Leptospira sp. PHYSICAL AND CHEMICAL PARAMETERS RELATIONSHIP AND ITS AVAILABILITY AT LATA JANGGUT, JELI, KELANTAN

ANIS AMALINA BINTI RAHIM

FACULTY OF EARTH SCIENCE
UNIVERSITI MALAYSIA KELANTAN

2017

ANIS AMALINA BINTI RAHIM

B. App. Sc. (SUSTAINABLE SCIENCE) with Hons.

2017

Leptospira sp. PHYSICAL AND CHEMICAL PARAMETERS RELATIONSHIP AND ITS AVAILABILITY AT LATA JANGGUT, JELI, KELANTAN

by

ANIS AMALINA BINTI RAHIM

A report submitted in fulfillment of the requirements for the degree of Bachelor of Applied Science (Sustainable Science) with Honours

FACULTY OF EARTH SCIENCE

UNIVERSITI MALAYSIA KELANTAN

2017

DECLARATION

I declare that this thesis entitled "*Leptospira* sp. Physical and Chemical Parameters Relationship and its Availability at 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.

Signature: ___________________________

Name: ANIS AMALINA BINTI RAHIM

Date: ___________________________

ACKNOWLEDGEMENT

I would like to express my deep sense of gratitude to one and all that helped me and motivated me during the duration of my project. I would like to thank my supervisor, Dr. Mohamad Faiz bin Mohd Amin for his invaluable and constant guidance, understanding, support, advices, assistant and discussion throughout this research as without it I would not be succeeded in carrying out this research. I would like also to sincerely thank Madam Farah Khaliz binti Kedri and Dr. Norrimi Rosaida Binti Awang as I am grateful and indebted to her for her understanding, encouragement, coaching and discussion in every aspect of my research. Special thanks to Dr. Suganthi A/P Appalasamy for her personal generosity in making time and giving out information for a fruitful discussion in solving my difficulties.

Deepest appreciation to the Faculty of Earth Science, University Malaysia Kelantan for providing all the facilities and equipments needed in carrying out my research. Special thank to Madam Nur Izzati binti Salleh, Encik Rohanif bin Mohamed Ali, Miss Hasimah binti Hassan, Encik Muhammad bin Che Isa and other lab assistants for their understanding and sincere assistant during the laboratory works and in providing the materials and equipment to be used in the research.

Finally, I would like to take this oppurtunity to extend my gratitude to my family for their moral, spiritual and financial support throughout this research. Not to forget to my friends and all lecturers for their friendly and sincere advices, valuable suggestion, information and understanding.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

NO. **PAGE**

LIST OF ABBREVIATIONS

sp.	Species
DOE	Department of Environment
WQI	Water Quality Index
NTU	Nephelometric Turbidity Units
cm	Centimetre
mg/L	Milligram per litre
m/L	Mililitre
m	Metre
BOD	Biological Oxygen Demand
COD	Chemical Oxygen Demand
TSS	Total Suspended Solid
TDS	Total Dissolve Solid
DO	Dissolve Oxygen
HCl	Hydrochloric Acid
KI	Potassium Iodide
$KMNO_4$	Potassium Permanganate
AAS	Atomic Absorption Spectroscopy
SAP	Sodium Adsorption Ratio
μS/cm	Micro Siemens per Centimetre

μL	Microlitre
Ca	Calcium
Mg	Magnesium
Hg	Mercury
As	Arsenic
Na	Sodium
Pb	Lead
K	Potassium
Mn	Manganese
Zn	Zinc
Cu	Copper
Cr	Chromium
Al	Aluminium
Ni	Nickel
Fe	Iron
Cd	Cadmium
Fe	Silver
km	Kilometre
UiTM	Universiti Teknologi Mara
NH_3N	Ammoniacal Nitrogen

LIST OF SYMBOLS

>	More than
<	Less than
≥	Equal or more than
≤	Equal or less than
μ	Micro
%	Percentage
°C	Degree Celsius
*	Multiply
-	to
±	More or less

Leptospira sp. Physical & Chemical Parameters Relationship and its Availability at Lata Janggut, Jeli, Kelantan

ABSTRACT

The number of Leptospirosis cases and deaths in Kelantan from 2009-2015 showed increasing trends from year to year, with Jeli District held the highest record for the number of deaths. This study play an important role to give the right information about the causes of Leptospirosis that can be transmitted from a contaminated creature towards the publics. This research focusing about the physical and chemical parameters of *Leptospira* sp., at Lata Janggut. Lata Janggut is part of Sungai Long, and is recognized as the natural recreational area in Kelantan. *In-situ* data measurement, such as Conductivity, pH, Total Dissolved Solid, Dissolved Oxygen (DO), Turbidity, Temperature and Salinity were taken by using multiparameter (YSI 556) and HACH DR 900 Calorimeter as the physical parameters, meanwhile the chemical parameters such as Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Ammoniacal Nitrogen (NH_3N) and Total Suspended Solid were measured as *ex-situ* parameters by using APHA method spectrophotometer (DRB 2800) and DO probe meter (HQ40d). The characterization of the 16 elements of heavy metal was determined by using Atomic Absorption Spectrophotometer (AAS). Water Quality Index (WQI) of the Lata Janggut was analysed as slightly polluted, with 74.532 (Class III) and 72.133 (Class III) according to the WQI value suggested by DOE of Malaysia, at the direct and shaded sun area respectively. The area was slightly polluted due to the unhygienic conditions of the surrounding environment. Based on the gram staining procedures, it was indicated that Lata Janggut was free from Leptospirosis since there was no *Leptospira* sp. found in the study period that further discussed as the ecological parameters of *Leptospira* serovars at Lata Janggut.

Keywords*:* *Leptospira* sp., physical parameters, chemical parameters, gram staining, water quality

Leptospira sp. Hubungan Parameter Fizikal dan Kimia dan Ketersediaan di Lata Janggut, Jeli, Kelantan

ABSTRAK

Jumlah kes Leptospirosis dan kematian di Kelantan dari 2009-2015 menunjukkan peningkatan trend dari tahun ke tahun, dengan Daerah Jeli memegang rekod tertinggi bagi bilangan kematian. Kajian ini memainkan peranan yang penting untuk memberikan maklumat yang tepat tentang punca-punca Leptospirosis yang boleh dihantar dari makhluk yang tercemar ke arah orang awam. Kajian ini memberi tumpuan mengenai parameter fizikal dan kimia *Leptospira* sp., di Lata Janggut. Lata Janggut adalah sebahagian daripada Sungai Long, dan diiktiraf sebagai kawasan rekreasi semula jadi di Kelantan. Bagi pengukuran data *in-situ*, seperti konduktiviti, pH, jumlah terlarut pepejal, Oksigen terlarut, kekeruhan, suhu dan tahap kemasinan telah diambil dengan menggunakan multiparameter (YSI 556) dan HACH DR 900 Kalorimeter sebagai parameter fizikal, sementara itu parameter kimia seperti sebagai Permintaan Oksigen Biokimia, Permintaan Oksigen Kimia, Ammoniakal Nitrogen dan jumlah pepejal terampai diukur sebagai parameter *ex-situ* dengan menggunakan kaedah APHA spektrofotometer (DRB 2800) dan DO prob meter (HQ40d). Pencirian 16 unsur-unsur logam berat telah ditentukan dengan menggunakan Spektrofotometer Penyerapan Atom (AAS). Indeks Kualiti Air daripada Lata Janggut dianalisis sebagai sedikit tercemar, dengan masing-masing 74,532 (Kelas III) dan 72,133 (Kelas III) mengikut nilai Indeks Kualiti Air yang disyorkan oleh Jabatan Alam Sekitar Malaysia, di kawasan matahari langsung dan teduh. Kawasan itu sedikit tercemar disebabkan oleh keadaan yang tidak bersih daripada alam sekitar. Berdasarkan prosedur gram pewarnaan, ia telah menunjukkan bahawa Lata Janggut bebas daripada Leptospirosis kerana tiada *Leptospira* sp. dijumpai di dalam tempoh kajian yang dibincangkan dengan lebih lanjut sebagai parameter ekologi serovars *Leptospira* di Lata Janggut.

Kata kunci: *Leptospira* sp., parameter fizikal, parameter kimia, gram pewarnaan, kualiti air

CHAPTER 1

INTRODUCTION

1.1 Background of the study

Leptospirosis is one of the re-emerging zoonotic illnesses that be transmitted from a contaminated creature to human (Lim, Murugayah, Abdul, Mohamed, Shamsudin, Tan, 2011). Leptospirosis is spread by leptospires which are pathogenic or saprophytic. Moreover both pathogenic and non-pathogenic serovars can be ordered inside of the same species (Thayaparan, Robertson, Fairuz, Suut, Abdullah, 2013). Saprophytic strains can be disengaged from streams and lakes (Trueba, Zapata, Madrid, Cullen, Haake, 2004). The Leptospirosis causing microorganisms ordinarily contaminate animals, for example, rodents. It is a bacterial contamination brought on by pathogenic *Leptospira* genus called Spirochaetes (Thayaparan *et al*., 2013). Exposure to the bacteria in the urine of these animals enables the *Leptospira Spirochetes* to enter the body through cuts, wounds, and mucous membranes such as the eyes, mouth and nose (Lim *et al*., 2011). Generally, reasons related to the rising of infection are occupational exposure, poor sanitation, climate changes, recreational activities as well as management of wild animals (Lim *et al*., 2011). The increasing incidence of Leptospirosis in forested regions is associated with the bacteria infecting small wild mammals other than rats. Infection in wildlife could result in the introduction of new serovars to human and domesticated animals (Thayaparan *et al*., 2013). But, not all the serovars are unsafe to people. Leptospires can attack the body when in direct contact with leptospires through cuts and wounds. Moreover, it enters the human body through nasal, oral and conjunctiva mucosa if individuals are in contact with polluted water for a period of time (Lim *et al*., 2011). As rats are the significant hosts of these microbes, one of the progressions to control this infection is by controlling the rodent populace. In this way, honing great sanitation can control the rodent populace (Thayaparan *et al.*, 2013).

Pathogenic leptospiras confined from regular waters and wet soils in Malaysia contained 29 diverse serovars but all with the exception of two of the serovars had been discovered already in Malaysia. The extensive assortment of serovars found in wilderness territories was reliable with comparable past discoveries of assorted serovar contaminations in troops who had worked in Malaysian wildernesses (Alexander, Evans, Baker, Ellison, Marriapan, 1974). Flare-ups of Leptospirosis have been related with common water occasions, for example, rustic and urban flooding, swimming, and other water sports also included prevalently with cultivating and drinking debased water (Faine, Adler, Bolin, Perolat, 1999).

Since soil and water are easily polluted in some areas with urine, for example, rats, it is best to evade any immediate contact with this dirt and water. Introduction through water defiled by bacteria from tainted animals is the most widely recognized course of disease and rodents are animals that are perceived as the significant repositories in the transmission of Leptospirosis (Lim *et al*., 2011).

1.2 Problem statement

From the research, Kelantan had higher identification of leptospires most probably because of high occurrence of precipitation in the zone. The Northeast Monsoon as a rule conveys precipitation, especially toward the east drift conditions of Peninsular Malaysia. In this manner, overwhelming precipitation could encourage the spread of the living being and sully the earth. 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. This study of Leptospirosis will be done at Lata Janggut, Jeli Kelantan. A thorough and conscientious study about the ecological parameters of *Leptospira* sp. will be carried out as waterfalls become one of the potential breeding site for *Leptospira* sp. In this research, the effects of salinity on leptospiral survival in natural water can also be investigated (Saito, Miyahara, Villanueva, Aramaki, Ikejiri, Kaboyashi, 2014).

1.3 Objectives

The objectives of the study are as follow:

1. To investigate the physical and chemical parameters of *Leptospira* sp. in the study area.
2. To identify the presence of *Leptospira* sp. by morphological characterization in Lata Janggut, Kelantan.
3. To determine the water quality index and classification of Lata Janggut, Kelantan.

1.4 Significance of the study

The study was focusing on the ecological parameters of *Leptospira* sp. which cause its presence or absence in Lata Janggut, Kelantan. This study also play an important role to give the right information about the causes of Leptospirosis towards the publics especially at the waterfall ecosystem. Apart from that, this research investigate the presence of heavy metals contain which are harmful towards human and environment. Besides that, this research focus on the appropriate condition of physical parameters such as pH of water, temperature, salinity and turbidity which act as reservoirs that would sustain the presence of leptospires in the environment. Lata Janggut is recognized as the natural recreational area by the locals. From the observations, there are many locals from all levels of age 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 *Leptospira* in water samples especially in recreational area, may pose a health risk, especially to those who come into contact with contaminated water during spots activities (Ariyapruchya, Sungkanuparph, Dumrongkitchaipon, 2003). 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 Leptospires

Leptospirosis episodes have as of late been accounted for some creating nations including Malaysia. *Leptospira interrogans* is the primary causal living being of this irresistible illness and rats are accepted to be the significant supply for this microscopic organisms (Bahaman, Ibrahim, Adam, 1987). *Leptospira* sp. can survive in nature under specific conditions (Ridzlan, Bahaman, Khairani-Bejo, Mutalib, 2010).

2.2 Taxonomy and Classification

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

Table 2.1: The scientific classification of leptospires (Source: Paul, 2001)

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

2.2.1 Serological Classification

Serological and genotypic are two classification of *Leptospira* sp. Before 1989, the family *Leptospira* was separated into two species, *L. interrogans*, involving all pathogenic strains, and *L. biflexa*, containing the saprophytic strains secluded from the earth (Johnson & Faine, 1984). *L. biflexa* was separated from *L. interrogans* by the development of the previous at 13°C and development within the sight of 8-azaguanine (225 mg/mL) and by the failure of *L. biflexa* to form spherical cells in 1M NaCl Both L. interrogans and *L. biflexa* are isolated into various serovars characterized by agglutination after cross-retention with homologous antigen (Dikken & Kmety, 1978). Within the species *L. interrogans* over 200 serovars are recognized additional serovars have been isolated but have yet to be validly published. Serovars that are antigenically related have traditionally been grouped into serogroups (Kmety & Dikken, 1993).

2.2.2 Genotypic Classification

The phenotypic order of leptospires has been replaced by a genotypic one, in which a number of genomospecies incorporate all serovars of both *L. interrogans* also, *L. biflexa*. Hereditary heterogeneity was illustrated some time back (Brendle, Ragul, Elexander, 1974). Pathogenic and nonpathogenic serovars occur within the same species (Paul, 2001). Thus, neither serogroup nor serovar reliably predicts the species of *Leptospira*. Moreover, recent studies (Brenner, Kaufman, Sulzer, Steigerwalt, Rogers, Weyant, 1999) have included multiple strains of some serovars.

2.3 Biology Characteristics of Leptospires

Leptospires comprise of two unmistakable types of development which are translational and non-translational while it have a commonplace two fold layer structure in the same way as different spirochetes, in which the cytoplasmic film and peptidoglycan cell divider are nearly related and are overlain by an external layer (Paul, 2001).

Leptospiral lipopolysaccharide has a composition similar to that of other gram-negative bacteria, but has lower endotoxic activity. Leptospires may be stained using carbol fuchsin counterstain (Douadi, Pei, Siti, Fairuz, Kwai, 2013).

2.4 Morphological Identification of *Leptospira* sp.

The *Leptospira* sp. appear tightly coiled thin flexible Spirochetes 5 –15 microns long while the shape of *Leptospira* sp. is fine spiral of 0.1–0.2 microns (Srikanth, Samasekhar, Kanthi, Raghu Babu, 2013). *Leptospira* sp. could be look one end appears bent forms a hook (Figure 2.1). It is actively motile and could be seen best with dark field microscopy (Srikanth *et al.*, 2013). *Leptospira* sp. is a gram negative bacteria and it will show purple colour of staining under a microscope. Figure 2.2 show the surface of the *Leptospirosis Spirochete* at high magnification under the microscope.

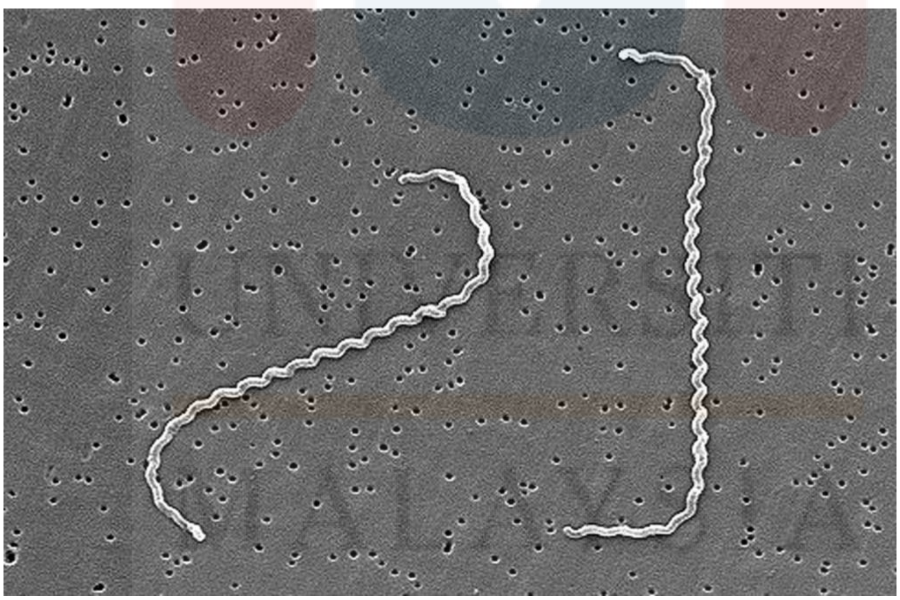


Figure 2.1: Characteristic hooked end with high resolution scanning electron micrograph of *Leptospira interrogans* serovar copenhageni (Source: Paul, 2001).

