Leptospira sp. Availability and Its Relationship with Ecological Parameters of Lata Janggut, Jeli, Kelantan

by

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DECLARATION

I declare that this thesis entitled "*Leptospira* sp. Availability and Its Relationship with Ecological Parameters of Lata Janggut, Jeli, Kelantan" is the result of my own research except as cited in the references. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree.

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LIST OF ABBREVIATIONS

BOD	Biochemical Oxygen Demand
COD	Chemical Oxygen Demand
DF	Dilution Factor
DNA	Deoxyribonucleic acid
DO	Dissolve Oxygen
DOE	Department of Environment
HCl	Hydrochloric acid
KESEDAR	South Kelantan Development Authority
KI	Potassium Iodide
$KMnO_4$	Potassium Permanganate
LERG	Leptospirosis Burden Epidemiology Reference Group
LPS	Lipopolysaccharide
MLEE	Multi-locus Enzyme Electrophoresis
MPS	Multiprobe System
mL	millilitre
mg/L	milligrams per Liter
NH_3N	Ammoniacal Nitrogen
NSC	National Security Council
NTU	Nephelometric Turbidity Unit
SAR	Sodium Adsorption Ratio
sp.	Species
SS	Suspended Solid
TSS	Total Suspended Solid
UPM	Universiti Putra Malaysia
WHO	World Health Organization
WQI	Water Quality Index
µm	Micrometre
µL	Microliter
µS/cm	micro-Siemens per centimeter

LIST OF SYMBOLS

%	Percentage
>	Greater than
<	Less than
x	Multiply
°C	Temperature (degree Celsius)

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ABSTRACT

The number of Leptospirosis cases and deaths in Kelantan from 2009-2015 showed increasing trends with Jeli District held the highest record for the number of fatalities, which indirectly creates an anxiety situation. This study provides information about the ecological parameters of *Leptospira* sp. at Lata Janggut, which is a natural, recreational area. However, waterfall ecosystem is also one of the potential sites of the bacteria, due to infected animals excrete the urine that contain pathogenic leptospires. In this research, water samples were collected at the upstream part of the waterfall, from the shade and direct sun area. The study was conducted for three months (July-September). Water samples were taken for the *Leptospira* sp. availability testing through the morphological characterization and staining technique. *In-situ* and *ex-situ* data were measured as the physical and chemical parameters of water samples. The biological parameter was taken based on the observation as well. Water Quality Index (WQI) of the upstream area of Lata Janggut was analysed with 64.50 (Class III) and 77.51 (Class II), in the shade and direct sun area, respectively, according to the WQI value suggested by DOE of Malaysia. Based on the identification, the study area was free from Leptospirosis during the observation period, which further correlates with the water properties at the upstream part of Lata Janggut.

Keywords: Leptospirosis, Ecological, Environment, Water, Characterization

Keberadaan *Leptospira* sp. dan Hubungannya dengan Parameter Ekologi Lata Janggut, Jeli, Kelantan

ABSTRAK

Jumlah kes Leptospirosis dan kematian di Kelantan menunjukkan trend peningkatan pada tahun 2009-2015 dengan Daerah Jeli memegang rekod tertinggi bagi bilangan kematian, secara tidak langsung mewujudkan rasa kebimbangan. Kajian ini menyediakan maklumat mengenai parameter ekologi *Leptospira* sp. di Lata Janggut, iaitu kawasan rekreasi yang semulajadi. Walau bagaimanapun, ekosistem air terjun juga satu daripada lokasi yang berpotensi untuk bakteria ini, datang daripada haiwan yang dijangkiti dan mengeluarkan air kencing yang mengandungi leptospires patogenik. Dalam kajian ini, sampel air telah diambil di bahagian hulu air terjun, dari kawasan terlindung dan terdedah kepada matahari. Kajian ini telah dijalankan selama tiga bulan (Julai-September). Sampel air telah diambil untuk mengenalpasti *Leptospira* sp. melalui teknik ujian pewarnaan dan karakter morfologi. Data *in-situ* dan *ex-situ* telah diukur sebagai parameter fizikal dan kimia sampel air. Parameter biologi juga diambil berdasarkan pemerhatian. Indeks Kualiti Air (WQI) kawasan hulu Lata Janggut dianalisis dengan 64.50 (Kelas III) dan 77.51 (Kelas II), masing-masing di kawasan terlindung dan terdedah kepada cahaya matahari, dikelaskan mengikut nilai WQI yang disyorkan oleh Jabatan Alam Sekitar Malaysia. Berdasarkan pengenalpastian, kawasan kajian ini adalah bebas daripada Leptospirosis sepanjang tempoh pemerhatian, yang dihubungkaitkan dengan sifat air di bahagian hulu Lata Janggut.

Kata kunci: Leptospirosis, Ekologi, Alam Sekitar, Air, Pencirian

CHAPTER 1

INTRODUCTION

1.1 Background of the Study

Leptospirosis is a silent killer of humans as the symptoms are hard to detect at an early stage but can be fatal. It typically happen in developing countries, however due to globalization and international travel make the developed countries are exposed as well (Lehmann, Matthias, Vinets & Fouts, 2014). It is a zoonotic disease that originated from animal 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 active infection and asymptomatic stage (Wahab, 2015).

Leptospira interrogans is the main causal organism of this infectious disease and rats are believed to be the major natural reservoir for this bacteria. Leptospires can survive for long period of time under convenient conditions in the environment. High occurrence of leptospirosis is influenced by the moisture, pH values of soil and surrounding temperature for the survivability of the pathogenic leptospires (Ridzlan, Bahaman, Khairani-Bejo, & Mutalib, 2010).

This disease commonly occurs in the epidemic potential countries with tropical or humid subtropical climates. Climate changes, recreational activities and poor urban slum communities are often linked to Leptospirosis outbreaks. Due to the difficult clinical diagnosis and laboratory services, it is usually underreported in many countries

(WHO, 2014). According to the Leptospirosis Burden Epidemiology Reference Group (LERG) of World Health Organization, they estimate over 500,000 human cases of critical leptospirosis every year, when the real value of the global disease burden not yet identified (Lehmann *et. al*, 2014).

Meanwhile in Malaysia, the Leptospirosis cases still at the anxiety phase. Even though the statistics from Ministry of Health show a decrease in mortality, but the numbers of cases increase every year especially in Kelantan state. Malaysia is one of the country that anticipated to reach the developed country status by 2020, and thus further studies need to be done to overcome the disease. This study will focus on the ecological parameters that cause the existence or absence of *Leptospira* serovars with the physical and chemical parameters of water at the study area. Ecological parameters shown to affect the epidemiology of Leptospirosis in our environment (Lau, 2008). By studying its ecological parameters, the environmental conditions that are suitable for *Leptospira* sp. can be identified throughout this research.

1.2 Problem statement

Recent studies of Leptospirosis in Malaysia has shown that Kelantan has the highest number of cases than other states. The number of Leptospirosis cases and deaths in Kelantan from 2009-2015 showed increasing trends with Jeli District held the highest record for the number of fatalities, which indirectly creates an anxiety situation. Due to the big flood that occurred in Kelantan in the end year of 2014, there were many places 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 disease has skyrocketed to the highest number compared than the previous months. The state recorded the highest number of Leptospirosis cases with

538 reported and 62 confirmed cases (Loon, n.d.). While according to Deputy Health Minister (Malaysian Digest, 2015), the cases of Leptospirosis increased threefold following the massive floods in Kelantan made it the worse in Malaysia.

In contrast to the common perception of Leptospirosis infected through flood, contaminated food and drink, the case involving the location of the waterfall began to provoke the question mark. In 2000, a healthy 32-year-old man presented to University of Malaya Medical Centre with fever, chest discomfort, and generalized myalgia. He had a history of a picnic at a waterfall about two weeks prior to admission, which later confirmed diagnosed as Leptospirosis (Thiruventhiran & Tan, 2000).

This study of Leptospirosis will be done at the upstream area of Lata Janggut, Jeli Kelantan, which is one of the waterfall areas nearby the university. A thorough and conscientious study about the ecological parameters of *Leptospira* sp. will be carried out as waterfalls become one of the potential breeding sites for the bacteria when the area is rocky and has water reservoir causing bacteria to spread.

1.3 Objectives

1. To identify the availability of *Leptospira* sp. at the study area by using morphological characteristics and staining technique.
2. To identify the ecological parameters, which are physical, chemical and biological parameter of the study area.

1.4 Significance of the Study

This study will provide the information about the ecological parameters of *Leptospira* sp. at the upstream area of Lata Janggut. This study will also play an important role to give the right information about the causes of Leptospirosis towards the publics especially at the waterfall ecosystem.

Lata Janggut is recognized as the natural recreational area by the locals. From the observations, there are many locals from all level of ages come to this place to have a leisure time together. Therefore, the present study can help the visitors to have further knowledges about the Leptospirosis disease, as well as to raise its awareness to the locals and publics. Detection of pathogenic leptospires in water samples especially in recreational area, may benefit to those who come into contact with contaminated water during sports activities and provide them with early alert about its detection. Necessary pre-cautions should be taken by the authorities to monitor water bodies and to alert the public of contaminated water bodies in view of this.

CHAPTER 2

LITERATURE REVIEW

2.1 Historical Perspectives of Leptospirosis

Based on the historical aspect, it is reported that *Leptospira* was first described in 1886, by Adolf Weil, by reporting three medical conditions, which were the enlargement of spleen, nephritis as the inflammation of kidneys, and jaundice or also known as the condition with yellowing of the skin, as the acute infectious diseases (Wahab, 2015). Back then, due to the similarity with the Leptospirosis, the syndrome is commonly referred as Weil's syndrome (Crustal, n.d.). Nevertheless, even though leptospires already discovered at that time, it was only after two decades, when Arthur Stimson became the first to observe the organism by demonstrating the existence of spirochetes in patient's kidney who had died due to jaundice in 1907. He later cultured and named it as *Spirocheta interrogans* because of its shape that resembles a question mark. While in 1914, Walbalch and Binger discovered a saprophytic *Leptospira* in the fresh water, and named it as *Spirocheta biflexa* (Sambasiva, Naveen, Bhalla & Agarwal, 2003).

After several years, the cause of Weil's syndrome was successfully found both in Japan and Germany in 1915, the second half of the twentieth century. The pathogen *Leptospira* was independently discovered by Ryokichi Inada and his colleague in the blood of jaundice patients who involved in coal mining, which they named it as *Spirochaeta icterohaemorrhagiae*. While in Germany, the spirochetes were detected in the infected soldiers' blood who got the disease in the battleground (Santiago, n.d.).

Later in 1917, another Japanese scientist, Hideyo Noguchi proposed and suggested the name of genus *Leptospira* which means a slender coil. Subsequently, there were many serovars have been discovered all around the world whilst at the moment, there are 23 serogroups containing more than 200 serovars in total (Abdollahpour, 2013).

It was also reported that some years earlier than Weil's discovery, Leptospirosis was already known as an occupational hazard while harvesting rice by ancient people in China. Based on the past experience, it was believed to appear as early as in 19th century. Till date, further studies still need to be done as leptospires can cause an extremely wide spectrum of human diseases, from pre-infection with no signs at all to mortality due to the serious damage of multi-organs (Levett, 2001).

2.2 Taxonomy and Classification of Leptospires

Leptospires are spirochaetes that have three genera which are *Leptospira, Leptonema* and *Turneria*. Since the nomenclature and classification of *Leptospira* is quite complicated, scientists come out with two different classification systems to classify them; which are based on the serological characterization and on the genetic relatedness. The scientific classification of leptospires are shown in Table 2.1.

Table 2.1: The scientific classification of leptospires.

Phylum	Class	Order	Family	Genus
Spirochaetes	Spirochaetia	Spirochaetales	Leptospiraceae	*Leptospira*

2.3 Biology and Characteristic Features of Leptospires

The spirochetes of leptospires and both of Gram-positive and Gram-negative share the same features. Leptospires are also very mobile and flexible obligate aerobes which exhibit translational and non-translational distinct forms of movement. Their size are about 0.26 x 6 – 25 µm and can go through the 0.45 µm filters. A distinctive hook can be identified either or both at the cells' pointed ends. It have the regular double membrane structure, which the peptidoglycan cell and cytoplasmic membrane are closely related. They are catalase and oxidase positive as well (Levett, 2001).

2.4 Morphological Identification through Gram Staining Technique

Leptospires belong to the order of Spirochaetales, family Leptospiraceae and genus *Leptospira.* Their size is about 0.1 µm in diameter and by 6-20 µm in length. Specifically, they are corkscrew-shaped bacteria, which differ from other spirochaetes by the presence of end hooks. Since leptospires are mobile and have small bodies, the use of phase contrast, highest resolution or dark field microscopy is required for the morphological identification. They have a Gram-negative-like cell envelope consisting of a cytoplasmic and outer membrane.

Leptospires have distinctive hooked ends (Figure 2.1). Leptospires have a typical double membrane and peptidoglycan cell wall are closely associated, which overlaid by an outer membrane that constitutes the main antigen for *Leptospira* (Mohammed, Nozha, Hakim, Abdelaziz & Rekia, 2011).

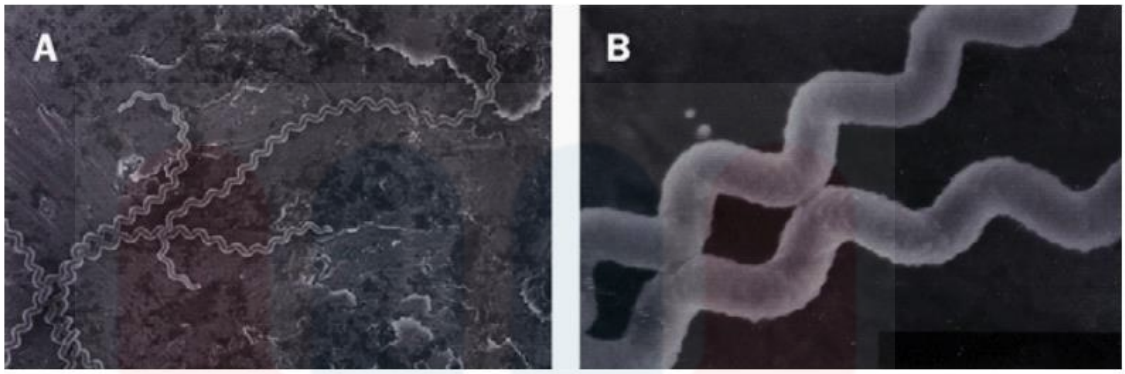


Figure 2.1: High-resolution scanning electron micrograph of *Leptospira interrogans* serovar *copenhageni* with A) Note characteristic hooked ends and B) The surface of the spirochete seems ruffled and beaded (Source: Mohammed *et al.,* 2011).

The Gram staining method, which is named after the Danish bacteriologist is one of the most important staining techniques in microbiology history. The test can differentiate the bacteria into two fundamental varieties of cells and always performed for the identification of bacteria, which also useful for the initial classification of unknown isolates. The primary stain of the Gram's method is crystal violet, which the microorganisms that are stained by the Gram's method are commonly classified as Gram-positive. While others that are not stained by crystal violet are referred to as Gram-negative, and appear pink in colour.

Gram staining is based on the ability of bacteria cell wall to retaining the crystal violet dye during solvent treatment. The cell walls for Gram-positive microorganisms have a higher peptidoglycan and lower lipid content than gram-negative bacteria. Iodine is subsequently added as a mordant to form the crystal violet-iodine complex so that the dye cannot be removed easily. As for their characteristics, Gram-positives have a thick, relatively impermeable wall that resists decolourization and is composed of peptidoglycan and secondary polymers. While Gram-negatives have a thin peptidoglycan layer plus an overlying lipid-protein bilayer known as the outer membrane, which can be disrupted by decolourization.

The Gram stain is an important light microscopy stain for microbiology because it differentiates bacteria into two fundamental varieties of cells. The Gram stain is still a primary tool for identification even though there are a lot of modern techniques to discover molecular biological probes, genera and even species quickly. This is due to the staining technique that does not alter the shape and form of bacteria, which is also an easy method for determining the overall structure of the cells such as cocci, rods, spirals, filaments, and cubic packets (Beveridge, 2001).

