PHYTOREMEDIATION OF SEMBRONG RIVER WATERS USING *NEPTUNIA OLERACEA* AND *PISTIA STRATIOTES*

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DEDICATION

This thesis is first and foremost dedicated to Almighty Allah for seeing me through. Then to my brother Alhaji Mohasen and my wife for their unwavering support, advice, encouragement and prayers which guided me towards this achievement, I am very proud of them and may Almighty Allah (S.W.T) reward them abundantly. The thesis is also dedicated to my siblings, uncles and aunties for their prayers and support.

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ABSTRACT

Water quality of Sungai Sembrong is in poor condition but it is an important source of water for people in Parit Raja. Water has to be treated intensively resulting in high cost. This study aims to determine the water quality index (WQI) and the efficiency of phytoremediation as well as the effect on the two plant species (*Neptunia oleracea* and *Pistia stratiotes*) due to bio mineralization of heavy metals. Water quality parameters measured were conductivity, turbidity, pH, Dissolved Oxygen (DO), Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), NH_3N, TP, Zn, Fe, and Al. From this study, Sungai Sembrong is classified as Class IV according to DOE-WQI. Elements with high concentration were Al (61mg/L), Fe (33mg/L), and Zn (1.5mg/L) making it one of the most contaminated river in Malaysia. The condition for the water quality of the river was related to various land use along the river banks. *N. oleracea* performed better because for example on day 10 the COD is 60 mg/L as compared to *P. stratiotes* with COD at 78 mg/L *N. oleracea* was also in good condition for longer period of time. Uptake of the three trace elements (Al, Fe and Zn) in plants tissues were shown using AAS. The concentration of elements in plant tissue that were cultivated in river water were up to 254 times higher than the control plants except for Zn. Results of the biological studies suggested that the plants could be used for phytostabilization and phytoextraction of Al and Fe. However, the plants were not hyperaccumulators of Zn. Using photomicrography transverse sections of plant tissues cultivated in river water showed toxic symptoms like distortion, increase in the number of layers of cells and damages. The control plants did not exhibit any symptoms of damage. SEM-EDS analysis showed bio mineralized heavy metals distribution in different plant tissues which was supported by results from morpho-anatomical changes. The study concluded that cheaper ways of water treatment could be possible with the two species; *N. oleracea* and *P. stratiotes*.

ABSTRAK

Kualiti air Sungai Sembrong berada dalam keadaan yang tercemar, sedangkan ia merupakan sumber air bagi masyarakat Parit Raja yang penting. Air perlu dirawat secara intensif sehingga menyebabkan kos yang tinggi. Kajian dilakukan untuk menentukan piawai kualiti air dan kecekapan proses fitoremediasi dan menentukan bioremediasi oleh dua spesies tumbuhan (*Neptunia oleracea* dan *Pistia stratiotes*) akibat biomineralisasi logam berat. Kajian ini bertujuan mencari rawatan alternatif menggunakan tumbuhan akuatik yang ada. Parameter yang diukur ialah kekonduksian, kekeruhan, pH, Oksigen Terlarut (DO), Permintaan Oksigen Biologi (BOD), Permintaan Oksigen Kimia (COD), NH_3N, TP, Zn, Fe, dan Al. Daripada kajian ini, Sungai Sembrong termasuk dalam Kelas IV menurut DOE-WQI. Unsur-unsur logam surih yang berkepekatan tinggi ialah Al (61mg/L), Fe (33mg/L), dan Zn (1.5mg/L); menjadikannya salah satu sungai tercemar di Malaysia. Keadaan sungai yang demikian dikaitkan dengan jenis guna tanah di sepanjang tebing sungai. Pengambilan tiga unsur logam surih Al, Fe dan Zn ke dalam tisu tumbuhan telah diukur menggunakan AAS. Kepekatan unsur dalam tisu tumbuhan yang dibiakkan dalam air sungai meningkat sehingga 254 kali ganda berbanding tumbuhan kawalan kecuali bagi Zn. Hasil kajian biologi mencadangkan bahawa tumbuhan boleh digunakan dalam fitostabilasi dan fitoekstraksi Al dan Fe. Dengan menggunakan fotomikrografi hirisan melintang, tisu tumbuhan yang dipelihara dalam air sungai menunjukkan simptom toksik seperti distorsi, pertambahan lapisan sel dan kerosakan. Tumbuhan kawalan tidak menunjukkan sebarang simpton kerosakan. Kajian ini dapat merumuskan tisu tumbuhan mana yang banyak dirosakkan. Analisis SEM-EDS menunjukkan taburan logam berat yang terbiomineralisasi dalam berbagai tisu tumbuhan dan ini disokong oleh hasil dari perubahan morfo-anatomi tisu tumbuhan. Kajian juga menunjukkan potensi perawatan air dengan menggunakan dua spesies tumbuhan *N. oleracea* and *P. stratiote*s.

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CHAPTER 1

INTRODUCTION

Presently, water and land pollution remain the major global problem, because it is the leading cause of deaths and diseases, as reported during the United Nations World Water Day released on March 22, 2010. Around 2.2 million people a year die from diarrheal diseases caused by drinking contaminated water and poor hygiene (Hunter *et al*., 2010). About 97% of the world's water are saline (seawater), whereas freshwater represents only 3% of the total global water resources. However, only one-third of the freshwater is accessible for human activities due to the fact that the 2% occurs as snow and ice in the polar and the alpine region of the world. Moreover, the most part of the freshwater (98%) is locked in the ground as 'groundwater', with only about 2% of it easily available as surface water (rivers and lakes), for human consumption, agriculture and industrial activities. As a result, freshwater is seen as a finite and limited resource, especially in the arid regions (Christensen, 2013; Awang *et al.*, 2015).