Figure 2.2 : The surface of the *Leptospirosis Spirochete* seems ruffled and beaded at high magnification (Source: Cameron, Zuerner, Raverty, Colegrove, Norman, Lambourn, Jeffries, Gulland, 2008).

2.5 Epidemiology of Leptospirosis

There are three epidemiological patterns of Leptospirosis (Paul, 2001). As a matter of first importance, it happens in mild atmospheres where few serovars are included and human disease perpetually happens by direct contact with tainted animals however cultivating of cows and pigs (Feresu, Bolin, Van de Kemp, Korver, 1999). Control by inoculation of animals and/or people is conceivable. Besides, it happens in tropical wet territories, inside of which there are numerous more serovars tainting people and animals and bigger quantities of store species, including rodents, ranch animals, and canines (Vinth, Adler, Faine, 1986). Control of rat populaces, waste of wet territories, and word related cleanliness are all essential for the prediction of the presence of Leptospirosis. The third example involves rat borne contamination in the urban environment.

2.5.1 Leptospirosis Outbreaks in Malaysia

The first case in Malaysia was reported by Fletcher in 1925. There has been a significant increase in recent years (Lim *et al.,* 2011). One of the cases in Malaysia is in Maran Pahang state during 2010. One hundred and fifty people were exposed to this outbreak, 85 (55.5%) were professional rescuers from various government agencies and 68 (44.5%) were villagers. 21 fulfilled the case definition. Ten cases were confirmed melioidosis, six melioidosis alone and four infected with Leptospirosis. There were eight deaths in this outbreaks, seven were villagers and one professional rescuer.

According to the online newspaper dated 21 May 2016, Saturday. A student, Muhd Syazwan Ismail, 21, from Mantin, Nilai, confirmed infected with Leptospirosis or rat urine while bathing in a recreation area Jeram Toi, Taipei and was died after receiving treatment as a result of prolonged fever.

2.5.2 Leptospirosis Outbreaks in Kelantan

Number of Leptospirosis cases by state in Malaysia 2013 and 2014 had shown that Kelantan was the second highest number of cases after Selangor in 2013 and 2014. However, the number of Leptospirosis cases by state in Malaysia 2014 and 2015 shows that Kelantan had the highest number of cases in 2015. This is possible due to the part of the contaminated area, not clean and the lack of monitoring and intensive care from any party in the certain area of Kelantan state. Such conditions will become contributors to the existence of *Leptospira* sp. immediately bring the disease known as Leptospirosis to the human. Leptospirosis has been nearly connected with rice development in different parts of the world. Field examines led in five Kelantan rice fields demonstrated that the paddy grower there have been tainted. Additionally, the *R. argentiventer* rats pervasive in such zones, have been found to discharge leptospires at a high rate into the rimfields (Paul, 2001). However, the water and soil tests gathered from the five Kelantan rice fields concentrated on were observed to be acidic, the mean pH of the water being 5.9 and of the dirt 5.2 amid the stormy season (Ridzlan, *et al.*, 2010). The pH of the dirt alone amid the dry season was 4.8 and the dirt sort was fundamentally mud which under research facility conditions adsorb leptospires and render them helpless (Ridzlan, *et al.*, 2010).

2.6 Mode of Transmission of Leptospirosis

Transmission of Leptospirosis was initially perceived as a word related in commercial enterprises identified with horticulture, sewer support, and tainted animals cultivation (Wynwood, Graham, Weier, Collet, McKay, Craig, 2014). There are three types of modes of transmission. Firstly, contact with urine or tissue of tainted animals through skin scraped spots, in place bodily fluid film. Also, contact which are broken skin with tainted soil, water or vegetation and ingestion of defiled nourishment and water. Thirdly, spread contamination. It is through spreading of urine. The animals that can spread the bacteria are rats, mice, wild rodents, mutts, swine, cows are guideline wellspring of contamination. The *Leptospira* survive and stay feasible for a few weeks in stagnant water. In addition, nourishment that is tainted by urine can likewise transmit the pathogenic leptospires to human (Lim *et al.*, 2011). Other regular methods of transmission incorporate introduction to urine contaminated water through recreational exercises, experience travel, and ingestion of polluted water supplies (Cacciapuoti, Ciceroni, Maffei, Stanislao, Calegari, Lupidi, 1987).

2.7 Ecology Parameters of Lata Janggut

The ecological parameters that cause the presence and absence of *Leptospira* serovars such as the rainfall distribution and humidity at the study area. Besides that, ecological variables have as often as possible been appeared to impact the improvement of Leptospirosis in animals and individual (Khairani-Bejo, Bahaman, Zamri-Saad, Mutalib, 2004). For example, pathogenic leptospires get by in clammy soil and new water for a period of time (Karaseva, Chermukha, Aiskunova, 1973). pH estimations of water and encompassing temperature will impact the survivability of the pathogenic leptospires which can prompt high frequency of Leptospirosis (Gordon & Tuner, 1961). The condition of pathogenic leptospires to survive outside the host requires a warm, moist domain and pH of water (Michna, 1970). In the other word, high precipitation, warm and wet atmosphere condition make Leptospirosis endemic in Malaysia (Khairani-Bejo *et al.*, 2004). Areas with high rainfall and warm climatic conditions provide optimal environments for the survival of leptospires (Wynwood *et al.*, 2014). In addition, the variables that appeared to influence Leptospirosis the study of disease transmission incorporate the stickiness, shaded range, direct sun region and turbidity. Poor hygienic condition at the recreational area was identified as a contribution factor for the occurrence of the outbreak (Weese, Peregrine, Armstrong, 2002). A few elements can impact the consistent presence of pathogenic leptospires as they cannot spread outside the host. Leptospires require crisp water to stay suitable in the environment and can get by for a while in running water yet just a few weeks in stagnant water, while some halophilic strains might be recouped from salty and salt water (Faine, 1999). *Leptospira*, as different spirochetes, is all around adjusted to thick situations (Petrino & Doetsch, 1978).

2.8 Recommended Guidelines for Lata Janggut Water Quality Analysis

For this research, the following standard guidelines (Table 2.2, 2.3, 2.4 and 2.5) already been used to evaluate and characterize the quality of water in freshwater river. The standards to be used as reference would be:

(a) Malaysia's Department of Environment (DOE) Water Classes And Uses

(b) Malaysia Department of Environment (DOE) Water Quality Index (WQI) Classification

(c) National Water Quality Standards of Malaysia (Physical and Chemical Parameters)

(d) National Water Quality Standards of Malaysia (Heavy Metals Parameters)

Table 2.2: Malaysia's Department of Environment (DOE) Water Classes and Uses (Source: Malaysia Environment Quality report, 2010)

Class	Uses
I	Conservation of natural environment Water supply I – Practically no treatment necessary Fishery I – Very sensitive aquatic species
IIA	Water supply II – Conventional treatment required Fishery II – Sensitive aquatic species
IIB	Recreational use with body contact
III	Water supply III – Extensive treatment required Fishery III – Common of economic value and tolerant species; livestock drinking
IV	Irrigation
V	None of the above

Table 2.3: Malaysia Department of Environment (DOE) Water Quality Index Classification

(Source: Malaysia Environment Quality report, 2010)

Sub Index And Water Quality Index	**Index Range**		
	Clean	Slightly Polluted	Polluted
Biochemical Oxygen Demand (BOD)	91-100	80-90	0-79
Ammoniacal Nitrogen (NH_3N)	92-100	71-91	0-70
Suspended Solid (SS)	76-100	70-75	0-69
Water Quality Index (WQI)	81-100	60-80	0-59

Table 2.4: National Water Quality Standards of Malaysia (Physical and Chemical Parameters)

(Source: Malaysia Environment Quality report, 2010)

Parameter	Unit	Class					
		I	IIA	IIB	III	IV	V
Ammoniacal Nitrogen	mg/L	0.1	0.3	0.3	0.9	2.7	>2.7
Biochemical Oxygen Demand (BOD)	mg/L	1	3	3	6	12	>12
Chemical Oxygen Demand	mg/L	10	25	25	50	100	>100
Dissolved Oxygen	mg/L	7	5-7	5-7	3-5	<3	<1
pH		6.5-8.5	6-9	6-9	5-9	5-9	-
Electrical conductivity	μS/cm	1000	1000	-	-	6000	-
Salinity	%	0.5	1	-	-	2	-
Total Suspended Solid	mg/L	25	50	50	150	300	300
Temperature	^{o}C	-	Normal + 2^{o}C	-	Normal +2^{o}C	-	-
Total Dissolved Solid	mg/L	500	1000	-	-	4000	-
Turbidity	NTU	5	50	50	-	-	-
Water Quality Index (WQI)		>92.7	76.5-92.7	76.5-92.7	51.9-76.5	31.0-51.9	<31.0

Table 2.5: National Water Quality Standards for Malaysia (Heavy metals) (Source: Malaysia Environment Quality report, 2010)

Parameter	Unit	Class					
		I	IIA	IIB	III#	IV	V
Ca	mg/L	N	-	-	-	-	
Mg	mg/L	A	-	-	-	-	L
Hg	mg/L	T	0.001	0.001	0.004(0.0001)	0.002	E
As	mg/L	U	0.05	0.05	0.4(0.05)	0.1	V
Na	mg/L	R	-	-	-	3SAR	E
Pb	mg/L	A					L
K	mg/L	L	-	-	-	-	S
Mn	mg/L		0.1	0.1	0.1	0.2	
Zn	mg/L	O	5	5	0.4*	2	A
Cu	mg/L	R	0.02	0.02	-	-	B
Cr (III)	mg/L		-	-	2.5	-	O
Cr (IV)	mg/L		0.05	0.05	1.4(0.05)	0.1	V
Al	mg/L	A	-	-	(0.06)	0.5	E
Ni	mg/L	B	0.05	0.05	0.9*	0.2	
Cd	mg/L	S	0.01	0.01	0.01*(0.001)	0.01	
Ag	mg/L	E	0.05	0.05	0.0002	-	IV
Fe	mg/L	N T	1	1	1	1(leaf)5(others)	

Notes:

* = At hardness 50 mg/L $CaCO_3$

= Maximum (unbracked) and 24-hour average (bracketed) concentrations

N = Freedom visible film sheen, discolouration and deposits

2.9 Lata Janggut as an Ecotourism Place

The proposed study area is in the area of Lata Janggut, Jeli. Jeli is located at the eastern part of Kelantan. Jeli is located within central belt in map of Peninsular Malaysia. Jeli 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. Jeli is one of few district in Kelantan that due to its ecological superior status enjoys a wide variety of scenic beauty and there in Lata Janggut can be considered as the place that have a potential of ecotourism. This study is mainly focusing on the ecotourism potential of Lata Janggut, Jeli. The areas of Sungai Long and Lata Janggut have high value of of recreational. This is due to suitable site for recreational activities such as picnics and jungle trekking. Lata janggut becomes the attractive place to the visitor to go there.

The people around the Jeli area like to go to Lata Janggut to spend their time. Visitors like to take bath and swim (Figure 2.3). During the jungle trekking, the visitors can take a fresh air and enjoy the scenery.

Lata Janggut is a part of Long River. These cascade is very attractive to tourism and suitable for the recreation. So, the uniqueness and significance of the place should be enjoyed by the next generation. Long River flows through Lata Janggut village. The direction in which a river travels is determined by topography, which in turn is controlled by composition, configuration and distribution of the underlaying rock. The river is suitable for recreation. In addition, the formulation of the Long River can be reveals. The river is quite shallow. So the place is suitable for the children to take swim here.

The common magnificence of greenery has pulled in neighborhood and remote voyagers to appreciate the nature, otherwise called Eco-tourism. Lata Janggut, which is as high as 3 levels, was created by Head of Jeli locale in a joint effort with the Ministry of Tourism and the South Kelantan Development Authority (KESEDAR). Ecotourism is a sustainable tourism activity and has contributed more economically than other tourism to local people who are taken the place in tourism.

Figure 2.3: The view of Lata Janggut taken on 24th of July 2016.

CHAPTER 3

MATERIALS AND METHODS

3.1 Study area

The research was conducted at Lata Janggut in Jeli, Kelantan. In this research, Lata Janggut is selected as a study area for ecotourism because it is to investigate whether the place has ecotourism potential or otherwise. The coordinate of study area is between N5’40’0” to N 5’42’30” and E 101’44’00” to E 101’47’00”. The distance located 12 kilometres southwest of Jeli and approximately seven kilometres from the Jeli town. While from UMK Jeli Campus, it is about 10-15 km. Figure 3.1, 3.2 and 3.3 show the location of Lata Janggut in Jeli Kelantan.

Within this area, there was protected forest named Hutan Rizab Gunung Basor. The study area consists of two main rivers in Kelantan, Sungai Pergau and Sungai Long. Based on the map, the high peak of the study area is 720 m above the sea level meanwhile the lowest is 80 m above the sea level.

All the tools and materials is brought from the laboratory. The Global Positioning System (GPS) tools was used to get the coordinate of the location for the sample to be taken. The water sample has been taken randomly at of Lata Janggut according to the ecological parameters.

Figure 3.1.: Location of Kelantan state in Peninsular Malaysia

Figure 3.2: Location of Lata Janggut Village in Jeli District

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

Figure 3.3: Lata Janggut (research area) taken on 24th of July 2016.

3.2 Sample Collection of Physical and Chemical Parameters

The water samples were collected according to each parameter sampling procedures. The sampling bottles being used are from plastic and glass. The water sample has been added a respective preservation chemical in order to preserve the content of the sample matrix and to lengthen the holding time.

3.2.1 Collection of Water Sample

The sampling activities was carried out starting from July until September. Samples of water were collected at two areas in Lata Janggut and the current condition during the sampling day such as hygenic condition, rainfall availability, humidity and temperature.

The water sample were collected randomly in surrounding the river in which in a river, two checkpoints which are direct sun area and shaded sun area has been choose in 3 times water sample that taken in a week. Two different type of bottles for Biochemical Oxygen Demand analysis and water quality parameters analysis is prepared. The bottles were immersed below the water surface and filled to capacity to ensure no air bubbles, brought out of the water and properly closed.

Preservation chemicals were added into the samples respected to the parameters that required it. During transportation, all of the sampling bottles then were kept into an icebox which filled in with ice in order to give cold temperature to the samples. The samples is brought to laboratory and kept in chiller to 4^{o}C.

The ecological water tests are acquired from the Lata Janggut. A measure of water test were selected at every inspecting site as per every parameter examining strategies and safeguarding. The inspecting bottles that were utilized are non-straightforward container for biochemical oxygen demand (BOD) test, while the others will utilize the polyethylene or glass bottle. While the return trip, all the example containers were kept into the refrigerator that loaded with ice so that the temperature keep at 4°C and beneath.

The water sample has been taken just below the surface for the detection of the *Leptospira* at six points. The points were selected with the area that have high probability of the presence of the *Leptospira* sp. at the stagnant water with a shaded regions and direct sun areas. For the ecological parameters analysis, the sample were taken also at the six point with different areas. The mark is prepared and placed at each of the point so that, the sample were selected at the same place along the three times sampling in a week. Apart from that, it is to avoid any biases while also assuring that entire site is appropriately represented.

3.3 Morphological Identification and Determination

For the Leptospirosis identification, the experiment was conducted by using morphological characteristics to determine the presence of *Leptospira* sp.

3.3.1 Media culture preparation

Nutrient agar was used for bacteria culture. Nutrient agar powder about 23 grams were added in conical flask with 1000 mL distilled water. The stock was sterilized by autoclaving at 121°C for 15 minutes. The sterile agar was added into a series of Petri dishes when it is ready. The plates were allowed to cool and were 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 was about 10 minutes and the slant nutrient agar was stored in the chiller.

3.3.2 Serial dilution and bacteria isolation

For bacteria isolation, 1 mL water sample was pipetted and added with 9 mL of distilled water. Both of the liquid then were diluted onto serial dilution of 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}. Next, 2 µL of water sample was pipetted and spread plate onto the nutrient agar. The sample were incubated in room temperature (37°C) for 24-48 hours in the incubator. Each bacteria colony were isolated to get pure culture, and has been kept in the chiller.

3.3.3 Microscopic Techniques

3.3.3 (a) Slide preparation and observation

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

3.3.3 (b) Simple Stain

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

3.3.3 (c) Gram Stain

The smears was stain with Crystal Violet for one minute. After that, the smears was stain with Gram's Iodine for one minute. The smears was rinsed carefully with acetone. It is rinsed only until blue colour stops coming out of the smear. Next, the smear has been counter-stain with Safranin for at least 10 seconds. For the precaution step, the slide must be completely dry before putting it on microscope. The low power (10X) lens was used to find a good field. A drop of oil was added and the 100X oil immersion lens was immersed into the oil by rotating the nosepiece. The fine adjustment is used to bring the image into clear focus.