2.5 Epidemiology of Leptospirosis

Leptospirosis is assumed as the most endemic widespread zoonosis and their huge quantity depends on its ability infect various species of animals and humans. Favourable environmental conditions also become the factors in order to sustain outside the hosts (Thayaparan *et al.*, 2013). The disease often associated with seasonal distribution such as rainfall season, but also can happen throughout the year. It commonly occurs in the tropical, subtropical and temperate zones distributed in all inhabited continents (Sambasiva *et al.*, 2003). The urine of infected and carrier animals always become as the major sources of this infection. Human can be infected with it either direct or indirect contact with their excretions.

2.5.1 Leptospirosis Outbreaks in Malaysia

Leptospirosis may also present with variety of manifestations. The disease can mimic another disease symptoms such as dengue and typhoid fever and it become the solid reason why it is hard to be recognized at the early stage. Laboratory test is needed for the diagnosis which the facility is not available at everywhere. Therefore, the cases are always being ignored and fail to be reported efficiently. However nowadays, due to the high science, technology and innovation, there are already some institutions in

the world, which invent the product or kit that can diagnose the disease faster. For instance, in Malaysia, the scientists from Universiti Putra Malaysia (UPM) invented a simple test kit named Leptoscan2 in 2013, which can detect the presence of the leptospirosis bacteria in the blood of the patient in less than 15 minutes even in the early stages of the disease, unlike the other products in the market which can only provide the results based on the antibodies produced after seven days or more of the infection. LeptoScan2 is an improvement over earlier invention of LeptoScan, a rapid DNA-based detection kit for Leptospirosis, which was unveiled in 2006.

In 1928, Fletcher was the first person who began working and discovered Leptospirosis on human in Malaysia, which before known as *Tanah Melayu.* The first fatal case on human Leptospirosis was reported by him in 1925, which happened due to *Leptospira icterohemorrhagiae.* There were three serovars managed to be discovered which were *icterohemorrhagiae, hebdomadis and pyrogenes* from 21 patients.

Later in 1926, Galloway detected four cases in Singapore, which back then was a part of Malaysia. Researchers managed to demonstrate leptospires from Kuala Lumpur General Hospital in the body of four patients. Therefore, more investigations of Leptospirosis were made in Malaysia, as typical cases with jaundice were also well recognized. In addition, among military personnel and civilians, cases of febrile illness appeared frequently and these cases catch the eyes as an emerging disease in the country (El Jalii & Bahaman, 2004).

In 2016, Kedah recorded the first case of death from rat urine infection this year, which involving an 18 years old Polytechnic Kulim's student and a teenager aged 17, where both cases took place in Lata Sedim and Lata Bukit Perak respectively. According to State Health Director, the situation of the patient was still critical and

require intensive care physicians. While the recent outbreak which involving death was happened in Seremban, Negeri Sembilan, when a student of a private college confirmed infected with Leptospirosis after bathing at a recreation area of Jeram Toi. The victim died after receiving treatment as a result of prolonged fever, as a result from the spreading of pathogenic bacteria all over the body (Harian Metro, 2016).

2.5.2 Leptospirosis Outbreaks in Kelantan State of Malaysia

Annually, during the months of November to March, there are many parts of peninsular Malaysia especially the eastern states of Kelantan, Terengganu and Pahang will experience floods due to the Northeast monsoon season. Usually, frequent flash floods happen due to sudden heavy downpour, which also bring consequences about health due to few causes such as poor sanitation and contaminated water supply.

The number of Leptospirosis cases has tripled in Kelantan following massive floods in 2014. Due to that, Kelantan recorded the highest number of cases in Malaysia. According to this, the public, residents, and volunteers were advised to be more careful and to avoid using water from unhygienic sources. The disease can spread easily when the flood victims clean their houses or surroundings carelessly. The use of boots and masks during clean-up are crucial as the safety measures during the cleaning.

Occupations also have the potential risk as the exposure to the infection. Workers in the agriculture and farming industry are the examples to be exposed based on their environment and life-style basis. Survivors of natural disasters such as flooding and recreational activities enthusiasts are also another groups that face high risk of leptospirosis (Wynwood, Graham, Weier, Collet, McKay, & Craig, 2014)

2.6 Mode of Transmission of Leptospires

After circulating abundances of the spirochete in the blood, infected animals will contaminate their surrounding and the environment, by shedding the spirochete in their urine. According to Wahab, (2015) there are three type modes of transmission. The first one is direct contact with urine or tissue of the infected animal, second as through the indirect contact, and third one is through the droplet infection. Outside the hosts, they are all facilitated by the favourable conditions in the environment to ensure its pathogenic leptospires survival (Wynwood *et al.*, 2014).

Direct contact transmission enable the pathogenic organisms to enter the human body via abrasions and waterlogged skin, and mucous membranes such as nose, mouth and eyes. The spirochetes will infect the body tissue after moving through the aid of circulatory systems. They likely to settle down in the kidney, and thus the patient will release the urine that shed the spirochete as well. The transmission from person-to-person might also happen as urine from a patient considered to be infectious. However it is absolute rare, and thus there is no abundant data related to it (Santiago, n.d.). While for indirect contact transmission, the exposure can occur through water, soil, or foods as their routes contaminated by urine from infected hosts. As for droplet infection, the transmission can happen when there is inhalation of droplet aerosols of polluted fluid contaminated by urine (WHO, 2014).

2.7 Ecological Parameters of Study Area

Several parameters have to be considered in order to develop the ecological parameters such as the water quality index (WQI) of the study area. WQI serves as a mean indication of water quality assessment through the determination of physico-chemical parameters of surface water. The parameters are physical, chemical and

biological. Each of the parameters has significant impact on the water quality. The physical parameters include temperature, pH, turbidity, total dissolved solids, dissolved oxygen, salinity and conductivity. While Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Ammoniacal Nitrogen (NH_3N) and total suspended solid are considered as chemical parameters. Faecal coliform such as *Escherichia Coli (E.coli*) and groups of organism are the examples classified under biological parameters (Nithyanandam, Tsu & Thy, 2015).

Leptospires need suitable conditions in order for them to survive in the environment. By studying its ecological parameters, the appropriate circumstance for pathogenic *Lepstospira* sp. can be recognized. This infectious disease also provide such a fine example that involve the interaction between environment, animals and humans (Lau, 2008). In Malaysia, the majority cases of Leptospirosis reported in was related to the exposure of humans to an environment contaminated by the pathogenic bacteria. For instance, there was an outbreak of leptospirosis occurred during the Eco-Challenge in Sabah, Malaysia in 2000. Eighty out of 189 competitors diagnosed with Leptospirosis and twenty-nine people were hospitalized, but there were no fatalities (Benacer, Woh, Mohd Zain, Amran, & Thong, 2013).

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 and appropriate niche for this organism include the physical and chemical parameters such as the temperature, pH and water quality of the area. Typically, the leptospires are sensitive to heat and dryness, acids and basics disinfectants. The high humidity and warm temperature of tropical and subtropical countries like Malaysia are ideal for *Leptospira* to survive for long periods in the environment.

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 at Andaman Island, leptospires were frequently present in waters of pH 6.9 or more, but were absent from those of pH 6.6 or less (Bejo, Bahaman, Saad, & Mutalib, 2004). While for temperature parameter, pathogenic leptospires reproduce best at body temperature, but they appear to be unable to tolerate temperatures over about 32°C in water.

As leptospires do not have a waterproof membrane they must remain immersed in water to survive, are killed immediately if their environment dries out unless they are freeze-dried, which intentionally prepared. The inability to survive out of water is an important control factor in the natural environment, as it means they are unable to create infection risks from dry surfaces. Pathogenic leptospires are extremely sensitive to chemicals of all kinds, and therefore they are quite easy to be killed. Detergents, acids and heavy metals are all sufficient to cause death at very low concentrations and this means that pathogenic colonies find it difficult to survive in much polluted water (Stoddard, Bui, Haberling, Wuthiekanun, Thaipadungpanit, & Hoffmaster, 2014). 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 (Wynwood *et al.*, 2014).

2.8 Recommended Guidelines for Lata Janggut Water Quality Analysis

Currently, there is no specific standard guideline for the assessment of water quality in waterfall ecosystem. However, most of the research regarding the assessment of water quality at waterfall are using the following guideline (Table 2.2). It will be used in order to evaluate and characterize the quality of water at the upstream area of Lata Janggut. The standards to be used as the references will be the National Water Quality Standards of Malaysia (Physical and Chemical Parameters) and Malaysia's Department of Environment (DOE) Water Quality Index (WQI) Classification as shown in Table 2.2.

2.9 Lata Janggut as an Ecotourism Location

The ecotourism potential of Lata Janggut encompasses the tourists looking at the natural landscape including the landforms and rocks. The earth's geological wonders have always fascinated people and are fundamental part of a culture's identity. In order to describe the nature-tourism phenomenon, the term 'ecotourism' was adopted. Historically, it is well-known that the first formal and one of the most widely accepted definitions of ecotourism was introduced by Ceballos-Lascurain in the 1980s. It stated that ecotourism is traveling to relatively undisturbed or uncontaminated natural areas with the specific objective of studying, admiring, and enjoying the scenery and its wild plants and animals, as well as any existing cultural manifestations for both past and present found in these areas (Donohoe & Needham, 2006).

Lata Janggut is part of Sungai Long, and one of the captivating cascades exists in Jeli district, Kelantan. Jeli is located at the eastern part of Kelantan, within central belt in map of Peninsular Malaysia.

Table 2.2: National Water Quality Standards of Malaysia (Physical and Chemical Parameters) and DOE Water Quality Index Classification

Parameter	Unit	Class				
		I	II	III	IV	V
Ammoniacal Nitrogen	mg/L	<0.1	0.1-0.3	0.3-0.9	0.9-2.7	>2.7
Biochemical Oxygen Demand	mg/L	<1	1-3	3-6	6-12	>12
Chemical Oxygen Demand	mg/L	<10	10-25	25-50	50-100	>100
Dissolved Oxygen	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	-
Total Suspended Solid	mg/L	25	50	150	300	300
Total Dissolved Solid	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: http://www.wepadb.net/policies/law/malaysia/eq_surface.htm

The district is bordered by the state of Perak to the west, Thailand to the north, Tanah Merah district to the north east and Kuala Krai district to the south east. Due to its geographically superior status, Jeli is one a few districts that enjoys a wide variety of scenic beauty and geological graces, and there is Lata Janggut that can be considered as the place that have a potential of recreational area and geotourism. The information board of the study area is shown as in Figure 2.2.

The cascade is formed on the exposed body of the intruding igneous rock, which is batholith that can be seen from the geological mapping. Other than just a cascade, some parts of Lata Janggut are exposed to faults, joints and potholes. In the upper part of this cascade, there is a waterfall as high as 5 meters; meanwhile the downstream of the cascade is shallow which resembles a pool (Padzin, 2013). Lata Janggut was developed by Head of Jeli district in collaboration with the Ministry of Tourism and the South Kelantan Development Authority (KESEDAR). Gazebos and camping grounds are among the facilities available at the recreation area.

Figure 2.2: Information Board of Lata Janggut

CHAPTER 3

MATERIALS AND METHODS

3.1 Study Area

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 UMK Jeli Campus. On map, the coordinate is N5 40’05”, E101°46’17”. Figure 3.1 until 3.6 shows the map, the base map, and the location of the study area.

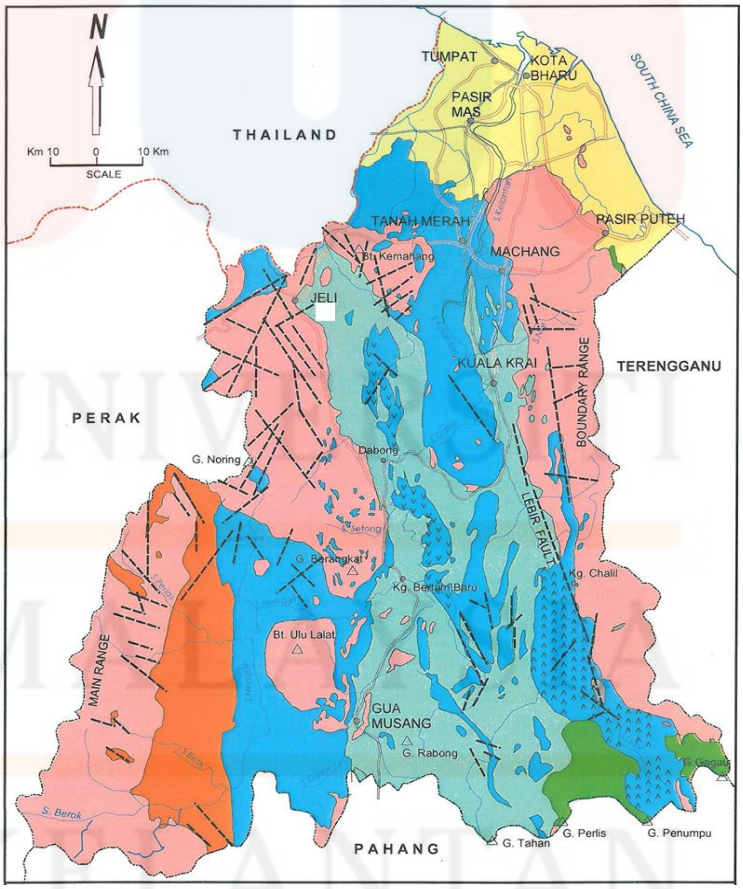


Figure 3.1: Map of Kelantan State (shape box for study area). Source of map: Department of Minerals and Geoscience Malaysia (2003) Quarry Resource Planning for the State of Kelantan

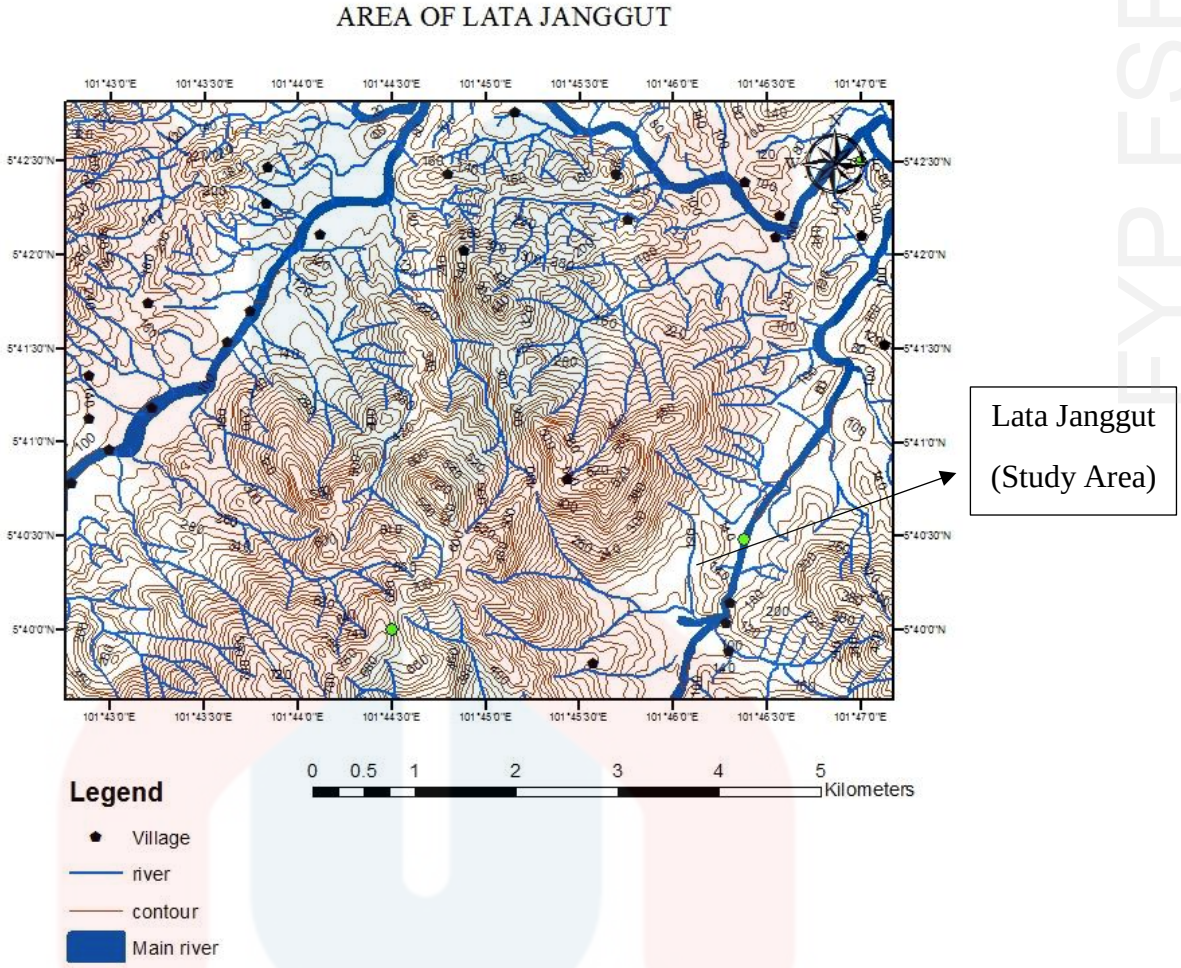


Figure 3.2: The base map of Lata Janggut (10km x 6 km)

Source: Esri, DigitalGlobe, GeoEye, Earthstar Geographics, CNES/Airbus DS, USDA, USGS, AEX, Getmapping, Aerogrid, IGN IGP's wisstopo, end the GIS User Community.

