REMOVAL OF SELECTED POLYCYCLIC AROMATIC HYDROCARBONS USING Phragmites AND Vetiver IN HORIZONTAL SUB –SURFACE FLOW CONSTRUCTED WETLAND

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By

RABIA DAW ALI

Thesis Submitted to the School of Graduate Studies Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Doctor of Philosophy

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DEDICATION

"And they ask you about the Spirit. Say: "The Spirit by command of my Lord: and you are not given aught of knowledge but a little."

(Al-Quran Alkareem, Surat Al-Israa, 85)

Every challenging work needs self-effort as well as the guidance of elders especially those who are close to our hearts. Whose affection, love, encouragement and praise through day and night make me able to reach such success and honor and the reason of what I become today. I dedicate my humble effort I to my sweet and loving

My father Daw Alsghayer, and my mother Aisha Amar

My first guides and teachers

My Husband

Whom always have been my epitomes of strength

My Kids, My Family, My Friends

I am really grateful to you all!

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

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July 2020

Chairman : Associate Professor Salmiaton binti Ali, PhD
Faculty : Engineering

The research and investigation on the removal of polycyclic aromatic hydrocarbons (PAHs) with high concentration (10000µg/L) in constructed wetlands under field conditions has not been explored prior to this. Hence, in this study, Horizontal Subsurface Flow Constructed Wetland (HSFCWs) was used to remove three polycyclic aromatic hydrocarbons. The synthetic PAHs that were used in the experiments include Phenanthrene, Pyrene, and Benzo [a]Pyrene in percentages that reflected their actual contents in the industrial wastewater. The CWs sustainable treatment technique incorporates two plants namely *Phragmites Karka* (*Phragmites*) and *Vetiver Zizanioides* (*Vetiver*) where they were tested for their ability in PAHs tolerance and uptake in pot experiments for 20 days using two different concentrations (2500 and 10000µg/L). Then, it was followed by treatment of high PAHs concentration (10000µg/L) using eight Horizontal Subsurface Flow Constructed Wetland (HSFCWs), each with dimensions of (length=90cm x width=30cm x depth=50cm) and planted with *Phragmites* and *Vetiver*. While, the other twelve constructed wetlands were smaller in size (length = 45cm, width = 15cm and height = 30cm) and were used mainly to replace plants in the big CWs after sampling. The experiments on the CWs were conducted to measure plant growth, PAHs concentration in plants, PAHs removal efficiency, accumulation of PAHs in the soil of CW, and lipid effect on PAHs accumulation during sampling days according to a predetermined pattern (7, 14, 28, 42, and 72 days). The quantitative analysis of PAHs concentration was conducted by GC-FID. The mass balance technique was conducted to determine the distribution pathways of PAHs in HSFCWs. The effect of PAHs on the surface structure of different parts of *Phragmites* and *Vetiver* was investigated by using scanning microscopy. The capacity of the HSFCWs to address the wastewater contaminated with PAHs was modelled by using multiple regression stepwise method. The results revealed that the growth parameter was significantly different among the two plants. The highest concentrations of three PAHs were found to be in *Phragmites*

shoot and root system with 229µg/g and 192µg/g, for Phenanthrene, 69µg/g and 59µg/g for Pyrene, and 25µg/g and 20µg/g for Benzo [a]Pyrene respectively. While the greatest concentrations of the same compounds in the *Vetiver* shoot and root systems were 88µg/g and 64µg/g for Phenanthrene, 63µg/g and 42µg/g for Pyrene, and 21µg/g and 27µg/g for Benzo[a]Pyrene respectively. The maximum difference in removal rates between planted constructed wetland and unplanted constructed wetland was found to be 21% Phenanthrene, 13% Pyrene, and 30% Benzo[a]Pyrene. Both selected plants demonstrated high tolerance, uptake, and accumulation of PAHs in different proportions. The HSFCWs planted with *Phragmites* showed high removal capacity of PAHs than other HSFCWs. Under scanning electron microscopy some notable changes were observed in the internal composition of both plants. The mass balance calculations of the HSFCWs provided a clear picture of the key constructed wetland processes and helped to identify the components that are most important for PAHs treatment using HSFCW.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

PENYINGKIRAN HIDROKARBON AROMATIK POLISIKLIK TERPILIH MENGGUNAKAN *Phragmites* DAN *Vetiver* DALAM TANAH BENCAH BUATAN ALIRAN SUBPERMUKAAN MENDATAR

