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

MOHAMED.B.E.BOGELIL

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Universiti Tun Hussein Onn Malaysia

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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

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