



DEVELOPMENT OF LANDSCAPE ECOLOGICAL
MODEL SYSTEM USING *BACOPA MONNIERI*,
LILAEOPSIS BRASILIENSIS, *HEMIANTHUS*
CALLITRICHOIDES AND *GLOSSOSTIGMA*
ELATINOIDES FOR AQUATIC ENVIRONMENT
CONTAMINANTS REMEDIATION

BY

ANIS FATIHAH BINTI ABU YAZID

A thesis submitted in fulfilment of the requirement for the
degree of Master of Science (Built Environment)

Kulliyyah of Architecture and Environmental Design
International Islamic University Malaysia

FEBRUARY 2017

ABSTRACT

The pollution of aquatic ecosystems in Malaysia has emerged as a major ecological problem coinciding with rapid industrialisation and urbanisation. The contamination is caused predominantly by industries bearing heavy metals, such as Cd, Cr, Cu, Ni, and Pb which can cause significant ecological and environmental problems. Due to their high solubility in a water environment, they can easily enter the food chain, thus making it a serious health hazard for human and other organisms. Although conventional membrane filtration technologies are widely known as an alternative to treating the contamination, it is not always a convenient option due to its costly operations and maintenance. Presently, the study on phytoremediation using macrophytes is on-going and appears to be a prospective alternative and innovative technology that can be applied to contaminated aquatic environments. However, careful evaluation of its applicability and effectiveness will be required. Therefore, this study aims to assess Cd, Cr, Cu, Ni, and Pb remediation in water through selected aquatic plant species via the in-vitro model system. The study will assess four selected aquatic species, which are *Bacopa monnieri*, *Lilaeopsis brasiliensis*, *Hemianthus callitrichoides* and *Glossostigma elatinoides*. The four aquatic species have distinctive qualities which resulted in having different remediation rates and tolerance towards heavy metals in water. Metal characteristics, mixed metals interaction, pH and other environmental factors can also influence the remediation rate. Phytoremediation of the five heavy metals will be assessed within a mixed metals contamination model system in four different periods (week 1 until week 4) at three different concentrations (1ppb, 2ppb, and 5ppb). The findings indicate that all of the plant species have the capability to remediate all the heavy metals and *Bacopa monnieri* stood out as a species that can withstand and remediate heavy metals even at 4 weeks of exposure to heavy metals at all three concentrations. The highest recorded bioconcentration factor (BCF) was not the *Bacopa monnieri* sequestration rate, but Cd and Pb sequestration by *Glossostigma elatinoides*, which were 48.6 and 36.0 respectively. All of the plants may have shown vary signs of stress and symptoms of toxic poisoning due to high concentration level exposure to the heavy metals at extended periods. This study identified metal excluders and metal accumulators as the two main mechanisms of heavy metals uptake. For example, *Bacopa monnieri* and *Hemianthus callitrichoides* may have shown signs of tolerance mechanism, in which they both can accumulate heavy metals while maintaining its growth development by restricting metals translocation on their plant tissue, however there is a high heavy metal concentrate in their roots. Whereas, *Glossostigma elatinoides* and *Lilaeopsis brasiliensis* can accumulate significant amount of heavy metals but with lesser levels of tolerance towards higher concentrations of heavy metals. The expected outcome of this research is to introduce the potentials of phytoremediation as an innovative strategy to remediate aquatic environment contaminants.

