under 100× magnification of a microscope, it can be concluded that there is no *Leptospira* sp. present in the research area. There were many more bacteria observed under the microscope, and these were only a few of them to show that no leptospires were found during the study period. The results for morphological identification from the gram staining technique for selected weeks were as shown in Table 1. Gram-negative bacteria, like other spirochetes, have a classic double-membrane structure. The cytoplasmic membrane and the peptidoglycan cell wall are intimately linked, with an outer membrane covering them both [11]. These bacteria do not retain crystal violet dye in the Gram staining protocol. In a Gram stain test, a counterstain that is commonly safranin is added after the crystal violet, colouring all Gram-negative bacteria with a red or pink colour.

3.2 Ecological Parameters

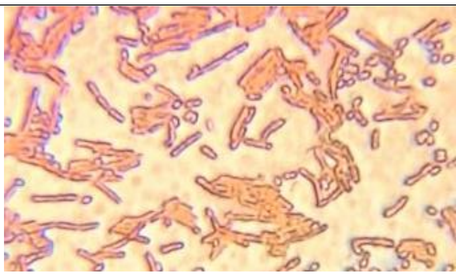
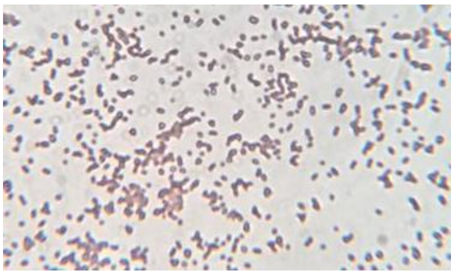
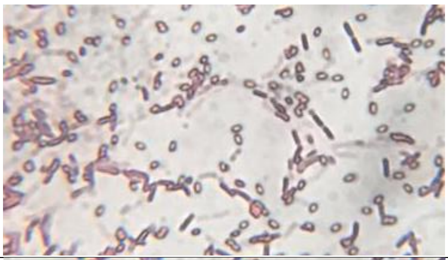
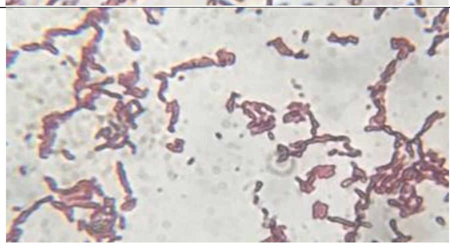
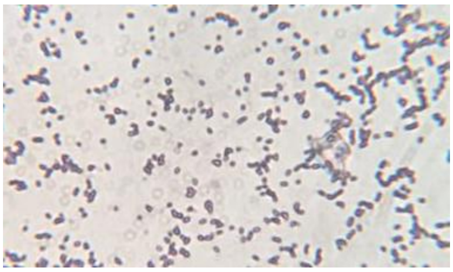
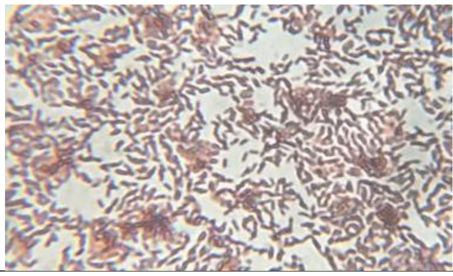
pH: At the upstream, the pH concentration at the shaded sun area for six continuous weeks ranged between 5.75 – 6.95 with a mean concentration of 6.22 ± 0.43 (Appendix A). The pH concentration at each week was 6.95, 6.58, 6.26, 5.94, 5.82, and 5.75, respectively. The pattern for pH at direct sun area was also the same as the shaded sun area as the mean pH concentration was 7.42 ± 0.57, ranging from 6.43 – 8.37 (Appendix B). However, unlike at the shaded sun area, the pH at the direct sun area indicated slightly higher readings, which were 8.37, 7.58, 6.43, 7.31, 7.32, and 7.49, respectively. Environmental temperature and pH values were important factors influencing the survival time of leptospires in water [12]. According to Bejo et al. [13], the longest survival time of serovar hardjo was recorded in river water with a pH between 6.7 and 7.3 placed in the shaded area. They were recorded to survive in river water for 11 days. Previously published studies [6] showed that pH levels outside the neutral zone are not favourable for the survival of leptospires. These findings also showed that the river could be a source of leptospiral infection. It is also important that many activities like military operations, jungle tracking, picnicking and fishing are related to rivers. Duration of survival of pathogenic leptospires outside the host requires the pH of water that is near neutral. In 1931, Taylor and Goyle recorded that leptospires were frequently present in waters of pH 6.9 or more at Andaman Island but were absent from pH 6.6 or less [13].