Oleh

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Penyelidikan dan penyiasatan terhadap penyingkiran hidrokarbon aromatik polisiklik (PAH) dengan kepekatan tinggi (10000µg/L) dalam tanah bencah buatan dalam keadaan lapangan belum pernah diterokai secara mendalam sebelum ini. Oleh itu, dalam kajian ini, Tanah Bencah Buatan Aliran Subpermukaan Mendatar (HSFCWs) digunakan untuk menyingkirkan tiga hidrokarbon aromatik polisiklik. PAH sintetik yang digunakan dalam eksperimen ini termasuklah Phenanthrene, Pyrene, dan Benzo [a]Pyrene dalam peratusan yang mencerminkan kandungan sebenar dalam air sisa industri. Teknik rawatan lestari CW menggabungkan dua tumbuhan iaitu *Phragmites Karka* (*Phragmites*) dan *Vetiveria Zizanioides* (*Vetiver*) di mana tumbuhan tersebut digunakan diuji kemampuan mereka dalam toleransi PAH dan pengambilan PAH dalam eksperimen tanaman pasu selama 20 hari menggunakan dua kepekatan berbeza (2500 dan 10000µg/L). Proses ini diikuti dengan rawatan kepekatan PAH tinggi (10000µg/L) menggunakan lapan tanah bencah buatan aliran subpermukaan mendatar (HSFCWs), masing-masing dengan dimensi (panjang = 90cm x lebar = 30cm x kedalaman = 50cm) yang ditanam dengan *Phragmites* dan *Vetiver*. Dua belas tanah bencah buatan yang lain adalah lebih kecil ukurannya (panjang = 45cm, lebar = 15cm dan tinggi = 30cm) dan digunakan terutamanya untuk menggantikan tanaman di CW besar selepas penyampelan. Eksperimen pada CW dilakukan untuk mengukur pertumbuhan tanaman, kepekatan PAH dalam tanaman, kecekapan penyingkiran PAH, pengumpulan PAH di dalam tanah CW, dan kesan lipid ke atas pengumpulan PAH semasa hari penyampelan mengikut sela masa yang telah ditentukan (7, 14, 28, 42, dan 72 hari). Analisis kuantitatif kepekatan PAH dilaksanakan oleh GC-FID. Teknik keseimbangan jisim dilakukan untuk menentukan laluan pengedaran PAH dalam HSFCWs. Kesan PAH terhadap struktur permukaan bahagian yang berbeza *Phragmites* and *Vetiver* disiasat dengan menggunakan mikroskopi pengimbas. Kapasiti HSFCW dalam menangani air sisa tercemar dengan PAH dimodelkan menggunakan kaedah berperingkat regresi berganda. Hasil kajian menunjukkan bahawa parameter pertumbuhan sangat berbeza di antara kedua tanaman tersebut.

Kepekatan tertinggi tiga PAH didapati dalam sistem pucuk dan akar *Phragmites* dengan 229µg/g dan 192µg/g, untuk Phenanthrene, 69µg/g dan 59µg/g untuk Pyrene, dan 25µg/g dan 20µg/g untuk Benzo [a] Pyrene masing-masing. Manakala kepekatan yang paling hebat untuk kompaun yang sama pada sistem pucuk dan akar *Vetiver* adalah 88µg/g dan 64µg/g untuk Phenanthrene, 63µg/g dan 42µg/g untuk Pyrene, dan 21µg/g dan 27µg/g untuk Benzo [a] Pyrene masing-masing. Perbezaan maksimum dalam kadar penyingkiran antara tanah bencah buatan dengan tanaman dan tanah bencah buatan tanpa tanaman didapati 21% Phenanthrene, 13% Pyrene, dan 30% Benzo [a] Pyrene. Kedua-dua tanaman terpilih menunjukkan toleransi yang tinggi, sementara pengambilan dan pengumpulan PAH pula dalam perkadaran yang berbeza. HSFCW yang ditanam dengan *Phragmites* menunjukkan kapasiti penyingkiran PAH yang tinggi berbanding dengan HSFCW yang lain. Di bawah mikroskopi elektron pengimbas, terdapat beberapa perubahan ketara dalam komposisi dalaman kedua-dua tumbuhan tersebut. Pengiraan keseimbangan jisim HSFCW memberikan gambaran yang jelas untuk proses tanah bencah buatan dan membantu dalam mengenalpasti komponen yang paling penting untuk rawatan PAH menggunakan HSFCW.