Figure 3.3: Location of Lata Janggut Village in Jeli District

Figure 3.4: The study area at Lata Janggut (Upstream)

Figure 3.5: Shaded sun area

Figure 3.6: Direct sun area

Source: Google Earth (Lidas @ US Dept of State Geographer, Image Landsat, Data SIO, NOAA, U.S, Navy, NGA, GEBCO) 2014.

3.2 Collection of Water Sample

The water samples were collected according to each parameter sampling procedures. There were two samples will be taken randomly at the two potential sites, which are the shaded and direct sun area. The points were chosen with the area that have high probability of the presence of the *Leptospira* sp. at the stagnant water areas. The water samples were taken weekly, for six weeks, starting from July 26 until August 28. The sampling bottles that used were non-transparent or polyethylene bottle. During transportation, all the sample bottles were kept into the icebox that filled with ice so that the temperature kept at 4°C and below.

The samples were tested directly in the laboratory after the sampling procedures, therefore no preservation was done towards the samples for the morphological identification and determination procedure. This was because the chemical added for preservation might affect and denatured the leptospires inside the water samples. However, for water quality and heavy metal analysis, preservation method were applied in order to ensure the results to become valid by adding two mL 50% of Nitric acid in the sampling bottles.

3.3 Morphological Identification and Determination

3.3.1 Media Culture Preparation

Nutrient agar were used for bacteria culture. Nutrient agar powder about 28 grams were added in conical flask with 1000 mL distilled water. The stock was sterilized by autoclaving at 15 lbs pressure (121°C) for 15 minutes. The sterile agar was added into a series of Petri dishes when it was ready. The plates were allowed to cool and placed in the 35°C incubator for at least 24 hours. The nutrient agar was

poured into the half of small bottle for the slant agar. The solidification took about 10 minutes and the slant nutrient agar was stored in the chiller.

3.3.2 Serial Dilution and Bacteria Isolation

For bacteria isolation, one mL of water sample was pipetted and added with nine mL of distilled water. Both of the liquid then were diluted onto serial dilution of 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, and $10^{-}5$. Next, 200 µL of water sample were pipetted and spread plate onto the nutrient agar. The samples were incubated in room temperature (37°C) for 24-48 hours in the incubator. Each bacteria colony was isolated to get pure culture, and kept in the chiller.

3.3.3 Slide Preparation and Observation

The morphological identification of *Leptospira* sp. was observed by using a compound microscope. First of all, slide preparation was prepared, by placing a small drop of specimen on the center of the slide. A clean coverslip was placed over the drop while avoiding bubbles. The prepared slide was observed first under the microscope to record all the morphology, color and mobility of the bacteria that can be seen.

3.3.4 Simple Staining

For the simple staining procedure, the smears were covered with Crystal Violet for at least 10 seconds. Then it were rinsed with water and air-dried. The slide was observe first with low power (10X) to locate a good field. Only the fine focus was used to bring the image into clear focus.

3.3.5 Gram Staining

Next, the smears were stained with Crystal Violet again for one minute, for the gram staining procedure. After being rinsed with water, the smears were stained with Gram's Iodine for one minute. The smears were rinsed carefully with acetone until the blue color stops coming out from the smear. The smears were rinsed with water, and counter-stained with Safranin for at least 10 seconds. The slide were rinsed with water and air-dried again before putting under the microscope lens. The low power (10X) lens were used to find a good field. A drop of oil was added to immerse the 100X oil immersion lens into the oil by rotating the nosepiece.

3.4 Physical Parameter Analysis of Water

3.4.1 *In-situ* data (pH, Temperature, Salinity, Conductivity, Total Dissolved Solid, Turbidity, Dissolved Oxygen)

The testing of the above mentioned parameters were undertaken *in-situ,* and analysed immediately on site. The instrument used was YSI 556 MPS (Multiprobe System). This multiparameter could read and gave result for pH, temperature, salinity, and conductivity, total dissolved solid and dissolved oxygen automatically. The probe of the multiparameter were immersed in the water, and then the readings were appeared on the device screen. While for the turbidimeter of the water sample, it was tested by using turbidimeter.

3.5 Chemical Parameter Analysis of Water

The collected water samples were undergo several analytical procedures for Biochemical Oxygen Demand, Chemical Oxygen Demand, Ammoniacal Nitrogen and Total Suspended Solid by following the guidelines for probe meter (HQ40d) and HACH DR 900 Calorimeter. Below were the methods that used to test all the chemical parameters selected in this study.

3.5.1 Biochemical Oxygen Demand (BOD)

The water samples from the study area were collected in plastic bottle. The bottle was filled in to exclude air. As the sample may degraded during storage, thus the reduction could be minimise trough analyse the sample promptly or cool it near to freezing temperature during storage. The maximum holding time recommended between collection and analysis was 48 hours. Before starting the analysis, the sample needed to be warm chill to 20-27 ± 3°C. The method used in texting the BOD was Dilution Method (Method 8043), adapted from Adapted from Standard Methods for the Examination of Water and Wastewater.

Firstly, the dilution water was prepared by using a BOD Nutrient Buffer Pillow. Then, the bottle was filled in dilution water to just below the lip. For blank, the BOD bottle was filled in with dilution water only. The DO for each bottle was measured by using probe meter (HQ40d). The bottles were stoppered carefully to prevent air bubbles and water seals were made. A plastic cap was placed over the bottle lip, then incubated at 20±1 °C for five days. After five days, the remaining DO concentration in each bottle was measured. The BOD values then were calculated by using calculation method, as by using the equation below (Equation 3.1).

BOD_5, mg/L = (DO_1 – DO_5) x DF Equation (3.1)

where:

BOD_5 is BOD value from the five day test;

DO_1 is DO of diluted sample immediately after preparation, in mg/L;

DO_5 is DO of diluted sample after five day incubation at 20°C, in mg/L;

DF is bottle volume (300 ml) / Sample volume (10 ml)

3.5.2 Chemical Oxygen Demand (COD)

The water samples from study area were collected with glass bottles. The method used in testing COD was Reactor Digestion Method (Method 8000) (Jirka & Carter, 1975). Firstly, the sample was preheated to 150° by using reactor. The cap of a COD Digestion Reagent Vial was removed for the appropriate range. Next, two ml samples were pipetted into the vial and the outside vial was rinsed with deionised water, then wiped with towel paper. The vial was hold over the cap and inverted gently several times to mix the contents. Then, the vial was placed in the preheat reactor. The blank was prepared by using the same steps by substituting sample with deionized water. The vials were heated for two hours. The vials were cooled down for 20 minutes to 120°C or less. The vials were inverted several times while still warm and waited until it cooled down to room temperature. Then, a colorimetric determination was used to measure the COD in mg/L.

3.5.3 Ammoniacal Nitrogen (NH_3N)

The water samples from study area were collected by using polyethylene bottle. The method used in testing the ammoniacal nitrogen was Salicylate Method (Method 8155) that adapted from Clin. Chim. Acta., (1966).

Firstly, a sample cell was filled with 10 mL of water sample. The contents of one Ammonia Salicylate powder pillow was added to the sample cell. The sample cell was closed and shaken well to dissolve the reagent. By using instrument timer, three-minute reaction was started. After the timer expires, an Ammonia Cyanurate powder pillow was added to the sample cell. The sample cell was closed and shaken well again to dissolve the reagent. A 15-minutes reaction was started and a green colour appeared if ammonia-nitrogen was present. The prepared sample was inserted into the cell holder, and the result showed in mg/L NH_3-N.

3.5.4 Total Suspended Solid (TSS)

The sample was collected by using polyethylene bottle. The container was filled to exclude the air. The method used was Photometric Method (Method 8006) that adapted from Sewage and Industrial Wastes (1959). Firstly, 10 mL of water samples were poured into a sample cell. The prepared sample cell needed to be swirled to remove any gas bubbles and uniformly suspended any residue. The prepared sample was cleaned and inserted into the cell holder. The result showed in mg/L TSS.

3.6 Correlation among Physical and Chemical Parameters of Water

Correlation analysis is used when both the variables are experimental and measured with error. The parameters of physical and chemical parameters of water in the research were correlated by using IBM SPSS Statistics Version 20. The analysis was carried out to determine the relationship of parameters between each other's. The value of r is such that $-1 \leq r \leq +1$. The + and – signs were used for positive linear correlations and negative linear correlations, respectively.

3.7 Ecological Parameters Analysis

All the gathered data were merged as the ecological parameters at the upstream area of Lata Janggut. All the parameters, which were the physical, chemical and biological parameters, were taken into considerations.

The data was taken and gathered weekly according to weather conditions for six consecutive weeks. Every result from the morphological identification analysis of *Leptospira* sp. at two different sites was discussed along with the properties of water to identify the suitable environmental conditions of this Leptospirosis causing bacteria at the upstream area of Lata Janggut. Meanwhile, the negative presence of *Leptospira* sp. at the selected areas was also considered as the results. The research flow of this study was as in the Figure 3.6 below.

RESEARCH FLOW

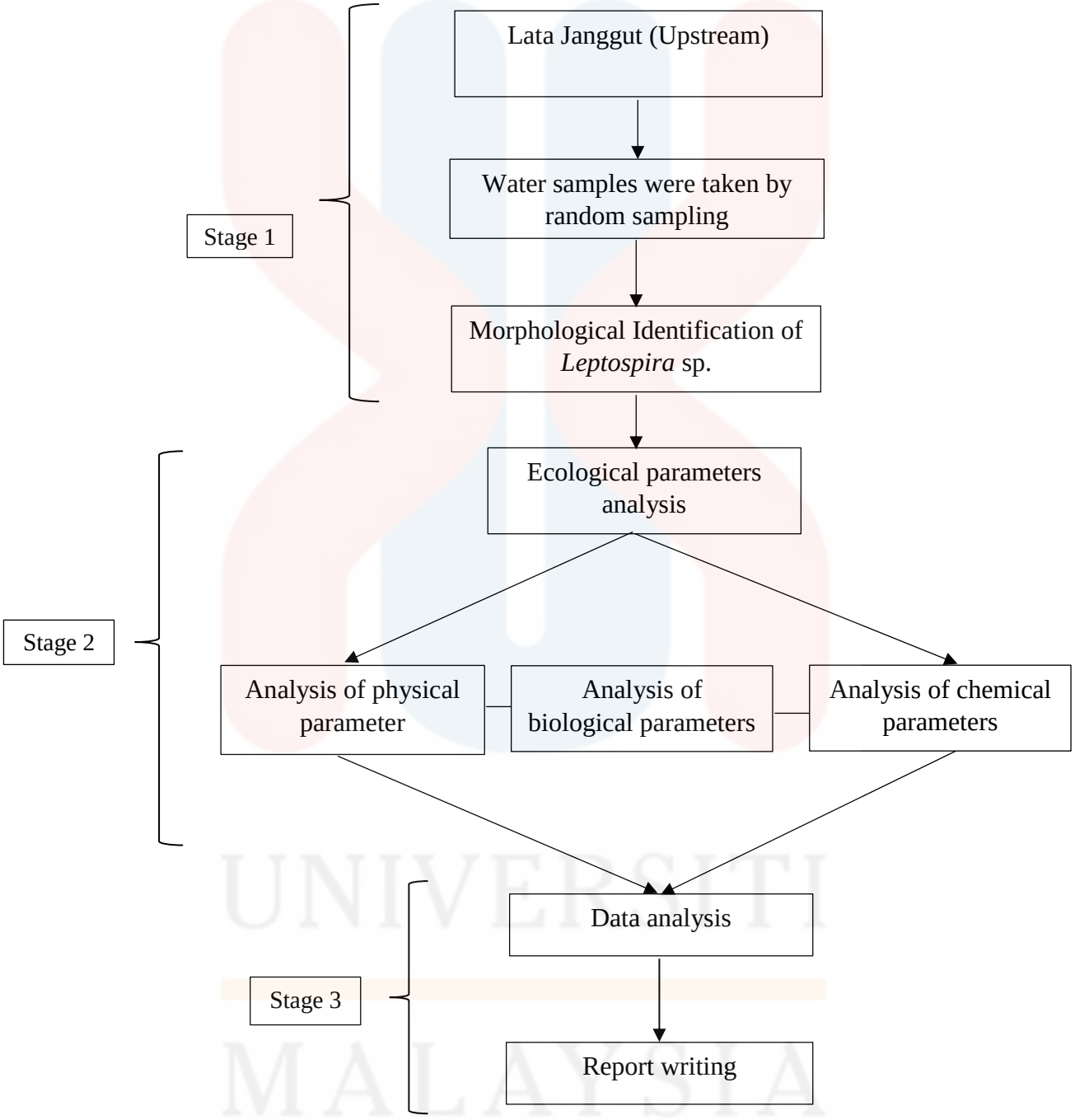


Figure 3.7: Research Flow of *Leptospir*a sp. Availability and Its Relationship with Ecological Parameters of Lata Janggut, Jeli, Kelantan.

Stage 1: Apparatus preparation and identification of *Leptospira* sp.

Stage 2: *In-situ* and further experiments in the laboratory

Stage 3: Analyse all the gathered data and report writing

CHAPTER 4

RESULT AND DISCUSSION

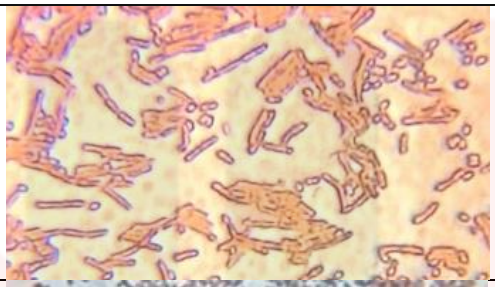
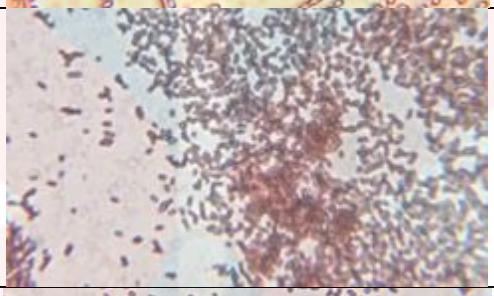
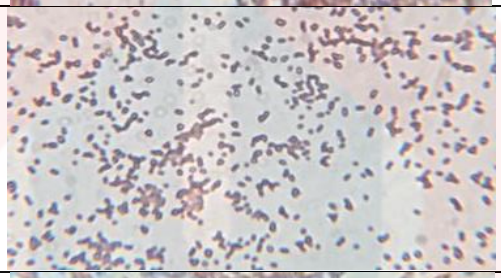
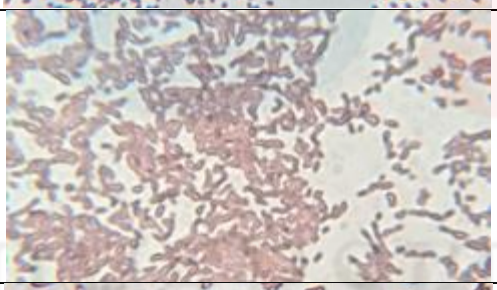
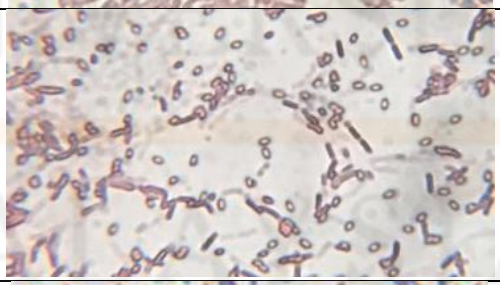
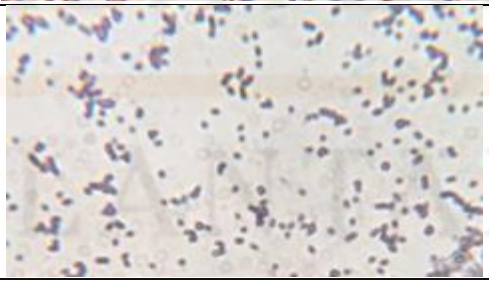
4.1 The Morphological Identification of *Leptospira* sp. from Gram Staining Technique