Temperature: The mean temperature of water at the shaded sun area of Lata Janggut's upstream for the present study of the upstream part was 25.54 ± 0.67 °C that was the range from 24.92 – 26.96 °C (Appendix A). The temperature reading from the first week was the highest while the fourth week was the lowest temperature. They showed that the pattern decreased until the fourth week and increased at the fifth and sixth weeks. Meanwhile, for the direct sun area, significant variation values were found rather from the shaded sun area as the study area was exposed to and received the direct light from the sun. The temperature ranges from 25.87 – 27.28 °C with a mean of 26.51 ± 0.42 °C from the continuous six weeks of the study period (Appendix B). The pattern was also the same as at the shaded sun area, with the values recorded decreasing from the first week until the third week, and later increasing started from the fourth week.

Based on the result, it can be interpreted that the intensity of sunlight radiation and evaporation had influenced surface water temperature. The most common physical assessment of water quality is the measurement of temperature [14]. According to the previous study made by Bejo et al. [13], the survival time of serovar hardjo in water placed under the shaded sun area was longer than the direct sun area. Leptospires can easily be killed in direct sun areas with high temperatures (32 ºC) and strong ultraviolet rays in the environment. In comparison, the environmental factors in the jungle, such as unpolluted water, the temperature of around 27 ºC and the canopy of trees, may increase the ability of the bacteria to survive longer in the particular area. The temperature pattern at the study area was due to the canopy structure from those tall trees presented at Lata Janggut. Although pathogenic leptospires multiply optimally at body temperature, they can survive at a wide variety of temperatures. However, they do not able to sustain temperatures over approximately 42 °C, which may explain why bacterial growth is inhibited or reversed in individuals with extremely high fevers, with no cases of survival at temperatures above 55 °C [13].

Biochemical Oxygen Demand (BOD): The concentration of BOD at the upstream was ranged from 18.2 – 26.4 mg/L with a mean concentration of 21.5 ± 2.44 mg/L, which was found at the shaded sun area of Lata Janggut (Appendix C). The variation of BOD concentration during the study period was relatively high compared to the National Water Quality Standards of Malaysia. While at the direct sun area, the mean concentration was 22.32 ± 2.38 mg/L (Appendix D), which was slightly higher than the shaded sun area. Among the six consecutive weeks, the BOD value ranged from 19.2 – 25.6 mg/L, which the third and the sixth week had the lowest and highest BOD concentration, respectively. BOD is a parameter to access the organic load in a waterbody [15]. Many researchers have recorded higher BOD values in polluted water. Low DO, high BOD and high nitrate concentrations indicate the eutrophic status of the waterbody and the unsuitability of water for domestic use [16]. According to Offem [17], BOD values less than 5 mg/L indicated the absence of organic matter pollution sources in the area. Therefore, based on the values taken, it could be analysed that the pollution sources of organic matter were present in the study area.

Table 1 - Morphological identification from gram staining technique

Area	Week/Date	Observation Under Microscope	Description
Shaded Sun	Week 1		Magnification: 100× Dilution Factor: 10^{-2} Colour: Pink Gram: Negative Shape: Curved-rod
	Week 3		Magnification: 100× Dilution Factor: 10^{-4} Colour: Pink Gram: Negative Shape: Cocci
	Week 5		Magnification: 100× Dilution Factor: 10^{-1} Colour: Pink Gram: Negative Shape: Bacilli
Direct Sun	Week 1		Magnification: 100× Dilution Factor: 10^{-4} Colour: Pink Gram: Negative Shape: Spirillum
	Week 3		Magnification: 100× Dilution Factor: 10^{-1} Colour: Purple Gram: Positive Shape: Cocci
	Week 5		Magnification: 100× Dilution Factor: Master Plate Colour: Pink Gram: Negative Shape: Bacilli