3.4 Physical parameters analysis

At the same time, *in-situ* experiment was also carried out, to analyze the physical parameters at the study area by using YSI 556 MPS (Multiprobe System). Physical parameters including the temperature, pH, conductivity, salinity, Total Dissolved Solid and Dissolved Oxygen (DO) were measured by using HACH DR 900 Calorimeter. A total water samples were taken for this experiment to get the result of the presence of pathogenic leptospires. The ecological parameter has been considered as reservoirs that sustain the presence of leptospires in the environment. For the result analysis, the physical parameters was linked with their related to the presence of *Leptospira* sp. in Lata Janggut.

3.5 Chemical Parameters Analysis

In this analysis, the collected water sample were analyzed for the chemical parameters which are biochemical oxygen demand (BOD), chemical oxygen demand (COD), ammoniacal nitrogen (NH_3N) and total suspended solid (TSS). The collected water samples were undergo several analytical procedures, by using APHA method spectrophotometer (DRB 2800) and DO probe meter (HQ40d). The water quality at Lata Janggut has been analyzed from the chemical parameters above. Below are the methods that were used to test all the parameters selected in the research. Chemical parameters analysis were undergo sample duplication to get an accurate value.

3.5.1 Biochemical Oxygen Demand (BOD)

The water samples were collected in plastic bottle. The bottle was filled in to exclude air means that to prevent bubbles from forming. As the sample may degraded during storage, thus the reduction could be minimize through 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 need to be warm chill to 20-27 ± 3°C.

The method used in texting the BOD was Dilution Method (Method 8043), adapted from Standard Methods for the Examination of Water and Wastewater (Klein, R.L; Gibbs, C. Journal of Water Pollution Control Federation,1979, 51(9), 2257). First of all, sample dilution water was prepared using a BOD Nutrient Buffer Pillow. A serological pipette is used to measure a graduated series of five portions of well-mixed sample and transfer to separate, glass-stoppered, 300 mL BOD bottles. After that, the bottle was stopper without any trap of air bubbles then invert several times to mix. Then, the bottle was filled in dilution water to just below the lip. For blank preparation, the BOD bottle was filled in with dilution water only. The initial dissolved oxygen values was determined using probe meter (HQ40d). An enough dilution water is added to the lip of the BOD bottle to make a water seal. A plastic overcap is placed over the lip of each bottle and bottle is placed in an incubator at 21^{O}C. The bottle is incubated in the dark for five days. When the five days incubation period is complete, the dissolved oxygen content is determined in each bottle. The results is in (mg/L DO remaining).

After five days, the remaining DO concentration in each bottle is measured. The BOD value then has been calculate by using calculation method:

BOD_5, mg/L = (DO_1 – DO_2) X DF Equation (1)

Where:

BOD_5 is BOD value from the 5-day test;

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

DO_5 is DO of diluted sample after 5 day incubation at 20^{o}C, in mg/L;

DF is bottle volume (300mL) / Sample volume

3.5.2 Chemical Oxygen Demand (COD)

The sample was collected with glass bottles. The method that is used in testing COD is Reactor Digestion Method (Method 8000) (Jirka & Carter, 1975). First of all, the reactor is turned on and preheat to 150 ^{O}C. The cap of a COD Digestion Reagent Vial is removed for the appropriate range. After that, the 2.0 mL of sample is carefully pipetted into the vial. Then, the vial is placed in the preheated DRB200 Reactor. The protective lid is closed. After that, the reactor is turned off and wait until the vial to cool to 120^{o} C or less for about 20 minutes. The vial is inverted in several times while still hot. The vial is placed into a rack to cool to room temperature. Then, a calorimetric determination was used to measure the COD mg/L.

3.5.3 Ammoniacal Nitrogen

The sample was collected by using polyethylene bottle. The method that is used in testing the ammoniacal nitrogen is Salicylate Method (Method 8155) that adapted from Clin. Chim. Acta, 14, 403 (1996) for water, wastewater and seawater.

Firstly, for the sample preparation, a square sample cell is filled to the 10mL mark with sample. The contents is added of one to each cell. The contents is closed and shaken well to dissolve the reagent. By using an instrument timer, a three-minute reaction period will begin. When the timer expires, the contents will be added with one Ammonia Cyanurate Reagent Powder Pillow to each cell. The contents is closed and shaken well to dissolve the reagent. A 15-minute reaction period is begin until a green colour develop if ammonia-nitrogen is present. The prepare sample is inserted into the cell holder and the result will show in mg/L HH_3N.

3.5.4 Total Suspended Solid (TSS)

The samples were collected by using polyethylene bottle. The container is filled to exclude the air. The method that is used is Photometric Method (8006) that is adapted from Sewage and Industrial Wastes (1959). First of all, 10 mL of water sample is poured into a sample cell. The gas bubbles is removed in the water by swirling or tapping the bottom of the cell on a table. The blank is inserted into the cell holder with the fill line facing right. The prepared sample is swirled to remove any gas bubbles and uniformly suspend any residue. The results will show in mg/L TSS.

The TSS value then was calculated by using the formula below:

$$\text{TSS mg/L} = \frac{(W_2 - W_1) \times 1000}{\text{mL sample}} \qquad \text{Equation (2)}$$

3.5.5 Heavy Metal Analysis of Water Sample

The collected water samples is analyzed by using Atomic Absorption Spectrophotometer (Pin AAcle 900F) (AAS). AA Spectrophotometer is to analyse the elements of heavy metals in water samples at Lata Janggut. The methods that were used is the method for analysis natural waters. Sixteen heavy metals has been analyzed at the initial characterization are Calcium (Ca), Magnesium (Mg), Mercury (Hg), Arsenic (As), Sodium (Na), Lead (Pb), Potassium (K), Manganese (Mn), Zinc (Zn), Copper (Cu), Chromium (Cr), Aluminium (Al), Nickel (Ni), Iron (Fe), and Cadmium (Cd) and Silver (Fe). This method is preserved with an amount of 50 % of Nitric acid (HNO_3) which 2 mL of Nitric acid is placed into 500 mL of water sample bottle. After that, the water sample is filtered through syringe filter of a 0.45 micron micropore membrane into the 50 mL of the falcon tube. The filtered water sample is transfered into 15 mL of falcon tube. Then, 1 mL of water sample is transfered into the other falcon tube to undergo a serial dilution of 10^{-1}, 10^{-2}, 10^{-3} and 10^{-4}. While for the procedure of sample preparation for Mercury and Arsenic, each was undergo dilution of 10^{-1}. For Mercury, the water sample is mixed with 1 mL of 5 % of Ascorbic acid and 5 % Potassium Iodide (KI) solutions while dilution process. For the arsenic, the water sample is mixed with concentrated Hydrochloric Acid (HCl) and 5 % of Potassium Permanganate ($KMNO_4$) solutions. Then, the water sample has been run by AAS and the result was recorded.

3.6 Ecological Parameters Analysis

The data was taken three times a week according to weather conditions starting from July until September. All the gathered data was merged as the ecological parameters at the Lata Janggut was divided into two categories which are physical parameter analysis and chemical parameters analysis. Physical parameters has been analyzed according to primary data that conducted at the site as known as *in-situ* and further experiment will be conducted in laboratory. From the morphological identification analysis of *Leptospira* sp. at two different sites has been discussed along with all the obtained ecological parameters to identify the suitable conditions of the presence of Leptospirosis causing bacteria at the Lata Janggut.

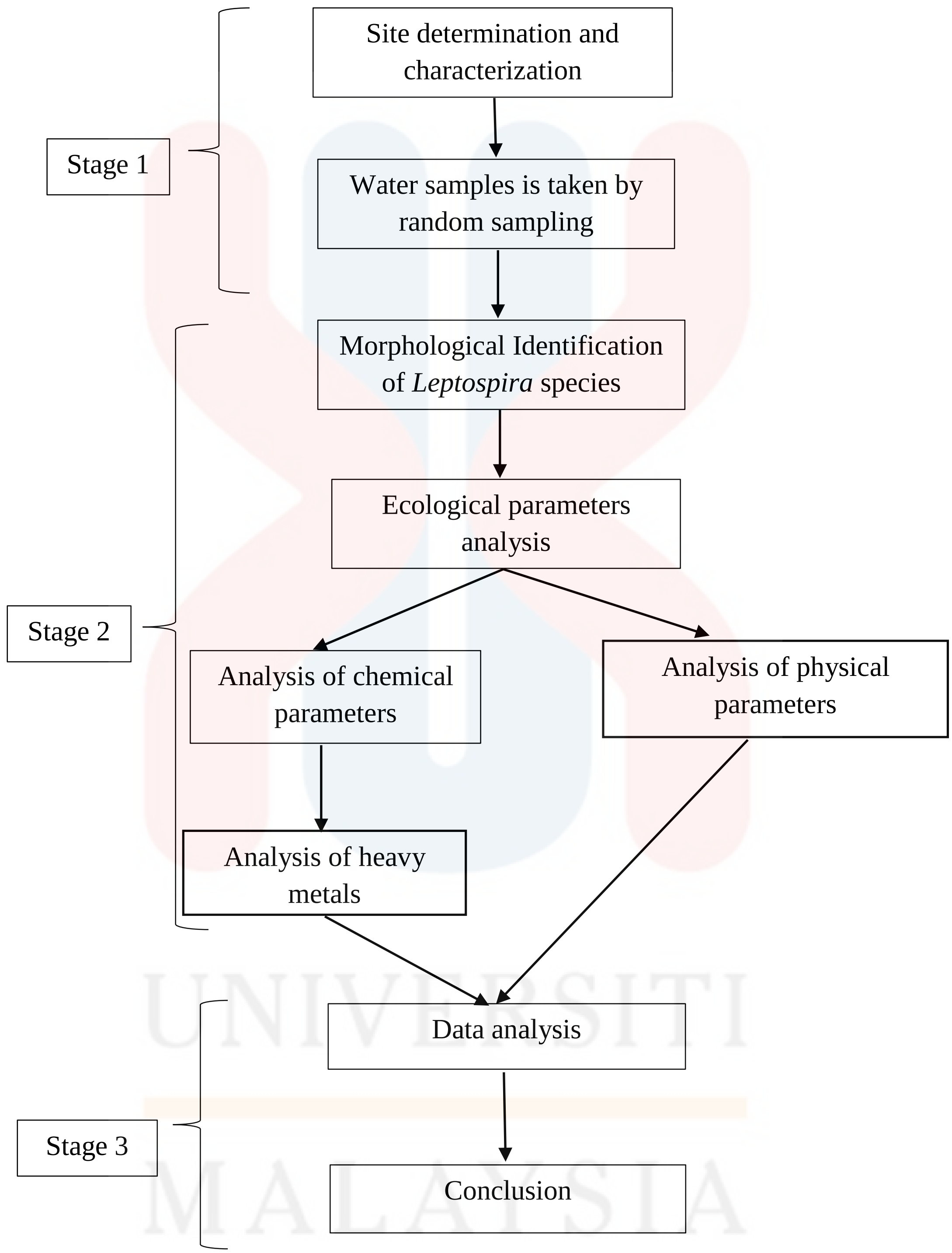


Figure 3.4: Research flow chart

Stage 1: Apparatus preparation, *in-situ* experiment, dividing the samples

Stage 2: Further experiments in the laboratory and ecological parameters study

Stage 3: Analyse all the gathered data and report writing

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Outline of Chapter Four

This chapter represents the results of gram staining procedure to identify the existence of *Leptospira* sp. based on the morphological identification at the study area. The result and the comparison with morphological characteristics of *Leptospira* sp. were shown in Table 4.9. The water quality parameters obtained from water samples collected from Lata Janggut are also being interpreted.

4.2 Physico-chemical Properties for Water Samples

The variety of physical and chemical properties of water samples which are pH, temperature, conductivity, DO, salinity, total dissolved solid were measured as *in-situ* parameters, while BOD, COD, NH_3N, TSS and heavy metals were measured as *ex-situ* parameters. The summary of the physical and chemical properties were showed in Figure 4.1, 4.2, 4.3 and 4.4, respectively.

4.2.1 The Physical Properties of Water Samples

The concentration of pH at direct sun area for six continuous weeks were ranged between from 7.31 - 7.97 with a mean concentration of 7.43 ± 0.09. The concentration of pH at each individual week were 7.63, 7.62, 7.61, 7.61, 7.57 and 6.55, respectively. From this observation, the pattern of pH from direct sun area was decrease from the first week until the last week (Figure 4.1) as the mean concentration of pH was at 7.06 ± 0.02 that range from 6.42 - 7.98 However, unlike at the direct sun area, the pH at shaded sun area slightly lower readings, which were 7.95, 7.77, 6.95, 6.82, 6.46 and 6.43, respectively.

The mean temperature of water at direct sun area of Lata Janggut for the present study was 28.28 ± 0.15. That were range from 25.65 - 29.78 ^{o}C. From the observation, the temperature reading from the first week was the lowest while the fourth and fifth week were the highest temperature. Meanwhile for the shaded sun area in the graph (Figure 4.1), it showed that the pattern was increasing until the fifth week, the temperature range from 25.11 - 26.05 ^{o}C which made the mean to become 25.83 ± 0.03. From the continuous weeks of study period. The graph indicated that the pattern was decreasing until fourth week and increase until sixth week. Based on the result, it can be interpret that the intensity of sunlight radiation and evaporation had influenced surface water temperature.

As for dissolved oxygen (DO), its concentration of direct sun area were ranged from 7.80 - 9.09 mg/L with a mean 7.48 ± 0.01 mg/L for the study period. From the recorded readings, it was noticed that the DO reading for the fourth week is the highest with 9.04 mg/L. There was huge gap and variation that could be seen among the other DO values within the six weeks. However, the mean concentration of DO values was just intermediate, neither not too low nor too high. While for the shaded sun area, it was recorded that the mean concentration of DO was lower than the direct sun area, with 7.48 ± 0.01 mg/L within the study period. The range of DO value for shaded sun area is which 3.00 - 9.52 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, it can be seen that the pattern for the shaded sun area is more fluctuated than direct sun area

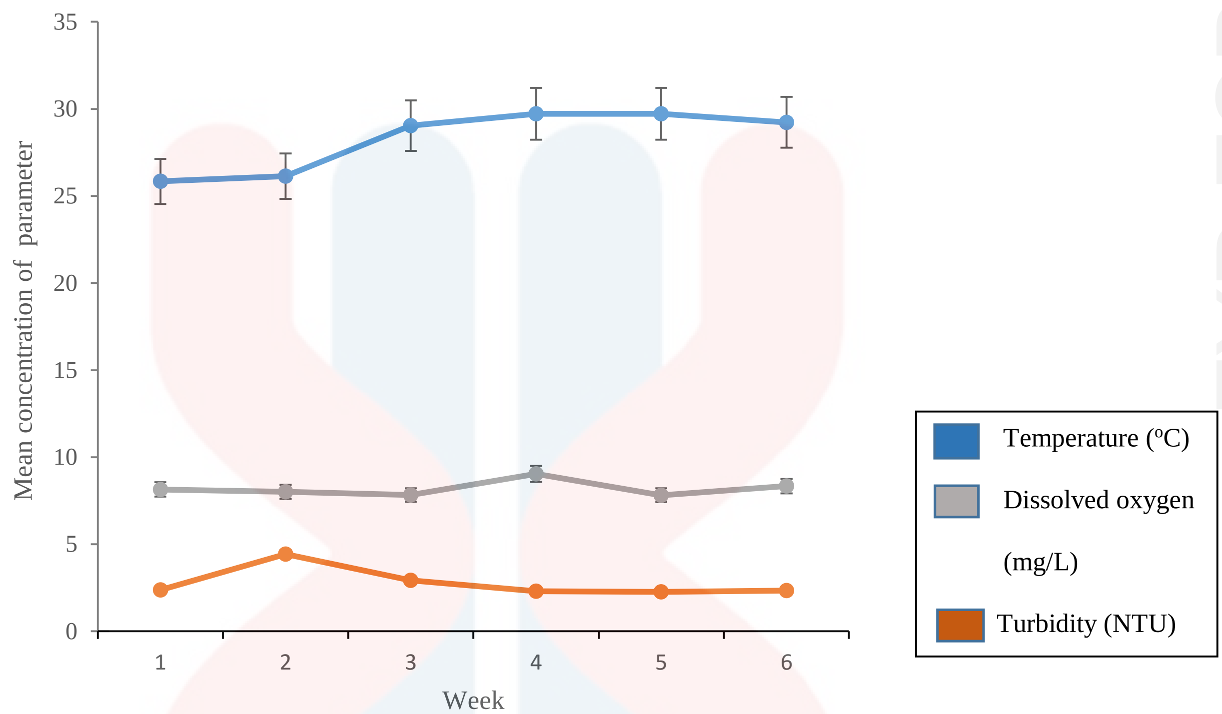


Figure 4.1: The trends for turbidity, temperature and dissolved oxygen for direct sun area.