Currently, over 80% of the world population faces intricate water security problems. Nearly all countries in the world are affected by the water security threat of consuming water resources that are not safe through either endemic water diseases due to lack of proper water treatment capabilities and/or decreased in annual precipitation due to severe climatic change (Hanjra & Qureshi, 2010). Generally, the global water resources are polluted mainly through human activities (anthropogenic), because the industrial revolution contributed immensely to the global environmental degradation (Sayyed & Sayadi, 2011). Correspondingly, the natural water is also under severe stress as a result for the rising demand of freshwater caused by the increase in world population, urbanization and industrialization (Gleick & Palaniappan, 2010). It was estimated that the world population would increase to 9 billion at the end of this century and more than 80% of this population would live in the cities (DESA, 2009; Godfray et al., 2012). These could lead to a remarkable growth of both urban and industrialized areas and the possibility of providing enough water for the growing population will be very challenging. The rapid growth in population coupled with the massive industrialization and agricultural activities have raised the water demand to a greater extent, even countries with sufficient quantities began considering sustainable water resource management to avoid water insecurity in the near future (Peasey *et al.*, 2000). At the moment, the demand for freshwater and world population growth are at the rate of 64 billion cubic meters and 80 million people per annum, respectively (Godfray et al., 2012). However, the Malaysian water demand and population growth increase annually at the rate of 12% and 1.8, correspondingly (Reed, 2015). Consequently, all these variables have direct or indirect impacts on the water problems as experienced by several developing countries. Therefore, improved awareness of harnessing water resources is a crucial component in addressing current world water security which is the only sustainable goal of living in the 21st century (Nature *et al*., 2011).

The discharge of domestic and industrial effluents into water bodies without adequate removal of the unwanted constituents results in water pollution. The three major sources of river pollution in Malaysia are domestic sewage, agricultural and industrial effluents (Rafia Afroz *et al.*, 2014). Based on the Department of Environment (DOE) registration conducted in 2006, a total number of 18,956 water pollution point sources were identified in the country. The data reveal that sewage treatment plants (47.79%) and manufacturing industries (45.07%) together accounted for more than 90% of the total number of water pollution sources. Meanwhile, animal farms and agro-based industries accounted for only 4.50% and 2.55%, respectively (Malaysian 1st Mathematics in Industry Study Group, 2011). Similarly, a survey of industrial water pollution source distribution from agro-based and manufacturing industries in each state were conducted by DOE (2006) and the results indicated that Selangor (20.49%) and Johor (19.65%) have more than 40% of the total number identified (9,027) (Malaysian 1st Mathematics in Industry Study Group, 2011).

The Sungai Sembrong located in Batu Pahat district of Johor is among the most significant rivers in the state. Currently, the river serves as the potable water source for more than 500,000 people in the area, particularly to the population of Parit Raja (Latiff, *et al*., 2009). However, the activities along the river bank include industries, agricultural activities (like oil palm plantations and paddy fields) and residential areas (Mohiyaden, *et al*., 2014). Consequently, the water from the river has been characterized as highly acidic with high concentrations of metals such as aluminum (Al), iron (Fe) and manganese (Mn). Though the recommended effluent discharge limit from the industrial, agricultural and domestic sewerages were unambiguous in the country's environmental guideline, the activities along the river had direct contributions to the level of pollution observed by the river (Latiff, *et al*., 2009).

In order for all living things to live in a safer environment, there is a need to address the severe damage done to the environment. This is due to the continuous increase in pollutant agents such as heavy metals and endocrine disruptors in the environment that make the environment unfavorable and causes dangerous health distress to the population (Jodeh *et al*.,2015).

1.1 Techniques for the treatment of wastewater

The major purpose of wastewater treatment is to reduce the physical, chemical and biological constituents to a level recommended for drinking and other daily life activities and subsequently to avoid health related problems associated with contaminated water. The applications of treatment technologies such as itation, coagulation, flocculation, activated sludge, etc. in wastewater remediation have been documented extensively in the literature (Akpor & Muchie, 2010). However, the emergence of thousands of new chemical compounds in our water systems makes the earlier technologies to be impotent in eradicating all the undesirable materials in the wastewater.

Nevertheless, the physicochemical methods used in heavy metals and other pollutant treatment are extremely costly and labor-intensive (Karami & Sahmsuddin, 2010). In addition, these methods use the enormous quantity of chemicals and nutrients and magnify the amount of chemical concentrations in the sludge which required further treatment (Akpor & Muchie, 2010). However, the physicochemical procedure could be used beneficially if the volume of the wastewater is small, specifically for in-house treatment for smaller industries (Singh *et al.,* 2012). Recently, phytoremediation has been acknowledged as a novel technology for efficient wastewater treatment which is well accepted by the people, for the reason that it is ecofriendly and cost-effective (Ali *et al*., 2013).

1.2 Problem statement

Higher concentrations of metals in the Sungai Sembrong are causing serious health concern to the population that the river serves as the only source of freshwater. Basically, the water from the river is highly acidic and with high level of metals concentrations such as aluminum (61.0 mg/L), Iron (33.0mg/L), and manganese (1.5mg/L) making it one of the most contaminated rivers in Malaysia (Ab. Aziz *et al.*, 2009). Although the water is treated first before being discharged for human consumption, to remove the metals to meet the recommended level using the current traditional methods is quite challenging (Awang *et al*., 2015). Phytoremediation has several promising abilities for cost effective and reliable performance in removing organic and inorganic contaminants from surface water and soil (Nwoko, 2010). Therefore, the aim of this study is to treat Sungai Sembrong water using two different plants namely: *Pistia stratiotes* and *Neptunia oleracea*.