Chemical Oxygen Demand (COD): The COD measures the oxygen consumed in the chemical oxidation of organics [18]. The mean concentration for COD at the upstream was measured as 3.0 ± 0.77 mg/L (Appendix C) and 3.02 ± 1.33 mg/L (Appendix D) for shaded and direct sun area, respectively, which showed no big difference between both areas. By looking at its trends, the values for COD at both study areas were smaller than BOD values, which is a rare finding among the study of water quality parameters. The COD values should always be higher than BOD values since they include biodegradable and non-biodegradable substances while BOD only contains bio-degradable substances. However, the high presence of microbiologically oxidised chemicals such as ammonium may boost the BOD readings. The basis for the COD test is that nearly all organic compounds can be fully oxidised to carbon dioxide with a strong oxidising agent (dichromate) under acidic conditions. Dichromate does not oxidise ammonia into nitrate, as is the case in BOD measurement. Therefore, there is a possibility of getting a higher BOD value than COD for water samples rich with ammonia (NH_3N) and have very low organic carbon concentration as the COD values at the upstream. It is also fully dependent upon the containing organic species. Some organic species are bio-degradable, but it is difficult to be oxidised by the used oxidant used in COD analysis [19].

Ammonia nitrogen (NH_3N): The high level of NH_3N can cause toxicity to aquatic life and human beings when the source of pollution leaks to water resources [20], [21]. At the upstream, it is noticed that both values of the mean concentration of NH_3N for shaded sun and direct sun area were 0.29 ± 0.11 mg/L (Appendix C) and 0.28 ± 0.069 mg/L (Appendix D), respectively. The same trend was also found in both areas as the value kept decreasing from the first week until the third week but increasing again on the fourth week. Based on the result, the mean concentration of NH_3N is lower than COD in both direct and shaded sun areas. COD and NH_3N are closely related because the sources of both target parameters were organic matters. COD measure the amount of organic compound in water, while NH_3N was produced by bacteria when the decomposed dead plant and animal matter. Based on the comparison between the NH_3N readings at the upstream and downstream of Lata Janggut, it was found out that the downstream area indicated higher readings of NH_3N, which led to the higher readings of COD values for the six consecutive weeks of the study period [22]. Certain bacteria rapidly oxidise ammonia in natural water systems to nitrite and nitrate, which requires the presence of dissolved oxygen.

Total Suspended Solids (TSS): TSS comprise both inorganic and organic materials, of which the organic fraction accounts for about 25 to 35% [23]. At the upstream, the TSS values from each area ranged widely from 5.0 – 10.67 mg/L with the mean concentration of TSS 8.28 ± 1.84 mg/L. The highest TSS value was found at the sixth week (10.67 mg/L), followed by the fifth week, the first and the fourth week shared the same value (8.33 mg/L), the second week (7.33 mg/L) and the third week (5.0 mg/L) (Appendix C). As for direct sun area, 7.89 ± 1.84 mg/L was found as the mean concentration of TSS value, which ranges from 7.33 – 10.33 mg/L (Appendix D). The results show that the mean concentration for TSS at the direct sun area was relatively smaller than the other one. The results show that the mean concentration for TSS at the direct sun area was relatively smaller than the other one. TSS is usually due to the introduction of external factors carried by runoff rain waters that could increase the concentration of this parameter [24]. Contributions from natural phenomena, such as tidal effects and anthropogenic activities such as municipal, industrial, agricultural and aquaculture, have also influenced the TSS concentration [25]. The increase of TSS not only increase the cost of water treatment but it is also caused some impacts of ecological degradation on aquatic environments [26].

3.2 Heavy Metal Properties of Water Samples

Heavy metals are the essential micronutrient for plant metabolism; but become extremely toxic when present in excess [27], [28]. The analysis result (Fig. 3) at the upstream found out that the concentration of Na element was the highest at 6.475 ppm, followed by Ca (1.473 ppm) and K (1.397 ppm). While Hg, Mn, and As indicated the lowest concentration in ppm found at the upstream of Lata Janggut. Generally, leptospires are very sensitive to the presence of heavy metals, even in the smallest concentration. According to Offem [17], the higher-level concentration of heavy metals may be due to the water-sediment mixing process and effluents from runoff that is at maximum due to wind and storm, especially in the wet season samples. The heavy metals that were not found in the analysis might fall due to the turbulence nature of the study area, which brings up heavy metals lost to the sediments. It is known that the concentration of Na was the highest. Na is often naturally found in groundwater contained in most rocks and soils, which is easily dissolved. Erosion of salts deposits and sodium bearing rock minerals become the most common sources of elevated sodium levels at the waterfall [29].