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This thesis was submitted to the Senate of the Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

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LIST OF ABBREVIATIONS

PAH	Polycyclic aromatic hydrocarbons
HMW	High Molecular
LMW	Low Molecular Weight
Phe	Phenanthrene
Pyr	Pyrene
B[a]P	Benzo[a]Pyrene
HSSFCW	Horizontal Sub Surface Flow Constructed Wetland
CW	Constructed wetland
SE	Standard Error
TF	Translocation Factor
SCF	Shoot concentration Factor
RCF	Root concentration Factor
RGR	Relative Growth Rate
GC-FID	Gas chromatography-flame ionization detector
EPA	Environmental Protection Agency
SEM	Scanning electron microscope
U.S.D.A	United State Department of Agriculture
EDAX	Energy-Dispersive X-Ray
MLR	Multiple Linear Regression

CHAPTER 1

INTRODUCTION

1.1 Background

Industrialisation contributes towards negative environmental casualties such as pollution, global warming and many other adverse impacts. The rapid increase in industrialisation over the last century has led to elevated releases of anthropogenic chemicals into the environment. Consequently, there is a group of substances called polycyclic aromatic hydrocarbons (PAHs) which are the results of contamination in almost all environmental resources. Particularly, in the industrial zones where petrochemical and petroleum refinery industries are located, as these are the main sources of PAHs releases. In general, PAHs characteristics are very harmful as they are toxic, mutagenic and carcinogenic organic compounds, and will increase in ecotoxicity with increasing in molecular weight of PAHs (Liu et al., 2016), and hence must be remove from the environment.

The PAH family pollutants have been found in different water bodies all over the world, and these compounds pose, even at very low concentrations, a great threat to ecological and human health due to their benzene structures (Muff & Søgaard, 2010). PAHs are listed as US-EPA and EU priority pollutants, and their concentrations, therefore, need to be controlled. However, only 16 are currently being monitored by US-EPA and the Environmental Commission of European Community (Manoli et al., 2000). PAHs are hydrophobic compounds, which means that once they enter into the water systems it will be difficult to remove through conventional methods, as PAHs are persistent, and non-reactive in the water mainly due to their low water solubility and are part of numerous organic contaminants that are persistent in the environment. In addition, PAH are ubiquitous in the environment, have long transport potential and can cause adverse environmental effects (Abdel-Shafy et al., 2016).

One of the major threats and main issue that is impacting the water quality today is chemical pollution, especially organic challenges which need to be addressed because it includes hundreds of compounds. Among those are hydrocarbon compounds, which are found mostly in industrial effluents, and they can impose a significant threat to the environment and humans. Due to the toxic, mutagenic and carcinogenic natures of PAHs, numbers of methods such as physical, chemical, thermal, biological, surfactant enhanced, phytoremediation and combined technology (constructed wetland) have been developed in order to remove PAHs from contaminated soil, sediment and water (Al-Sbani et al., 2016; He et al. 2014; Li et al.2014; Li et al., 2015; Peng et al., 2015; Sun et al., 2014; Wang et al. 2014; Xu et al., 2016; Yi et al.,2016; Zhou et al., 2013).

Constructed wetland (CW) treatment systems are eco-friendly technologies that mimic the function of natural wetland to improve water quality together with cost-effective method, simple operation guide and more sustainable technique (Chen et al., 2014; Vymazal, 2013; Wu et al., 2013). These eco-technologies offer direct and indirect potential benefits to society such as improving the controlling point and non-point water pollution, cleaning water to acceptable discharge levels, and protecting abiotic and biotic source as well as ecological balance in the ecosystems (Vymazal and Kropfelova, 2008). Therefore, there is little surprise that the research applications of wastewater purification by CWs have dramatically increased in the recent years in the scientific literatures (Wu et al., 2014). Moreover, there are a number of successful applications of CWs for the removal of PAH (Cottin & Merlin, 2008; Kang et al., 2018; Warężak et al., 2015).

The Horizontal Subsurface Flow Constructed Wetland (HSFCWs) beds are the most widely applied CW systems due to its strong advantages which uses simple technology, reliable operating conditions and excellent potential to remove moderate loads of pollutants. The removal rates and performance of HSFCW may vary over time and space and are dependent on multiple factors such as influent wastewater characteristics and wetland plants. Wetland plants are an integral part of those systems and the literature is rich with reports suggesting that plants have a positive role in the removal of pollutants in constructed wetlands through sophisticated interaction of plants with water body, media and microorganism (Chen et al., 2014; Leto et al., 2013; Mesquita et al., 2013; Türker et al., 2016).

The presence of vegetation in CWs has several functions in relation to the treatment process such as the provision of substrates for the growth of attached bacteria, the release of oxygen and exudates, uptake of nutrients, surface insulation and wind velocity reduction (Vymazal, 2013). Overall, there is plenty of evidence indicating that planted CWs are more efficient to remove hydrocarbon compounds with low concentrations as compared with unplanted CWs (Al-Baldawi et al., 2014; Braeckevelt et al., 2008; Mothes et al., 2010).