خلاصة البحث

التلوث لبيئة المائية في ماليزيا هو من أكبر المشكلة تتعلق بالبيئة. وهذا بسبب سرعة التصنيع وسرعة التحضر. ولكن من أكبر السبب يكون من صناعة الحديد الثقيلة كـ Cu و Cr و Ni و Pb التي ستسبب مشكلة بيئية. وفي نفس الوقت، أن هذه المواد صفتها ذوبان عالية نحو بيئة المائية. ولذلك، ستدخل في سلسلة غذائية ونتيجتها ستسبب الأمراض والوباء للبشر وغيره. مع وجود تكنولوجيا غشاء الترشيح التقليدية التي استخدمه موسعا كوسيلة أخرى لعلاج التلوث، ولكن باستخدامه لا يكون كاختيار المناسب مع ثمنه عالية في العملية والصيانة. حاليا، دراسة في علاج النبات (phytoremediation) باستخدام الأوراق الكبيرة (macrophytes) يكون في جاري التنفيذ والذي سيكون بديل محتمل والتكنولوجيا المبتكرة الذي سيفيد في علاج البيئة المائية الملوثة. ومع ذلك، هذا يحتاج الى تقييم دقيق في تطبيقه وفعالته. وبالتالي، يقيم هذا الدرس لتقييم معالجة في الماء بواسطة أنواع النباتات المائية مختلفة نحو in-vitro نظام نمونجي. هذا الدرس يتعلق بدراسة أربعة النباتات المائية المختارة وهي باستخدام البيبي باستخدام *Bacopa monnieri* و *Lilaeopsis brasiliensis* و *Hemianthus callitrichoides* و *Glossostigma elatinoides*. وهذه النباتات المائية لها خصائص متميزة والذي سينتاج بقدر المعالجة المختلفة والتسامح تجاه نحو معدن ثقيل في الماء. خصائص المعادن و تفاعل المعدن مختلطة والرقم الهيدروجيني (pH) والعامل البيئي أخرى يتأثر على قدر المعالجة. علاج النبات (phytoremediation) لخمس المعدن الثقيلة سيتم تقييم في المعادن المختلطة بنظام نمونجي التلوث نحو أربعة فترة مختلفة (أسبوع الأول حتى أسبوع الرابع) في ثلاثة تراكيز مختلفة (1 ppb, 2 pbb, 5 pbb). يشير هذا الاكتشاف أن جميع أنواع النباتات لديها القدرة على معالجة جميع المعادن الثقيلة و *Bacopa monnieri* ووقت كنوع التي يمكن أن يعالج المعادن الثقيلة حتى في أربعة أسابيع من التعرض للمعادن الثقيلة في جميع التركيزات الثلاثة. أعلى معامل التركيز المسجلة (bioconcentration factor) ليس *Bacopa monnieri* ولكن *Glossostigma elatinoides* برقم 48.6 و 36.0. كل من هذه النباتات قد أظهرت علامات الإجهاد وأعراض التسمم السامة مختلفة بسبب تعرض التركيز لمستوى عال لهذا المعدن الثقيل في فترة طويلة. هذه الدراسة التي تم تحديدها استبعاد المعادن و تراكم المعادن كاثنين الآلية الرئيسية لامتناس المعادن الثقيلة. مثلا، *Bacopa monnieri* و *Hemianthus callitrichoides* قد أظهرت علامات على آلية التسامح الذي كلاهما يمكن أن تتراكم المعادن الثقيلة مع الحفاظ على التنمية نموها عن طريق تقييد المعادن النبات على الأنسجة النباتية الحية. ولكن هناك تركيز المعادن الثقيلة عالية في جذورها. في حين، *Glossostigma elatinoides* و *Lilaeopsis brasiliensis* يمكن أن تتراكم كمية كبيرة من المعادن الثقيلة لكن مستويات أقل من التسامح نحو تركيزات أعلى من المعادن الثقيلة. النتيجة المتوقعة من هذا البحث هو تقديم إمكانيات علاج النبات بوصفه استراتيجية مبتكرة لإصلاح ملوثات البيئة المائية.

APPROVAL PAGE

I certify that I have supervised and read this study and that in my opinion, it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a thesis for the degree of Master of Science (Built Environment)

.....
Rashidi Othman
Supervisor

.....
Maheran Yaman
Co-Supervisor

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a thesis for the degree of Master of Science (Built Environment)

.....
Mohd Ramzi Mohd Hussain
Internal Examiner

.....
Ahmad Ainuddin Nuruddin
External Examiner

This thesis was submitted to the Department of Landscape Architecture and is accepted as a fulfilment of the requirement for the degree of Master of Science (Built Environment)

.....
Khalilah Zakariya
Head, Department of
Landscape Architecture

This thesis was submitted to the Kulliyyah of Architecture and Environmental Design and is accepted as a fulfilment of the requirement for the degree of Master of Science (Built Environment)

.....
Abdul Razak Sapian
Dean, Kulliyyah of Architecture
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DECLARATION

I hereby declare that this thesis is the result of my own investigations, except where otherwise stated. I also declare that it has not been previously or concurrently submitted as a whole for any other degrees at IIUM or other institutions.

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ACKNOWLEDGEMENTS

Alhamdulillah, in the name of Allah, the Most Gracious and the Most Merciful, all the praises and thanks to Allah S.W.T, on whom we ultimately depend for our sustenance.

First and foremost, I would like to express my special gratitude to my supervisor, Assoc. Prof. Dr. Rashidi Othman and co-supervisor, Asst. Prof. Dr. Maheran Yaman for their guidance, enthusiasm and academic support which tremendously have broadened my knowledge in landscape architecture. I am very fortunate to be under their supervision. Their enthusiasm, dedication and willingness to aid in solving any academic, thesis related issues and problems were a constant source of encouragement throughout this research.

I would also like to thank my family especially my loving parents, Sharina binti Osman and Abu Yazid bin Ahmad as well as my siblings for their unconditional support and prayers throughout the challenging journey of finishing this thesis. They have been a constant source of inspiration and motivation towards self-improvement.