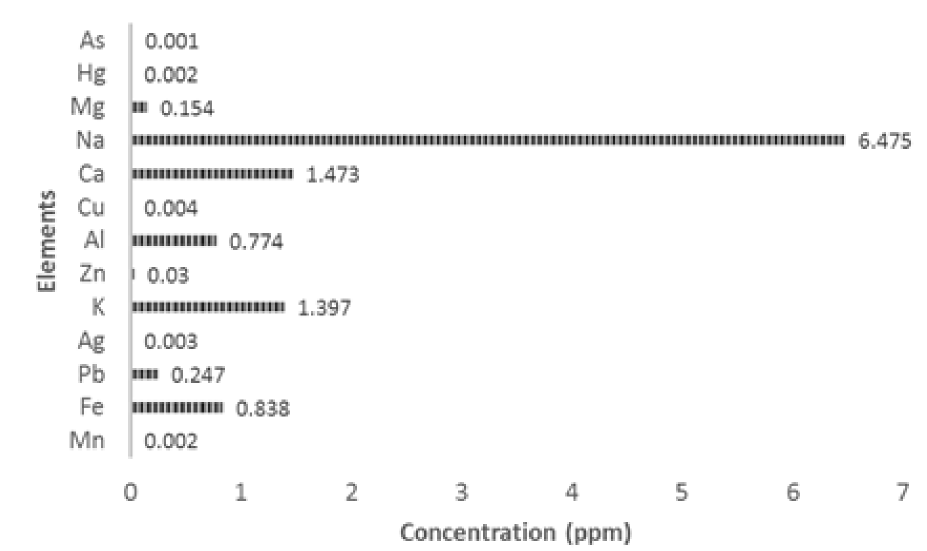


Fig. 3 - The concentration of heavy metals at Lata Janggut's upstream

3.2 Analysis of Ecological Parameters and Water Quality Index

The survival of leptospires in the environment depends on many conditions. *Leptospira* sp. exists in two groups, i.e. the free-living saprophytes and the pathogenic parasitic types [1]. Both groups have different food requirements and life cycles, although they need the similar foundations to sustain, such as temperature, stable pH, oxygen and water. Compared to others, leptospires are relatively slow-growing bacteria, therefore the colony densities in a water body are likely to be identical [30]. The presence of saprophytic leptospires in the water body does not indicate that it is unclean or contaminated. Bacteria prefer water that is free of chemical pollution. Pathogenic kinds, on the other hand, require a host to complete their life cycle. The bacteria's survival inside and outside the host is determined by the immunological response and species of the host [31]. As for the upstream area of Lata Janggut, the WQI of the area during the study period was indicated as slightly polluted, and this might be the reason for the absence of leptospires.

Water quality status classification was determined by using the Water Quality Index (WQI) . WQI value for the upstream of Lata Janggut was calculated by entering the six water quality parameters mean values of DO, BOD, COD, pH, NH_3N and TSS. First of all, the values were converted to Sub-Indices according to the equation guided by DOE. Currently, there is no specific standard guideline for the assessment of water quality in the waterfall ecosystem. However, most of the research regarding the assessment of water quality at waterfall uses the National Water Quality Standards of Malaysia (Physical and Chemical Parameters) and Malaysia's Department of Environment (DOE) Water Quality Index (WQI) Classification (Appendix E) and the National Water Quality Standards for Malaysia (Heavy Metal Parameters) (Appendix F) to evaluate and characterise the quality of water. Based on Table 2, the WQI of the study area averagely was classified in Class III (71.01, slightly polluted). This outcome correlated with the absence of leptospires during the study period since the bacteria prefer clean water without any chemical pollution for their survival. The WQI value of shaded and direct sun area at the upstream was 64.50 and 77.51, respectively (Table 2). However, the WQI value for the direct sun area indicated better quality status than the shaded sun area. The analysis determined that the water quality of the upstream of Lata Janggut was slightly polluted for the study period, which was taken into consideration for six consecutive weeks. Also, based on the physical observations, it was monitored that not enough dustbin was placed at the recreational area, leading to the unhygienic conditions. Table 2 shows the WQI value and classification for the upstream of Lata Janggut.