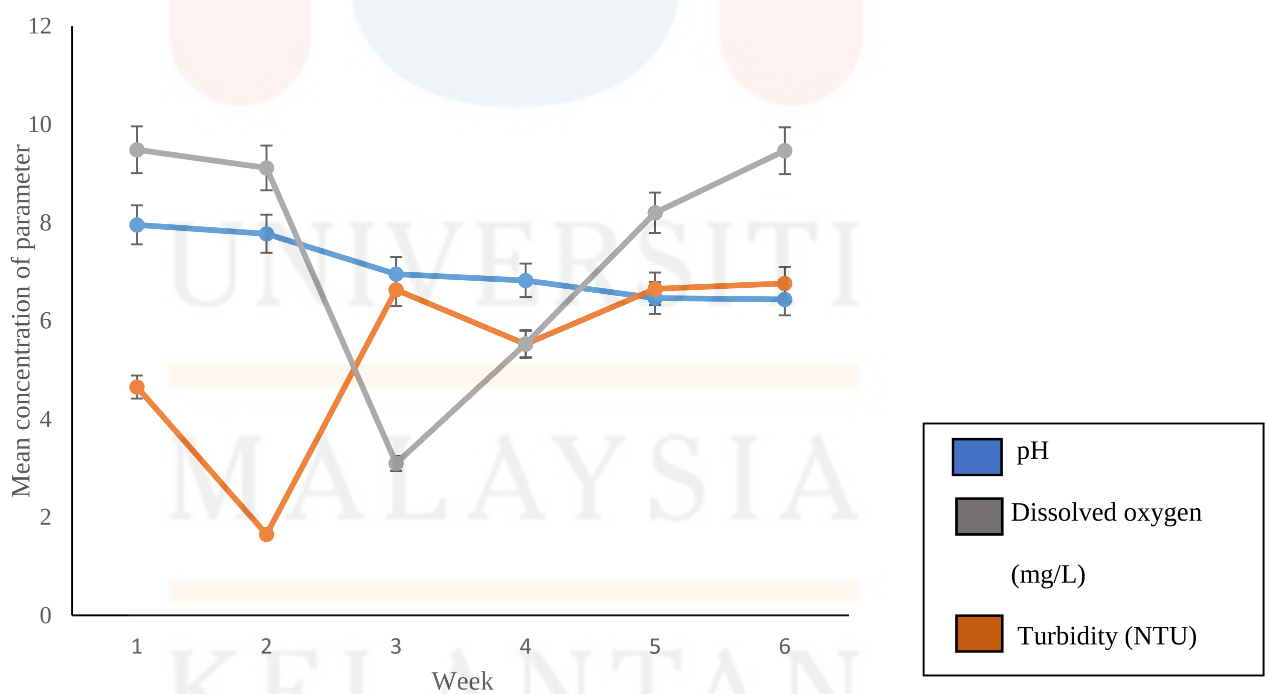


Figure 4.2: The trends for turbidity, pH and dissolved oxygen for shaded sun area.

The next physical parameters are for turbidity and total dissolved solid. At shaded sun area, it was found that 2.77 ± 0.03 NTU as the mean concentration of turbidity and 0.03 ± 0.11 g/L as the mean concentration of total dissolved solid. It was recorded that the lowest and the highest turbidity was from second week with 1.65 NTU and from sixth week with 6.76 NTU, respectively. While for total dissolved solid, the range varied from 0.02 – 0.04 g/L, with the lowest and the highest values came from first and third week as well. From the results, it is observed that the turbidity shows the trend of fluctuated with the trend decreasing during the second week then the value increasing until sixth week.

While for direct sun area, the mean concentration of turbidity was found at 2.77 ± 0.03 NTU that range from 2.26 – 4.43 NTU from the six consecutive weeks of study period. At the same time, the mean concentration for total dissolved solid was recorded at 0.03 ± 0.001 g/L with range 0.02 – 0.03 g/L within the study period. It can be noticed that both parameters have the different trends which, the value keep decreasing and increase back at different week.

4.2.2 The Chemical Properties of Water Samples

The concentration of BOD were ranged from 1.2 – 25.5 mg/L with a mean concentration of 11.77 ± 6.29 mg/L, was found at the direct sun area of Lata Janggut (Table 4.3). The variation of BOD concentration among the study period were relatively high compared to the National Water Quality Standards of Malaysia. While at the shaded sun area, the mean concentration was found as 16.81 ± 8.85 mg/L which was slightly higher than direct sun area. Among the six consecutive weeks, the BOD value range from 0.90 – 29.60 mg/L, which the first week had the lowest and highest BOD concentration was at the fourth week. Both of the areas shared the same trend of BOD concentrations as it were observed that the value increase from the first week until the fourth week then it decrease until the sixth week.

Next, the values of TSS for direct sun area were ranged widely from 2.00 – 28.30 mg/L with the mean concentration of TSS 0.18 ± 0.006 mg/L. The highest TSS value was found both at the third week and fourth week which shared the same value (28.30 mg/L), followed by the first week (16.00 mg/L), the fifth week (15.00 mg/L) and the sixth week (9.33 mg/L) and the second week (2.00 mg/L). As for shaded sun area, 16.16 ± 3.47 mg/L was found as the mean concentration of TSS value that range from 2.00 – 32.30 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.

While it is noticed that both value of mean concentration of COD for direct sun and shaded sun area were relatively low with 14.20 ± 2.01 mg/L and 13.91 ± 0.42 mg/L, respectively. The same trend was also found at both area as the value keep increasing from first week until fifth week, but decreasing again on the sixth week.

Generally, COD concentration is higher than BOD concentration where it was shown from the direct sun area result. However, it 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. Based on the figure 4.3, there were four weeks that represents COD concentration is lower than BOD concentration in shaded sun area. This is probably because of the presence of microbiologically oxidisable chemicals such as ammonium that could boost BOD readings. Thus, ammonia being a source of nitrogen is also a nutrient for algae and other forms of plant life and thus contribute to overloading of natural systems and cause pollution. Futhermore, high presence of microbiologically oxidisable chemicals such also could increase the BOD readings.

As for Ammoniacal Nitrogen, COD and NH_3N are closely related because the sources of both target parameter was organic matter. In fact, COD measure the amount of organic compound in water, while ammonia was produced by bacteria of the decomposed dead plant and animal matter. Based on the result, the mean concentration of NH_3N is lower than COD in both direct and shaded sun area. Ammonia is rapidly oxidized by certain bacteria in natural water systems to nitrite and nitrate where a process that requires the presence of dissolved oxygen.

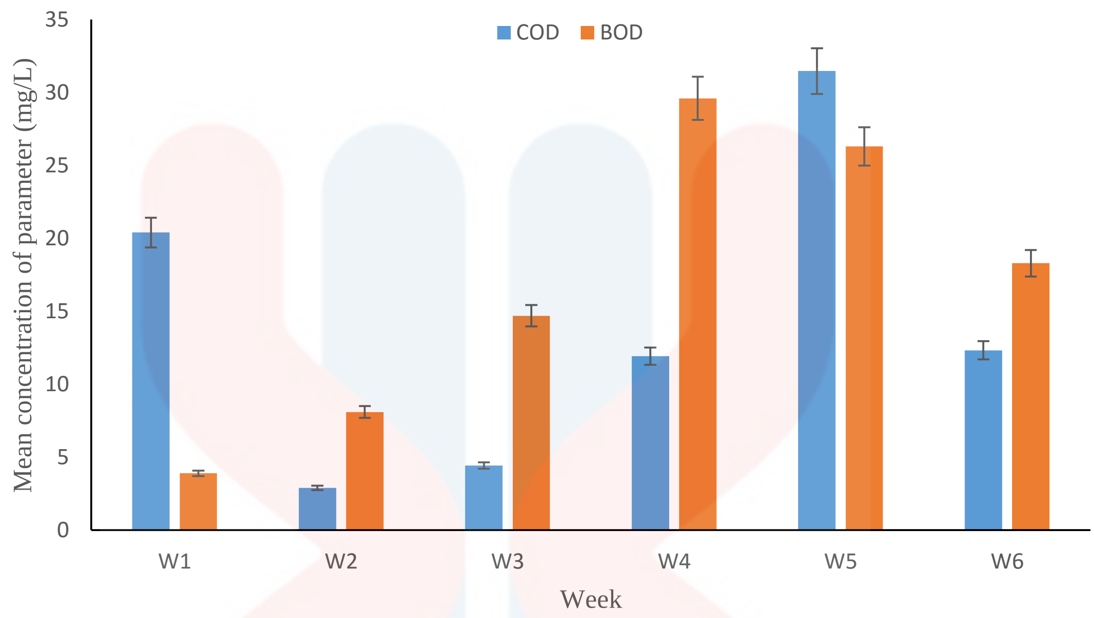


Figure 4.3: The comparison of Chemical Oxygen Demand and Biochemical Oxygen Demand values in shaded sun area of Lata Janggut

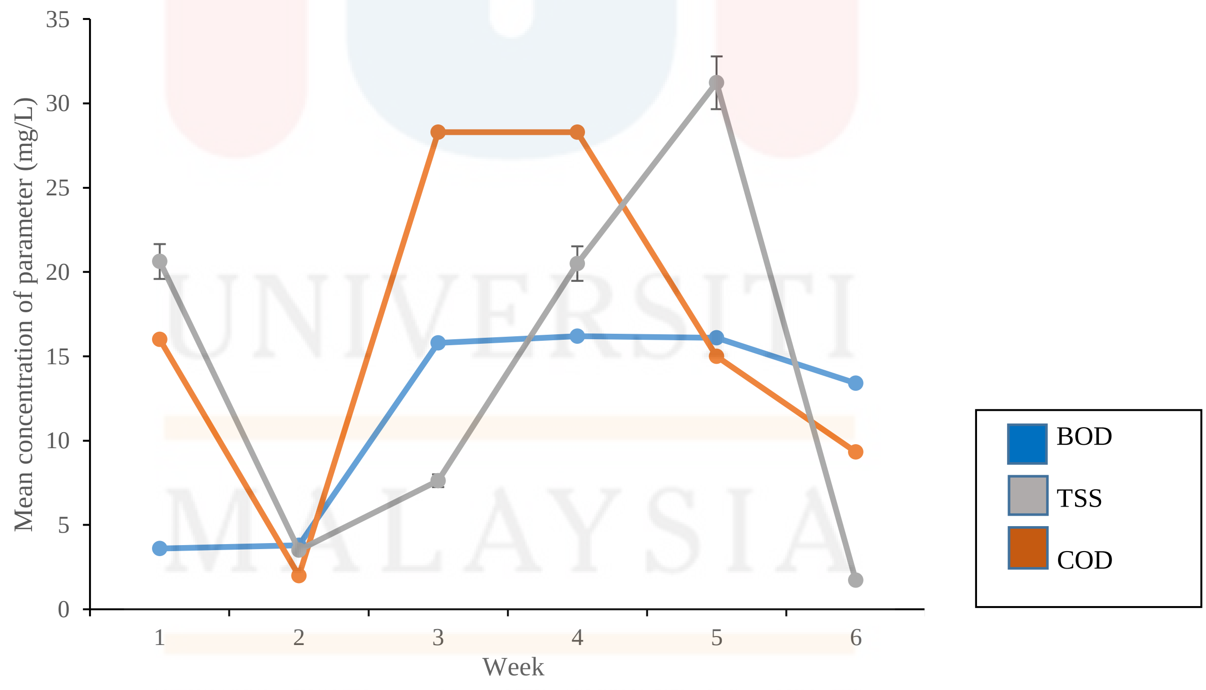


Figure 4.4: The trends for biochemical oxygen demand, total suspended solid and chemical oxygen demand for direct sun area.

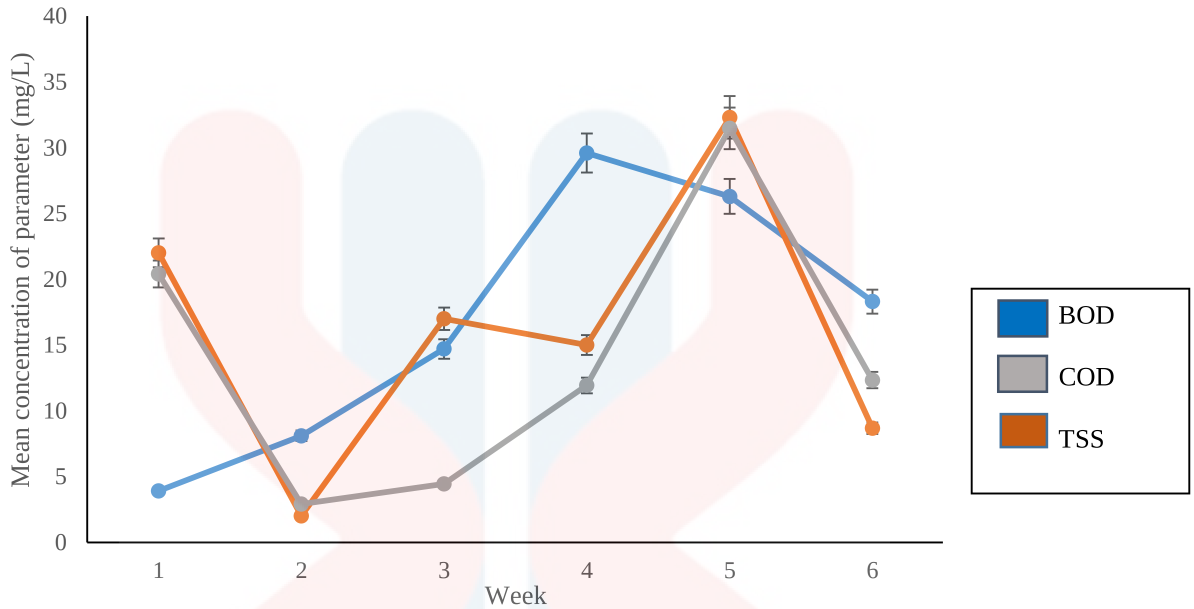


Figure 4.5: The trends for biochemical oxygen demand total suspended solid, and chemical oxygen demand for shaded sun area

4.2.3 The Heavy Metal Properties of Water Samples

The presence of heavy metal in the study area were also being taken into consideration to determine the water characteristics at Lata Janggut. Based on the AAS analysis, it was found out that among all the 16 elements tested, there were only two element of heavy metals that could not be detected, which were Cd and Ni. While the other 14 elements managed to be detected even though in the small concentrations.

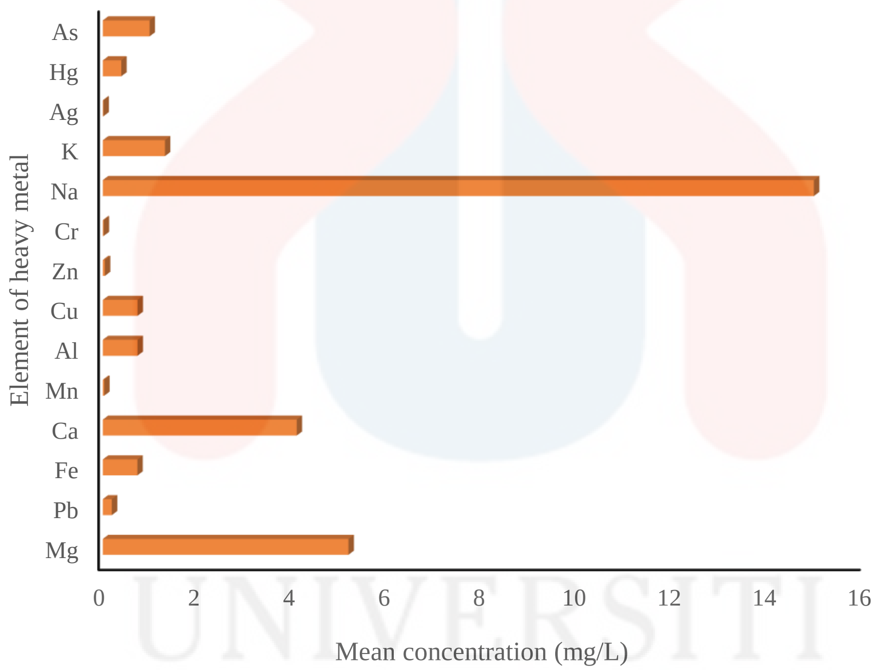


Figure 4.6: The concentration value of heavy metals initial characterization at Lata Janggut

Generally, leptospires are very sensitive towards the presence of heavy metal even in the smallest concentration. It is to be noted that only two elements of heavy metals does not exist which are Cadmium and Nickel (Table 4.6). The highest concentration of heavy metal obtained at Lata Janggut is Sodium with 14.94 mg/L followed by Magnesium (5.15

mg/L) and Calcium (4.68 mg/L). At the same time, Silver was recorded at 0.01 mg/L, which showed the lowest concentration of heavy metal.

4.3 Correlation Analysis among the Physical and Chemical Parameters

Coefficient of correlation was worked out to understand the relationship between the parameters of water samples (Table 4.7 and 4.8). By using Pearson's correlation method, analysis among the physical and chemical parameters at the direct and shaded sun area revealed that there were significant positive and negative relationship between them.