1.3 Scope of the study

The aim of this study is to use locally available plants to remove heavy metals concentrations of Sungai Sembrong. The process would help in reducing the organic constituents of the water such as biological oxygen demand (BOD) and chemical oxygen demand (COD). The research intends to use two plants from Malaysia *Neptunia oleracea* (Water mimosa) and *Pistia stratiotes* (Water lettuce). The heavy metal and organic constituents of the water will be determined using water and wastewater standard method (APHA, 2012). The treatment performance of the two plants would be evaluated to ascertain their heavy metal removal efficiency. The best plant will be recommended to be used for pretreatment option in the water treatment plant located in the area. For the analysis, atomic absorption spectroscopy and other tests were carried out following a standard method. Their efficiency as treatment agents of the river water will be compared. The better species will be promoted to provide possible recommendations to improve the water quality of Sungai Sembrong.

1.4 Objectives

The general aim of this research is to investigate the water quality of Sungai Sembrong at Parit Raja, Batu Pahat, Johor, Malaysia. Then, the research intends to evaluate the efficiency of two native plants which could be used to treat the water especially heavy metals concentration of the water. The specific objectives of the research are;

1. To determine the water quality of Sungai Sembrong at Parit Raja based on 6 parameters which are pH, Dissolved Oxygen (DO), Suspended Solids (SS), Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD) and Ammonia Nitrate (NH_3- N) in order to classify the river;
2. To determine the efficiency of *Neptunia oleracea* and *Pistia stratiotes* as phytoremediation agent for pH, DO, conductivity, BOD, COD, NH_3-N, total phosphorus and the metals (Zn, Fe, and Al);
3. To evaluate the effect of heavy metal in the river water to morpho-anatomical changes of *Neptunia oleracea* and *Pistia stratiotes* tissues;
4. To analyse biominerals distribution among different tissues and structures of *Neptunia oleracea* and *Pistia stratiotes*.

1.5 Significance of the study

This study found that Sungai Sembrong is polluted due to human activities especially in the residential areas. It is acidic and high in metals such as Al, Fe and Zn. The two aquatic plants available in Malaysia, *Neptunia oleracea* and *Pistia stratiotes* do have potentials to treat the water of this river. This offers an excellent opportunity to bioremediation the river water, as the river is crucial in providing water supply to the population of Parit Raja. However, one factor that would require further study is the management of the plants as they grow easily and may cause other environmental problem such as eutrophication.

CHAPTER 2

LITERATURE REVIEW

Pollution occurs when objectionable substances accumulate in the environment beyond the recommended levels which lead to environmental degradation. The pollutants might be in the form of energy or matter that cause adverse effects on the overall conditions of people (Elaine Baker, 2004). Generally, pollution is everything that makes the environment unclean and unhealthy due to its physical, chemical or biological appearance in the ecosystem (Joseph *et al*., 2013). The environment is mainly degraded through the exploitation of natural resources in order to improve human being living conditions. Thus, the impact of environmental pollution extends to our living premises, farmland, atmosphere, water bodies and the natural forests.

Water is the weakest resource ruined by the anthropogenic actions of human being on the surface of earth. The speed surface water deteriorate was between the industrial revolution (1820 - 1840). The agricultural and industrial sectors are the major consumers of ground and surface water, with the respective withdrawal volume of 67% and 23% (Hanjra & Qureshi, 2010). Nevertheless, the alarming effect of these two sectors does not depend on the over usage of the limited water resource only, but rather disposing their wastes into the water bodies (rivers and lakes). These water bodies are the only natural reservoirs for freshwater storage. Those heavy pollutants discharged back to water surface have a greater potential of health susceptibility due to their composition which includes viruses and many traces of toxic compounds.

2.1 Freshwater

Surface water bodies remain as the only easy way of accessing freshwater for our daily needs. Instead of preserving the waterways as the most valuable natural resource to mankind, it becomes the dumping ground for liquid wastes. The deposition of any new materials into the water through useful applications of water (residential areas, institutions, agricultural activities and industrials outlets) leads to water pollution (Schwarzenbach *et al*., 2010). In general, water pollution occurs due to deposition of chemicals and hazardous substances into the water such as domestic sewage, pesticides from agricultural runoff (nitrites, phosphates) and heavy metals (Paper & Faculty, 2015). As a result, the quality of the natural water becomes degraded either by changing the physical, biological and/or chemical properties and make it unsuitable for consumption (Joseph *et al.*, 2013). Generally, materials that usually cause water pollution are divided based on their resulting effects on the water quality such as oxygen demanding wastes, disease-causing agents, organic and inorganic chemicals, sediments, radioactive materials and energy. Although their polluting mechanisms vary, their collective objectives cause the objectionable alteration of the water quality and thus prevent the maximum utilization of the water by living creatures (Black, 1977).

Various causes of environmental degradation might be as a result of rapid urbanization, affluence which increases materials consumption and wastes, poverty, which limits choices on how to sustain the use of environmental resources and non-eco-friendly technologies and processes which use energy and national resources. Similarly, human attitude toward economic development through agricultural and industrial activities has given way to the production of huge amounts of chemicals. It is very difficult to destroy hazardous chemicals completely, however, the substances are only changing from one form to another and ultimately enter the environment through various means. The most vulnerable part of the environment is water bodies because all the pollutants deposited either on the land or in the atmosphere are transported into the water through heavy precipitation. For example, both agricultural land and atmosphere have great influences on the river pollution due to nutrients, because the atmosphere contains about 78% dry nitrogen, which can be easily brought to the ground by rain and then collectively run into the rivers with the excess fertilizers in the agricultural area (Castillo, 2010).