1.2 Problem Statement

The presence of polycyclic aromatic hydrocarbons in the environment (air, soil, and water) with concentrations higher than the required environmental standards poses a significant risk to the ecosystem and human health (Yang et al., 2015). Many researchers had reported that the concentrations of PAHs in wastewater of several industries had exceeded permissible limits which was set by the environment standards (Al Zarooni and Elshorbagy, 2006; Oh et al., 2016; Sponza and Oztekin, 2010). Regardless of the sources of these pollutants, whether they are pyrogenic or petrogenic, these problems need to be overcome at their sources.

In many countries, modern sewage treatment systems have been used successfully for pollution control (Li et al., 2014). However, application of these techniques for wastewater treatment that include membrane separation, adsorption material, solvent extraction incineration, photocatalysis, and ultrasonic is rather expensive and requires sophisticated heavy machinery, high energy consumption and could cause massive air pollution (Zheng et al., 2013). Nevertheless, other treatment approaches are still limited and insufficient to comply with stringent water and sanitation standards (Wu et al., 2013).

Therefore, other sustainable methods are urgently required in order to remove a high concentration of PAHs from wastewater which include methods like natural or constructed wetlands. There are many advantages associated with using constructed wetlands (CWs) such as low construction and maintenance costs, environmentally friendly, sustainable, and easy to operate when compared to the conventional wastewater treatment technologies (Puigagut et al., 2008). Constructed wetlands for treating polycyclic aromatic hydrocarbon have been investigated by a large number of studies such as (Anderson, 2013; Fountoulakis et al., 2009; Terzakis et al., 2008; Wang et al., 2014), however, the effectiveness of constructed wetlands in treating industrial wastewater with only low PAHs concentrations by using wetland plants were limited.

In CWs, plants play an important role in treatment processes and polishing the quality of treated wastewater (Ko et al., 2011; Liu et al., 2012 and Al-Sbani et al., 2016). However, no information was available in regards to the plant's tolerance and uptake in CWs that are subjected to high concentrations of PAHs. The effects of wastewater contaminated with PAHs on wetland plant morphologies such as *Phragmites* and *Vetiver* were found missing in the literature. The mass balance technique for analyzing the distribution of PAHs concentrations in various components of the constructed wetland systems is one of the gaps that was identified from the literature. In addition, there is lacking in the mathematical model for predicting the concentrations of various PAHs components in the effluent of the constructed wetland that is used for treating high strength industrial wastewater.

In this study, the synthetic wastewater with low and high concentrations (2500 and 10000µg/L) of three compounds of PAHs will be treated using constructed wetland models. These concentrations were selected to reflect the actual concentrations in the industrial wastewater effluent from petroleum refineries and petrochemical industries. Synthetic industrial wastewater which contain PAHs is prepared based on their presence in actual industrial wastewater with ratios of 74.61% for Phenanthrene, 17.11% for Pyrene and 8.28% for Benzo[a]Pyrene (Lu et al., 2013; Sponza & Oztekin, 2010). The investigation will be done to find out the removal efficiency of PAHs, plant uptake, PAHs accumulation in CWs soil, and lipid effect on PAHs accumulation in horizontal subsurface flow constructed wetlands (HSFCWs). The effects of PAHs on the surface structure of different parts of *Phragmites* and *Vetiver* as wetland plants will be evaluated using SEM and EDX. The contributions of different PAHs removal pathways in CWs will be quantified based on the mass balance technique. While, the multiple regression stepwise method will be used for modeling the capacity of the constructed wetland to address the contaminated wastewater with high PAHs concentration (10000µg/L). Initial hypothesis outlined that constructed wetland with the horizontal subsurface flow and presence of the plants would significantly treat a high concentration of PAHs in contaminated wastewater. While, the null hypothesis was that constructed wetland with the horizontal subsurface flow would not address a high concentration of PAHs

1.3 Research Objectives

The main objective of the current study is to assess the performance of a horizontal sub-surface flow constructed wetland system with two plants (*Phragmites* and *Vetiver*) used for treating polycyclic aromatic hydrocarbons (PAHs) that exist in industrial wastewater. While, the specific objectives of this study are outlined as follows:

1. To examine in detail the two wetland plants, namely *Phragmites Karka* and *Vetiver Zizanioides* for their ability to tolerate and uptake low and high concentrations (2500 and 10000µg/L) of PAHs in industrial wastewater.
2. To evaluate the capability of horizontal sub-surface flow constructed wetlands (HSFCWs) in treating contaminated wastewater with a high concentration (10000µg/L) of PAHs using two plants, *Phragmites* and *Vetiver*.
3. To investigate the pathway of PAHs in constructed wetlands based on the components of constructed wetlands using mass balance calculations, the PAH's effect on the surface structure of different parts of *Phragmites* and *Vetiver*, and the capacity model of the constructed wetland.