Table 2 - WQI value and classification for the upstream of Lata Janggut

	Study Area	**WQI Value**	**Classification**
Upstream	Shaded Sun Area	64.50	Class III: Slightly Polluted
	Direct Sun Area	77.51	Class II: Slightly Polluted
	Mean	71.01	Class III: Slightly Polluted

Leptospires are immediately killed in the dry environment or surfaces, and hence the moisture is important for them. They cannot thrive outside of water due to the waterproof membrane unavailability. The inability of leptospires to survive outside water is one of the crucial control factors in the natural environment. Pathogenic leptospires are extremely sensitive to all kinds of chemicals even though at very low concentrations since they are very fragile little bacteria. Acids, detergents and heavy metals are lethal and difficult for pathogenic colonies to survive in polluted water bodies. This happens due to the compounds attacking the bacterial envelope and damaging the inner chemical activities needed for the bacterium's survivability [30]. Lipopolysaccharide, a substance found in the envelope's outer layer, can be damaged by detergents, alkalis, and soaps [31]. Acids and heavy metals, on the other hand, tend to destroy the bacterium's internal elements and cell metabolism. Therefore, this toughens the research outcome that leptospires were not found in the study area; either they were being killed, or the study area was free from animals that excrete the leptospires, both in active infection and asymptomatic stage.

4. Conclusion

Lethal conditions of the bacteria itself support the absence of leptospires at the study area of Lata Janggut. Environmental conditions are lethal to the bacteria when in acid conditions at $pH < 6.5$ and alkali conditions at $pH > 8.4$. Leptospires also can be easily killed at the direct sun area with high temperature, as high as 32 ºC in the environment. Based on the low concentration salinity levels, it was the favour condition for the survival of the bacteria. Although pathogenic leptospires cannot tolerate the high salt concentrations, they can gradually adapt to thrive in solutions as low as 1%. Therefore, it was concluded that leptospires were absent from the study area during the study period since the bacteria prefer clean environment for their survival, as supported by the studied ecological parameters, water quality status and physical observation at the upstream of Lata Janggut.

It is possible that persons who come into contact with contaminated water during sport activities may be at danger from the presence of pathogenic *Leptospira* sp. Monitoring of water bodies, public awareness campaigns, and proper rubbish management are all necessary steps that should be performed by authorities for better control of this water-borne disease.

Acknowledgement

The authors are grateful to the Faculty of Earth Science, Universiti Malaysia Kelantan, for the research facilities, and Faculty of Civil Engineering and Built Environment, Universiti Tun Hussien Onn Malaysia, for the publication of this research.

Appendix A: Physical Water Quality Parameters at Shaded Sun Area

Week	pH	Temperature (ºC)	DO (mg/L)	Salinity (%)	Conductivity (µS/cm)	Turbidity (NTU)	TSS (mg/L)
1	6.95 ± 0.07	26.96 ± 0.53	8.91 ± 0.89	0.02 ± 0.00	0.04 ± 0.01	2.56 ± 0.01	8.33 ± 0.58
2	6.58 ± 0.03	25.54 ± 0.02	2.64 ± 0.01	0.01 ±0.00	0.03 ± 0.01	2.45 ± 0.01	7.33 ± 0.58
3	6.26 ± 0.05	25.23 ± 0.03	2.67 ± 0.05	0.01 ± 0.00	0.03 ± 0.00	2.35 ± 0.07	5.00 ± 1.00
4	5.94 ± 0.07	24.92 ± 0.02	2.81 ± 0.09	0.03 ± 0.00	0.06 ± 0.01	1.87 ± 0.15	8.33 ± 0.58
5	5.82 ± 0.06	25.07 ± 0.04	3.48 ± 0.06	0.03 ± 0.00	0.06 ± 0.02	2.51 ± 0.04	10.00 ± 1.00
6	5.75 ± 0.05	25.57 ± 0.18	4.80 ± 0.13	0.03 ± 0.00	0.07 ± 0.01	2.59 ± 0.03	10.67 ±1.53
Mean	6.22 ± 0.43	25.54 ± 0.67	4.22 ± 2.23	0.02 ± 0.09	0.05 ± 0.02	2.39 ± 0.24	8.28 ± 1.84