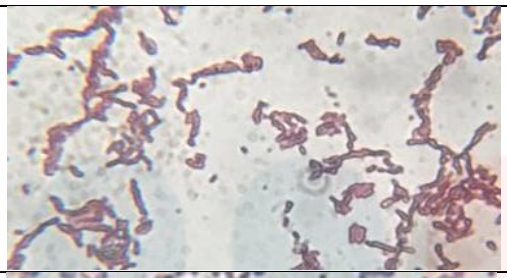
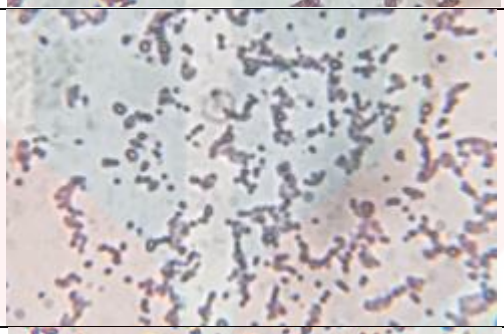
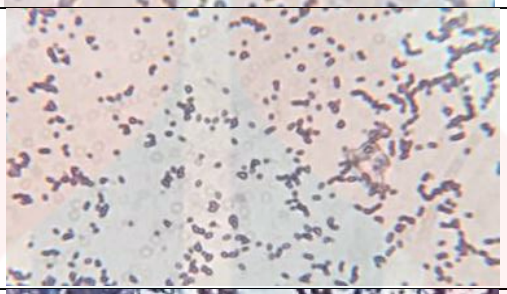
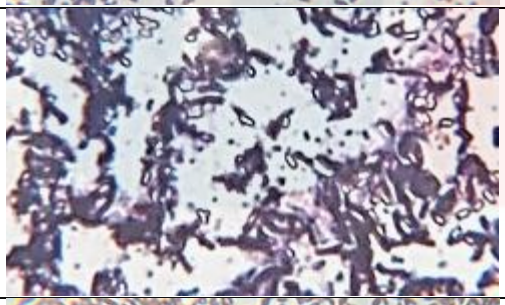
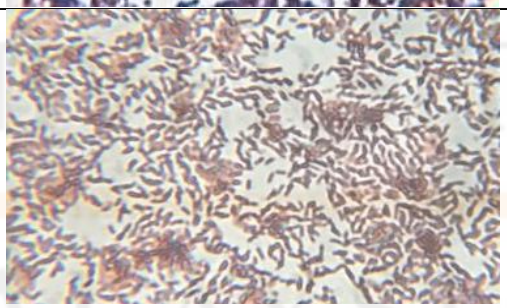
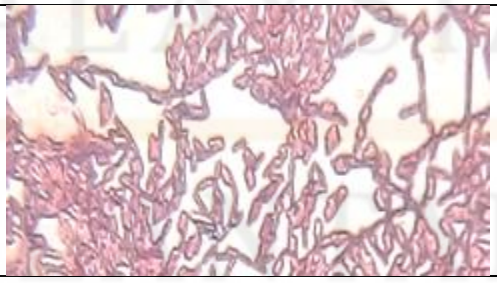
Leptospires specifically have corkscrew-shaped at its end and a Gram-negative-like cell envelope consisting of a cytoplasmic and outer membrane (Mohammed *et al.*, 2011).

Based on the results and observations with its morphological characteristics as the references, supported by gram staining technique with under 100x magnification of microscope, it can be concluded that there is no *Leptospira* sp. present in the research area. There were actually much more of bacteria observed under the microscope, and these just only a few of them (Table 4.1) to show that no leptospires found during the study period. The results for morphological identification from gram staining technique were as shown in Table 4.1.

From the morphological characteristics analysis at the shaded sun area, it were identified that the water sample from the first week contained Gram-negative bacteria with Vibrio shaped or curved-rod shaped, the second and third week with Gram-negative and cocci shaped, the fourth and fifth week with Gram-negative and bacilli shaped and the sixth week with Gram-positive and cocci shaped bacteria.

Table 4.1: Morphological Identification from Gram Staining Technique

Area	Week/Date	Observation Under Microscope	Description
Shaded Sun Area	Week 1		Magnification: x100 Dilution Factor: 10^{-2} Colour: Pink Gram: Negative Shape: Curved-rod
	Week 2		Magnification: x100 Dilution Factor: 10^{-3} Colour: Pink Gram: Negative Shape: Cocci
	Week 3		Magnification: x100 Dilution Factor: 10^{-4} Colour: Pink Gram: Negative Shape: Cocci
	Week 4		Magnification; x100 Dilution Factor:10^{-4} Colour: Pink Gram: Negative Shape: Bacilli
	Week 5		Magnification: x100 Dilution Factor: 10^{-1} Colour: Pink Gram: Negative Shape: Bacilli
	Week 6		Magnification: x100 Dilution Factor: 10^{-5} Colour: Purple Gram: Positive Shape: Cocci

Direct Sun Area	Week 1		Magnification: x100 Dilution Factor: 10^{-4} Colour: Pink Gram: Negative Shape: Spirillum
	Week 2		Magnification: x100 Dilution Factor: Master Plate Colour: Pink Gram: Negative Shape: Cocci
	Week 3		Magnification: x100 Dilution Factor: 10^{-1} Colour: Purple Gram: Positive Shape: Cocci
	Week 4		Magnification: x100 Dilution Factor: 10^{-4} Colour: Purple Gram: Positive Shape: Coccobacilli
	Week 5		Magnification: x100 Dilution Factor: Master Plate Colour: Pink Gram: Negative Shape: Bacilli
	Week 6		Magnification: x100 Dilution Factor: 10^{-4} Colour: Pink Gram: Negative Shape: Spirillum

Meanwhile from the direct sun area, it were observed that the water samples from the first week contained Gram-negative and spirillum shaped, the second and third week with Gram-negative and cocci shaped, the fourth week with Gram-positive and coccobacilli shaped, while the fifth and sixth week consist of Gram-negative with bacilli and spirillum shaped bacteria respectively.

4.2 Physical and Chemical Properties of Water

The variety of physical and chemical properties of water samples which are pH, temperature, conductivity, DO, conductivity, salinity, total dissolved solid were measured as *in-situ* parameters, while BOD, COD, NH_3N and TSS were measured as *ex-situ* parameters. The results were as shown in Table 4.2, 4.3, 4.4 and 4.5. Based on the physical observation of the weather, there could be seen that it was raining during two or one night before the water sampling, logically known as the wet seasons.

4.2.1 The Physical Properties of Water

4.2.1.1 pH

The intensity of acidity or alkalinity and the concentration of hydrogen ions in water can be measured by pH. A change in pH also can indirectly alter the aspects of water chemistry that affects the aquatic life (Prommi & Payakka, 2015).

The concentration of pH at shaded sun area for six continuous weeks were ranged between from 5.75 – 6.95 with a mean concentration of 6.22 ± 0.43. The concentration of pH at each individual week were 6.95, 6.58, 6.26, 5.94, 5.82, and 5.75 respectively. From this observation, the pattern of pH from shaded sun area was decreasing from the first three weeks, and increasing at the fourth until sixth week

(Figure 4.1). The pattern for pH at direct sun area was also the same as shaded sun area (Figure 4.2) as the mean concentration of pH was at 7.42 ± 0.57 that range from 6.43 – 8.37. However, unlike at the shaded sun area, the pH at direct sun area indicated slightly higher readings, which were 8.37, 7.58, 6.43, 7.31, 7.32, and 7.49 respectively.

One of the factors on the survival time of leptospires is affected by the acidity or alkalinity of the water. Environmental conditions shown to be lethal to the bacteria when are in acid conditions (pH <6.5) and alkali conditions (pH >8.4). According to previous studies made by Smith and Turner (1961), leptopires survived longer in alkaline than in acid water, and significant differences between the serotypes were found in response to pH. Survival at pH's under 7.0 ranged from 10 to 117 days and at pH's over 7.0 from 21 to 152 days.

Environmental temperature and pH values were important factors that influence the survival time of leptospires in water. According to Bejo *et al.*, (2004), the longest survival time of serovar hardjo was recorded in river water pH between 6.7 and 7.3 placed in shaded area. They were recorded to survive in river water for 11 days. Previously published studies (Stoddard *et al.*, 2014) that showed pH levels outside the neutral zone is not favourable for survival of leptospires. These findings also showed that, river could be a source of leptospiral infection. It is also important that many activities like military operation, jungle tracking, picnicking and fishing are related to rivers.

4.2.1.2 Temperature

The most common physical assessment of water quality is the measurement of temperature. The mean temperature of water at shaded sun area of upstream of Lata Janggut for the present study was 25.54 ± 0.67 °C that were range from 24.92 – 26.96

°C. From the observation, the temperature reading from the first week was the highest while the fourth week was the lowest temperature. Through the graph (Figure 4.1), it showed that the pattern was decreasing until the fourth week and increasing at the fifth and sixth week. The trends for pH, temperature and dissolved oxygen at shaded sun area were shown as in Figure 4.1.

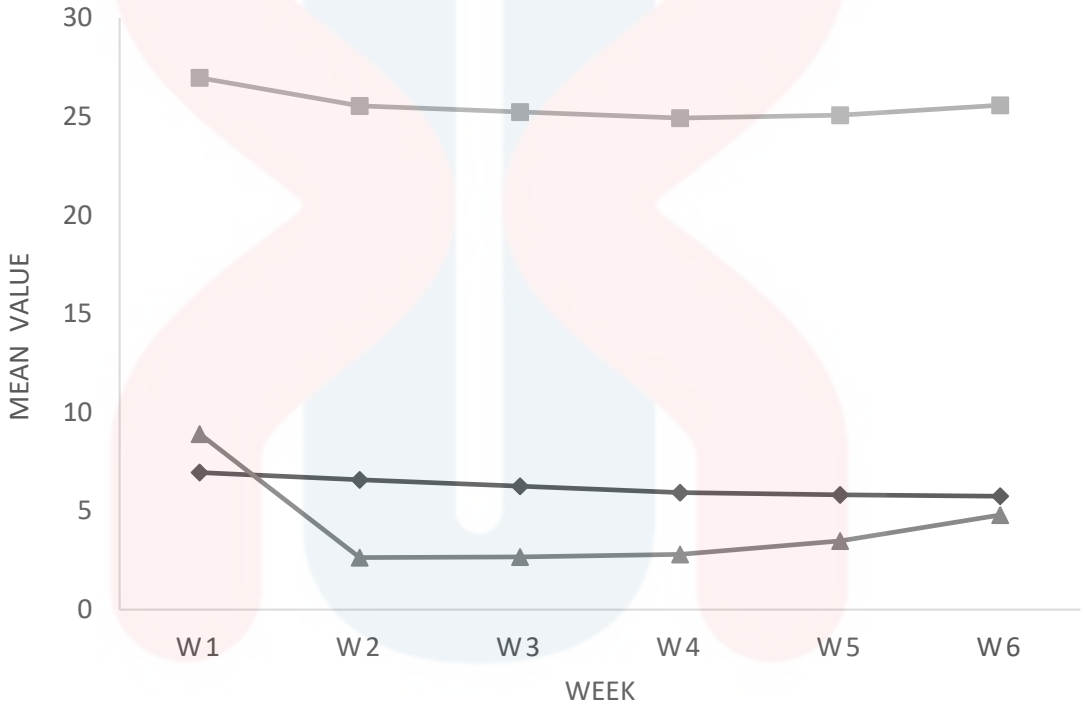


Figure 4.1: The trends for pH (◆), temperature (■, ºC) and dissolved oxygen (▲, mg/L) for shaded sun area.

This was due to the canopy structure from those tall trees presented at the upstream area of Lata Janggut. Meanwhile for direct sun area, big 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 range from 25.87 – 27.28 °C which made the mean to become 26.51 ± 0.42 °C from the continuous six weeks of study period. The graph indicated that the pattern was also 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 (Figure 4.2). The intensity of sunlight

radiation and evaporation had influenced surface water temperature. The temperature is one of the important physical factors, which affects the chemical and biological reactions in water. It regulates the rate of photosynthesis in aquatic ecosystem. (Manikannan, Asokan, Hameed & Samsoor, 2011). The trends for pH, temperature, and dissolved oxygen at direct sun area were shown as in Figure 4.2.

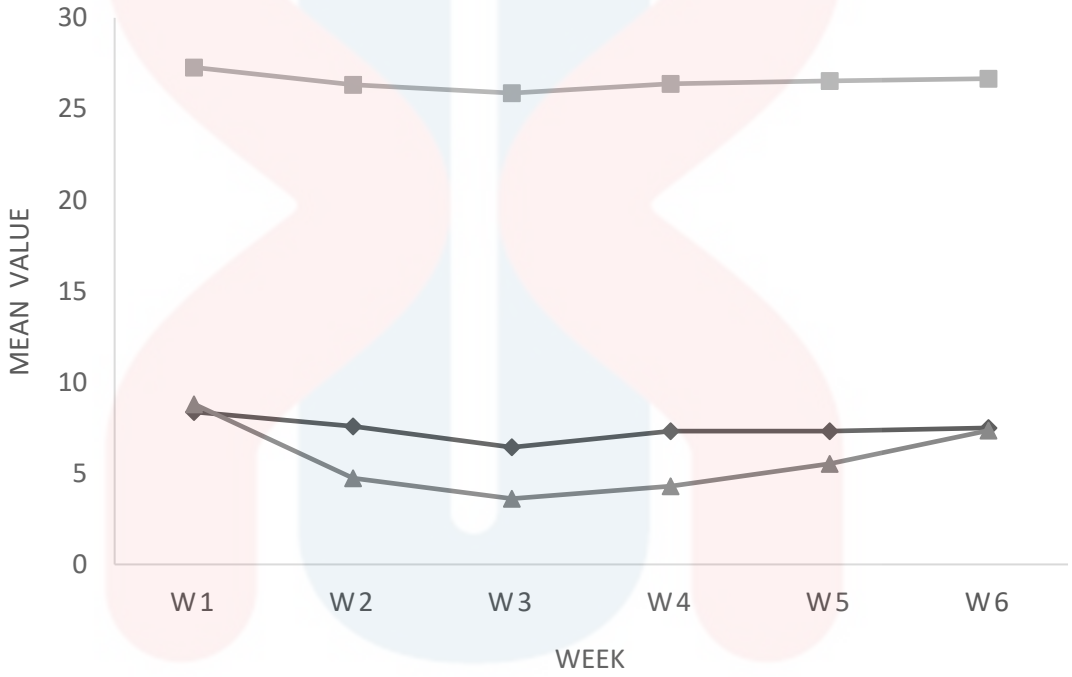


Figure 4.2: The trends for pH (◆), temperature (■, ºC) and dissolved oxygen (▲, mg/L) for direct sun area.

According to the previous study made by Bejo *et al.* (2004), the survival time of serovar hardjo in water placed under shaded sun area was longer than direct sun area. Leptospires can easily be killed at direct sun area with high temperature (32ºC) and strong ultraviolet ray in the environment. While the environmental factors in the jungle such as unpolluted water, temperature of around 27º C and canopy of trees may increase the ability of the bacteria to survive longer in the particular area.

4.2.1.3 Dissolved Oxygen

As for dissolved oxygen (DO), its concentration of shaded sun area were ranged from 2.64 – 8.91 mg/L with a mean of 4.22 ± 2.23 mg/L for the study period. From the recorded readings, it was noticed that the DO reading for the first week is the highest with 8.91 mg/L. There was huge gap and variation that could be seen among the other DO values within the six weeks. However, due to other lower DO values, the mean concentration of DO values was just intermediate, neither not too low nor too high. This indicated that the present of organic matter were less in the shaded sun area of the upstream area at Lata Janggut. While for direct sun area, it was recorded that the mean concentration of DO was higher than the shaded sun area, with 5.72 ± 1.81 mg/L within the study period. The range of DO value for direct sun area was 3.61 – 8.80 mg/L which showed the value for the first week was the highest and therefore made a huge gap for other values. From the graph plotted (Figure 4.2 and 4.3), it can be seen that both areas had the same pattern of DO, which was decreasing until the third week, and the values increasing from the fourth week.