2.2 Importance of water

Water is essential for the existence of all living creatures on earth because the human body is made of about 60% water (Herman, 2016). Basically, living things can only survive for a few days without water and this clearly shows how significant freshwater is to human physiological health (White *et al.*, 2010). The polar nature of the water molecules makes it a ‘universal solvents’ because it dissolves many substances than any other liquid and this is responsible for its easy attraction to many foreign substances. Consequently, it is found to be useful in many capacities in the environment ranging from manufacturing, domestic purpose, farming, building, and recreational activities. The world water demand increases at the rapid rate due to population growth, excessive industrialization and movement of people to urban areas. Currently, the demand of freshwater for sustainable development of the human being is increasing at the rate of 64 billion cubic meters annually due to an increase in human population of 80 million per year (Godfray *et al.*, 2012). This rapid increase in the global water demand was first observed during the period of industrial revolution after 1940 and agricultural mechanization in the early 1900’s (Godar *et al.*, 2009).

In Malaysia, the water demand is also increasing at the annual rate of 12% and this is possible since the country’s economy was transformed from agricultural to industrial-based. Despite the challenges of getting affordable clean water at this era, the country’s main focus is toward providing safe drinking water as enshrined by the World Health Organization (WHO) standard (Sumber & Makanan, 2011). According to United Nation (UN), water security is the ability of protecting the sustainable access to sufficient amounts of suitable quality water for livings, human welfare, and socio-economic growth, for guaranteeing protection against water-borne contamination and water-related tragedies, and for conserving environment in a climate of peace and political stability (Baumgartner & Pahl-Wostl, 2013).

2.3 Water pollution in Malaysia

Malaysia is one the countries in the world in which water are abundantly accessible through surface water, rainfall and groundwater, with an average annual rainfall between 1000 to 3000mm. At the moment, about 98% of the country's total water supply comes from rivers to which about 70% are utilized in the agricultural sector (Huang *et al.,* 2015). Heavy industrial and agricultural activities located near the rivers increasied the pollution indices of these rivers, which require additional treatment cost for safer utilization. Furthermore, the heavy pollutants cause the death of aquatic living organisms, for example, eutrophication of rivers induced by the discharge of nutrients and phosphates from agricultural runoff encourage the growth of phytoplankton plants that depletes water oxygen (whereby fish and other living organisms suffocate to death). Similarly, hazardous chemicals and compounds are being transported to human beings through the food chain, because the substances accumulate in fishes and other water-related human diet. Thus, the accumulation of unwanted materials in the water bodies has negative impacts on the ecological systems in terms of health and recreational activities (Najah & Elshafie, 2009).

The river water quality index conducted in 2012 discovered that 34 rivers were categorized as contaminated (Huang *et al.*, 2015). The quality of the rivers is mostly affected by organic and inorganic constituents, however, the inorganic elements have more effects on the treatment performance and health-related damages. Basically, the discharge of water pollution from point sources such as industrial effluents, domestic sewerages and animal farms are termed as point sources, because their origins could be easily traceable in case of any regulation abuse. Moreover, according to the Environmental Protection Agency (EPA) the term point source means "any discernible, confined and discrete conveyance, including but not limited to any, pipe, ditch, channel, tunnel, conduit, well, discrete fissure, container, rolling stock, concentrated animal feeding operation, or vessel or other floating craft, from which pollutants are or may be discharged". This term does not include agricultural storm-water discharges and returns flows from irrigated agriculture" (US EPA, 2014). However, non-point source water pollution is among the principal cause of water quality degradation, because the overflow of water due to rainfall usually sweeps excess fertilizers, herbicides and insecticides from agricultural lands, oil, grease and toxic chemicals from residential estates are difficult to control. Similarly, nonpoint water pollution encompasses total water pollution sources that do not meet the standard characterization of “point source” as in section 502(14) of the Clean Water Act (US EPA, 2014).

In Malaysia, about 1,662,329 water pollution point sources were identified in 2012, whereby the food service outlets dominated the number with 192,710 channels followed by domestic treatment plants (9,883), manufacturing (4, 595), wet market (865), animal farm (754) and agro-based industries (508) (Huang *et al.*, 2015). In Johor, Sungai Sembrong is among the worst rivers rated for high heavy metals pollution, mainly due to agricultural and industrial point sources and nonpoint sources of water pollution (Awang *et al.*, 2015).

2.4 River classification in Malaysia

In Malaysia, the Department of Environment (DOE) developed a Water Quality Index system (WQI) to analyze trends in water quality of rivers in the country based on 6 parameters which are Dissolved Oxygen (DO), Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Total Suspended Solids (TSS), Ammonia Nitrogen (NH3-N) and pH. WQI, in common with many other indices systems, relates a group of water quality parameters to a common scale and combines them into a single number in accordance with a chosen method or model of computation.

The main objective of the WQI system is to be used as a preliminary means of assessment of a water body for compliance with the standards adopted for five designated classes of beneficial uses. The desired used of WQI to an assessment of water quality trends for management purposes even though it is not meant specially as an absolute measure of the degree of pollution or the actual water quality. The river classification based on the DOE-WQI is given in Table 2.1 and 2.2. Rivers are usually classified according to their beneficial uses as shown in Table 2.3. Table 2.4 shows the calculation of sub-index properties for each parameter in order to obtain the WQI value while Table 2.5 shows the classification based on INWQS and the parameters involved.