Based on at the direct sun area, for pH, the weak positive correlation relationship were found between pH:TDS (r = 0.101). Meanwhile, weak negative relationships were found both at pH:conductivity (r = -0.236) and pH:temperature (r = -0.195). The other moderate positive relationships that could be found were through turbidity:TDS (r = 0.691) and turbidity:temperature (r = 0.558). All the correlations mentioned were significant at the 99% and 95% confident level.

Meanwhile for the correlation between physical parameters at the shaded sun area, there were different trend of relationships that could be indicated for pH, where the strongest positive and negative relationships were found between salinity:TDS (r = 0.843) and turbidity:pH (r = 0.793). Meanwhile, positive moderate relationships were found both at pH:salinity (r = 0.676) and turbidity:TDS (r = 0.423). The other strong negative relationships that could be found were through TDS: DO (r = -0.781) and the other moderate positive values are temperature:conductivity (r = 0.403). All the correlations mentioned were significant at the 99% and 95% confident level.

Next, the correlation analysis between the chemical parameters at the direct and shaded sun area were analysed. As for the direct sun area, the moderate negative relationship was found between NH_3N: COD (r = -0.666) and NH_3N: BOD (r = -0.426) Meanwhile at the shaded sun area, it was indicated that the correlation between TSS:COD had the strong positive relationship with (r = 0.785). While the correlation between COD and NH_3N was considered as moderate positive relationship at (r = 0.412). All the correlations were analysed significant at 99% and 95% confident level.

Table 4.1: Correlation Analysis for Physical Parameters

Area	Parameter	pH	Temperature	turbidity	TDS	DO	Salinity	Conductivity
Direct Sun Area	pH	1	-.195	.101	.101	.017	-	-.236
	Temperature	-.195	1	-.558	.139	.257	-	-.179
	Turbidity	.101	-.558*	1	-.691**	-.319	-	-.092
	TDS	110	.139	-.691**	1	286	-	-.250
	DO	.017	.257	-.319	286	1	-	.393
	Salinity	-	-	-	-	-	-	-
	Conductivity	-.236	-.179	-.092	-.250	-.393	0	1
Shaded Sun Area	pH	1	.308	-.793**	-.478*	262	.-.516	-.229
	Temperature	.308	1	-.238	-.032	-.415	-.110	.403
	Turbidity	-.793**	-.238	1	.423	-.375	.272**	.168
	TDS	-.478*	-.032	.423	1	-.781**	.843*	.034
	DO	.262	-.415	-.375	-.781*	1	-.653	-.507
	Salinity	-.516	-.110	.272	.843*	-.653**	1*	.219
	Conductivity	-.229	.403	.168	.034	-.507*	.219	1

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

Table 4.2: Correlation Analysis for Chemical Parameters

Area	Parameter	BOD	TSS	NH_3N	COD
Direct Sun Area	BOD	1	.415	-.426	.149
	TSS	.415	1	-.197	.309
	NH_3N	-.426	-.197	1	-.666**
	COD	.149	.309	-.666**	1
Shaded Sun Area	BOD	1	.243	.059	-.018
	TSS	.243	1	.033	.785**
	NH_3N	.059	.033	1	.41
	COD	-.018	.785**	.412	1

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

4.4 Water Quality Index (WQI) and Classification at Lata Janggut

Water quality status classification was determined by using the Water Quality Index (WQI). WQI value for 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 follows:

$$WQI = (0.22*SIDO) + (0.19*SIBOD) + (0.16*SICOD)$$

$$+ (0.15*SIAN) + (0.16*SISS) + (0.12*pH)$$

According to Table 4.9, the WQI value of direct and shaded sun area were 74.53 and 72.13, respectively. Based on the calculation provided, the WQI value for direct sun area indicated higher reading than the shaded sun area.

Through the analysis, it was determined that the water quality of the direct and shaded sun area at Lata Janggut was slightly polluted for the study period, which were taken into consideration for six consecutive weeks, from July 24 until August 28 of 2016.

Table 4.3: WQI Value and Classification for Each Studied Area

Study Area	WQI value	Classification
Direct Sun Area	74.53	Slightly polluted (Class III)
Shaded Sun Area	72.13	Slightly polluted (Class III)

The WQI value of the present study area was calculated as follows:

WQI (Direct Sun Area): = (0.22*115.50) + (0.19*55.35) + (0.16*80.21)

+ (0.15*0.12) + (0.16*88.04) + (0.12*97.23)

WQI (Shaded Sun Area): = (0.22*101.50) + (0.19*41.16) + (0.16*47.46)

+ (0.15*55.80) + (0.16*88.22) + (0.12*99.19)

A comparison of NH_3N, BOD, COD, DO, pH, TSS and WQI at the Lata Janggut were compared with the value provided by DOE were summarized in Table 4.10. WQI values for the Lata Janggut were measure through two different areas, which are direct and shaded sun area. Both of the WQI values fall under Class III, respectively.

As for direct sun area, for the compared parameters, NH_3N was categorized in Class II, BOD in Class IV, COD in Class II, DO in Class III, pH in Class IV, and TSS in Class I. Meanwhile for shaded sun area, NH_3N was identified in Class III, BOD in Class V, COD in Class II, DO in Class II, pH in Class I, and TSS in Class I.

Table 4.4: Mean Chemical Water Quality Parameters for direct sun area at Lata Janggut for Six Continuous Weeks from July 24 to August 28 of 2016

Week	Dissolved Oxygen : D1	Dissolved Oxygen : D2	Biochemical Oxygen Demand (mg/L)	Total Suspended Solid(mg/L)	Ammoniacal Nitrogen(mg/L)	Chemical Oxygen Demand (COD)	pH
Week 1	8.56±0.07	8.44±0.01	3.6±2.16	16.00±1.00	0.17±0.01	20.63±1.08	7.63±0.33
Week 2	8.56±0.14	8.18±0.15	3.8±8.59	2.00±0.58	0.20±0.01	3.50±0.27	7.62±0.02
Week 3	8.64±0.20	8.11±0.18	15.8±10.78	28.30±14.84	0.02±0.00	7.63±0.15	7.61±0.14
Week 4	8.65±0.04	8.12±0.25	14.6±6.24	28.30±1.53	0.18±0.01	20.50±1.15	7.61±0.00
Week 5	8.70±0.13	8.05±0.04	19.4±3.35	15.00±0.58	0.04±0.01	31.23±5.40	7.57±0.04
Week 6	8.55±0.08	8.12±0.03	13.4±7.14	9.33±3.06	0.09±0.01	1.73±0.32	6.55±0.03
Mean	8.61±0.11	8.17±0.11	11.77±6.29	16.49±3.60	0.12±0.01	14.20±2.01	7.43±0.09

Table 4.5: Mean Chemical Water Quality Parameters for shaded sun area at Lata Janggut for Six Continuous Weeks from July 24 to August 28 of 2016

Week	Dissolved Oxygen : D1	Dissolved Oxygen : D2	Biochemical Oxygen Demand (mg/L)	Total Suspended Solid(mg/L)	Ammoniacal Nitrogen(mg/L)	Chemical Oxygen Demand (COD)	pH
Week 1	8.70±0.06	8.57±0.04	3.9±2.08	22.00±0.58	0.14±0.02	20.40±0.75	7.95±0.33
Week 2	8.43±0.17	8.16±0.04	8.1±6.35	2.00±0.58	0.23±0.01	2.90±0.530	7.77±0.02
Week 3	8.79±0.03	8.30±0.34	14.7±11.17	17.00±12.29	0.78±0.00	4.43±0.42	6.45±0.14
Week 4	8.55±0.08	7.57±0.47	29.6±11.17	15.00±2.65	0.05±0.01	11.93±0.12	6.82±0.00
Week 5	7.85±0.08	8.73±0.01	26.3±11.17	32.30±1.53	0.34±0.02	31.47±1.17	6.46±0.04
Week 6	8.78±0.07	8.12±0.03	18.3±11.17	8.67±3.22	0.36±0.01	12.33±1.16	6.43±0.03
Mean	8.52±0.08	8.24±0.17	16.81±8.85	16.16±3.47	0.32±0.00	13.91±0.42	6.98±0.02

Table 4.6: 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	D: 0.12 S: 0.32
BOD	mg/L	<1	1-3	3-6	6-12	>12	D: 11.77 S: 16.81
COD	mg/L	<10	10-25	25-50	50-100	>100	D: 14.20 S: 13.91
DO	mg/L	<7	5-7	3-5	1-3	<1	D: 8.19 S: 7.48
pH	-	>7.0	6.0-7.0	5.0-6.0	<5.0	>5.0	D: 7.43 S: 7.06
TSS	mg/L	<25	50	150	300	>300	D: 16.49 S: 16.16
WQI		>92.7	76.5-92.7	51.9-76.5	31.0-51.9	<31.0	D: 74.53 S: 72.13

Note: D-Direct Sun Area, S-Shaded Sun Area

4.4.1 The comparison of the range value for pH, temperature, salinity and Heavy Metal with lethal conditions parameters

Lethal condition	Direct Sun Area	Shaded Sun Area
Acid condition (pH < 6.5)	**6.55-7.63**	**6.43-7.95**
Alkali condition (pH > 8.4)		
High temperature ($32^{o}C$)	**25.65-29.78 ^{o}C**	**25.11-26.05 ^{o}C**
Seawater salt level >1%	**0.02 %**	**0.01-0.03%**
All kinds of chemical	***Clustered bar chart**	

4.5 The morphological Identification of *Leptospira* sp. from Gram Staining procedure

Leptospires are phylogenetically related to other spirochetes. Leptospires are tightly coiled spirochetes, usually 0.1 mm by 6 to 0.1 by 20 mm, but occasional cultures may contain much longer cells. The cells have pointed ends, either or both of which are usually bent into a distinctive hook. Leptospiral lipopolysaccharide has a composition similar to that of other gram-negative bacteria (Vinth *et al.*, 1986), but has lower endotoxic activity.

From the six consecutive weeks of study period, three water samples from the first, third and fifth week were tested for the morphological identification of *Leptospira* sp. at both direct and shaded sun area from the method of gram staining.

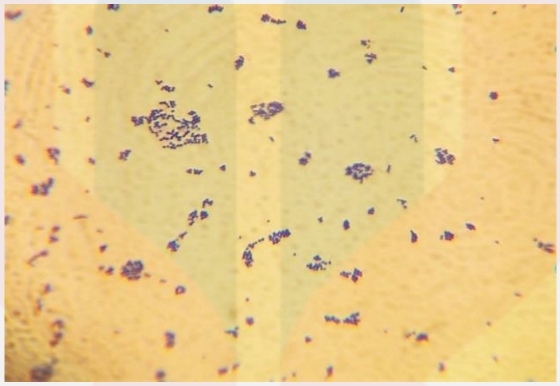
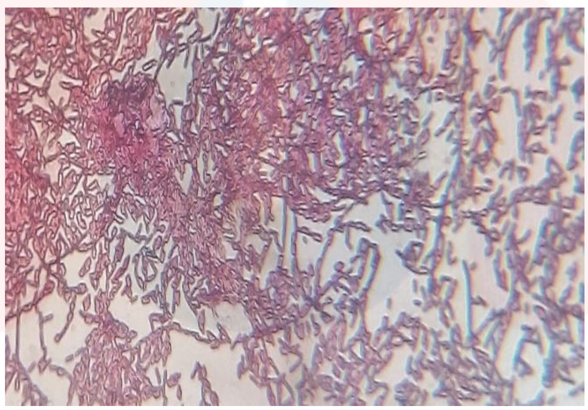
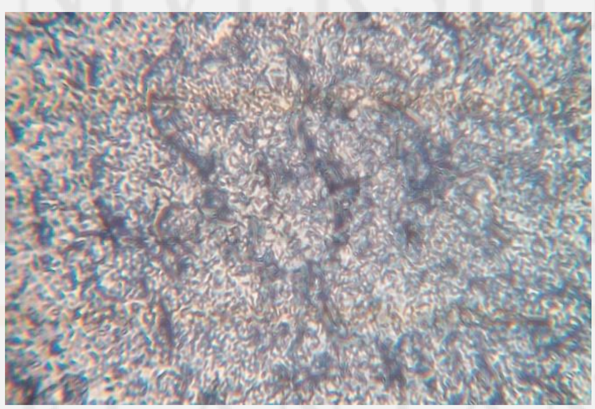
From the results and observation under 10x, 40x and 100x magnification of microscope, it can be concluded that there is no *Leptospira* sp. present in the research area for the six consecutive weeks of study period based on its morphological characteristics. The WQI values outcome in direct and shaded sun area, Lata Janggut correlated with the absence of leptospires during the study period as in fact, the bacteria prefer clean water without any chemical pollution for their survival.

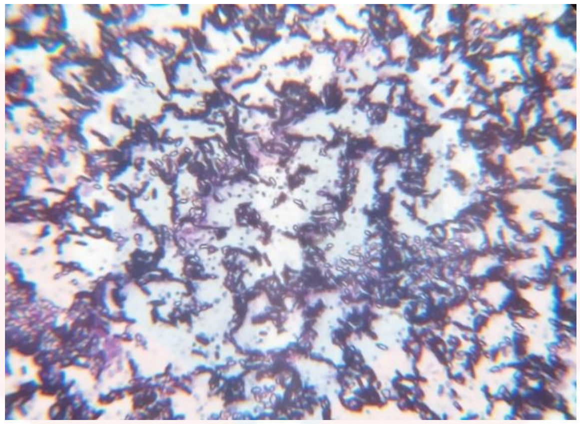
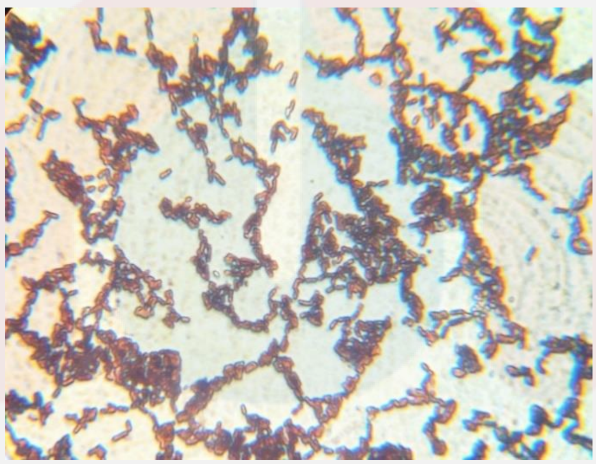
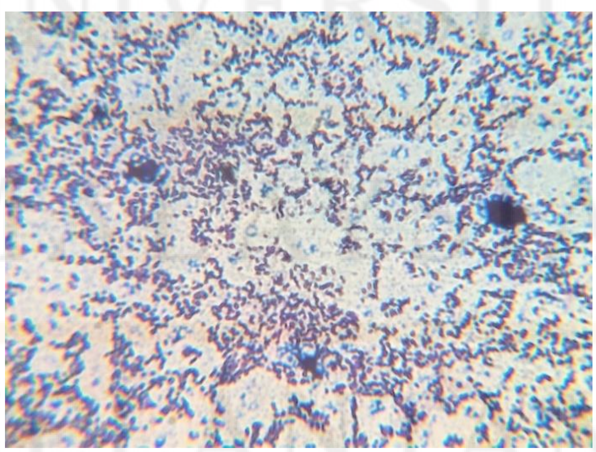
Gram-negative bacteria have a typical double membrane structure in common with other spirochetes, in which the cytoplasmic membrane and peptidoglycan cell wall are closely associated and are overlain by an outer membrane (Paul, 2001), these bacteria do not retain crystal violet dye in the Gram staining protocol. In a Gram stain test, a counterstain that is commonly safranin is added after the crystal violet, colouring all

Gram-negative bacteria with a red or pink colour. In this study, it was observed that all the water samples from direct and shaded sun area were contained the Gram-positive bacteria except the water sample from the third week at direct sun area that showed the presence of Gram-negative bacteria.

On the other hand, Gram-positive bacteria will retain the crystal violet dye when washed in a decolorizing solution because they do not have an outer membrane rather, they have a thickened peptidoglycan layer. From the morphological characteristics at the direct sun area, it was identified that the water sample from the first week contain Gram-positive bacteria with sarcinae shape. The third week showed the different type of bacteria with the identification of bacillus shape with pink colour that presence Gram-negative bacteria. Meanwhile from the fifth week, it was observed that the water sample from the same research area contained bacillus shape.

Table 4.7: Morphological identification from Gram Staining for direct and shaded sun area

Area	Week	Observation under microscope	Description
Direct Sun Area	Week 1		Magnification : 40x Dilution factor : 10^{-5} Colour : purple Shape : sarcinae Gram : positive
	Week 3		Magnification : 100x Dilution factor : 10^{-4} Colour :pink Shape : bacillus Gram : negative
	Week 5		Magnification : 100x Dilution factor : 10^{-5} Colour : purple Shape : bacillus Gram :positive

Shaded Sun Area	Week 1		Magnification : 40x Dilution factor : 10^{-4} Colour : purple Shape : coccobacilli Gram : positive
	Week 3		Magnification : 40x Dilution factor : 10^{-4} Colour : purple Shape : coccobacilli Gram : positive
	Week 5		Magnification : 40x Dilution factor : 10^{-5} Colour : purple Shape : vibrios Gram : positive

4.6 The Ecological Parameters Analysis and Its Relationship with the Identification of *Lepstospira* Sp. at Lata Janggut

The survival of leptospires in the environment depend on many conditions and here it stated as the ecological parameters. *Leptospira* exist in two groups, known as the free-living saprophytes and the pathogenic parasitic types. Based on the previous study, it can be classified that stable pH is an important factor for leptospiral survival in fresh water (Trueba *et al.*, 2004) However, it also depends on a few requirements basics to survive such as water, oxygen, and temperature.