Appendix B: Physical Water Quality Parameters at Direct Sun Area

Week	pH	Temperature (ºC)	DO (mg/L)	Salinity (%)	Conductivity (µS/cm)	Turbidity (NTU)	TSS (mg/L)
1	8.37 ± 0.12	27.28 ± 0.24	8.80 ± 0.66	0.02 ± 0.00	0.04 ± 0.01	2.81 ± 0.02	10.33 ± 0.58
2	7.58 ± 0.05	26.32 ± 0.06	4.73 ± 0.11	0.02 ± 0.00	0.04 ± 0.02	2.36 ± 0.04	7.33 ± 0.58
3	6.43 ± 0.04	25.87 ± 0.10	3.61 ± 0.18	0.01 ± 0.00	0.03 ± 0.00	2.27 ± 0.02	4.33 ± 0.58
4	7.31 ± 0.17	26.38 ± 0.13	4.30 ± 0.23	0.01 ± 0.00	0.03 ± 0.06	1.72 ± 0.03	8.00 ± 1.00
5	7.32 ± 0.18	26.54 ± 0.20	5.53 ± 0.28	0.01 ± 0.00	0.03 ± 0.01	4.64 ± 0.02	8.33 ± 0.58
6	7.49 ± 0.26	26.66 ± 0.33	7.36 ± 0.04	0.01 ± 0.00	0.03 ± 0.02	5.14 ± 0.05	9.00 ± 0.00
Mean	7.42 ± 0.57	26.51 ± 0.42	5.72 ± 1.81	0.013 ± 0.05	0.039 ± 0.03	3.16 ± 1.27	7.89 ± 1.84

Appendix C: Chemical Water Quality Parameters at Shaded Sun Area

Week	BOD (mg/L)	COD (mg/L)	NH_3N (mg/L)	TDS (mg/L)
1	21.20 ± 0.17	1.67 ± 0.25	0.41 ± 0.03	27.00 ± 0.00
2	21.00 ± 0.30	2.20 ± 0.20	0.24 ± 0.03	22.00 ± 0.00
3	18.20 ± 1.76	3.47 ± 0.15	0.10 ± 0.01	18.00 ± 0.00
4	20.90 ± 0.17	3.43 ± 0.50	0.25 ± 0.03	12.00 ± 0.00

5	21.30 ± 0.60	3.57 ± 0.15	0.36 ± 0.04	26.00 ± 0.00
6	26.40 ± 1.04	3.63 ± 0.31	0.40 ± 0.01	28.00 ± 0.00
Mean	21.50 ± 2.44	3.00 ± 0.77	0.29 ± 0.11	22.17 ± 5.67

Appendix D: Chemical Water Quality Parameters at Direct Sun Area

Week	BOD (mg/L)	COD (mg/L)	NH_3N (mg/L)	TDS (mg/L)
1	22.90 ± 0.46	3.17 ± 1.63	0.32 ± 0.01	27.00 ± 0.00
2	21.60 ± 0.30	0.40 ± 0.10	0.24 ± 0.02	27.00 ± 0.00
3	19.20 ± 0.79	3.30 ± 0.36	0.17 ± 0.02	25.00 ± 0.00
4	19.80 ± 0.52	3.43 ± 0.67	0.25 ± 0.01	25.00 ± 0.00
5	24.80 ± 0.17	2.90 ± 0.36	0.36 ± 0.05	34.00 ± 0.00
6	25.60 ± 2.13	4.90 ± 0.10	0.36 ± 0.02	42.00 ± 0.00
Mean	22.30 ± 2.38	3.02 ± 1.33	0.28 ± 0.07	30.00 ± 6.16

Appendix E: National Water Quality Standards of Malaysia (Physical and Chemical Parameters) and DOE Water Quality Index Classification

Parameter	Unit	Class				
		I	II	III	IV	V
NH_3-N	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	-	<7.0	6.0-7.0	5.0-6.0	<5.0	>5.0
Conductivity	µS/cm	1000	1000	-	6000	-
Salinity	%	0.5	1	-	2	-
TSS	mg/L	25	50	150	300	300
TDS	mg/L	500	1000	-	4000	-
Turbidity	NTU	5	50	-	-	-
Temperature	°C	-	Normal +2°C	Normal +2°C	-	-
WQI		>92.7	76.5-92.7	51.9-76.5	31.0-51.9	<31.0

Source: [32]

Appendix F: National Water Quality Standards for Malaysia (Heavy Metal Parameters)