The major factor controlling dissolved oxygen concentration is biological activity such as photosynthesis that producing oxygen while respiration and nitrification consume oxygen (Yap Chee, Shamarina & Edward, 2011). The high organic enrichment and turbulence nature of waterfall has become the possible reason responsible for low oxygen values in certain weeks. The water may also lacked aquatic plants which produced oxygen through respiration as well as having decomposing activities of organic compounds by aerobic organisms which consumed oxygen thus resulting in low DO (Yap *et al*., 2011).

4.2.1.4 Salinity and Conductivity

There were no big variation could be seen at the concentration of salinity for both shaded and direct sun area. However, shaded sun area showed higher trend than the opposite area with the mean concentration at 0.022 ± 0.009 % and 0.013 ± 0.005 % respectively for the study period. Based on the low concentration levels, it was the favour condition for the survival of the bacteria. In fact, pathogenic leptospires do not tolerate the salt levels in seawater but can slowly adapt to survive in solutions up to 1%.

The mean conductivity value was found 0.048 ± 0.017 µS/cm at upstream shaded sun area of Lata Janggut. The values varied from 0.025 – 0.065 µS/cm among the six consecutive weeks which, the highest conductivity value was obtained from the fifth week of study period while the third week had the lowest conductivity. Similar trends of results were obtained for the salinity at the shaded sun area, since conductivity and salinity are closely related to each other in which higher conductivity values indicate the higher presence of dissolve salts content in the water. For direct sun area, the mean conductivity value at this area was found at 0.039 ± 0.003 µS/cm. The highest and lowest conductivity value were found at the first and sixth week respectively, which range from 0.036 – 0.044 µS/cm. It can be observed that the direct sun area has small variation of conductivity compared to the shaded sun area but both still got the same trends for the relationship of conductivity and salinity. Electrical conductivity is not a human or aquatic health concern, but it can serve as an indicator of other water quality problems. If the conductivity of a stream suddenly increases, it indicates that there is a source of dissolved ions in the vicinity. Therefore, conductivity measurements can be used as an efficient way to locate potential water quality problems (Prommi & Payakka, 2015).

4.2.1.5 Turbidity and Total Dissolved Solid

Turbidity measures the scattering effect that suspended solids have on light, which the higher the intensity of scattered light, the higher the turbidity. The next physical parameters are for turbidity and total dissolved solid. At shaded sun area, it was found that 2.39 ± 0.24 NTU as the mean concentration of turbidity and 22.17 ± 5.67 mg/L as the mean concentration of total dissolved solid. It was recorded that the highest and the lowest turbidity was from sixth week with 2.59 NTU and from fourth week with 1.87 NTU, respectively. While for total dissolved solid, the range varied from 12.0 – 28.0 mg/L, with the highest and the lowest values came from sixth and fourth week as well. From the results, it is observed that the total dissolved solid and turbidity parameters have correlation with each other, with the trend decreasing until the fourth week, and the value increasing at fifth and sixth week.

While for direct sun area, the mean concentration of turbidity was found at 3.16 ± 1.27 NTU that range from 1.72 – 5.14 NTU from the six consecutive weeks of study period. At the same time, the mean concentration for total dissolved solid was recorded at 30.0 ± 6.16 mg/L with range 25.0 – 42.0 mg/L within the study period. It can be noticed that both parameters have the same trends which, the value keep decreasing until the fourth week, and increase back at the fifth and sixth week respectively. Turbidity is the amount of particulate matter that is suspended in water. Materials that cause water to be turbid include clay, silt, finely divided organic and inorganic matter, soluble coloured organic compounds, plankton and microscopic organisms. High turbidity and the associated suspended solid concentrations have important ecological impacts, because of light suppression effects (Yap *et al.,* 2011). The mean value of physical water quality parameters for six continuous weeks at shaded and direct sun area were shown as in Table 4.2 and 4.3.

Table 4.2: Mean Value of Physical Water Quality Parameters for Six Continuous Weeks (Shaded Sun Area)

Week/ Date	pH	Temperature (ºC)	Dissolved Oxygen (mg/L)	Salinity (%)	Conductivity (µS/cm)	Turbidity (NTU)	Total Dissolved Solid (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	27.00 ± 0.00
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	22.00 ± 0.00
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	18.00 ± 0.00
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	12.00 ± 0.00
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	26.00 ± 0.00
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	28.00 ± 0.00
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	22.17 ± 5.67

Table 4.3: Mean Value of Physical Water Quality Parameters for Six Continuous Weeks (Direct Sun Area)

Week/ Date	pH	Temperature (ºC)	Dissolved Oxygen (mg/L)	Salinity (%)	Conductivity (µS/cm)	Turbidity (NTU)	Total Dissolved Solid (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	27.00 ± 0.00
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	27.00 ± 0.00
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	25.00 ± 0.00
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	25.00 ± 0.00
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	34.00 ± 0.00
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	42.00 ± 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	30.00 ± 6.16

4.2.2 The Chemical Properties of Water

4.2.2.1 Biochemical Oxygen Demand (BOD)

BOD is a parameter to access the organic load in a waterbody. Many researchers have recorded higher BOD values in polluted water. Low dissolved oxygen, high BOD and high nitrate concentrations indicate the eutrophic status of the waterbody and the unsuitability of water for domestic use (Yogendra & Puttaiah, 2008).

The concentration of BOD were ranged from 18.2 – 26.4 mg/L with a mean concentration of 21.5 ± 2.44 mg/L, was found at the shaded sun area of Lata Janggut (Table 4.4). The variation of BOD concentration among the study period were relatively high compared to the National Water Quality Standards of Malaysia. While at the direct sun area, the mean concentration was found as 22.32 ± 2.38 mg/L which was slightly higher than shaded sun area. Among the six consecutive weeks, the BOD value range from 19.2 – 25.6 mg/L, which the third and the sixth week had the lowest and highest BOD concentration respectively.

Both of the areas shared the same trend of BOD concentrations as it were observed that the lowest and the highest concentrations were found at the third and sixth week respectively. According to Offem (2011), BOD values that were 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.

4.2.2.2 Chemical Oxygen Demand (COD)

COD is one of the parameters needed in the WQI calculation. It is a measure of the equivalent of the organic matter susceptible to oxidation by strong chemical oxidants.

The mean concentration for COD was measured as 3.0 ± 0.77 mg/L and 3.02 ± 1.33 mg/L for shaded and direct sun area respectively, which showed no big difference between both areas. As for the shaded sun area, the concentration value were range from 1.67 - 3.63 mg/L while for direct sun area it were ranged from 0.4 – 4.9 mg/L. By looking on its trends, the values for COD at the both of study areas were smaller than BOD values, which stated as the rare finding among the study of water quality parameters. The COD values should be always higher than BOD values since it includes both biodegradable and non-biodegradable substances while BOD contains bio-degradable substance only.

However, 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 oxidized to carbon dioxide with a strong oxidizing agent (dichromate) under acidic conditions. Dichromate does not oxidize ammonia into nitrate as the case in BOD measurement. Therefore, there is a possibility of getting higher BOD value than COD for water samples that rich with ammonia (NH_3N) and have very low organic carbon concentration. It is also fully depended upon the containing organic species. Some organic species are bio-degradable but it is difficult to be oxidized by the used oxidant used in COD analysis.

4.2.2.3 Ammonical Nitrogen (NH_3N)

NH_3N is a toxic pollutant defined as the amount of ammonia and ammonium compounds. These compounds are transferred into the environment out of the different sources such as waste incineration, sewage treatment and cattle excrement. Water quality degradation due to ammoniacal nitrogen remains a crucial environmental and public concern worldwide because it can cause eutrophication (Othman, M.E, & Mohamed, 2012).

It is noticed that both value of mean concentration of NH_3N for shaded sun and direct sun area were 0.29 ± 0.11 mg/L and 0.28 ± 0.069 mg/L respectively. The same trend was also found at both area as the value keep decreasing from first week until third week, but increasing again on the fourth week. COD and NH_3N are closely related because the sources of both target parameter was organic matter. COD measure the amount of organic compound in water, while ammonia 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 area of Lata Janggut, it was found out that the downstream area indicated higher readings of NH_3N, which lead to the higher readings of COD values, for the six consecutive weeks of study period (Rahim, 2016).

Natural source that contribute elevated nitrogen compound includes rainfall and runoff. Besides, domestic and industrial waste, run off from agricultural land, urban runoff, and farm animal was also another factor that influence the ammonia nitrogen level in water (Nik Azme, 2014). The comparison NH_3N values at the direct sun area between the upstream and downstream area of Lata Janggut were shown as in Figure 4.3.

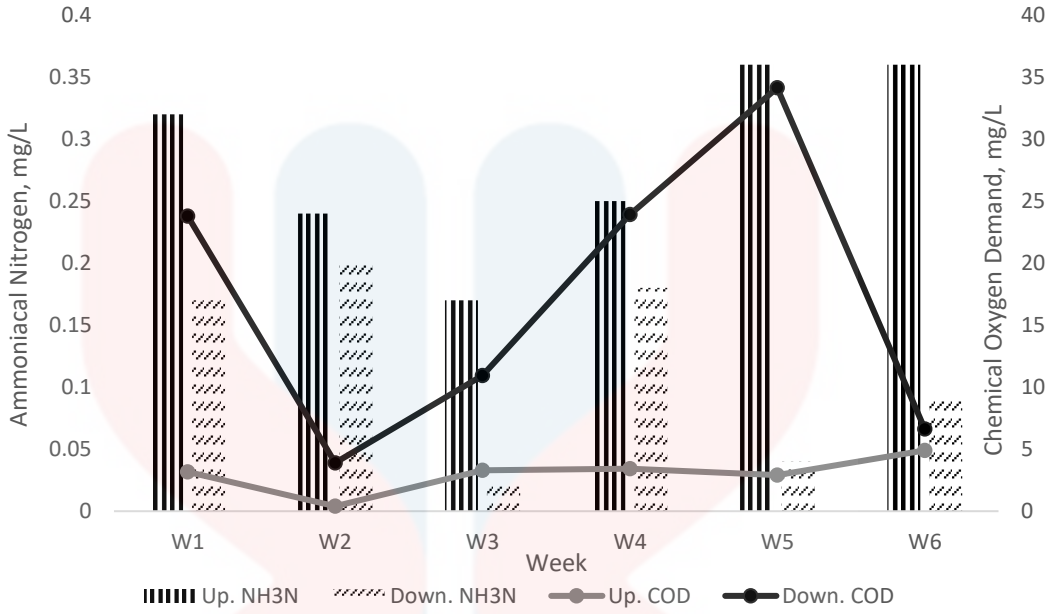


Figure 4.3: The comparison NH_3N reading at the direct sun area between the upstream and downstream area of Lata Janggut. (Source: Rahim, 2016).

4.2.2.4 Total Suspended Solid (TSS)

TSS is usually due to the introduction of external factors carried by runoff rain waters which can help increase the concentration of this parameter. Contributions from natural phenomena, such as tidal effects and anthropogenic activities such as municipal, industrial, agricultural and aquaculture have also been reported to influence the TSS concentration (Suratman, Sailan, Hee, Bedurus & Latif, 2015). The increase of TSS not only increase the cost of water treatment, it is also caused some impacts of ecological degradation on aquatic environments (Nithyanandam *et al*., 2014).

Next, the values of TSS from each area were 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). As for direct sun area, 7.89 ± 1.84 mg/L was found as the mean concentration of TSS value, which range from 7.33 – 10.33 mg/L. From the results, it

can be noticed that the mean concentration for TSS at the direct sun area was relatively smaller than the other one. The mean value of chemical water quality parameters for six continuous weeks at shaded and direct sun area were shown as in Table 4.4 and 4.5.

4.3 Biological Parameter based on the Observation

During the study period, it was observed that there were some insects found at the surface of the water while the samples were taken from the study area. The presence of biotic organisms such as water striders, grasshopper and dragonflies indicated that the area was clean and free from any serious pollution, especially at the shaded sun area due to its location that far and unexposed from any outer activities.

However, the condition of surrounding area was not that satisfying, when there were a lot of trash could be seen due to anthropogenic events such as picnicking, fishing as well as barbeque activities. This irresponsible behaviour of human could lead to the unhygienic environment and encourage the spreading of other diseases.

While *Escherichia coli* is abundantly found in sewage, treated effluents, all natural waters and soils subject to recent faecal contamination, whether from humans, wild animals, or agricultural activity. Recently, it has been suggested that *E. coli* may be present or even multiply in tropical waters not subject to human faecal pollution. However, even in the remotest regions, faecal contamination by wild animals, including birds, can never be excluded because presumption remains that the water has been contaminated and treatment has been ineffective (Nithyanandam *et al.*, 2015).

Table 4.4: Mean Value of Chemical Water Quality Parameters at Lata Janggut for Six Continuous Weeks (Shaded Sun Area)

Week/ Date	Biochemical Oxygen Demand (mg/L)	Chemical Oxygen Demand (mg/L)	Ammoniacal Nitrogen (mg/L)	Total Suspended Solid (mg/L)
1	21.20 ± 0.17	1.67 ± 0.25	0.41 ± 0.03	8.33 ± 0.58
2	21.00 ± 0.30	2.20 ± 0.20	0.24 ± 0.03	7.33 ± 0.58
3	18.20 ± 1.76	3.47 ± 0.15	0.10 ± 0.01	5.00 ± 1.00
4	20.90 ± 0.17	3.43 ± 0.50	0.25 ± 0.03	8.33 ± 0.58
5	21.30 ± 0.60	3.57 ± 0.15	0.36 ± 0.04	10.00 ± 1.00
6	26.40 ± 1.04	3.63 ± 0.31	0.40 ± 0.01	10.67 ±1.53
Mean	21.50 ± 2.44	3.00 ± 0.77	0.29 ± 0.11	8.28 ± 1.84

Table 4.5: Mean Value of Chemical Water Quality Parameters at Lata Janggut for Six Continuous Weeks (Direct Sun Area)

Week/ Date	Biochemical Oxygen Demand (mg/L)	Chemical Oxygen Demand (mg/L)	Ammoniacal Nitrogen (mg/L)	Total Suspended Solid (mg/L)
1	22.90 ± 0.46	3.17 ± 1.63	0.32 ± 0.01	10.33 ± 0.58
2	21.60 ± 0.30	0.40 ± 0.10	0.24 ± 0.02	7.33 ± 0.58
3	19.20 ± 0.79	3.30 ± 0.36	0.17 ± 0.02	4.33 ± 0.58
4	19.80 ± 0.52	3.43 ± 0.67	0.25 ± 0.01	8.00 ± 1.00
5	24.80 ± 0.17	2.90 ± 0.36	0.36 ± 0.05	8.33 ± 0.58
6	25.60 ± 2.13	4.90 ± 0.10	0.36 ± 0.02	9.00 ± 0.00
Mean	22.30 ± 2.38	3.02 ± 1.33	0.28 ± 0.07	7.89 ± 1.84

4.4 Correlation Analysis among the Physical and Chemical Parameters

Coefficient of correlation was worked out to understand the relationship between the parameters of water samples as were shown in Table 4.5 and 4.6. By using Pearson's correlation method, analysis among the physical and chemical parameters at the shaded and direct sun area revealed that there were significant positive and negative relationship between them.

Based on at the shaded sun area, for pH, the strongest positive relationship were found between the physical parameters, such as pH:temperature (r = 0.759) and moderate positive correlation relationship between pH:DO (r = 0.549). Meanwhile, negative moderate relationships were found both at pH:salinity (r = -0.664) and pH:conductivity (r = -0.669). The other strong positive relationships that could be found were through salinity:conductivity (r = 0.997), turbidity:TDS (r = 0.925), temperature:DO (r = 0.915). All the correlations mentioned were significant at the 99% level, p-value at 0.01.

Meanwhile for the correlation between physical parameters at the direct sun area, there were different trend of relationships that could be indicated for pH, where the strongest positive relationships were found between pH:temperature (r = 0.816) and pH:DO (r = 0.812). Meanwhile, positive moderate relationships were found both at pH:salinity (r = 0.676) and pH:conductivity (r = 0.484). The other strong positive relationships that could be found were through salinity:conductivity (r = 0.806), turbidity:TDS (r = 0.950), temperature:DO (r = 0.881). All the correlations mentioned were significant at the 99% level, except for the correlation between pH and conductivity, which was found significant at 95% level, p-value at 0.05.