Compared to others, leptospires are relatively slow growing bacteria and so colony densities in a body of water tend to be uniform (Srikanth *et al.*, 2013). 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 but, also considering the other ecological parameters surrounding the area (Khairani-Bejo *et al.*, 2004). Whilst for pathogenic types, they require a host in order to complete their life cycle and the host's immune response and species determine the survivability of the bacteria inside and outside the host (Srikanth *et al.*, 2013).

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 (Wynwood *et al.*, 2014). The inability of leptospires to survive outside water is one of the crucial control factor in the natural environment (Khairani-Bejo *et al.*, 2004)

Through the research, it was found that some ecological parameters studied were compatible with leptospires to survive. However, due to the other parameters that were enconvenient, still intrude them to survive longer or not exist at all. In fact, pathogenic leptospires are extremely sensitive to all kinds of chemicals even though at very low concentrations since they are very fragile little bacteria. Hence, it is difficult for pathogenic colonies to survive in polluted water bodies (Khairani-Bejo *et al.*, 2004).

. Therefore, this tought in 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 asymptomtic stage.

CHAPTER 5

5.1 Conclusion

The present study focused on the assessment of ecological parameters which involves the physical and chemical properties of water sample in order to determine the probability of presence or absence of *Leptospira* sp. at the research area. Relevantly, different location of each studied islands have given the different values for each tested parameters.

Based on the data presented in the research, one of the major finding is WQI of the study area. According to WQI and Classification provided by DOE, the mean water quality was calculated in slightly polluted for direct and shaded sun area.

Through the research, it was also acknowledged that Lata Janggut was free from leptospires for the first, third and fifth week of the study period. In fact, leptospires prefer clean environmental to survive. Although there are some parameters that appropriate for the leptospires to survive, but there are still some factors that interfere leptospires to survive. The absence of leptospires was associated and explained by all the ecological parameters studied.

With regard to the results, it is clear that the water and sediments of the Lata Janggut were contaminated by heavy metals and, pathogenic leptospires are very sensitive to all kinds of heavy metal even though at very low concentrations. Based on the above mentioned points, it gets clear that using the water for recreational purposes, washing, and fishing is detrimental to human health and the environment. Thus, necessary to take serious and essential measures to control the entry of the sewage, manage the quality of water and the sediments of the river, and utilize water for various purposes.

5.2 Limitation of the Study and Future Dimension of the Work

The present study was involved in determining the leptospires through the Gram staining method for three 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 *Leptospira* sp. Futhermore, the growth of bacteria may take more than one week to grow or to show its characteristics. In addition, future dimension of the work could be emphasize on higher technology methods or tools in order to determine the bacteria species, since it is tough to determine the identity of the bacteria just by looking through its morphological characteristic only.

REFERENCES

Alexander, A. D., Evans, L. B., Baker, M. F., Baker, H. J., Ellison, D. & Marriapan, M. (1975). Pathogenic Leptospiras isolated from Malaysian surface waters. *Applied Microbiology* 29(1):30-33.

Ariyapruchya, B., Sungkanuparph, S. & Dumrongkitchaiporn, S. (2003). Clinical presentation and medical complication in 59 cases of laboratory confirmed Leptospirosis in Bangkok. *Southeast Asian Journal Tropical Medicine Public Health* 34(1):159-64.

Bahaman, A. R., Ibrahim, A. L. & Adam, H. (1987). Serological prevalence of leptospiral infection in domestic animals in West Malaysia. *Epidemiology and Infection* 99(2):379-392.

Brendle, J. J., Rogul, M. & Alexander, A. D. (1974). Deoxyribonucleic acid *Bacteriology* 24:205–214.

Brenner, D. J., Kaufmann, A. F., Sulzer, K. R., Steigerwalt, A. G., Rogers, F. C. & Weyant, R. S. (1999). Further determination of DNA relatedness between serogroups and serovars in the family Leptospiraceae with a proposal for *Leptospira* alexanderi species and four new *Leptospira* genomospecies. *International Journal Systematic Bacteriology* 49:839–858.

Cacciapuoti, B., Ciceroni, L., Maffei, C., D, Stanislao, F., Calegari, L. & Lupidi, R. (1987). A waterborne outbreak of Leptospirosis. *AmJournal Epidemiology* 126(3):535–45.

Cameron, C. E., Zuerner, R. L., Raverty S., Colegrove K. M., Norman, S. A., Lambourn, D. M., Jeffries, S. J. & Gulland, F. M., (2008). Detection of Pathogenic *Leptospira* Bacteria in Pinniped Populations via PCR and Identification of a Source of Transmission for Zoonotic. *Leptospirosis in the Marine Environment* 46(5): 1728-1733.

Dikken, H. & Kmety, E. (1978). Serological typing methods of leptospires. *Methods in Microbiology Academic Press, London, U.K.* 11:259–307.

Douadi, B., Pei, Y. W., Siti, N. M. Z., Fairuz, A. & Kwai, L. T. (2013). Pathogenic and saprophytic *Leptospira* species in water and soils from selected urban sites in peninsular Malaysia. *Microbes Environment* 28(1):135-140.

Faine, S., Adler, B., Bolin C. & Perolat, P. (1999). *Leptospira* and Leptospirosis. *Second Edition Melbourne: Medical Science:* 17-66.

Feresu, S. B., Bolin, C. A., Van de Kemp, H. & Korver, H. (1999). Identification of a serogroup Bataviae *Leptospira* strain isolated from an ox in Zimbabwe. *Zentral Bacteriology* 289:19–29.

Fishman, M. J. & Downs S. C. (1996). Methods for analysis of selected metals in water by atomic absorption, "*Geological Survey Water Supply Paper 1540-C*, U. S. *Government Printing office, Washington, D. C.''*

Gordon, C. E. & Turner, L. H. (1961). The effect of pH on the survival of leptospires in water. *World Health Organisation* 24:35-43.

Johnson, R. C. & Faine, S. (1984). *Leptospira*. *In N. R. Krieg and J. G. Holt (ed.), Bergey's Manual Of Systematic Bacteriology, Williams and Wilkins, Baltimore* 1:62–67.

Karaseva, E. V., Chernukha, Y. G. & Piskunova, L. A. (1973). Results of studying the time of survival of pathogenic *Leptospira* under natural conditions. *Journal Epidemiology Microbiology Immunology* 17:339–345.

Khairani-Bejo, S., Bahaman, A. R., Zamri-Saad, M. & Mutalib, A. R. (2004). The survival of *Leptospira interrogans* Serovars *Hardjo* in the Malaysian environment. *Journal of Animals and Veterinary Advances* 3(3):123-129.

Kmety, E. & H. Dikken. (1993). Classification of the species *Leptospira* interrogans and history of its serovars. *University Press Groningen, Groningen, the Netherlands.*

Kosari, S. & Vanae M. (2007). Survey of the source and amount of pollutants concentration of heavy metal in Tembi River and Bohlol Lake. *Proceedings of the first Conference on Geological and Environmental and Medical Tehran, Iran*.

Lim, J. J., Murugayah, V. A., Abdul, R. H., Mohamed, N., Shamsudin, N. & Tan, J. C. (2011). A case study: A *Leptospira* in Malaysia. *Webmedical Central infectious diseases* 2(12): WMC002764.

Michna, S. W. (1970). Leptospirosis. *Veterinary Rec*. 86:484-496.

Paul, N. L. (2001). Leptospirosis. *Clinical Microbiology Reviews* 14:296–326.

Petrino, M. G. & Doetsch, R. N. (1978). "Viscotaxis", a new behavioral response of *Leptospira interrogans* (biflexa) strain B16. *Journal Generation Microbiology* 109:113-117.

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.

Saito, M., Miyahara, S., Villanueva, S., Y., Aramaki, N., Ikejiri, M., Kobayashi, Y. & (2014). Polymerase Chain Reaction and culture identification of pathogenic *Leptospira* from coastal soil in Leyte, Philippines after a storm surge during Super Typhoon Haiyan (Yolanda). *Application Environment Microbiology*. PII: AEM.02568-14.

Srikanth. P., Somasekhar, S. A., Kanthi, G. K. & Raghu Babu, K. (2013). Analysis of heavy metals by using atomic absorption spectroscopy from the samples taken around Visakhapatnam. *International journal of environment, ecology, family and urban studies (IJEEFUS)* 3(1):127-132.

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

Trueba, G., Zapata, S., Madrid, K., Cullen, P. & Haake, D. (2004). Cell aggregation: A mechanism of pathogenic *Leptospira* to survive in freshwater. *International Microbiology* 7:35–40.

Vinh, T., Adler, B., and Faine, S. 1986. Ultrastructure and chemical composition of lipopolysaccharide extracted from *Leptospira* interrogans serovar copenhageni. *Journal Genetic Microbiology* 132:103–109.

Weese, J. S., Peregrine, A. S. & Armstrong, J. (2002). Occupational health and safety in small animal veterinary practice: Part I-Nonparasitic zoonotic diseases. *Veterinary Journal* 43(8):631–636.

Wynwood, S. J., Graham, G. C., Weier, S. L., Collet, T. A., McKay, D. & Craig, S. B. S. (2014).Leptospirosis from water sources. *Pathogens and Global Health* 108(7).

APPENDIX A

RAW DATA

Table A.1: Physical Parameters for Six Continuous Weeks in Direct Sun Area

Date	pH	Temperature (ºC)	Turbidity (NTU)	Total Dissolved solid (mg/L)	Dissolved oxygen (mg/L)	Salinity (%)	Conductivity (μS/cm)
24/7/16	7.97	25.72	2.38	29	8.21	0.02	0.044
26/7/16	7.61	25.72	2.30	29	8.04	0.02	0.043
28/7/16	7.31	26.08	2.45	29	8.20	0.02	0.045
Mean	7.63	25.84	2.38	29	8.15	0.02	0.044
SD	0.330	0.208	0.075	0	0.095	0	0.001
31/7/16	7.64	25.65	4.43	23	8.00	0.02	0.043
2/8/16	7.60	26.70	4.42	22	8.01	0.02	0.043
4/8/16	7.63	26.08	4.43	22	8.01	0.02	0.043
Mean	7.62	26.14	4.43	22	8.01	0.02	0.043
SD	0.021	0.528	0.006	0.001	0.006	0	0
7/8/16	7.72	29.05	2.99	29	7.84	0.02	0.043
9/8/16	7.65	29.01	2.90	25	7.82	0.02	0.044
11/8/16	7.46	29.08	2.89	26	7.84	0.02	0.044
Mean	7.61	29.04	2.93	26	7.83	0.02	0.044
SD	0.135	0.035	0.005	0.002	0.012	0	0.001
14/8/16	7.61	29.70	2.29	28	9.09	0.02	0.042
16/8/16	7.61	29.73	2.30	28	9.01	0.02	0.042
18/8/16	7.61	29.75	2.30	28	9.01	0.02	0.042
Mean	7.61	29.72	2.30	28	9.04	0.02	0.042
SD	0	0.025	0.006	0	0.046	0	0
21/8/16	7.61	29.74	2.28	28	7.81	0.02	0.042
23/8/16	7.56	29.78	2.25	25	7.80	0.02	0.044
25/8/16	7.54	29.65	2.25	26	7.86	0.02	0.044
Mean	7.57	29.72	2.26	6	7.82	0.02	0.043
SD	0.036	0.067	0.017	0.002	0.032	0	0.001
28/8/16	7.54	29.27	2.38	27	8.33	0.02	0.042
30/8/16	7.53	29.20	2.30	24	8.30	0.02	0.045
1/9/16	7.58	29.24	2.32	24	8.40	0.02	0.045
Mean	6.55	29.23	2.33	25	8.34	0.02	0.044
SD	0.026	0.035	0.042	0.002	0.051	0	0.002

Table A.2: Physical Parameters for Six Continuous Weeks in Shaded Sun Area

Date	pH	Temperature (^{O}C)	Turbidity (NTU)	Total Dissolved solid (g/L)	Dissolved oxygen (mg/L)	Salinity (%)	Conductivity ($\mu S/cm$)
24/7/16	7.97	25.72	4.65	18	9.52	0.01	0.027
26/7/16	7.90	25.71	4.65	14	9.46	0.01	0.027
28/7/16	7.98	25.73	4.64	16	9.48	0.01	0.029
Mean	7.95	25.72	4.65	16	9.48	0.01	0.028
SD	0.044	0.01	0.006	0.025	0.001	0	0.001
31/7/16	7.79	25.65	1.65	25	9.18	0.02	0.027
2/8/16	7.76	25.60	1.64	24	9.16	0.02	0.027
4/8/16	7.76	25.63	1.67	23	9.00	0.02	0.029
Mean	7.77	25.63	1.65	24	9.11	0.02	0.038
SD	0.017	0.025	0.015	0.099	0	0	0.001
7/8/16	6.94	25.65	6.62	39	3.25	0.03	0.039
9/8/16	6.95	25.60	6.63	39	3.00	0.03	0.035
11/8/16	6.95	25.63	6.65	39	3.01	0.02	0.038
Mean	6.95	26.80	6.63	39	3.09	0.03	0.037
SD	0.006	0.025	0.015	0.142	0.002	0	0.002
14/8/16	6.82	26.04	5.52	25	5.65	0.02	0.060
16/8/16	6.80	26.03	5.52	24	5.60	0.02	0.059
18/8/16	6.83	26.05	5.53	24	5.35	0.02	0.059
Mean	6.82	26.04	5.52	0.024	5.53	0.02	0.059
SD	0.015	0.01	0.006	0.161	0.001	0	0.001
21/8/16	6.48	25.64	6.64	29	8.29	0.02	0.044
23/8/16	6.45	25.64	6.64	30	8.00	0.02	0.045
25/8/16	6.46	25.64	6.67	30	8.30	0.02	0.045
Mean	6.46	25.64	6.65	0.030	8.20	0.02	0.045
SD	0.021	0	0.017	0.170	0.001	0	0.001
28/8/16	6.43	25.11	6.76	24	9.51	0.02	0.037
30/8/16	6.43	25.12	6.78	23	9.36	0.02	0.037
1/9/16	6.42	25.12	6.75	24	9.50	0.02	0.037
Mean	6.43	25.12	6.76	0.024	9.46	0.02	0.037
SD	0.006	0.006	0.015	0.087	0	0	0

Table A.3: Mean Physical Parameters at Lata Janggut for direct sun area Six Continuous Weeks from July 24 to August 28 of 2016

Week	pH	Temperature (^0C)	Turbidity (NTU)	Total Dissolved Solid (g/L)	Dissolved Oxygen (mg/L)	Salinity (%)	Conductivity ($\mu S/cm$)
Week 1	7.63±0.330	25.84±0.208	2.38±0.075	29±0	8.15±0.095	0.01±0	0.044±0.001
Week 2	7.62±0.021	26.14±0.528	4.43±0.006	22±0.001	8.01±0.006	0.02±0	0.043±0
Week 3	7.61±0.135	29.04±0.035	2.93±0.005	26±0.002	7.83±0.012	0.03±0	0.044±0.001
Week 4	7.61±0	29.72±0.025	2.30±0.006	28±0	9.04±0.046	0.02±0	0.042±0
Week 5	7.57±0.036	29.72±0.067	2.26±0.017	26±0.002	7.82±0.032	0.02±0	0.043±0.001
Week 6	6.55±0.026	29.23±0.035	2.33±0.042	25±0.002	8.34±0.051	0.02±0	0.044±0.002
Mean	7.432±0.091	28.28±0.149	2.772±0.025	26±0.001	8.19±0.040	0.043±0	0.043±0.001

Table A.4: Mean Physical Parameters at Lata Janggut for shaded sun area Six Continuous Weeks from July 24 to August 28 of 2016

Week	pH	Temperature (^{0}C)	Turbidity (NTU)	Total Dissolved Solid (g/L)	Dissolved Oxygen (mg/L)	Salinity (%)	Conductivity ($\mu S/cm$)
Week 1	7.95±0.044	25.72±0.01	4.65±0.006	16±0.025	9.48±0.001	0.01±0	0.028±0.001
Week 2	7.77±0.017	25.63±0.025	1.65±0.015	24±0.099	9.11±0	0.02±0	0.038±0.001
Week 3	6.95±0.006	26.80±0.025	6.63±0.015	39±0.142	3.09±0.002	0.03±0	0.037±0.002
Week 4	6.82±0.015	26.04±0.01	5.52±0.006	24±0.161	5.53±0.001	0.02±0	0.059±0.001
Week 5	6.46±0.021	25.64±0	6.65±0.017	30±0.170	8.20±0.001	0.02±0	0.045±0.001
Week 6	6.43±0.006	25.12±0.006	6.76±0.015	24±0.087	9.46±0	0.02±0	0.037±0
Mean	7.06±0.018	25.83±0.025	5.31±0.012	26±0.114	7.478±0.001	0.02±0	0.041±0.001