Parameter (mg/L)	Class				
	I	11A/11B	III#	IV	V
Ag		0.05	0.0002	-	
Al		-	(0.06)	0.5	
As		0.05	0.4(0.05)	0.1	
Ca		-	-	-	
Cd		0.01	0.01*(0.001)	0.01	
Cr (III)		-	2.5	-	
Cr (IV)		0.05	1.4(0.05)	0.1	
Cu	Natural levels	0.02	-	-	Level
Fe	or absent	1	1	1(Leaf) 5(Others)	above IV
Hg		0.001	0.04(0.0001)	0.002	
K		-	-	-	
Mn		0.1	0.1	0.2	
Mg		-	-	-	
Na		-	-	3 SAR	
Ni		0.05	0.9*	0.2	
Pb		0.05	0.02*(0.01)	5	
Zn		5	0.4*	2	

Notes

* = At hardness 50 mg/L CaCO3

= Maximum (unbracketed) and 24-hour average (bracketed) concentrations N = Free from visible film sheen, discolouration and deposits
Source: [32]

References

[1] Lehmann, J. S., Matthias, M. A., Vinetz, J. M. & Fouts, D. E. (2014). Leptospiral Pathogenomics. *Pathogens, 3*(2), pp. 280-308.
[2] Wahab, Z. A. (2015). *Epidemiology and Current Situation of Leptospirosis in Malaysia*. Persidangan Kesihatan Persekitaran Pihak Berkuasa Tempatan 2015. WP Labuan, Malaysia. 8 – 9 September 2015.
[3] Department of Health, Kelantan (2015). Centers for Disease Control and Prevention (CDC) Unit, Disease Control Section.
[4] Lau, C. (2008). Urbanisation, climate change, and leptospirosis: environmental drivers of infectious disease emergence. *Universitas 21 International Graduate Research Conference: Sustainable Cities for the Future*. Melbourne & Brisbane. November 29 – December 5, 2009. pp. 83-87.
[5] Benacer, D., Woh, P. Y., Mohd Zain, S. N., Amran, F. & Thong, K. L. (2013). Pathogenic and saprophytic Leptospira species in water and soils from selected urban sites in peninsular Malaysia. Microbes and Environments, *Japanese Society of Microbial Ecology*, 28(1), pp. 135-140.
[6] Stoddard, R. A., Bui, D., Haberling, D. L., Wuthiekanun, V., Thaipadungpanit, J. & Hoffmaster, A. R. (2014). Viability of Leptospira isolates from a human outbreak in Thailand in various water types, pH, and temperature conditions. *The American Society of Tropical Medicine and Hygiene Viability. 91*(5), pp. 1020–1022.
[7] Wynwood, S. J., Graham, G. C., Weier, S. L., Collet, T. A., McKay, D. B. & Craig, S. B. (2014). Leptospirosis from water sources. *Pathogens and global health*, *108* (7), pp. 334-338.
[8] American Public Health Association (2017). *Standard Methods for the Examination of Water and Wastewater*, (21th ed.). Washington DC, USA: APHA 2017
[9] Mohd-Salleh, S. N. A., Shaylinda, M. Z. N., Othman, N., Azizan, M. O., Yashni, G. & Afnizan, W. M. W. (2020). Sustainability analysis on landfilling and evaluation of characteristics in landfill leachate: A case study. *IOP Conference Series: Materials Science and Engineering*, *736*(072002), pp. 1-13.
[10] Mohammed, H., Nozha, C., Hakim, K., Abdelaziz, F. & Rekia, B. (2011). Bacteriology & Parasitology Leptospira: Morphology, classification and pathogenesis. *Journal of Bacteriology and Parasitology, 2*(6), pp. 6-9.
[11] Paul, N. L. (2001). Leptospirosis. *Clinical Microbiology Reviews,* 14, pp. 296–326.
[12] Rostern, N. T. (2017). The effects of some metals in acidified waters on aquatic organisms. *Oceanography & Fisheries, 4*(4), pp. 1–7.
[13] Bejo, S. K., Bahaman, A.R., Saad, M. Z. & Mutalib, A.R. (2004). The Survival of Leptospira interrogans Serovars Hardjo in the Malaysian Environment. *Journal of Animal and Veterinary Advances, 3*(3), pp. 123-129.
[14] Babakhani, M., Zehtabian, G., Keshtkar, A. & Khosravi, H. (2016). Trend of groundwater quality changes, using geo statistics (case study: Ravar Plain). *Pollution, 2*(2), pp. 115–129.
[15] Uwidia, I. E. & Ejeomo, C. (2013). Characterisation of textile wastewater discharges in Nigeria and its pollution implications. *Global Journal of Researches in Engineering General Engineering, 13*(4) pp. 1–4.
[16] Yogendra, K. & Puttaiah, E. T. (2008) Determination of Water Quality Index and Suitability of an Urban Waterbody in Shimoga Town, Karnataka. *Proceedings of Taal 2007: The 12th World lake Conference*, pp. 342-346.
[17] Offem, B. O. (2011). Water quality and environmental impact assessment of a tropical waterfall system. *Environment and Natural Resources Research, 1*(1), pp. 63-74.
[18] Groele, J. & Foster, J. (2019). Hydrogen peroxide interference in chemical oxygen demand assessments of plasma treated waters. *Plasma 2*(3), pp. 294–302.
[19] Patel, H. & Vashi, R. T. (2015). Characterisation and column adsorptive treatment for cod and color removal using activated neem leaf powder from textile wastewater. *Journal of Urban and Environmental Engineering 9*(1), pp. 45–53.
[20] Chen, K. T., Bai, M. D., Yang, H. Y., Chen, Y. C., Lu, W. J. & Huang, C. (2020). Removal of ammonia from leachate by using thermophilic microbial fuel cells equipped with membrane electrode. *Sustainable Environment Research, 30*(5), pp. 2-9.
[21] Mehmood, M. K., Adetutu, E., Nedwell, D. B. & Ball, A. S. (2009). In situ microbial treatment of landfill leachate using aerated lagoons. *Bioresource Technology, 100*(10), pp. 2741–2744.
[22] Rahim, A. A. (2016). *Leptospira sp. Physical & Chemical Parameters Relationship and Its Availability at Lata Janggut, Jeli, Kelantan.* Universiti Malaysia Kelantan: Degree Thesis
[23] Vigneshpriya, D. & Shanthi, E. (2015). Physicochemical characterization of textile wastewater. *International Journal of Innovative Research and Development, 4*(10), pp. 48-51.
[24] Tafesse, T. B., Yetemegne. A. K. & Kumar, S. (2015). The physico-chemical studies of wastewater in Hawassa textile industry. *Journal of Environmental Analytical Chemistry, 2*(4), pp. 2–7.