Table 2.1 Classification WQI-DOE

WQI-DOE Value	**Condition**
90-100	Very Good
75-90	Good
45-75	Average
20-45	Polluted

Table 2.2 The river classification based on the DOE-WQI (DOE, 1986)

Parameter	Unit	Classes				
		I	II	III	IV	V
Ammonium Nitrogen	mg/L	< 0.1	0.1-0.3	0.3-0.9	0.9-2.7	> 2.7
Biochemical Oxygen Demand	mg/L	< 1	1 - 3	3 - 6	6 - 12	> 12
Chemical Oxygen Demand	mg/L	10	10 - 25	25 - 50	50-100	> 100
Dissolved Oxygen	mg/L	> 7	5 - 7	3 - 5	1 - 3	< 1
pH	-	> 7.0	6.0-7.0	5.0-6.0	< 5.0	> 5.0
Total Suspended Solids	mg/L	< 25	25 - 50	50-150	150-300	> 300
Water Quality Index (WQI)		> 92. 7	76.5-92.7	51.9 - 76.5	31.0 - 51.9	< 31.0

Table 2.3 Interim National River Water Quality Standards River Classification (DOE, 1986)

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

Table 2.4 Subindex parameters to calculate DOE-WQI (DOE, 1986)

Parameter	Value	Subindex equation
COD	For X = < 20 For X > 20	$SI_{COD} = 99.1 – 1.33*X$ $SI_{COD} = 103 * [exp]^{-0.0157*X} – 0.04*X$
BOD	For X = <5 For X >5	$SI_{BOD} = 100.4 – 4.32*X$ $SI_{BOD} = 108 * [exp]^{-0.055*X} – 0.1*X$
NH_3-N	For X = < 0.3 For 0.3 < X < 4 For X = > 4	$SI_{AN} = 100.5 – 105*X$ $SI_{AN} = 94 * [exp]^{-0.573X} – 5*(X-2)$ $SI_{AN} = 0$
SS	For X = < 100 For 100 < X < 1000 For X= > 1000	$SI_{SS} = 97.5 * [exp]^{-0.00676*X} + 0.05*X$ $SI_{SS} = 71 * [exp]^{-0.0016*X} – 0.015*X$ $SI_{SS} = 0$
pH	For X < 5.5 For 5.5 = < X < 7 For 7 = < X <8.75 For X = > 8.75	$SI_{pH} = 17.2 – 17.2*X + 5.02*X^2$ $SI_{pH} = -242 + 95.5*X – 6.67*X^2$ $SI_{pH} = -181 + 82.4*X – 6.05*X^2$ $SI_{pH} = 536 – 77*X + 2.76*X^2$
DO	X = DO*12.6577 For X = < 8 For X = > 92 For 8 < X < 92	$SI_{DO} = 0$ $SI_{DO} = 100$ $SI_{DO} = -0.395 + 0.030*X^2 - 0.00020*X^3$

The formula used in the calculation of the DOE's WQI is:

WQI = (0.22 X SIDO) + (0.19 X SIBOD) + (0.16 X SICOD) + (0.15 X SIAN) + (0.16 X SISS) + (0.12 X SIpH).

Where: X is the concentration of the parameters in mg/L, except for pH and DO. SIDO, SIBOD, SICOD, SIAN, SISS and SIpH are the Sub Indices (SI) of the respective water quality parameters which is used to calculate the Water Quality Index (WQI).

Table 2.5 Interim National River Water Quality Standards (INWQS) for Malaysia (DOE, 1986).

PARAMETER	UNIT	CLASS					
		I	IIA	IIB	III#	IV	V
Ammonia Nitrogen	mg/l	0.1	0.3	0.3	0.9	2.7	>2.7
BOD_5	mg/l	1	3	3	6	12	>12
COD	mg/l	10	25	25	50	100	>100
DO	mg/l	7	5-7	5-7	3-5	<3	<1
pH	-	6.5-8.5	6-9	6-9	5-9	5-9	-
Color	TCU	15	150	150	-	-	-
Conductivity	µmhos/cm	1,000	1,000	-	-	6,000	-
Floatables	-	N	N	N	-	-	-
Odour	-	N	N	N	-	-	-
Salinity	ppt	0.5	1	-	-	2	-
Taste	-	N	N	N	-	-	-
Total Dissolved Solid	mg/l	500	1,000	-	-	4,000	-
Total SS	mg/l	25	50	50	150	300	>300
Temperature	^{o}C	-	Normal ± 2	-	Normal ± 2	-	-
Turbidity	NTU	5	50	50	-	-	-
Fecal Coliform	counts/100ml	10	100	400	5,000	5,000	-
Total Coliform	counts/100ml	100	5,000	5,000	(20,000)* 50,000	(20,000)* 50,000	>50,000

2.5 The importance of Sungai Sembrong

Sungai Sembrong is the major source of freshwater supply for the community around the area as the river supplies three-quarter of the areas' water demand. The remaining one-quarter requirement of the area is covered by River Bekok. In 1984, Sembrong dam (130.0 km^2) was completed at the cost of RM 24 million for the flood prevention. However, the dam currently serves dual purposes since it serves as a storage facility for Syarikat Air Johor (SAJ) for water supply. Presently, about 2,000,000 liters of water is extracted from the dam per day for domestic consumption. Therefore, the dam provides a source of freshwater supply for about 250,000 consumers around the Kluang district for their domestic applications (Awang *et al*., 2015). However, the prolong drought experienced by the area between December 2004 to July 2005 had reduced the volume of the dam water to the lowest minimum. These affected more than 500,000 dwellers of Batu Pahat and Kluang that usually depend on the dam as source of freshwater supply (Latiff, *et al*., 2009).

Sungai Sembrong is basically characterized as having low pH mainly due to the oxidation of the pyrite compounds found in the areas (Latiff, *et al*., 2009). These affect the cost of the raw water treatment since large quantities of neutralizing chemicals are required to reduce the acidity to achieve drinking quality level (Teresa *et al.*, 2011). Furthermore, the corrosion of water pipes of the treatment plants by the low pH will result in accumulation of numerous toxic constituents in the water that might need additional treatments to meet the average quality for human consumption. For example, in 2009, the water quality of the river in terms of pH, aluminum, iron and manganese was reported to exceed the Malaysian standard for drinking water quality (Latiff, *et al*., 2009). These subsequently affected the normal operation of the water treatment plant and thus upset the normal supply of drinking water to the community.