Table A.5: Mean Chemical Parameters for direct sun at Lata Janggut for Six Continuous Weeks from July 24 to August 28 of 2016

Week	Biochemical Oxygen Demand (mg/L)	Total Suspended Solid(mg/L)	Ammoniacal Nitrogen(mg/L)	Chemical Oxygen Demand (mg/L)
Week 1	3.6±2.163	16.0±1.00	0.22±0.006	20.63±1.079
Week 2	3.8±8.585	2.0±0.577	0.24±0.01	3.5±0.265
Week 3	15.8±10.776	28.3±14.844	0.16±0	7.63±0.153
Week 4	16.2±8.238	28.3±1.528	0.21±0.012	20.5±1.153
Week 5	16.1±1.345	15.0±0.577	0.04±0.006	31.23±5.400
Week 6	13.4±7.144	9.33±3.055	0.21±0.006	1.733±0.321
Mean	11.77±6.29	16.49±3.597	0.18±0.006	14.204±2.009

Table A.6: Mean Chemical Parameters for shaded sun area at Lata Janggut for Six Continuous Weeks from July 24 to August 28 of 2016

Week	Biochemical Oxygen Demand (mg/L)	Total Suspended Solid(mg/L)	Ammoniacal Nitrogen(mg/L)	Chemical Oxygen Demand (mg/L)
Week 1	3.9±2.078	22.0±0.577	0.14±0.021	20.4±0.754
Week 2	8.1±6.350	2.0±0.577	0.23±0.006	2.9±0.529
Week 3	14.7±11.173	17.0±12.288	0.78±0	4.433±0.416
Week 4	29.6±11.173	15.0±2.646	0.05±0.012	11.933±0.115
Week 5	26.3±11.173	32.3±1.528	0.34±0.015	31.467±1.1676
Week 6	18.3±11.173	8.67±3.215	0.36±0.006	12.333±1.1590
Mean	16.81±8.853	16.16±3.472	0.317±0.002	13.911±0.420

Table A.7: Chemical Parameters for Six Continuous Weeks in Direct Sun Area

Week	Biochemical Oxygen Demand (mg/L)	Total Suspended Solid(mg/L)	Ammoniacal Nitrogen(mg/L)	Chemical Oxygen Demand(mg/L)
Week 1				
24/7/16	1.2	17	0.22	19.4
26/7/16	4.2	16	0.21	21.1
28/7/16	5.4	15	0.22	21.4
Mean	3.6	16.0	0.22	20.63
SD	2.163	1.00	0.006	1.079
Week 2				
31/7/16	15.9	2	0.25	3.4
2/8/16	16.8	2	0.24	3.3
4/8/16	1.5	1	0.23	3.8
Mean	3.8	2.0	0.24	3.5
SD	8.585	0.577	0.01	0.265
Week 3				
7/8/16	25.5	41	0.16	7.5
9/8/16	17.7	32	0.16	7.6
11/8/16	4.2	12	0.16	7.8
Mean	15.8	28.3	0.16	7.63
SD	10.776	14.844	0	0.153
Week 4				
14/8/16	17.1	30	0.22	19.2
16/8/16	19.2	28	0.20	20.9
18/8/16	12.2	27	0.20	21.4
Mean	16.2	28.3	0.21	20.5
SD	8.238	14.844	0.012	1.153
Week 5				
21/8/16	17.7	16	0.04	34.2
23/8/16	14..9	15	0.04	34.5
25/8/16	15.6	15	0.03	25.0
Mean	16.1	15.0	0.04	31.23
SD	1.345	0.577	0.006	5.400
Week 6				
28/8/16	15	12	0.22	1.5
30/8/16	17.7	10	0.21	1.6
1/9/16	4.2	6	0.21	2.1
Mean	13.4	9.33	0.21	1.733
SD	7.144	3.055	0.006	0.321

Table A.8: Chemical Parameters for Six Continuous Weeks in Shaded Sun Area

Week	**Biochemical Oxygen Demand (BOD)(mg/L)**	**Total Suspended solid(mg/L)**	**Ammoniacal Nitrogen(mg/L)**	**Chemical Oxygen Demand (mg/L)**
Week 1				
24/7/16	1.5	23	0.16	20.3
26/7/16	5.1	22	0.12	19.7
28/7/16	5.1	22	0.15	21.2
Mean	3.9	22.0	0.14	20.4
SD	2.078	0.577	0.021	0.754
Week 2				
31/7/16	12.9	3	0.24	2.7
2/8/16	10.5	2	0.23	2.5
4/8/16	0.9	2	0.23	3.5
Mean	8.1	2.0	0.23	2.9
SD	6.350	0.577	0.006	0.529
Week 3				
7/8/16	21	31	0.07	4.9
9/8/16	21.3	12	0.07	4.3
11/8/16	1.8	8	0.09	4.1
Mean	14.7	17.0	0.78	4.433
SD	11.173	12.288	0	0.416
Week 4				
14/8/16	21	17	0.05	12.0
16/8/16	21.3	16	0.05	11.8
18/8/16	1.8	12	0.05	12.0
Mean	29.6	15.0	0.05	11.933
SD	11.173	2.646	0.012	0.115
Week 5				
21/8/16	21	34	0.35	30.2
23/8/16	21.3	32	0.34	31.7
25/8/16	1.8	31	0.32	32.5
Mean	26.3	32.3	0.34	31.467
SD	11.173	1.528	0.015	1.1676
Week 6				
28/8/16	21	11	0.35	11.0
30/8/16	21.3	10	0.36	12.9
1/9/16	1.8	5	0.36	13.1
Mean	18.3	8.67	0.36	12.333
SD	11.173	3.215	0.006	1.1590

Table A.9: Heavy metal Initial Characterization Analysis

Element	Value(mg/L)	Element	Value(mg/L)
Magnesium, Mg		Calcium, Ca	
1	5.165	1	3.888
2	5.155	2	4.290
3	5.143	3	4.026
Mean	5.154	Mean	4.068
SD	0.0108	SD	0.2040
Manganese, Mn		Lead, Pb	
1	0.034	1	0.213
2	0.029	2	0.166
3	0.029	3	0.191
Mean	0.030	Mean	0.190
SD	0.0032	SD	0.0238
Iron, Fe		Cadmium, Cd	
1	0.734	1	-0.001
2	0.728	2	-0.001
3	0.712	3	-0.003
Mean	0.725	Mean	-0.002
SD	0.0114	SD	0.0013
Nickel, Ni		Aluminium, Al	
1	-0.018	1	0.726
2	-0.004	2	0.750
3	-0.024	3	0.708
Mean	-0.015	Mean	0.728
SD	0.0101	SD	0.0211
Copper, Cu		Chromium, Cr	
1	0.011	1	0.018
2	0.010	2	0.030
3	0.007	3	0.008
Mean	0.010	Mean	0.019
SD	0.0020	SD	0.0108
Sodium, Na		Zinc, Zn	
1	15.29	1	0.045
2	14.77	2	0.043
3	14.77	3	0.043
Mean	14.94	Mean	0.044
SD	0.299	SD	0.0014
Potassium, K		Silver, Ag	
1	1.299	1	0.015
2	1.316	2	0.012
3	1.295	3	0.011
Mean	1.304	Mean	0.013
SD	0.0111	SD	0.0018

Mercury. Hg		Arsenic, As	
1	0.426	1	0.918
2	0.203	2	0.989
3	0.535	3	1.038
Mean	0.388	Mean	0.982
SD	0.1694	SD	0.0607

Table A.10: Mean Concentration of Heavy Metal Initial Characterization at Lata Janggut

ELEMENT	Concentration (mg/L)
Magnesium, Mg	5.154 ± 0.0108
Lead, Pb	0.190 ± 0.0238
Iron, Fe	0.725 ± 0.0114
Calcium, Ca	4.068 ± 0.2040
Manganese, Mn	0.030 ± 0.0032
Cadmium, Cd	-0.002 ± 0.0013
Nickel, Ni	-0.015 ± 0.0101
Aluminium, Al	0.728 ± 0.0211
Copper, Cu	0.728 ± 0.0020
Zinc, Zn	0.044 ± 0.0014
Chromium, Cr	0.019 ± 0.0108
Sodium, Na	14.94 ± 0.299
Potassium, K	1.304 ± 0.0111
Silver, Ag	0.013 ± 0.0018
Mercury, Hg	0.388 ± 0.1694
Arsenic, As	0.982 ± 0.0607

Table A.11: Water Quality Parameters for the First Week

Area	Date	Dissolved Oxygen D1 (mg/L)	Dissolved Oxygen D2 (mg/L)	BOD (mg/L)	TSS (mg/L)	AN (mg/L)	COD (mg/L)	pH
Direct Sun Area	24/7/16	8.49	8.45	1.2	17	0.22	19.4	7.97
	26/7/16	8.57	8.43	4.2	16	0.21	21.1	7.61
	28/7/16	8.63	8.45	5.4	15	0.22	21.4	7.31
	Mean SD	8.56 0.070	8.44 0.012	3.6 2.163	16.0 1.00	0.22 0.006	20.63 1.079	7.63 0.330
Shaded Sun Area	24/7/16	8.64	8.59	1.5	23	0.16	20.3	7.97
	26/7/16	8.70	8.53	5.1	22	0.12	19.7	7.90
	28/7/16	8.76	8.59	5.1	22	0.15	21.2	7.98
	Mean SD	8.70 0.06	8.57 0.035	3.9 2.078	22.0 0.577	0.14 0.021	20.4 0.754	7.97 0.044

Table A.12: Water Quality Parameters for the Second Week

Area	Date	Dissolved Oxygen D1 (mg/L)	Dissolved Oxygen D2 (mg/L)	BOD (mg/L)	TSS (mg/L)	AN (mg/L)	COD (mg/L)	pH
Direct Sun Area	31/7/16	8.64	8.11	15.9	2	0.25	3.4	7.64
	2/8/16	8.65	8.09	16.8	2	0.24	3.3	7.60
	4/8/16	8.40	8.35	1.5	1	0.23	3.8	7.63
	Mean SD	8.56 0.142	8.18 0.145	3.8 8.585	2.0 0.577	0.24 0.01	3.5 0.265	7.62 0.021
Shaded Sun Area	31/7/16	8.55	8.12	12.9	3	0.24	2.7	7.79
	2/8/16	8.50	8.15	10.5	2	0.23	2.5	7.76
	4/8/16	8.23	8.20	0.9	2	0.23	3.5	7.76
	Mean SD	8.43 0.172	8.16 0.040	8.1 0.350	2.0 0.577	0.23 0.006	2.9 0.529	7.77 0.017

Table A.13: Water Quality Parameters for the Third Week

Area	Date	Dissolved Oxygen D1 (mg/L)	Dissolved Oxygen D2 (mg/L)	BOD (mg/L)	TSS (mg/L)	AN (mg/L)	COD (mg/L)	pH
Direct Sun Area	7/8/16	8.86	8.01	25.5	41	0.16	7.5	7.72
	9/8/16	8.59	8.00	17.7	32	0.16	7.6	7.65
	11/8/16	8.46	8.32	4.2	12	0.16	7.8	7.46
	Mean	8.64	8.11	15.8	28.3	0.16	7.63	7.61
	SD	0.204	0.182	10.776	14.844	0	0.153	0.135
Shaded Sun Area	7/8/16	8.81	8.11	21	31	0.07	4.9	6.44
	9/8/16	8.80	8.09	21.3	12	0.07	4.3	6.45
	11/8/16	8.76	8.70	1.8	8	0.09	4.1	6.45
	Mean	8.79	8.30	14.7	17.0	0.78	4.433	6.45
	SD	0.026	0.342	11.173	12.288	0	0.416	0.006

Table A.14: Water Quality Parameters for the Fourth Week

Area	Date	Dissolved Oxygen D1 (mg/L)	Dissolved Oxygen D2 (mg/L)	BOD (mg/L)	TSS (mg/L)	AN (mg/L)	COD (mg/L)	pH
Direct Sun Area	14/8/16	8.62	8.05	17.1	30	0.22	19.2	7.61
	16/8/16	8.64	8.00	19.2	28	0.20	20.9	7.61
	18/8/16	8.70	8.45	7.5	27	0.20	21.4	7.61
	Mean	8.65	8.12	14.6	28.3	0.21	20.5	7.61
	SD	0.042	0.247	6.238	1.528	0.012	1.153	0
Shaded Sun Area	14/8/16	8.64	7.99	19.5	17	0.05	12.0	7.61
	16/8/16	8.53	7.06	44.1	16	0.05	11.8	7.61
	18/8/16	8.49	7.65	25.2	12	0.05	12.0	7.61
	Mean	8.55	7.57	29.6	15.0	0.05	11.933	6.82
	SD	0.078	0.471	11.173	2.646	0.012	0.115	0.015

Table A.15: Water Quality Parameters for the Fifth Week

Area	Date	Dissolved Oxygen D1 (mg/L)	Dissolved Oxygen D2 (mg/L)	BOD (mg/L)	TSS (mg/L)	AN (mg/L)	COD (mg/L)	pH
Direct Sun Area	21/8/16	8.78	8.09	20.7	16	0.04	34.2	7.61
	23/8/16	8.76	8.03	21.9	15	0.04	34.5	7.56
	25/8/16	8.54	8.02	15.6	15	0.03	25.0	7.54
	Mean SD	8.70 0.133	8.05 0.038	19.4 3.345	15.0 0.577	0.04 0.006	31.23 5.400	7.57 0.036
Shaded Sun Area	21/8/16	8.80	7.94	25.8	34	0.35	30.2	6.48
	23/8/16	8.65	7.86	23.7	32	0.34	31.7	6.45
	25/8/16	8.73	7.75	29.4	31	0.32	32.5	6.46
	Mean SD	7.85 0.075	8.73 0.095	26.3 11.173	32.3 1.528	0.34 0.015	31.467 1.1676	6.46 0.021

Table A.16: Water Quality Parameters for the Sixth Week

Area	Date	Dissolved Oxygen D1 (mg/L)	Dissolved Oxygen D2 (mg/L)	BOD (mg/L)	TSS (mg/L)	AN (mg/L)	COD (mg/L)	pH
Direct Sun Area	28/8/16	8.64	8.14	15	12	0.35	1.5	7.54
	30/8/16	8.53	8.10	12.9	10	0.34	1.6	7.53
	1/9/16	8.49	8.08	12.3	6	0.32	2.1	7.58
	Mean SD	8.55 0.078	8.12 0.031	13.4 7.144	9.33 3.055	0.21 0.006	1.733 0.321	6.55 0.026
Shaded Sun Area	28/8/16	8.79	8.18	18.3	11	0.35	11.0	6.43
	30/8/16	8.70	8.19	15.3	10	0.06	12.9	6.43
	1/9/16	8.84	8.13	21.3	5	0.36	13.1	6.42
	Mean SD	8.78 0.071	8.12 0.032	18.3 11.173	8.67 3.215	0.36 0.006	12.333 1.1590	6.43 0.006

APPENDIX B

CALCULATION OF WATER QUALITY

WQI Formula and Calculation

Water quality status classification will be determined using the Water Quality Index (WQI). WQI value for Lata Janggut freshwater will be calculated by entering the six water quality parameters mean values such as DO, BOD, COD, pH, NH_3N and TSS. Firstly, the value were converted to SubIndices according to the equation guided by DOE as follows:

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

Where;

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;

$0 \leq WQI \leq 100$

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= $-0.39 + 0.030x^2 - 0.00020x^3$ 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 $x \leq 0.3$

$SIAN = 94 * \exp(-0.573x) – 5 * 1 x – 21$ 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 $100 < x < 100$