Next, the correlation analysis between the chemical parameters at the shaded and direct sun area were analysed. As for the shaded sun area, the strongest positive relationship was found between TSS:NH_3N (r = 0.788) and TSS:BOD (r = 0.758) followed by BOD:NH_3N (r = 0.681). Meanwhile at the direct sun area, it was indicated that the correlation between BOD:NH_3N had the strong positive relationship with (r = 0.886), followed by TSS:NH_3N (r = 0.775). While the correlation between TSS and BOD was considered as moderate positive relationship at (r = 0.594). All the correlations were analysed significant at 99% level.

4.5 Water Quality Index (WQI) and Classification at Lata Janggut (Upstream)

Water quality status classification was determined by using the Water Quality Index (WQI). WQI value for upstream area of Lata Janggut was calculated by entering the six water quality parameters mean values such as 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. Then, the WQI was derived from the calculation as below (Equation 4.1).

$$WQI = (0.22*SIDO) + (0.19*SIBOD) + (0.16*SICOD) + (0.15*SIAN) + (0.16*SISS) + (0.12*pH) \quad \text{(Equation 4.1)}$$

According to Table 4.8, the WQI value of shaded and direct sun area were 64.50 and 77.51 respectively. Based on the calculation provided, the quality of water from both areas showed that they were classified as slightly polluted. However, the WQI value for direct sun area indicated higher reading than the shaded sun area.

Table 4.6: Correlation Analysis between Physical Parameters

Area	Parameter	pH	Temperature	Salinity	Conductivity	Turbidity	TDS	DO
Shaded Sun Area	pH	1	0.759**	-0.664**	-0.669**	0.235	0.126	0.549*
	Temperature		1	-0.200	-0.195	0.505*	0.543*	0.915**
	Salinity			1	0.997**	-0.155	0.126**	0.128
	Conductivity				1	-0.114	0.165**	0.129
	Turbidity					1	0.925**	0.455*
	TDS						1	0.579*
	DO							1
Direct Sun Area	pH	1	0.816**	0.676**	0.484*	0.127	0.123	0.812**
	Temperature		1	0.452	0.462	0.290	0.245**	0.881**
	Salinity			1	0.806**	-0.318	-0.344**	0.404
	Conductivity				1	-0.479*	-0.565*	0.346
	Turbidity					1	0.950**	0.470*
	TDS						1	0.488**
	DO							1

**. Correlation is significant at the 0.01 level (2-tailed).
*. Correlation is significant at the 0.05 level (2-tailed).

Table 4.7: Correlation Analysis between Chemical Parameters

Area	Parameter	BOD	COD	NH_3N	TSS
Shaded Sun Area	BOD	1	0.160	0.681**	0.758**
	COD		1	-0.223	0.133
	NH_3N			1	0.788**
	TSS				1
Direct Sun Area	BOD	1	0.277	0.886**	0.594**
	COD		1	0.359	0.110
	NH_3N			1	0.775**
	TSS				1

**. Correlation is significant at the 0.01 level (2-tailed).

Through the analysis, it was determined that the water quality of the upstream area of Lata Janggut was slightly polluted for the study period, which were taken into consideration for six consecutive weeks. The WQI values of the study areas were calculated as in the equations below (Equation 4.2 and 4.3).

Table 4.8: WQI Value and Classification for Each Studied Area

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

WQI (Shaded Sun Area): = (0.22*30.92259) + (0.19*30.95324) + (0.16*95.11) + (0.15*70.05) + (0.16*92.60657) + (0.12*93.95837) (Equation 4.2)

WQI (Direct Sun Area): = (0.22*88.70096) + (0.19*29.41145) + (0.16*95.0834) + (0.15*71.1) + (0.16*92.83045) + (0.12*97.31678) (Equation 4.3)

A comparison of NH_3N, BOD, COD, DO, pH, TSS and WQI at the upstream area of Lata Janggut were compared with the value provided by DOE as summarized in Table 4.9. WQI values for the upstream area of Lata Janggut were measured through two different areas, which are shaded and direct sun area. Both of the WQI values fall under Class III and II respectively. As for shaded sun area, for the compared parameters, NH_3N was categorized in Class II, BOD in Class V, COD in Class I, DO in Class III, pH in Class II, and TSS in Class I. Meanwhile for direct sun area, NH_3N was identified in Class II, BOD in Class V, COD in Class I, DO in Class II, pH in Class I, and TSS in Class I.

Table 4.9: Comparison with DOE Water Quality Index and Classification

Parameter	Unit	Class					Present Study
		I	II	III	IV	V	
NH_3N	mg/L	<0.1	0.1-0.3	0.3-0.9	0.9-2.7	>2.7	S: 0.29 D: 0.28
BOD	mg/L	<1	1-3	3-6	6-12	>12	S: 21.50 D: 22.32
COD	mg/L	<10	10-25	25-50	50-100	>100	S: 3.00 D: 3.02
DO	mg/L	<7	5-7	3-5	1-3	<1	S: 4.22 D: 5.72
pH	-	>7.0	6.0-7.0	5.0-6.0	<5.0	>5.0	S: 6.22 D: 7.42
TSS	mg/L	<25	50	150	300	>300	S: 8.28 D: 7.89
WQI		>92.7	76.5-92.7	51.9-76.5	31.0-51.9	<31.0	S: 64.50 D: 77.51

S: Shaded Sun Area D: Direct Sun Area

4.6 Ecological Parameters Analysis with the Absence of *Lepstospira* sp. at the Upstream Area of Lata Janggut

The survival of leptospires in the environment depend on many conditions and here it stated as the ecological parameters. *Leptospira* sp. exist in two groups, known as the free-living saprophytes and the pathogenic parasitic types. Both groups have different food requirements and life cycles although they require same basics to survive such as water, oxygen, stable pH and temperature.

Compared to others, leptospires are relatively slow growing bacteria and so colony densities in a body of water tend to be uniform. The presence of saprophytic leptospires in the water body do not indicate that it is unclean or contaminated. In fact, the bacteria prefer clean water without any chemical pollution. Whilst for pathogenic types, they require a host in order to complete their life cycle. The host's immune response and species determine the survivability of the bacteria inside and outside the host. As for the upstream area of Lata Janggut, the WQI of the area during the study period was indicated as slightly polluted and this may the reason for the absence of leptospires.

Leptospires are immediately killed in the dry environment or surfaces, and hence the moisture is important for them. As they do not have a waterproof membrane, they must remain immersed in water to survive. The inability of leptospires to survive outside water is one of the crucial control factor in the natural environment. Besides, pathogenic leptospires are extremely sensitive to all kinds of chemicals even though at very low concentrations since they are very fragile little bacteria. This happen due to the compounds that attack the bacterial envelope, and damage the internal chemical processes that are needed for the bacterium's survivability. The chemicals will damage the envelope's outer layer that is made of a compound called lipopolysaccharide or LPS. Therefore, this support the outcome of the research that leptospires were not found in the study area, either they were being killed or the study area was totally free from animals that excrete the leptospires both in active infection and asymptomatic stage.

CHAPTER 5

CONCLUSION

5.0 Conclusion

The present study focussed on the availability testing of *Leptospira* sp. through morphological identification and gram staining technique, which found out to be absent during the study period. Based on this identification, the first objective is successfully achieved even though it was a negative result. While the second objective is accomplished by identifying the ecological parameters that includes physical, chemical and biological parameter of the study area.

The absence of leptospires at the upstream area of Lata Janggut is supported by lethal conditions of the bacteria itself. Environmental conditions shown to be lethal to the bacteria, when are in acid conditions pH < 6.5 and alkali conditions pH > 8.4. Leptospires also can be easily killed at the direct sun area with high temperature, as high as 32ºC in the environment. Based on the low concentration salinity levels, it was the favour condition for the survival of the bacteria. In fact, pathogenic leptospires do not tolerate the salt levels in seawater but can slowly adapt to survive in solutions up to 1%.

Based on the data presented in the research, the WQI of the study area averagely was 71.005, classified in Class II (slightly polluted). This outcome correlated with the absence of leptospires during the study period, as the matter of fact, the bacteria prefer clean water without any chemical pollution for their survival.

In a nutshell, detection of pathogenic *Leptospira* sp. in water samples especially in recreational lakes may pose a health risk, especially to those who come into contact with contaminated water during sports activities. Necessary precautions should be taken by the authorities to monitor water bodies, to alert the public for better control of the host reservoir population and appropriate garbage management.

5.1 Limitation of the Study and Future Dimension of the Work

The present study was involved in determining the leptospires trough the Gram staining method for a few weeks only. The future work should be done for more weeks according to the weather conditions in order to increase the possibility to find the desired bacteria. The next project also could involve longer study period that can cover up to the whole year's data of the study area. The data from this study also could be useful information towards the public who love natural activities, and to any related departments, regarding Leptospirosis.

REFERENCES

Abdollahpour, G. (2013). A review on Leptospirosis. Retrieved March 1, 2016 from http://leptolab.ut.ac.ir/Review-En.htm.

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), 123-129.

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 / JSME*, 135-140.

Bernama, (2015, January 26). 187 Leptospirosis Cases Reported At HRPZ II. *Malaysian Digest.* Retrieved February 2, 2016 from http://www.malaysiandigest.com/frontpage/29-4-tile/539378-187-leptospirosis

Beveridge, T. J. (2001). Use of Gram Stain In Microbiology. *Biotechnic & histochemistry : official publication of the Biological Stain Commission*, 76 (3), 111-118.

Crustal, P. (n.d.). Chapter 1 Review of Leptospirosis. *North*, 1-37.

Donohoe, H. M., & Needham, R. D. (2006). Ecotourism: The Evolving Contemporary Definition. *Journal of Ecotourism*, 5 (3), 192-210.

El Jalii, I. M. & Bahaman, A. R. (2004). A Review of Human Leptospirosis in Malaysia. *Tropical Biomedicine*, 21 (2), 113-119.

Lau, C. (2008). Urbanisation, Climate Change and Leptospirosis: Environmental Drivers of Infectious Disease Emergence. *School of Population Health, University of Queensland*, 83-87.

Lehmann, J. S., Matthias, M. A., Vinetz, J. M., & Fouts, D. E. (2014). Leptospiral Pathogenomics. *Pathogens* 280-308.

Levett, P. N. (2001). Leptospirosis. *Clinical Microbiology*, 14 (2), 296-326.

Loon, L. C. (n.d.). Flood : Recognizing and Managing the illusive Leptospirosis Post Disaster. Retrieved March 1, 2016 from http://www.ihm.moh.gov.my/images/files/MOHAMM/slide/12082015_Symposium1/DR_LEONG_CHEE_LOON_LEPTOSPIROSIS.pdf.

Manikannan, R., Asokan, S., Hameed, A. & Samsoor, M. (2011). Seasonal variations of physico-chemical properties of the Great Vedaranyam Swamp , Point Calimere Wildlife Sanctuary , South-east coast of India. *African Journal of Environmental Science and Technology* Vol. 5(9), pp. 673-681

Mansor, A. S. (2016, May 18). Remaja Kritikal Dipercayai Dijangkiti Leptospirosis. *Harian Metro,* p. 17.

Mohammed, H., Nozha, C., Hakim, K., Abdelaziz, F. & Rekia, B. (2011). Bacteriology & Parasitology LEPTOSPIRA : Morphology , Classification and Pathogenesis. *J Bacteriol Parasitol* 2 (6), 6-9.

Nik Azme, N.N.A (2014). Water Quality Assessment At Ranchan Waterfall, Serian Sarawak. *Undergraduated Project Report, Faculty of Resource Science and Technology*

Offem, B. O. (2011). Water Quality and Environmental Impact Assessment of a Tropical Waterfall System. *Environment and Natural Resources Research* Vol. 1, No. 1.

Othman, F., M E, A. E. & Mohamed, I. (2012). Trend analysis of a tropical urban river water quality in Malaysia. *Journal of Environmental Monitoring* : JEM 14 (12), 3164-3173.

Padzin, N. M. (2013). General geology, geoheritage resources and geotourism potential of Lata Janggut, Jeli District, Kelantan. *Undergraduated project report, Faculty of Earth Science.*

Prommi, T., & Payakka, A. (2015). Aquatic Insect Biodiversity and Water Quality Parameters of Streams in Northern Thailand. *Sains Malaysiana* 44(5)(2015): 707–717.

Rahim, A. A (2016). *Leptospira* sp. Physical & Chemical Parameters Relationship and Its Availability at Lata Janggut, Jeli, Kelantan. *Undergraduated Project Report, Faculty of Earth Science.*

Ridzlan, F. R., Bahaman, A. R., Khairani-Bejo, S. & Mutalib, A. R. (2010). Detection of pathogenic Leptospira from selected environment in Kelantan and Terengganu, Malaysia. *Tropical Biomedicine*, 27 (3), 632-638.

Sambasiva, R. R., Naveen, G., Bhalla P. & Agarwal, S. K. (2003). Leptospirosis in India and the rest of the world. *The Brazilian journal of infectious diseases : an official publication of the Brazilian Society of Infectious Diseases*, 7 (3), 178-193.

Santiago, J. (n.d.). Leptospirosis. Retrieved March 1, 2016 from http://www.austincc.edu/microbio/2993r/li.htm.

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), 1020–1022.

Smith, C. E. G. & Turner, L. H. (1961). The Effect of pH on the Survival of Leptospires in Water. *World Health Organization*, 24 (2), 35-43.

S. 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): 67–73.

Thayaparan, S., Robertson, I. D., Fairuz, A., Suut, L. & Abdullah, M. T. (2013). Leptospirosis, an emerging zoonotic disease in Malaysia. *Malaysian Journal of Pathology*, 35 (2), 123-132.

Wahab, Z. A. (2015). Epidemiology and Current Situation of Leptospirosis in Malaysia.

WHO. (2014). Leptospirosis. *Excerpt from "WHO recommended standards and strategies for surveillance, prevention and control of communicable diseases"*, 1-4.

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), 334-338.

Yap, C.K., Chee, M.W., Shamarina, S. & Edward, F.B. (2011). Assessment of Surface Water Quality in the Malaysian Coastal Waters by Using Multivariate Analyses. *Sains Malaysiana* 40(10)(2011): 1053–1064

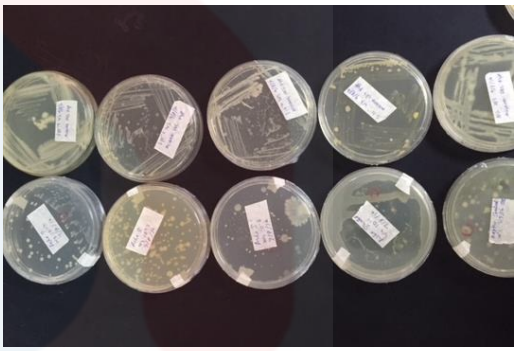
Yogendra, K. & Puttaiah, E. T. (2008) Determination of Water Quality Index and Suitability of an Urban Waterbody in Shimoga Town, Karnataka. *Proceedings of Taal 2007: The 12th World lake Conference*: 342-346.

APPENDIX A

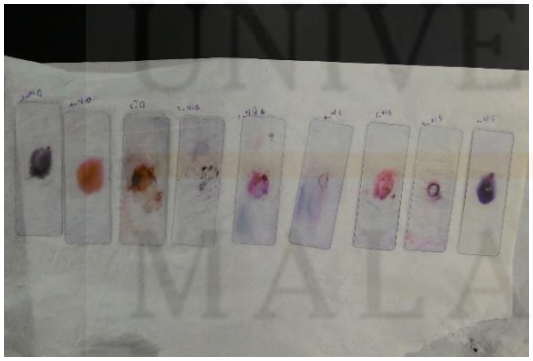
Unhygienic condition of downstream area

Unhygienic condition of upstream area

Nutrient agar preparation



Pure culture of growth bacteria



Gram staining technique

Chemical parameter analysis (NH_3N)

APPENDIX B

WQI Formula and Calculation

WQI = (0.22*SIDO) + (0.19*SIBOD) + (0.16*SICOD) + (0.15*SIAN) + (0.16*SISS) + (0.12*pH)

Where: $0 \leq WQI \leq 100$

SIDO is Subindex DO (% saturation);

SIBOD is Subindex BOD;

SICOD is Subindex COD;

SIAN is Subindex NH_3N;

SISS is Subindex SS;

SIpH is Subindex pH.