Pollution of this river by heavy metals is a serious problem because many of such pollutants are both highly toxic and not biodegradable in the natural environment (Morillo *et al*., 2002). Heavy metal contamination in aquatic and soil environments threatens aquatic ecosystems, agriculture and human health (Overesch *et al*., 2007). In addition, these elements can be accumulated in sediment and in the tissues of living organisms in the river. Aquatic plants absorb heavy metals from the water column and pore water because of the pressure exists between the plant's xylems and the groundwater pore water. These heavy metals can be incorporated into the food chain, and their levels can increase through biological magnification (Cardwell *et al.*, 2002).

2.6 Causes and sources of pollution at Sungai Sembrong

The major source of pollution at Sungai Sembrong is the overflow of nutrients and heavy metals mostly found on the agricultural land and nearby industries through the river tributaries once the area experiences heavy rainfall (Latiff, *et al*., 2009). Similarly, the soil composition (acid sulphate) of the area also contributes to the rivers' water quality degradation. The soil has been characterized as having a low pH and high concentrations of aluminum, iron and sulphate (Minh *et al*., 1997). Mostly, the acidic sulphate soils persistent nature results, when the natural neutralization ability of the area is below the level of oxidation of pyrite in acid (Shamshuddin *et al.*, 2004). Likewise, during prolonged dry season, the moisture contents of the soil drop rapidly which activates the oxidation of the pyrite sulphate to ions, thereby reducing the soil pH level (Auxtero *et al*., 1991; Tin & Wilander, 1995). However, the condition of the inner part of the soil remains stable and ecofriendly during the rainy season because high moisture content of the soil prevents the penetration of oxygen to trigger the reaction (Shamshuddin *et al*., 2004a). Moreover, the reaction remains active on the soil surface at all time of the season, since both water and oxygen have significant roles in pyridine oxidation as shown in equation 2.1 (Rassam *et al*., 2002).

$$\mathrm{FeS_{2(s)}} + \frac{15}{4}\mathrm{O_{2(g)}} + \frac{7}{2}\mathrm{H_2O} \rightarrow \mathrm{Fe\,(OH)_{3(s)}} + 4\mathrm{H^+}_{(aq)} + 2SO^{2-}_{4\,(aq)} \qquad \text{(Eq2.1)}$$

As a result, the sulphuric acid produced from the reaction destroys the clay mineral formation of the soil by liberating Iron, Aluminum irons and other heavy metals (Cook *et al*., 2000; Shamshuddin *et al*., 2004). Another effect of the acid sulphate soil is the prevention of water logging and draining of industrial and agricultural runoff, which expose the pyrites to the atmospheric conditions for further oxidation (Rassam *et al*., 2002; Shamshuddin *et al*., 2004). These dangerous compounds emanating from the oxidation of sulphate soil easily move into the rivers as rainfall runoff and thereby deteriorating the water quality. In addition, unless appropriate control measures are done to condition the soil, toxic substance will penetrate into the surface and ground water through leaching and causes more severe damage to the ecosystem (Lin *et al*., 1998). The major metals known to be associated with the acid sulphate soil are aluminum, iron and manganese and are all considered carcinogenic when their daily consumption are above the recommended levels. The high level of aluminum from the river is possible, since aluminum is naturally available in the earth's crust and is easily released into the environment either through chemical speciation, hydrological flow paths, soil-water interactions, and the composition of the underlying geological materials (World Health Organization, 2003). Iron is the second most plentiful metal found on earth's crust joined together with oxygen and sulfur containing compounds as oxides, hydroxides, carbonates and sulfides (World Health Organization, 2003).

Generally, the degradation of water quality of the Sungai Sembrong is highly related to the soil condition of the area. However, other sources of pollution like discharges of partially treated effluents from domestic and industrial activities in the river could not be ruled out as possible contributions in the reduction of the river water quality. Several industries such as fiberboard, electronics, food and textile industries and agricultural-based industries are located within the vicinity of the rivers and they usually discharge their treated effluents into the river. The situation transforms the natural state of the river catchment area, thus gradually changes the ecological condition of the river. Currently, there are about 718 hectares of palm oil production near the Sungai Sembrong and this could be the reason why the raw water pumped from the river to Sembrong water treatment plant has high content of manganese and iron (Awang *et al.*, 2015). The water treatment plants that draw raw water from the river shut down their operations during the monsoon period because of the level of toxic compounds such as ammonium, iron, aluminum and manganese. Moreover, the nutrients build-up in the soil caused mainly by plantation fertilizer leftover are easily flowing into the rivers (Smith *et al*., 2007). In most cases the soil is being saturated and these chemicals find their way either through underground leaching or surface water runoff and causes acute damages to the water system.

Additional concern for Sungai Sembrong is the construction of series of drainage systems close to the river which lead to further degradation of the river water. This is because, the river could easily be contaminated through nonpoint sources, especially when the river flows through an agricultural area (Petersen Jr *et al*., 1987; Katimon *et al*., 2004). Besides, the no nonpoint source water pollution is the main problem encountered when is found in agricultural and industrial areas. Thus, any leftover chemicals from agricultural activities around the river catchment area and the alteration of the waterfront area through unnecessary building of concentrated drainage structures could contribute adversely to the degradation of the surrounding surface water (Evans *et al.*, 1996).

High levels of water pollution in the rivers is recognized by the appearance of several invasive aquatic plants, such as *Eichhornia crassipes* (water hyacinth) and *Fallopia japonica* (Japanese knotweed) which covers the entire river and deny other plants access to sunlight. These plants usually become ecological adversity to any river or lake they occupied, because they suffocate the water body and causes the reduction of fishes in the water. Hence, the presence of these plant species signals the impacts of human activities to the rivers (Tan, 2011). However, the plants were also identified as among the factors for the eutrophication and they are mostly found in the Sungai Sembrong where human activities are predominant (Tan, 2011).