I would also like to thank my dearest colleagues, Sr. Qurratu Aini binti Mat Ali, Sr. Fatimah Azzahra Zaifuddin, Sr. Razanah Ramya, Sr. Farah Ayuni Mohd Hatta, Sr. Nur Hanie Mohd Latiff, Sr. Ruhul Izzati Shahrudin, Sr. Azlen Hanifah and Sr. Wan Syibrah Hanisah Wan Sulaiman as well as Sr. Hafizana Mat Jusoh (Lab Assisstant, Herbarium Lab, KAED) for their assistance, sincere advise and support throughout my study.

I humbly dedicate this research to the Ministry of Higher Education, who granted me the scholarship to pursue my study at the Kulliyyah of Architecture and Environmental Design, International Islamic University Malaysia.

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LIST OF SYMBOLS

$^{\circ}\text{C}$ degree

= equal

> more than

\pm standard deviation

$\text{M}\Omega$ Mega-ohm

LIST OF ABBREVIATIONS

Al	aluminium	KOH	potassium hydroxide
ANOVA	analysis of variance	mg/L	milligram per litre
As	arsenic	mL	milliliter
BCF	bio-concentration factor	Mn	manganese
BOD	biochemical oxygen demand	NEB	nebulizer
Cd	cadmium	Ni	nickel
Co	cobalt	NGO	non-governmental organization
CO ₂	carbon dioxide	pH	power of hydrogen
COD	chemical oxygen demand	Pb	lead
Cr	chromium	ppb	parts perbillion
Cu	copper	QC	quality control
DI	deionized	STD	standard mode
EPA	Environmental Protection Agency	TOC	total organic carbon
Fe	iron	US EPA	united states environmental policy agency
g	gram		
H ₂ O ₂	hydrogen peroxide	UV	ultraviolet
HNO ₃	nitric acid	WWF	world wide fund for nature
HCL	hydrochloric acid	Zn	zinc
Hg	mercury		
ICP-MS	inductively coupled plasma mass spectrometry		
KED	kinetic energy discrimination		

CHAPTER 1

INTRODUCTION

1.1 RESEARCH BACKGROUND

The aquatic environment is a sensitive part of the earth easily susceptible to negative changes in the environment. It can easily collect organic and inorganic pollutants (heavy metal, trace metal, etc.) from direct or indirect sources. These pollutants may be naturally occurring pollutants (atmosphere, soil erosion and deposition, weathering of rock, forest fires, floods, etc.) and anthropogenic sources such as urban, agricultural and industrial discharge (Ren et al., 2015).

Due to rising concerns about metal contamination and its possible accumulation in the food chain, many studies on new aquatic plants (macrophytes) seek to determine their tolerance towards the toxic environment and their potential in phytoremediation or phytostabilisation (Bazihizina et al., 2015). So far, the use of macrophytes to treat water body contamination has shown positive outcomes mostly contributed by the strategic use of the natural and inherent traits of plants. Proper selection of plant species is important to achieve a successful phytoremediation (Subhashini and Swamy, 2014). According to Singh et al. (2012), one of the key aspects of the acceptance of phytoremediation pertains to the measurement of its performance, ultimate utilisation of by-products and its overall economic viability.

In urban areas, rapid changes in land use activities like residential development, deforestation, uncontrolled constructions and mining industries have significantly affected the quality of urban water bodies (Ujang et al., 2008). This has led to increasing public and local government awareness of the condition of urban water bodies leading to the search for a more sustainable development of water treatment

technology without adding more stress to human health and the environment (Shannon et al., 2008). It is important to improve the knowledge of aquatic biological and geochemical processes to improve the quality of urban water bodies.

1.2 ISSUES

The overwhelming inorganic and organic pollution of aquatic ecosystems is evident worldwide (Schwarzenbach et al., 2006). The threat of heavy metals contamination usually coincides with rapid industrialisation and urbanisation contributed by mobile contaminants resultant from open dumps, industrial discharges, urban storm water runoff, and agricultural drainage (pesticides and herbicides) (Onwughara et al., 2011). Unfortunately, Malaysia is not exempted from these threats where reports have shown deteriorating water quality especially in urban areas (Afroz et al., 2014). This might be due to the lack of strict governance and comprehensive and appropriate regulations (WWF, 2015). This pollution will not only jeopardise our ecological and environmental resources but also potentially pose serious health hazards to human beings (Ensley, 2000) due to their accretion in the human food chain.

