STUDY ON MANGROVE SPECIES AS PHYTOINDICATOR FOR INORGANIC CONTAMINANTS AND ASSESSMENT OF Zn, Fe AND Cu BIOACCUMULATION RATE THROUGH IN VIVO MODEL SYSTEM USING *Lemna minor* AND *Salvinia natans*

BY

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

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ABSTRACT

Heavy metals or inorganic contaminants exhibit in aquatic ecosystems including freshwater, mangrove as well as marine ecosystems due to anthropogenic activities that discharge directly without filtration or treatment system. This study proposes an landscape ecological indicator for specific inorganic contaminants using true mangrove species (*Sonneratia alba, Rhizophora apiculata* and *Avicennia alba*) and an experimental model to assess the capability of selected aquatic plant species (*Lemna minor* and *Salvinia natans*) to act as biosequester agents. Three types of true mangrove species were evaluated as potential landscape ecological indicators or bio-monitoring agents for five types of heavy metal contaminants (copper, iron, lead, manganese and zinc) and the level of toxicity in the mangrove ecosystem, especially in intertidal zones (shoreline) through plant leaf and root tissues as well as sediment collected from three different localities. Factors controlling heavy metal contaminant resistance and tolerance levels differ greatly with respect to types of plant species, biotic and abiotic components, source of pollution, localities, accumulation of heavy metals in plant tissues and plant interaction mechanisms. The analysis of variance established a significant to highly significant differences (P <0.001) between the three mangrove species, the three locations, the leaves, and roots and sediment samples and their interaction for all the five heavy metals content. From the findings, Zn and Pb concentration were found at toxic levels in mangrove tissues and sediment and was highly accumulated in *S. alba*, *R. apiculata* and *A. alba* at selected locations followed by Cu > Fe >Mn. The three mangrove species studied, namely *S. alba, R. apiculata* and *A. alba* were found to have great potential for landscape ecological indicator species to indicate specific heavy metals contaminants in mangrove ecosystems. Meanwhile, through the experimental model system, the sequestration rate of *L. minor* and *S. natans* showed that approximately 90% efficiency of copper, iron and zinc were sequestrated in different concentration ranges at different incubation periods except for iron by *L. minor* and dead at certain concentrations of copper. Interestingly, *S. natans'* ability and resistance over three types of heavy metals toxicity were much greater and stable compared to *L. minor*. In this study, *L. minor* and *S. natans* served as good potential biosequester agents to clean-up heavy metals pollutants in aquatic ecosystem due to the fact that they have been able to sequester all heavy metals in a linear relationship within the incubation period. The result revealed that the metal removal efficacies were increased initially through the increasing of time and concentration. Thus, both plants are good potential biosequester agents to clean-up heavy metals pollutants in aquatic ecosystems. In order for this finding to be applied on a large scale, certain factors need to be determined such as plant capabilities and tolerance towards toxicity level, aquatic plant species sequestration rate as well as type of contaminants that will determine the effectiveness of the plant sequestration rate.