2.7 Water quality parameters

The water quality parameters that are commonly used for pollution index identification in the water bodies are discussed as follows:

2.7.1 pH

Basically, pH level indicates the acidity or alkalinity of water and it measures the relative amount of free hydrogen and hydroxyl ions in the water. Normally, it varies across a scale of 0-14, with the average being the neutral water (pH=7) and values below and above the neutral point are acidic and alkaline, respectively. The instability of H^+ and OH^- ions concentrations of water due to other environmental conditions of the water storage medium or introduction of foreign materials into the water are responsible for the rise of pH variability in water (Carr *et al*., 2009). Hence, there is no specification pH level for general aquatic life requirement since each species adapts to different pH range. Perhaps, pH values between 6.5 to 9.0 is considered appropriate for freshwater aquatic life while the marine aquatic survival range is between 6.5 to 8.5 (Chin, 2006). Consequently, pH has a huge effect on the chemical, physical and/or biological transformations taking place which causes indirect pollution to the water system.

2.7.2 Dissolved Oxygen (DO)

Dissolved oxygen is well-known as the most significant factor that could influence the health of aquatic environment such as fish mortality, odors, and other aesthetic quality of surface waters (Chin, 2006). Usually, several aquatic organisms depend on oxygen for their basic metabolic activities and this is affected by the septic condition of the water, temperature, salinity and pressure. The solubility of oxygen and other gases are highly affected by the temperature and salinity, because low temperature increases the solubility of the oxygen while high salinity causes the decrease in oxygen solubility (Black, 1977). Similarly, the increase in atmospheric pressure causes the increase in the dissolved oxygen of the water (Vernberg & Vernberg, 2001).

In general, oxygen is found in the water through either aquatic plants photosynthesis (during daylight) or air-water interface interactions. However, the aquatic plants produce oxygen only during the daylight and consume it at night for more functions(Teresa *et al.*, 2011) On the other hand, the rate of air-water transfer depends largely on the surface area of the boundary and water movement. Thus, environmental agencies regularly monitor the amount of dissolved oxygen concentration in water bodies to avoid extreme cases of total oxygen depletion. Fundamentally, living creatures in water require oxygen for their metabolic activities and thus the insufficient amount of dissolved oxygen below the essential level in water bodies directly affects the living conditions of those organisms. For instance, when dissolved oxygen drops below 5mg/L for longer period causes the death of fishes and other shellfish species (Chin, 2006). This is due to the minimum amount of dissolved oxygen required for fish survival is in the range of 10-15 mg/l (Black, 1977). There are several mechanisms that reduce the concentration of dissolved oxygen in the water bodies which are the decrease in the rate of photosynthesis (especially at night), drop of water solubility along water profile, restriction of air-water interface transfer and increase in the biodegradable constituents.

2.7.3 Biochemical Oxygen Demand (BOD)

Biochemical oxygen demand (BOD) is another parameter that water and wastewater experts use to determine the amount of biodegradable substances in the water. It simply measures the amount of oxygen needed by aerobic microorganisms in order to decompose the organic materials in the water (Carr & Neary, 2009). The BOD_5 is the most commonly used protocol to test the quantity of oxygen required to biochemically oxidize the organic matter for a period of five days and it’s measured in mg/L.

Generally, BOD is a widely acceptable parameter in determining the oxygen demand of municipal and industrial effluent quality for final discharge to the receiving water bodies. However, the longer period needed for determining BOD (usually 5 days) make it unattractive and obliged water and wastewater personnel tend to look for alternative methods such as Chemical oxygen demand (COD) and total organic carbon (TOC). Therefore, elevated level of BOD in water streams has a harmful effect on the aquatic life, because the limited dissolved oxygen (DO) needed for their survival is being diverted for decomposition of organic matter (Weiner, 2000).

2.7.4 Chemical Oxygen Demand (COD)

Chemical oxygen demand (COD) is a water quality parameter to indicate the level of pollution in the water based on chemical characteristics and is a measure of the amount of oxygen required to oxidize the organic matter chemically by a strong oxidant known as dichromate and sulfuric acid. COD is therefore an estimate of the amount of organic and reduced matter presents in the water or better known as the amount of oxygen needed to chemically decompose the organic matter in the water (Varsha Gupta et al., 2017). Oxidizable chemicals introduced into natural water will initiate chemical reactions. Those chemical reactions create what is measured in the laboratory as the COD. Oxygen consumption by reducing chemicals such as sulfides and nitrites is typified as follows:

$$S^{2-} + 2O_2 \rightarrow SO_4^{2-} \quad \text{(Eq 2.2)}$$

$$NO_2 + ½\, O_2 \rightarrow NO^{3-} \quad \text{(Eq 2.3)}$$

Both the BOD and COD tests are a measure of the relative oxygen-depletion effect of a waste contaminant. Both have been widely adopted as a measure of pollution effect. The BOD test measures the oxygen demand of biodegradable pollutants whereas the COD test measures the oxygen demand of biodegradable pollutants plus the oxygen demand of non-biodegradable oxidizable pollutants. COD almost always exceeds BOD in water. The ratio of COD to BOD also indicates to what extent a wastewater is amenable to biological treatment methods (Tchobanoglous *et al.*, 2003).

2.7.5 Total Suspended Solids (TSS)

The amount of particles that suspend in a sample of water is called total suspended solids (TSS). It is important in drinking water quality and has significant impact on fish and other aquatic life. Total Suspended Solids are also a major carrier of inorganic and organic pollutants and other nutrients. To remain permanently suspended in the river, particles must have relatively low density, small in size, and have a surface area that is large in relation to their weight. The greater the TSS in the water, the higher its turbidity and the lower its transparency (McCaull & Julian, 1974). The source of TSS may be from loose soil brought by surface run off and also from particulates carried by domestic wastewater.