$SISS = 0$ for $x \geq 1000$

Subindex for pH

$SIpH = 17.2 – 17.2x – 5.02x^2$ for $x < 5.5$

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

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

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

Note: * means multiply with

APPENDIX C

MAP AND FIGURE

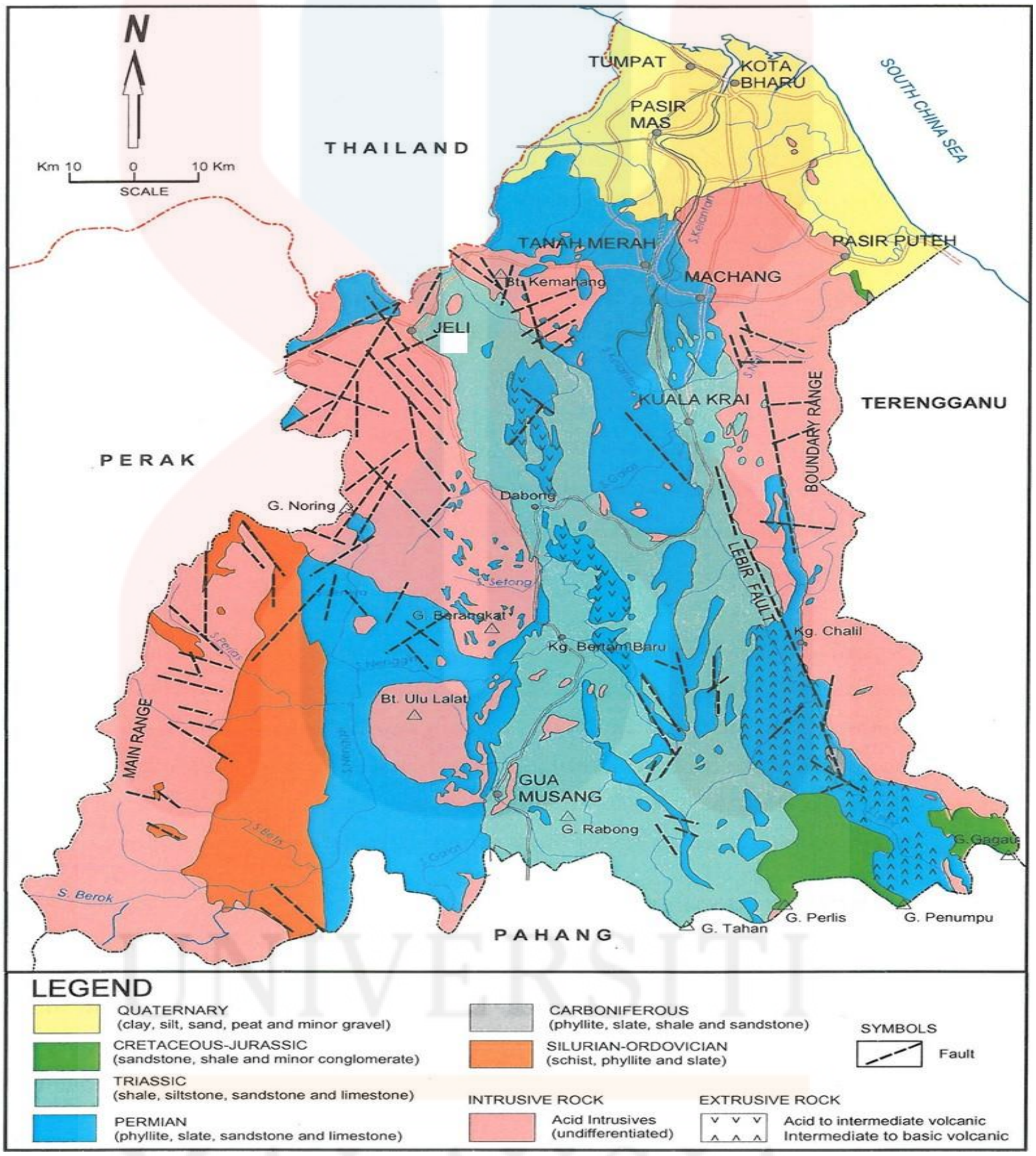


Figure C.1: Map of Kelantan State (Shape box for study area).

(Source: Department of Minerals and Geoscience Malaysia (2013) Quarry Resource Planning for the State of Kelantan.

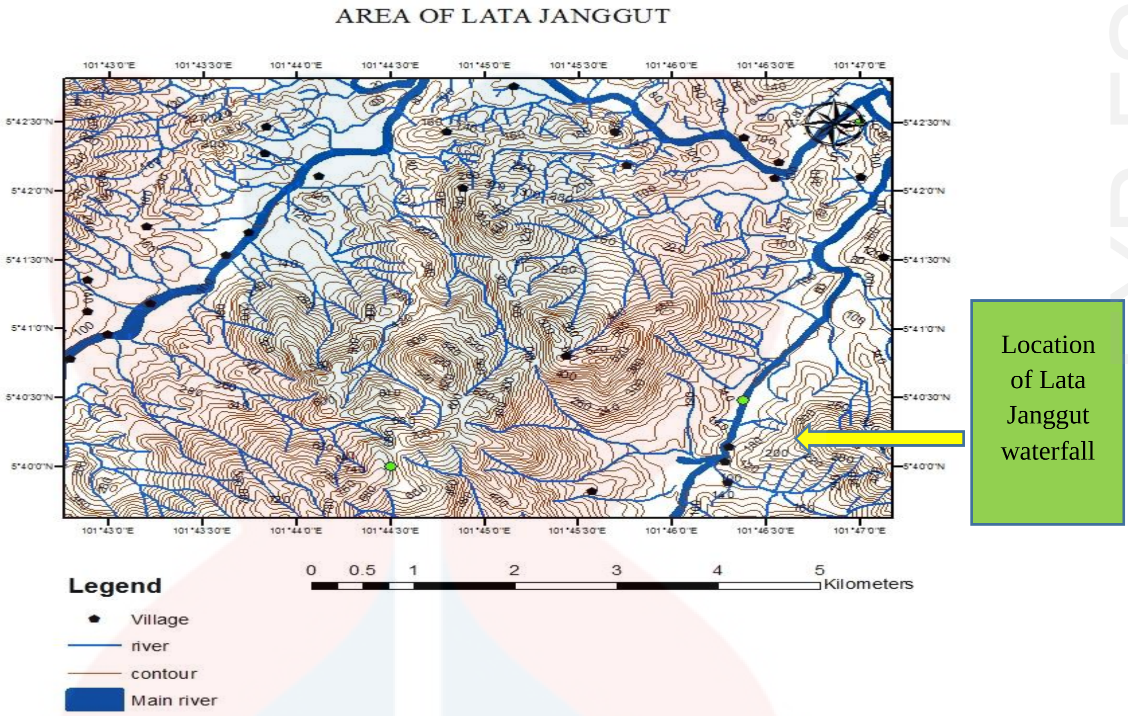


Figure C.2: The base map of Lata Janggut (10 km x 6 km)

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

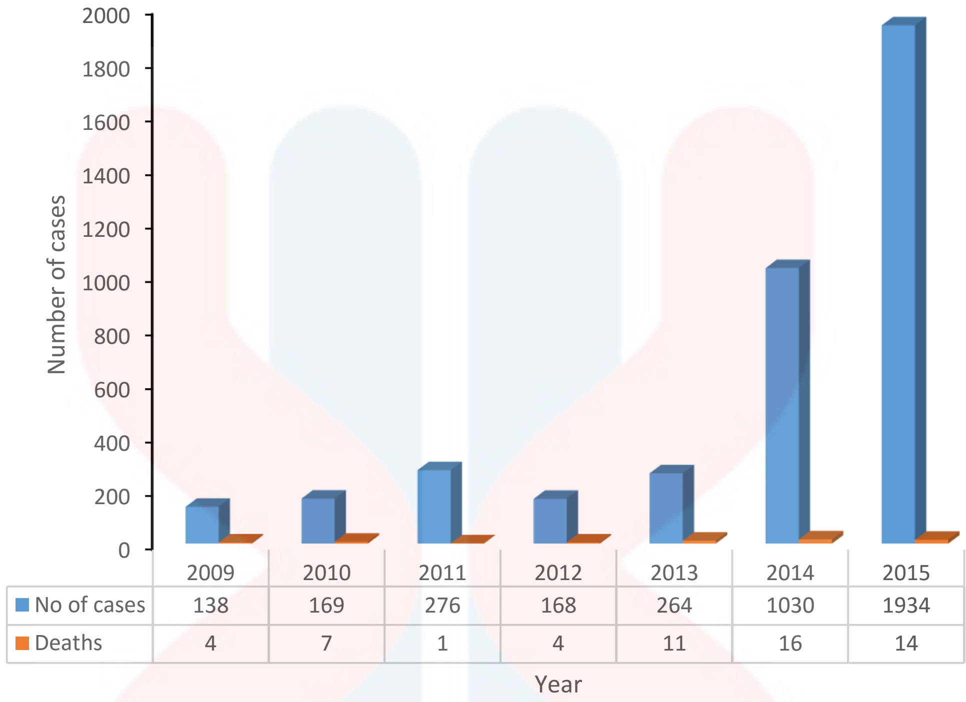


Figure C.3: The number of cases of Leptospirosis deaths in Kelantan

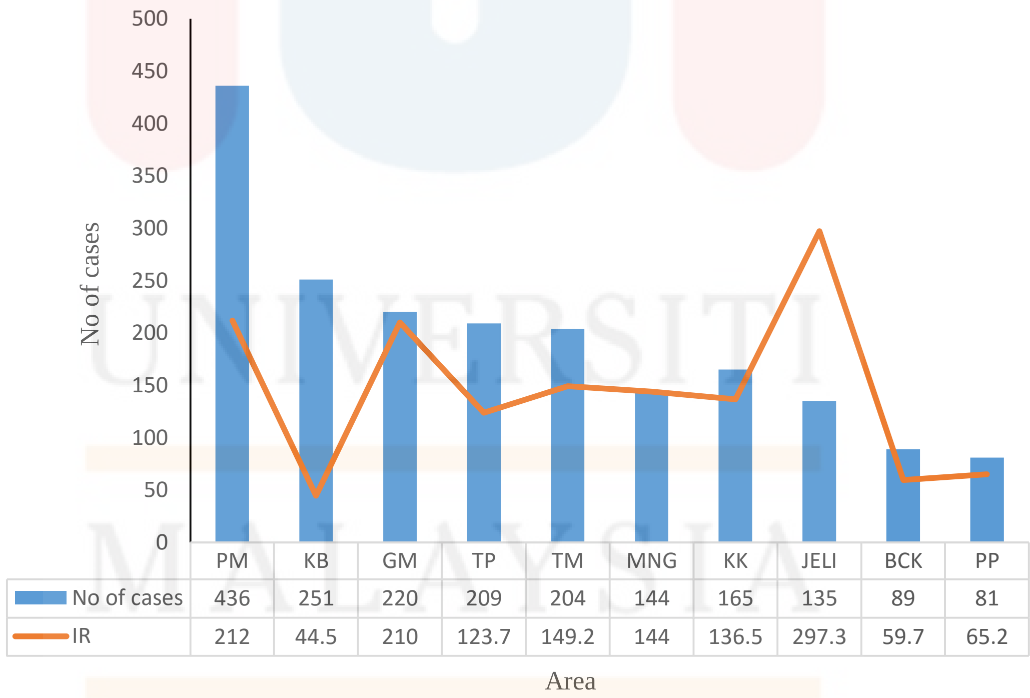


Figure C.4: The number of cases and incidence rate of Leptospirosis by regions

Figure C.5: The view of surrounding area in Lata Janggut taken on 20th of July 2016

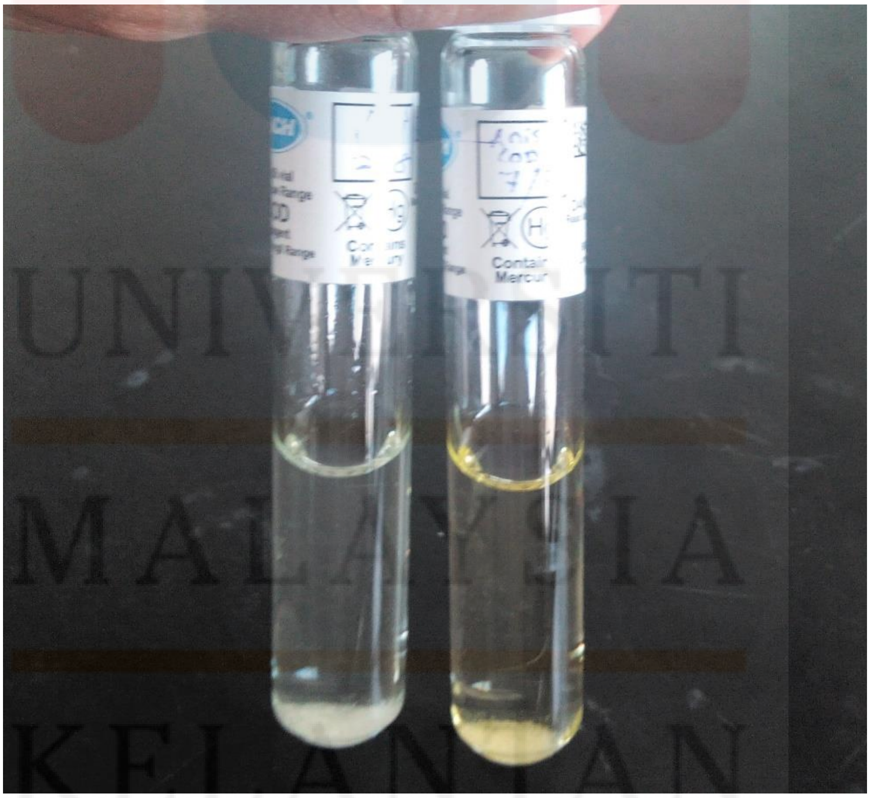


Figure C.6: COD reagent with water sample of Lata Janggut taken on 24th July 2016

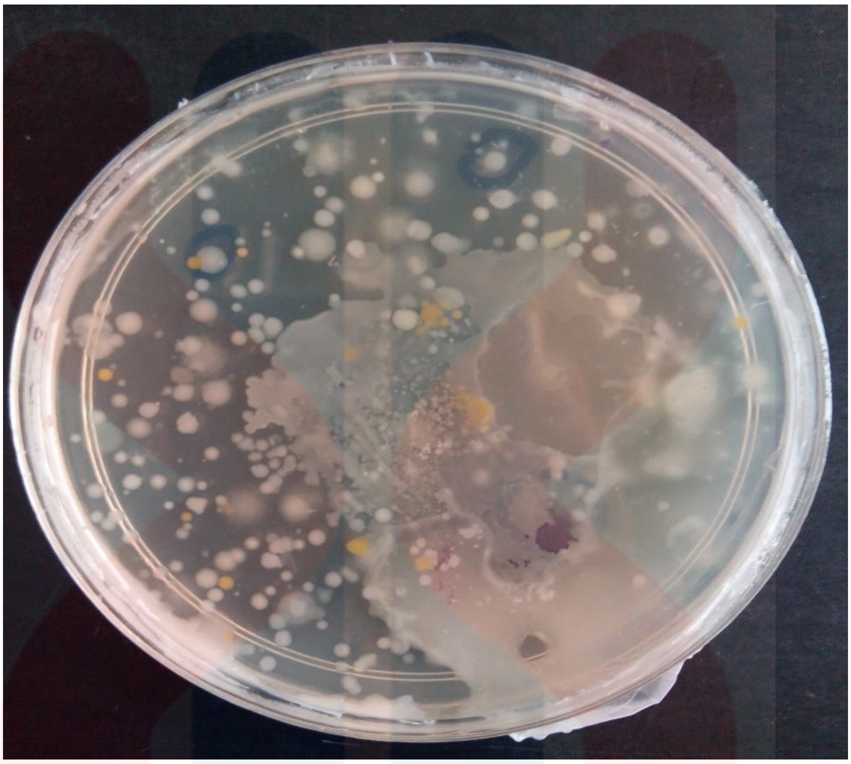


Figure C.7: The bacterial colonies using spreading method taken on 26th July 2016

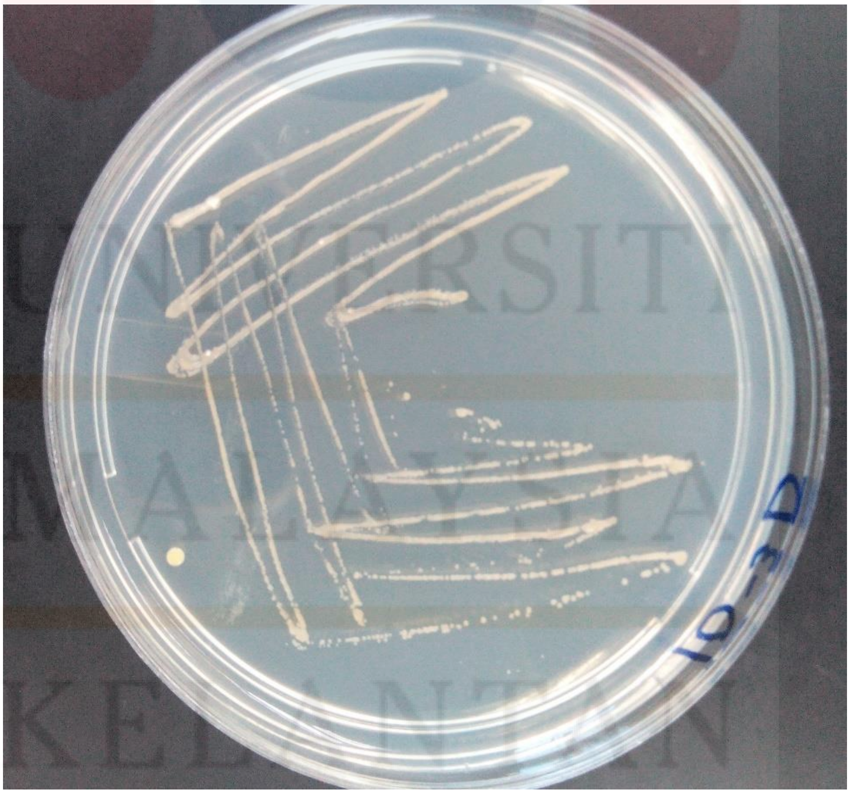


Figure C.8: The bacterial colonies using streaking method taken on 26th July 2016