Best Fit Equations for the Estimation of Various Subindex Values:

Subindex for DO (% in saturation)

SIDO = 0 for $x \leq 8$

SIDO = 100 for $x \geq 92$

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

Subindex for BOD

SIBOD = 100.4 – 4.23x for $x \leq 5$

SIBOD = 108* exp(-0.055x)-0.1x for $x > 5$

Subindex for COD

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

SICOD = 103* exp(-0.0157x)-0.04x for $x > 20$

Subindex for NH_3N

SIAN = 100.5 – 105x for $x \leq 0.3$

SIAN = 94* exp(-0.573x) – 5* I x-2 I for $0.3 < x < 4$

SIAN = 0 for $x \geq 4$

Subindex for SS

SISS = 97.5* exp(-0.00676x) + 0.05x for $x \leq 100$

SISS = 71* exp (-0.0061x) – 0.015x for $100 < x < 1000$

SISS = 0 for $x \geq 1000$

Subindex for pH

SlpH = 17.02 - 17.2x + 5.02x2 for $x < 5.5$

SlpH = -242 + 95.5x - 6.67x2 for $5.5 \leq x < 7$

SlpH = -181 + 82.4x - 6.05x2 for $7 \leq x < 8.75$

SlpH = 536 - 77.0x + 2.76x2 for $x \geq 8.75$

*Note: * means multiply with*

APPENDIX C

PHYSICAL PARAMETER ANALYSIS

SHADED SUN AREA

Week/ Date Mean, μ SD, σ	pH	Temperature (°C)	Salinity (%)	Conductiviy (μS/cm)	Turbidity (NTU)	Total Dissolved Solid (mg/L)	Dissolved Oxygen (mg/L)
Week 1							
24/7/16	7.02	27.55	0.02	0.044	2.56	27.0	9.83
26/7/16	6.88	26.54	0.02	0.045	2.55	27.0	8.87
28/7/16	6.94	26.78	0.02	0.044	2.57	27.0	8.04
Mean	6.95	26.96	0.02	0.044	2.56	27.0	8.91
SD	0.07	0.53	0.00	0.00058	0.01	0.00	0.89
Week 2							
31/7/16	6.56	25.52	0.01	0.027	2.44	22.0	2.64
2/8/16	6.58	25.53	0.01	0.026	2.44	22.0	2.64
4/8/16	6.61	25.56	0.01	0.028	2.46	22.0	2.65
Mean	6.58	25.54	0.01	0.027	2.45	22.0	2.64
SD	0.025	0.021	0.00	0.001	0.012	0.00	0.0058
Week 3							
7/8/16	6.21	25.21	0.01	0.025	2.43	18.0	2.63
9/8/16	6.31	25.26	0.01	0.025	2.32	18.0	2.66
11/8/16	6.27	25.22	0.01	0.025	2.29	18.0	2.72
Mean	6.26	25.23	0.01	0.025	2.35	18.0	2.67
SD	0.05	0.027	0.00	0.0	0.074	0.00	0.046
Week 4							
14/8/16	5.90	24.90	0.03	0.061	1.76	12.0	2.71
16/8/16	5.89	24.91	0.03	0.063	1.81	12.0	2.88
18/8/16	6.02	24.94	0.03	0.063	2.04	12.0	2.83
Mean	5.94	24.92	0.03	0.062	1.87	12.0	2.81
SD	0.072	0.021	0.00	0.0012	0.15	0.00	0.087
Week 5							
21/8/16	5.89	25.04	0.03	0.062	2.54	26.0	3.55
23/8/16	5.77	25.06	0.03	0.065	2.47	26.0	3.43
25/8/16	5.81	25.12	0.03	0.064	2.51	26.0	3.47
Mean	5.82	25.07	0.03	0.064	2.51	26.0	3.48
SD	0.06	0.04	0.00	0.0015	0.035	0.00	0.06
Week 6							
28/8/16	5.70	25.76	0.03	0.064	2.57	28.0	4.68
30/8/16	5.75	25.41	0.03	0.065	2.58	28.0	4.77
1/9/16	5.79	25.55	0.03	0.066	2.63	28.0	4.94
Mean	5.75	25.57	0.03	0.065	2.59	28.0	4.80
SD	0.045	0.18	0.00	0.001	0.032	0.00	0.13

APPENDIX D

	Week/ Date Mean, μ SD, σ	pH	Temperature (°C)	Salinity (%)	Conductivity (μS/cm)	Turbidity (NTU)	Total Dissolved Solid (mg/L)	Dissolved Oxygen (mg/L)
DIRECT SUN AREA	Week 1							
	1	8.50	27.49	0.02	0.043	2.83	27.0	9.20
	2	8.27	27.33	0.02	0.045	2.80	27.0	9.17
	3	8.33	27.01	0.02	0.043	2.80	27.0	8.04
	Mean, μ	8.37	27.28	0.02	0.044	2.81	27.0	8.80
	SD, σ	0.12	0.24	0.00	0.001	0.017	0.00	0.66
	Week 2							
	1	7.56	26.34	0.02	0.042	2.33	27.0	4.84
	2	7.54	26.25	0.02	0.041	2.35	27.0	4.74
	3	7.63	26.37	0.02	0.039	2.41	27.0	4.62
	Mean, μ	7.58	26.32	0.02	0.041	2.36	27.0	4.73
	SD, σ	0.047	0.062	0.00	0.0015	0.042	0.00	0.11
	Week 3							
	1	6.42	25.96	0.01	0.039	2.26	25.0	3.55
	2	6.39	25.88	0.01	0.039	2.29	25.0	3.47
	3	6.47	25.76	0.01	0.039	2.27	25.0	3.81
	Mean, μ	6.43	25.87	0.01	0.039	2.27	25.0	3.61
	SD, σ	0.04	0.10	0.00	0.0	0.015	0.00	0.18
	Week 4							
	1	7.51	26.27	0.01	0.039	1.72	25.0	4.13
	2	7.23	26.52	0.01	0.038	1.75	25.0	4.22
	3	7.19	26.34	0.01	0.038	1.70	25.0	4.56
	Mean, μ	7.31	26.38	0.01	0.038	1.72	25.0	4.30
	SD, σ	0.17	0.13	0.00	0.00058	0.025	0.00	0.23
	Week 5							
	1	7.15	26.72	0.01	0.039	4.64	34.0	5.24
	2	7.30	26.59	0.01	0.037	4.66	34.0	5.55
	3	7.51	26.32	0.01	0.037	4.63	34.0	5.79
	Mean, μ	7.32	26.54	0.01	0.038	4.64	34.0	5.53
	SD, σ	0.18	0.20	0.00	0.001	0.015	0.00	0.28
	Week 6							
	1	7.20	26.98	0.01	0.038	5.18	42.0	7.33
	2	7.56	26.32	0.01	0.036	5.15	42.0	7.35
	3	7.71	26.69	0.01	0.035	5.09	42.0	7.41
	Mean, μ	7.49	26.66	0.01	0.036	5.14	42.0	7.36
	SD, σ	0.26	0.33	0.00	0.0015	0.046	0.00	0.042

APPENDIX E

CHEMICAL PARAMETER ANALYSIS

SHADED SUN AREA

Week/ Date Mean, μ SD, σ	Biochemical Oxygen Demand (mg/L)	Chemical Oxygen Demand (mg/L)	Ammoniacal Nitrogen (mg/L)	Total Suspended Solid (mg/L)
Week 1				
1	21.30	1.70	0.41	8.00
2	21.00	1.90	0.44	9.00
3	21.30	1.40	0.39	8.00
Mean, μ	21.20	1.67	0.41	8.33
SD, σ	0.170	0.25	0.025	0.58
Week 2				
1	20.70	2.40	0.24	7.00
2	21.00	2.00	0.22	8.00
3	21.30	2.20	0.27	7.00
Mean, μ	21.00	2.20	0.24	7.33
SD, σ	0.30	0.20	0.025	0.58
Week 3				
1	18.90	3.30	0.09	5.00
2	19.50	3.50	0.10	6.00
3	16.20	3.60	0.11	4.00
Mean, μ	18.20	3.47	0.10	5.00
SD, σ	1.76	0.15	0.01	1.00
Week 4				
1	20.70	3.50	0.22	8.00
2	21.00	2.90	0.25	9.00
3	21.00	3.90	0.27	8.00
Mean, μ	20.90	3.43	0.25	8.33
SD, σ	0.17	0.50	0.025	0.58
Week 5				
1	21.30	3.40	0.39	10.00
2	20.70	3.70	0.36	9.00
3	21.90	3.60	0.32	11.00
Mean, μ	21.30	3.57	0.36	10.00
SD, σ	0.60	0.15	0.035	1.00
Week 6				
1	25.80	3.30	0.41	11.00
2	25.80	3.70	0.41	12.00
3	27.60	3.90	0.39	9.00
Mean, μ	26.40	3.63	0.40	10.67
SD, σ	1.04	0.31	0.012	1.53

APPENDIX F

	Week/ Date Mean, μ SD, σ	Biochemical Oxygen Demand (mg/L)	Chemical Oxygen Demand (mg/L)	Ammoniacal Nitrogen (mg/L)	Total Suspended Solid (mg/L)
DIRECT SUN AREA	Week 1				
	1	22.50	4.3	0.32	10.0
	2	22.80	1.3	0.33	11.0
	3	23.40	3.9	0.31	10.0
	Mean, μ	22.90	3.17	0.32	10.33
	SD, σ	0.46	1.63	0.01	0.58
	Week 2				
	1	21.60	0.40	0.22	7.00
	2	21.30	0.30	0.24	8.00
	3	21.90	0.50	0.25	7.00
	Mean, μ	21.60	0.40	0.24	7.33
	SD, σ	0.30	0.10	0.015	0.58
	Week 3				
	1	18.90	2.90	0.16	4.00
	2	18.60	3.40	0.17	5.00
	3	20.10	3.60	0.19	4.00
	Mean, μ	19.20	3.30	0.17	4.33
	SD, σ	0.79	0.36	0.015	0.58
	Week 4				
	1	19.50	2.70	0.24	9.00
	2	19.50	3.60	0.26	7.00
	3	20.40	4.00	0.25	8.00
	Mean, μ	19.80	3.43	0.25	8.00
	SD, σ	0.52	0.67	0.01	1.00
	Week 5				
	1	24.90	2.50	0.36	9.00
	2	24.60	3.00	0.36	8.00
	3	24.90	3.20	0.35	8.00
	Mean, μ	24.80	2.90	0.36	8.33
	SD, σ	0.17	0.36	0.0058	0.58
	Week 6				
	1	25.20	4.90	0.37	9.00
	2	27.90	4.80	0.35	9.00
	3	23.70	5.00	0.35	9.00
	Mean, μ	25.60	4.90	0.36	9.00
	SD, σ	2.13	0.10	0.012	0.00

APPENDIX G Correlation between Physical Parameters at Shaded Sun Area

		pH	Temperature	Salinity	Conductivity	Turbidity	TDS	DO
pH	Pearson Correlation	1	.759**	-.664**	-.669**	.235	.126	.549**
	Sig. (2-tailed)		.000	.003	.002	.347	.617	.018
	N	18	18	18	18	18	18	18
Temperature	Pearson Correlation	.759**	1	-.200	-.195	.505*	.543**	.915
	Sig. (2-tailed)	.000		.426	.438	.032	.020	.000
	N	18	18	18	18	18	18	18
Salinity	Pearson Correlation	-.664**	-.200	1	.997**	-.155	.126**	.128
	Sig. (2-tailed)	.003	.426		.000	.538	.620	.612
	N	18	18	18	18	18	18	18
Conductivity	Pearson Correlation	-.669**	-.195	.997**	1	-.114	.165**	.129
	Sig. (2-tailed)	.002	.438	.000		.652	.513	.609
	N	18	18	18	18	18	18	18
Turbidity	Pearson Correlation	.235	.505*	-.155	-.114	1	.925	.455*
	Sig. (2-tailed)	.347	.032	.538	.652		.000	.058
	N	18	18	18	18	18	18	18
TDS	Pearson Correlation	.126	.543*	.126	.165	.925**	1	.579*
	Sig. (2-tailed)	.617	.020	.620	.513	.000		.012
	N	18	18	18	18	18	18	18
DO	Pearson Correlation	.549*	.915**	.128	.129	.455	.579*	1**
	Sig. (2-tailed)	.018	.000	.612	.609	.058	.012	
	N	18	18	18	18	18	18	18

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

APPENDIX H Correlation between Physical Parameters at Direct Sun Area

		pH	Temperature	Salinity	Conductivity	Turbidity	TDS	DO
pH	Pearson Correlation	1	.816**	.676**	.484*	.127	.123	.812**
	Sig. (2-tailed)		.000	.002	.042	.614	.627	.000
	N	18	18	18	18	18	18	18
Temperature	Pearson Correlation	.816**	1	.452	.462	.290	.245**	.881
	Sig. (2-tailed)	.000		.060	.054	.244	.327	.000
	N	18	18	18	18	18	18	18
Salinity	Pearson Correlation	.676**	.452	1	.806**	-.318	-.344**	.404
	Sig. (2-tailed)	.002	.060		.000	.199	.162	.096
	N	18	18	18	18	18	18	18
Conductivity	Pearson Correlation	.484*	.462	.806**	1	-.479*	-.565*	.346
	Sig. (2-tailed)	.042	.054	.000		.045	.014	.160
	N	18	18	18	18	18	18	18
Turbidity	Pearson Correlation	.127	.290	-.318	-.479*	1	.950	.470
	Sig. (2-tailed)	.614	.244	.199	.045		.000	.049
	N	18	18	18	18	18	18	18
TDS	Pearson Correlation	.123	.245	-.344	-.565*	.950**	1	.448
	Sig. (2-tailed)	.627	.327	.162	.014	.000		.063
	N	18	18	18	18	18	18	18
DO	Pearson Correlation	.812**	.881**	.404	.346	.470*	.448**	1**
	Sig. (2-tailed)	.000	.000	.096	.160	.049	.063	
	N	18	18	18	18	18	18	18

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

APPENDIX I

Correlation between Chemical Parameters at Shaded Sun Area

		BOD	COD	NH3N	TSS
BOD	Pearson Correlation	1	.160	.681**	.758**
	Sig. (2-tailed)		.525	.002	.000
	N	18	18	18	18
COD	Pearson Correlation	.160	1	-.223	.133
	Sig. (2-tailed)	.525		.375	.600
	N	18	18	18	18
NH3N	Pearson Correlation	.681**	-.223	1	.788**
	Sig. (2-tailed)	.002	.375		.000
	N	18	18	18	18
TSS	Pearson Correlation	.758**	.133	.788**	1
	Sig. (2-tailed)	.000	.600	.000	
	N	18	18	18	18