2.7.6 Nutrients

Nutrients are essential for growth and maintenance of life for living organisms. They are generally classified into two: macronutrients and micronutrients according to their required level of living things.(Teresa *et al.*, 2011) Macronutrients are those nutrients that are needed in large quantities (e.g., carbohydrate, protein, fat, water and oxygen) for providing energy during the metabolic process, while the micronutrients are required in smaller quantities (iron, cobalt, chromium, copper, iodine, manganese, selenium, zinc and molybdenum and other organic compounds and vitamins) for building and repairing of tissues and body processes regulation. Therefore, the availability of excess amount of nutrients like nitrogen and phosphorus in the water system promotes the growth of unwanted plants (algae) which causes eutrophication of water bodies. Although nutrients are required in small amounts, however, their influence is responsible for total productivity in the environment (Chin, 2006). Therefore, brief explanation of nitrogen and phosphorus is discussed further, since major organic compounds found in water bodies contain them.

2.7.6.1 Phosphorus

Phosphorus is the eleventh most abundant mineral in the Earth's crust. However, phosphorus is highly reactive and it is difficult to obtain it as a free element in the environment. Moreover, it can combine with many elements and form phosphate compounds such as autunite (Ca $(UO_2)_2(PO_4)_2$10-12H_2O), carnotite (K_2 $(UO_2)_2(VO_4)_2 3H_2O$), (phosphophyllite Zn_2 (Fe, Mn) $(PO_4)_2 \cdot 4H_2O$), struvite ((NH_4) $MgPO_4 \cdot 6H_2O$). It has a significant role in living things such as promoting and controlling the growth rate of plant metabolic processes of animals, respectively. However, the consequences of high levels of phosphorus in the freshwater is the rapid growth of some organisms (e.g. algae) at the expense of others and the breakdown of inhabitants depressed of a vital source of livelihood such as oxygen and sunlight. Commonly, phosphate compounds are among the major constituents of total dissolved solids (TDS) in water pollution quality index determination, though not all phosphorus is in molecular form for easy breakdown by algae (Vanni & Layne, 1997).

2.7.6.2 Ammonia Nitrogen

Generally, ammonia nitrogen (NH_3-N) is a water quality parameter that measures the total amount of ammonia in liquid waste products such as domestic sewage, agro-based liquid wastes and other liquid organic wastes. Ammonia nitrogen is mostly found in rivers through the microbial degradation of nitrogenous organic matter such as human excrement from domestic sewage. The major source of ammonia in the environment is through the application of fertilizers on the farm land, because the excess amounts are washed into the streams and causes serious sanitary problems in the water bodies (Jafari, 2008). The amount of ammonia nitrogen concentrations depends on the original source, for example, liquid manure from dairy farm might contain NH_3-N of up to 1600 mg/L (Swensson, 2003), while sewage wastewater has an average of 250 mg/L of NH_3-N concentration (Manios *et al.,* 2003).

2.8 Metals

The rapid industrialization has increased the application of metals as raw materials for production of several products (e.g. paints, automobiles, electroplating, electrical and electronics). The effluent of those industries contains high levels of toxic heavy metals and their presence poses natural treatment difficulties due to their non-degradable and persistence nature (Ahluwalia & Goyal, 2007). Similarly, heavy metals can be found in the environment through soil and rock weathering, atmospheric deposition and metallurgical activities by human being (Chin, 2006). Moreover, the bioaccumulation of heavy metals by some aquatic organisms in the water is amongst the major channels through which they get into human body and cause health problems. Chronic exposure to heavy metals causes permanent disability like autism, schizophrenia, and mental retardation and in some cases lead to human and animal’s death. Recently, lead was found to be a silent killer due to its gradual accumulation in the human body through various environmental exposures and the resulting effects include interference to many organs and tissues like heart, kidney, and bones, reproductive and nervous organs. However, the most vulnerable human organ to lead exposure is the brain, because it has greater influence on retarding learning capability, especially in children causing permanent learning problems (Cecil *et al.*, 2008). However, sources of heavy metals in surface water bodies, specifically, Sungai Sembrong are from natural and anthropogenic activities like development of industrial, agricultural and mining sites along the river bank (Ahmad *et al.*, 2009).

2.8.1 Zinc

Zinc is typically available as the 24th most abundant element on earth with five stable isotopes. It is mostly used in electroplating of iron, steel and other metals against corrosion. Zinc is an important element found in living organisms and plants and its deficiency in human causes growth retardation, infection vulnerability, diarrhea and late sexual maturing (Hambidge & Krebs, 2007).

Exposure to free zinc ion in solution is extremely poisonous to living organisms, for example, only 6 micro molar zinc iron killed 93% of all *Daphnia* in water (Eisler, 1988; Muyssen *et al*., 2006). The dissolution of zinc ion in water increases with increased in pH (Frey & Reed, 2012). In humans, consumption of large quantity of zinc within a short period of time causes stomach disorder such as stomach cramps, nausea and vomiting, while long term ingestion causes anemia, pancreas damage and drop in high-lipoprotein cholesterol (Frey & Reed, 2012). Similarly, hazardous zinc compounds like zinc chloride, zinc oxide, zinc sulphide and zinc sulphate are mostly found in industrial wastes due to their utilizations in production of white paints, ceramics, rubber, dyeing fabrics and wood preservatives (Frey & Reed, 2012). On the other hand, zinc and its compounds found their way into water bodies through either natural practices and/or human activities such as metallic extraction and chemical industries, domestic sewage and runoff from soil containing zinc compounds (Tsang *at el*., 2013).

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