[25] Suratman, S., Sailan M. I., Hee, Y.Y., Bedurus, E. A. & Latif, M.T. (2015). A preliminary study of water quality index in Terengganu river basin, Malaysia. *Sains Malaysiana, 44*(1), pp. 67–73.
[26] Saharudin, N. F. A. & Nithyanandam, R. (2014). Wastewater Treatment by using Natural Coagulant. *2nd Eureca 2014*, pp. 2–3.
[27] Bakar, N. A., Othman, N., Yunus, Z. M., Daud, Z., Norisman, N. S. & Hisham, H. M. (2020). Physico-Chemical Water Quality Parameters Analysis on Textile. *IOP Conf. Series: Earth and Environmental Science IOP Publishing 498*(012077), pp. 1-9.
[28] Jiwan, S. & Kalamdhad, A. (2011). Effects of heavy metals on soil, plants, human health and aquatic life. *International Journal of Research in Chemical Environment, 1*(2), pp. 15–21.
[29] Wang, X., Li, C., Li, Z., Yu, G. & Wang, Y. (2019). Effect of pyrolysis temperature on characteristics, chemical speciation and risk evaluation of heavy metals in biochar derived from textile dyeing sludge. *Ecotoxicology and Environmental Safety, 168*, pp. 45–52.
[30] Goarant, C., Trueba, G., Bierque, E., Thibeaux, R., Davis, B. & De la Peña Moctezuma, A. (2019). *Leptospira and Leptospirosis.* In: J.B. Rose and B. Jiménez-Cisneros, (eds) Water and Sanitation for the 21st Century: Health and Microbiological Aspects of Excreta and Wastewater Management (Global Water Pathogen Project). (A. Pruden, N. Ashbolt and J. Miller (eds), Part 3: Specific Excreted Pathogens: Environmental and Epidemiology Aspects - Section 2: Bacteria), Michigan State University, E. Lansing, MI, UNESCO. pp.1-33.
[31] Rodriguez-Morales, A. J. & Castañeda-Hernández, D. M. (2019). Spirochetes: Leptospira. *Reference Module in Food Science 2019*, pp. 1-7.
[32] Malaysia Water Quality Standards (2018). *Interim National Water Quality Standard (INWQS).* Department of Environmental Malaysia, DOE, Malaysia: MWQS 2018.