1.4 Significance of the Study

This study will help tremendously in addressing the issue of wastewater contaminated with high concentration of polycyclic aromatic hydrocarbons (PAHs) through the use of eco-friendly method of constructed wetlands. The significance of the study can be summarized as:

1. In this study, low and high concentrations of PAHs (2500 and 10000µg/L) were used in early-stage pot experiments to test two species of wetland plants namely, *Phragmites* and *Vetiver* for their ability to tolerate and uptake of PAHs from synthetic wastewater to used in the constructed wetland models. The concentrations were selected based on a literature review that examined wastewater sources for PAHs compounds. Three compounds were specifically selected in these concentrations in different proportions (74.61%, for Phenanthrene, 17.11% for Pyrene and 8.28% for Benzo[a]Pyrene), owing to their high presence in wastewater and each represented a group of polycyclic aromatic hydrocarbons with different properties.
2. The models of the horizontal sub-surface flow constructed wetlands (HSFCWs) with two selected plants were examined for their efficiency in treating contaminated wastewater with high concentrations of PAHs.

3. Scanning electron microscopy (SEM) and Energy Dispersive X-ray (EDX) were used to examine the effects of the PAHs on the surface structure of different parts of *Phragmites* and *Vetiver*.
4. The contributions of different PAHs removal pathways in CWs were quantified by using the multiple regression stepwise method, the model of constructed wetlands that gave the best removal of three PAHs compounds was recommended.

1.5 Scope and Limitation of Research

This study mainly focuses on achieving the stated objectives by addressing the sustainable treatment of wastewater contaminated with high concentration of polycyclic aromatic hydrocarbons (PAHs) (10000µg/L) using constructed wetlands. The main limitations of the study are:

1. The effects of microorganisms as earlier documented in other researchers (Al-Baldawi et al., 2015; Al-sbani et al., 2016), and also the volatilization of PAH studied by Nesterenko-Malkovskaya et al. (2012) are not covered.
2. The plants' density and the aeration effects that were studied earlier by (Al-Baldawi et al., 2013; Liu et al., 2014) are not being considered. The horizontal subsurface flow constructed wetlands (HSFCW) is the only system being considered for the treatment in this study, with two wetland plants, namely *Phragmites* and *Vetiver* and unplanted system.
3. In this study, *Phragmites* and *Vetiver* were selected based on specific justifications such as resistance to contaminants, tolerance to environmental conditions, large biomass, fibrous root system, and a large root surface area.
4. This study had utilized eight plant pots for the first experiments and 20 pilots (HSFCW) for the second experiments, where two days theoretical hydraulic retention time was considered with hydraulic loading rate of 9.87 L/d, while the soil type was loamy.
5. The loading rate of three PAHs was 10000µg/L, percentage of the three compounds in the synthetic wastewater were 74.61% for Phenanthrene, 17.11% Pyrene and 8.28% Benzo[a]Pyrene and the percentages of these components were prepared according to the recommendations of earlier researchers (Lu et al., 2013; Sponza & Oztekin, 2010).

1.6 Organization of Thesis

This thesis was structured and arranged into five chapters

Chapter One entails the introduction and overview of the research study, the background information on the removal of PAHs from wastewater using constructed wetlands, research objectives, significance of research, and the scope and limitations of the study.

Chapter Two enumerates recent related works from the reviewed literature that relates to polycyclic aromatic hydrocarbons (PAHs), constructed wetland (CW), wetland plants, SEM for plants, the mass balance of PAHs in CWs, and multi regression with a stepwise method for models of CW.

Chapter Three contains the methodology of the research procedures, starting with the measurement of plants growth parameters, PAHs extraction analysis, removal efficiency, statistical analysis, scanning electron microscopic studies, PAH mass balance in CWs equation, and stepwise multiple regression to predict the dissipation of PAHs in CWs.

Chapter Four includes the results and discussions from the experiments and the implication divulged accordingly.

Chapter Five presents the conclusions of the research study by highlighting the novelty of the research study as well as considering the implication of the achieved result on the proposed objectives and recommend some potential future researches areas.

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