For example, recent uncontrolled bauxite mining activities in Pahang, Malaysia have drawn global concerns due to its massive environmental pollution. Water sampled different parts of the affected areas have shown that heavy metals such as cadmium, aluminium, silver, lead (plumbum), chromium, iron (ferum), nickel and copper have exceeded the permissible limits according to water quality assessments by local federal agencies. This will lead to toxic contamination in the aquatic ecosystem whereby the excess heavy metals will not only disrupt the aquatic biodiversity but also can be bioaccumulated in aquatic biomes and subsequently enter our food chain. Aquatic organisms such as fish, shellfish, shrimps and plants can

bioconcentrate and biomagnify the heavy metals in their tissues and systems, subsequently compromising human health upon consumption (Karim and Shah, 2015).

The importance of water quality is apparent for human and ecosystem survival. However, 80% of the world's population are exposed to the threats of water scarcity (Vörösmarty et al., 2010). The increasing global population has placed more strain on water supplies making water a rare commodity in some countries (Reddy and Lee, 2012). This is because the scarcity of accessible water can affect socioeconomic growth where it will affect ecotourism, industry, agricultural, fisheries/aquaculture activities and others.

Numerous technologies have been developed to remediate heavy metals contaminated water such as conventional membrane filtration technology, which is a common alternative used for large water volume treatment through ion exchange, reverse osmosis, microfiltration, precipitation, or flocculation (Fu and Wang, 2011). However, such technology can be exorbitantly expensive (Aksorn and Visoottiviseth, 2004) especially for developing countries like Malaysia and often do not achieve a sustainable nor provide any aesthetic improvements for the contaminated sites (Mulligan et al., 2001; Cao et al., 2002). Due to the high cost, many contaminated areas are left untreated, thus reducing the availability of water supplies.

The high cost is probably attributed to the constant need for maintenance to avoid fouling and to increase its feasibility period. For instance, membrane filtration technology requires operators (labour cost) to control its filtration process and possibly the need of membrane replacement when fouling occurs (Allgeier et al., 2005). This, in turn, creates a huge gap and limitations for water treatment efforts in countries like Malaysia.

Given this preliminary, there is an urgent need for introducing sustainable water treatment techniques, which can be achieved through innovative green technologies. Phytotechnology is currently a widely discussed effective and affordable technological solution used to trace environmental imbalance, immobilise, accumulate and degrade metal pollutants from contaminated water. This ecological technology is not only beneficial to the environment but is also cost effective (Ismail and Beddri, 2009) by capitalising on naturally occurring relationships among plants, microorganism and environment.

1.3 PROBLEM STATEMENT

In the case of Malaysia, the following are identified as problems affecting urban water bodies. For example, the number of contaminated urban water bodies left untreated continues to increase due to the high cost of conventional water treatment implementation as well as limited funding for water management (Kim, 2012). To make thing worst untreated sludge most probably directly discharged into the environment due to lack of sludge treatment facilities, hence worsen the quality of our water bodies. Subsequently, contaminated urban water bodies become unusable and less accessible due to its risks of health hazards towards human and other organisms.

Furthermore, only limited and selected aquatic plant species have been introduced on its potential to remediate unhealthy aquatic environments contaminated by heavy metals. For example, we can see in Malaysia the implementation of man-made wetland is usually limited to the use mangrove plant species, which is not quite practical for water bodies in urban areas. In Malaysia, green technologies such as phytotechnology are not well exposed to the public unlike more advance and developed country (Rajoo et al., 2016). Therefore, there is a lack of public

understanding on the potential of green technology resulted in limited sustainable greener alternatives introduced to replace the high cost of conventional methods. This also discourage public and private utilities to migrate to greener approach due to unwillingness of the public population to spend more on raw water supply that was treated with greener approach (Kim, 2012).

The increasing contamination of urban water bodies not only coincides with the constant increase in population but also by the lack of strict regulations and persistent monitoring by local authorities (Chin, 2008). For example, water bodies within proximity of agricultural areas are sensitive and more prone to contamination.

Malaysia as a developing and emerging country suffers from these following problems affecting its urban water bodies which is not unique and can be found in other developing countries. Financial concerns, lack of enforcement and understanding on the greener initiatives are among prevalent constraints in water management development. However, with sufficient studies on green technology, its endless benefits can be expanded and eventually address these problems.

1.4 RESEARCH AIM

To assess the selected aquatic plant species as potential heavy metal phytoremediation agents for Cd, Cr, Cu, Ni, and Pb contamination in unhealthy urban aquatic ecosystem through an in vitro model system.

1.5 RESEARCH OBJECTIVES

The following research objectives have been formulated to achieve the research aim:

- i. To assess the capability of *Glossostigma elatinoides*, *Hemianthus callitrichoides*, *Bacopa monnieri*, and *Lilaepsis brasiliensis* to remediate