**. Correlation is significant at the 0.01 level (2-tailed).

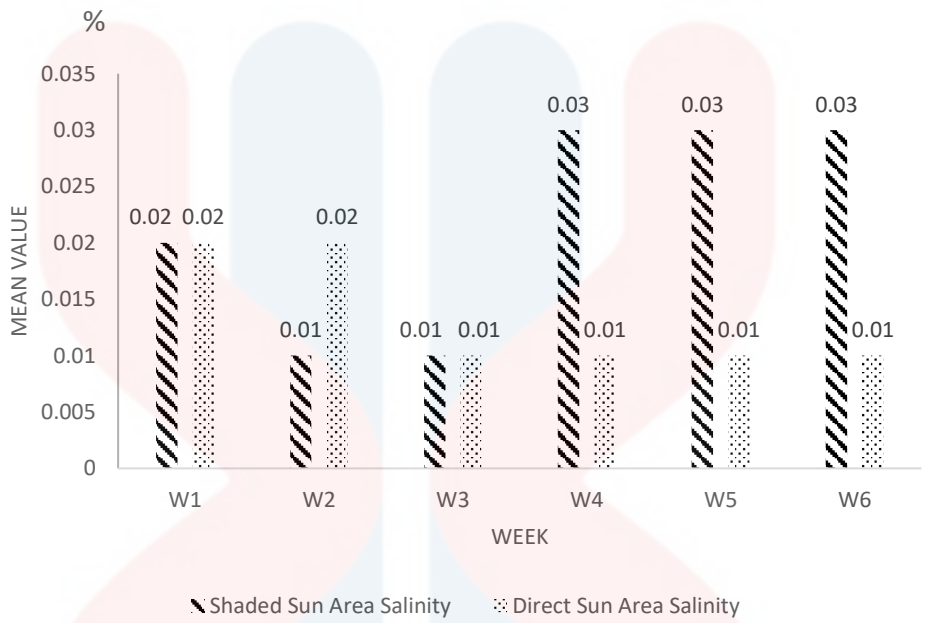
APPENDIX J

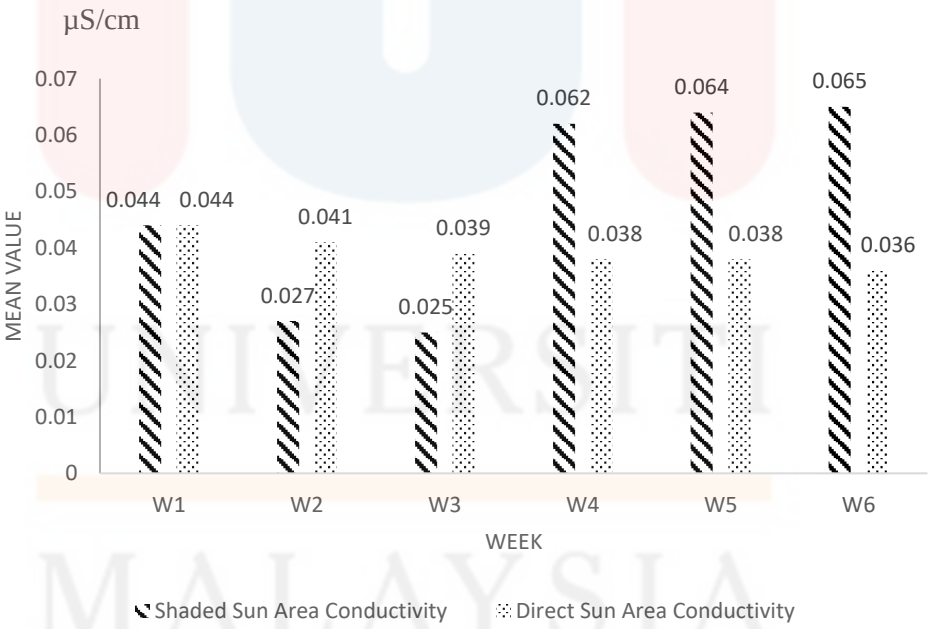
Correlation between Chemical Parameters at Direct Sun Area

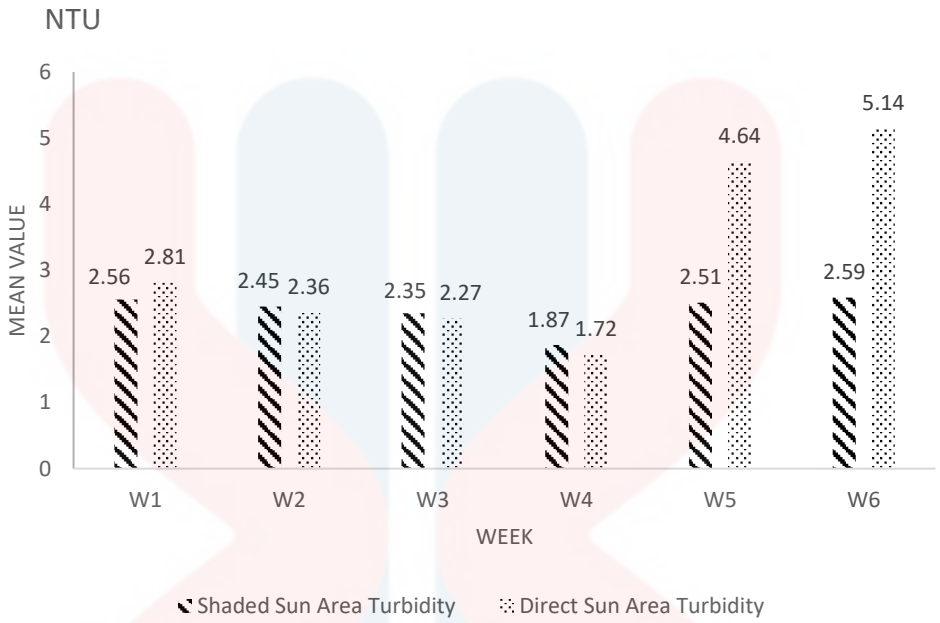
		BOD	COD	NH3N	TSS
BOD	Pearson Correlation	1	.277	.886**	.594**
	Sig. (2-tailed)		.266	.000	.009
	N	18	18	18	18
COD	Pearson Correlation	.277	1	.359	.110
	Sig. (2-tailed)	.266		.144	.665
	N	18	18	18	18
NH3N	Pearson Correlation	.886**	.359	1	.775**
	Sig. (2-tailed)	.000	.144		.000
	N	18	18	18	18
TSS	Pearson Correlation	.594**	.110	.775**	1
	Sig. (2-tailed)	.009	.665	.000	
	N	18	18	18	18

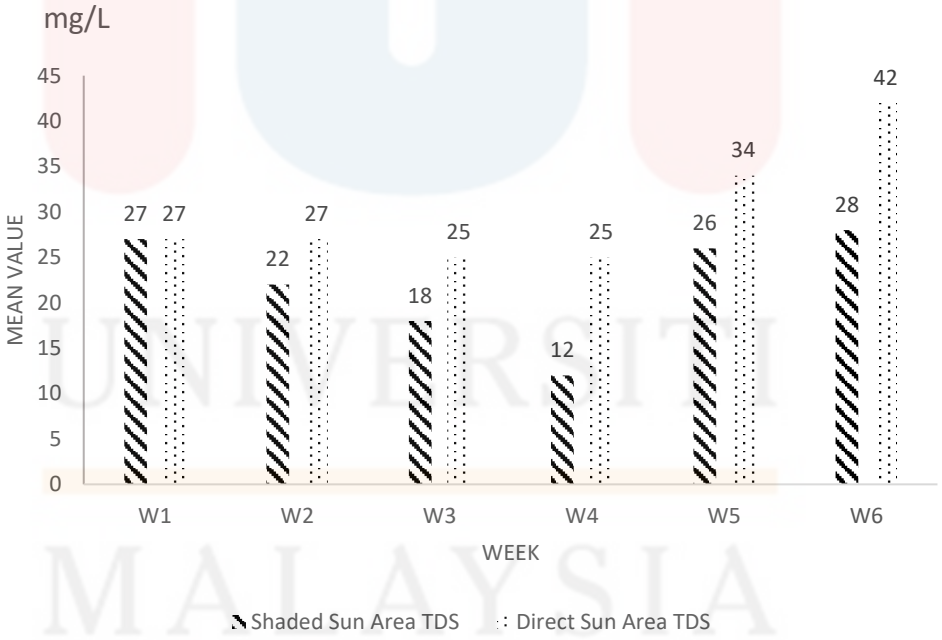
**. Correlation is significant at the 0.01 level (2-tailed).

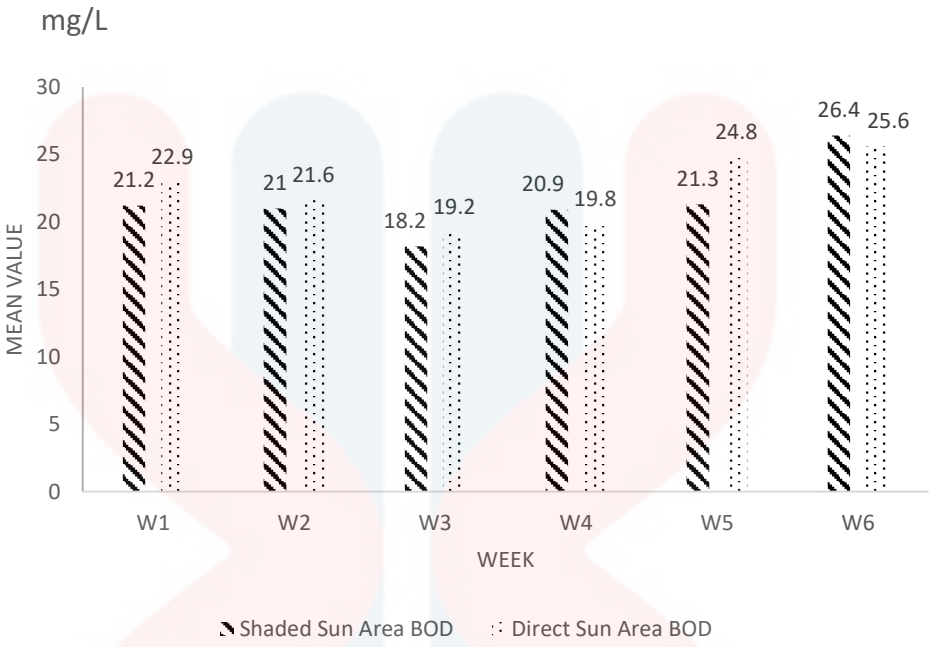
APPENDIX K

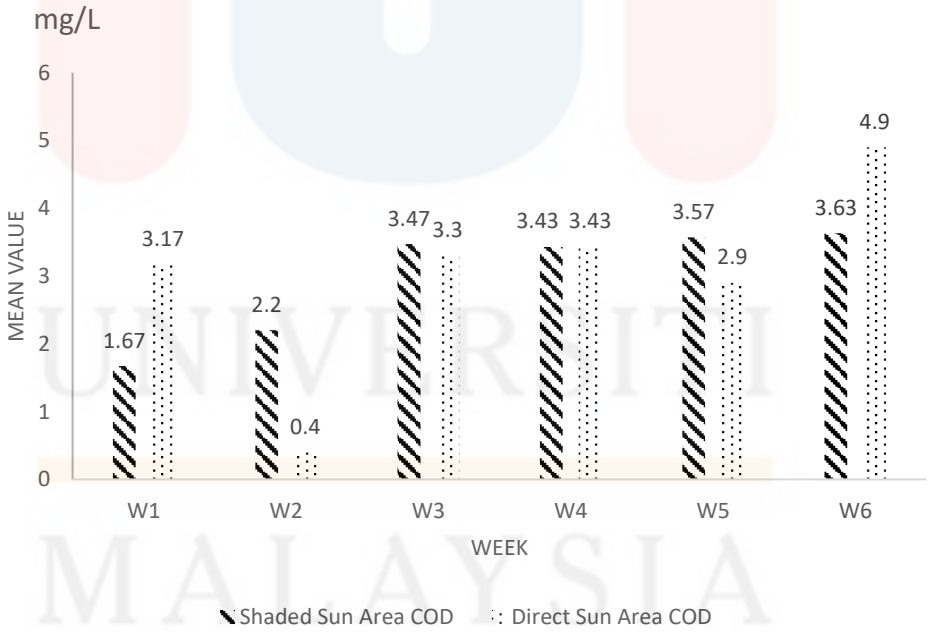


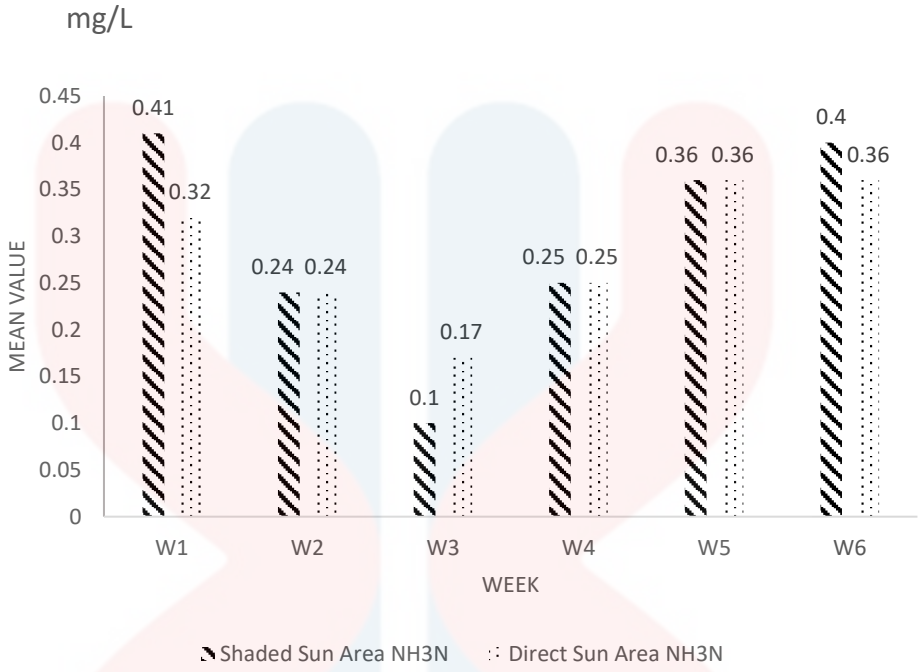


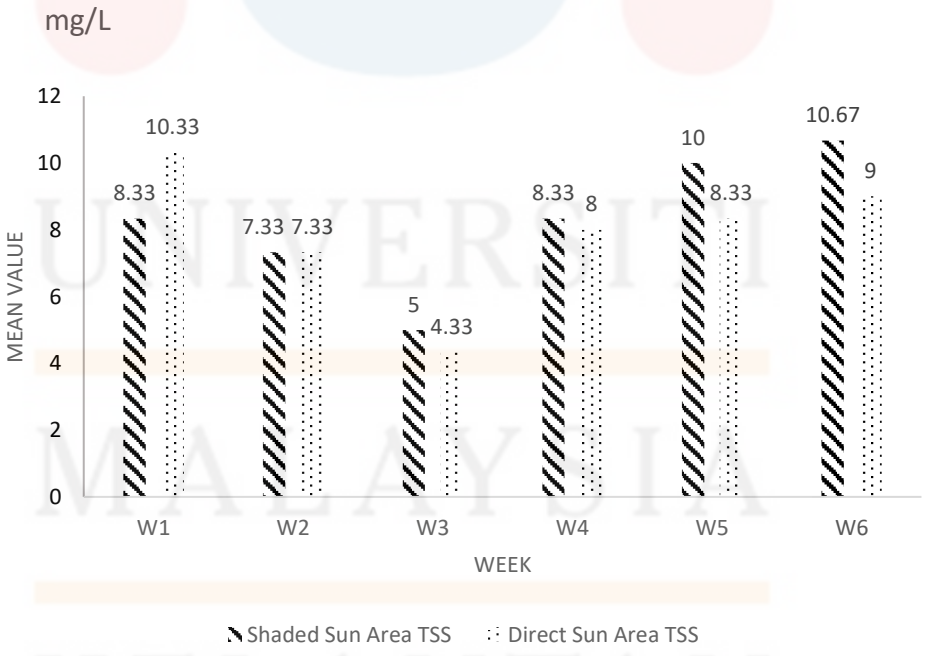




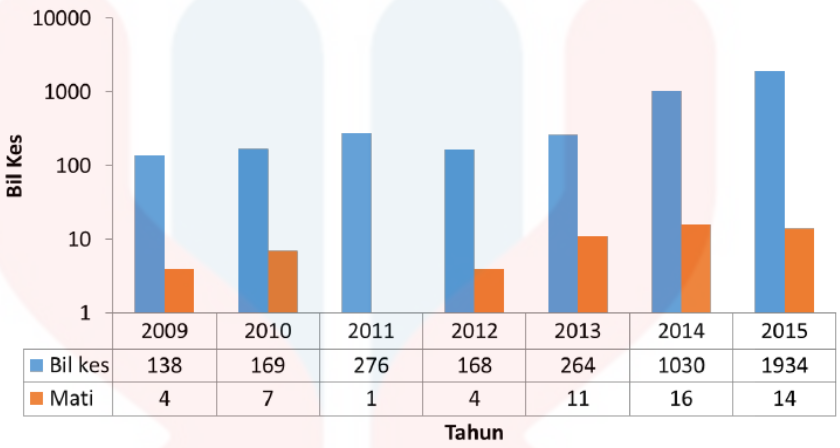








APPENDIX L



	2009	2010	2011	2012	2013	2014	2015
Bil kes	138	169	276	168	264	1030	1934
Mati	4	7	1	4	11	16	14

2015; Peningkatan kes berbanding tahun 2014 - 80% dan kematian menurun
14 kes berbanding 16 kes tahun 2014

The number of Leptospirosis cases and deaths in Kelantan (2009-2015)

	PM	KB	GM	TP	TM	MCG	KK	JELI	BCK	PP
Bil Kes	436	251	220	209	204	144	165	135	89	81
IR	212.0	44.5	210.0	123.7	149.2	144.0	136.5	297.3	59.7	65.2

Bil kes tertinggi - PM dan kadar insiden tertinggi - Jeli

The number of cases and incidence rates (IR) by districts (2015)

Source: EPID CDC Unit, Disease Control Section, Department of Health, Kelantan.