ملخص البحث

تتواجد المعادن الثقيلة أو الملوثات اللاعضوية في الأنظمة المائية ومن ضمنها المياه العذبة، والأشجار الاستوائية، بالإضافة إلى الأنظمة البيئية البحرية وذلك بسبب الأنشطة البشرية المتنوعة التي تُطرح في الأنظمة المائية مباشرة دون أية معالجة أو ترشيح. هذه الدراسة تقترح وجود مؤشر لمحميات بيئية طبيعية معينة للملوثات اللاعضوية وذلك باستخدام أصناف لأشجار إستوائيه حقيقية، وهو: "*Sonneratia alba, Rhizophora apiculata and Avicennia alba*"، ونموذج تجريبي لتقدير قابلية أصناف نباتية مائية، وهي: "*Lemna minor* and *Salvinia natans*"، كعوامل عازلة حيوية. وقد تمَّ تقييم ثلاثة أصناف حقيقية لأشجار استوائية كمؤشرات بيئية أو عوامل مراقبة حيوية لخمسة معادن ثقيلة ملوثة، وهي: "النحاس، والحديد، والرصاص، والمنغنيز، والزنك"، وكذلك مستوى السُّمية في النظام البيئي للأشجار الاستوائية خصوصاً في المناطق الساحلية عبر أوراق النباتات، وأنسجة الجذور بالإضافة إلى الترسبات التي تمَّ جمعها من ثلاث مناطق مختلفة. اختلفت العوامل المسيطرة على مقاومة التلوث بالمعادن الثقيلة، ومستويات السماحية بشدة بالنسبة لأصناف النباتات، وهي: المكونات الحيوية واللاحيوية، مصادر التلوث، الأماكن، تراكم المعادن الثقيلة في أنسجة النباتات، وميكانيكية تفاعل النباتات. وتحليل المتغيرات أساس واضح لاختلاف ($P <0.001$) بين أصناف الأشجار الاستوائية الثلاث، للمواقع الثلاثة، والأوراق، والجذور، ونماذج الرواسب لجميع المعادن الخمسة. أشارت النتائج أن تركيز الزنك، والرصاص كان بمستوى سمِّية في أنسجة الأشجار الاستوائية، والرواسب كانت متراكماً بشكل عالٍ في: "*S.alba, R. apiculata A. alba*" في مواقع مختارة، يليه النحاس، فالحديد، فالمنغنيز. يتبين من أنواع الأشجار الاستوائيه الثلاثة "*S. alba, R. apiculata A. alba*" بأن لديها إمكانات كبيرة لأنواع المؤشرات البيئية، لتحديد ملوثات المعادن الثقيلة، وفي الوقت نفسه من خلال نظام النموذج التجريبي أظهر معدل احتباس "*Lemna minor* وَ *Salvinia natans* "، أن ما يقرب من 90٪ من كفاءة النحاس والحديد والزنك تم حجزها في نطاقات مركزة في أزمنة الحضانة المختلفة باستثناء الحديد بواسطة " *L. minor* " بتركيزات معينة من النحاس. ومن المثير للاهتمام، أن قدرة " *S. natans*" والمقاومة على مدى ثلاثة أنواع من سمِّية المعادن الثقيلة أكبر بكثير ومستقرة مقارنة بِ " *L. minor* ". كشفت هذه الدراسة أن " *L. minor*وَ *S. natans* " يؤديان عملهما بشكل جيد كعاملين عازلين لحيوية تنظيف ملوثات المعادن الثقيلة في النظام البيئي المائي، ويرجع ذلك إلى قدرتهما الحقيقية على تنحية جميع المعادن الثقيلة في وجود علاقة خطية ضمن زمن الحضانة. وكشفت نتيجة الدراسة أيضاً أن كفاءة إزالة المعادن زادت في البداية من خلال زيادة الوقت والتركيز. وهكذا، فإن جميع النباتات تُعد عوامل عازلة حيوية لتنظيف ملوثات المعادن الثقيلة في النظم الإيكولوجية المائية. من أجل تطبيق هذه النتيجة على نطاق واسع، لابد من توفر عوامل معينة يتم تحديدها، مثل: قدرات النباتات، والسماحية تجاه مستوى السُّمية، وأنواع معدل امتصاص النباتات المائية، وكذلك نوع الملوثات التي من شأنها تحديد مدى فعالية معدل امتصاص النبات.

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).

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DECLARATION

I hereby declare that this dissertation 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.

Razanah Binti Ramya @ Abd Rahim

Signature ……………………………..………. Date …………………………….

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DECLARATION OF COPYRIGHT AND AFFIRMATION OF FAIR USE OF UNPUBLISHED RESEARCH

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Signature Date

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LIST OF ABBREVIATIONS

%	Percent	ppm	Parts permillion
<	Less than	µg	Microgram
>	More than	mg/kg	Milligram perkilogram
°C	Degree celcius	$CuSO_4.5H_2O$	Cupric sulfate pentahydrate
Al	Aluminium	$ZnSO_4.7H_2O$	Zinc sulfate heptahydrate
APHA	American Public Health Association	DMM	direct measurement method
ARIES	Artificial Intelligence for Ecosystem Services	DO	Dissolved oxygen
As	Arsenic	Mn	Manganese
BAC	Biological Accumulation Coefficient	Mo	Molybdenum
BCF	Bio-concentration Factor	N	Nitrogen
BOD	Biochemical Oxygen Demand	Ni	Nickel
BTC	Biological Transfer Coefficient	P	Phosphorus
Cd	Cadmium	Pb	Lead
Co	Cobalt		
CO_2	Carbon dioxide	Sr	Strontium
COD	Chemical Oxygen Demand	U	Uranium
Cr	Chromium	Zn	Zinc
Cs	Caesium	DEMNRE	Department of Environment in Ministry of Natural Resource and Environment
Cu	Copper	$FeSO_4.7H_20$	Ferrous sulfate heptahydrate
CVIF	Continues Vertical-Inlet Flow	H_2O_2	Hydrogen peroxides
Fe	Iron	HNO_3	Nitric acid
Fe^{2+}	Ferrous	EDXRF	X-ray fluorescence
Fe^{3+}	Ferric	EMM	Ecological modelling

			method
Hg	Mercury	EQR	Environmental Quality Report
ITRC	Interstate Technology & Regulatory Council	IUCN	International Union for the Conservation of Nature
MA	Millennium Ecosystem Assessment	NAHRIM	National Hydraulic Research Institute of Malaysia
mg/l	Milligram perlitre	FSPM	Functional-structural Plant Models
mgl^{-1}	Milligram perlitre	InVEST	Integrated Valuation of Ecosystem Services and Tradeoff
ml	Mililitre	IPCC	Intergovernmental United Panel on Climate Changes
NGTP	National Green Technology Policy	PCBs	Polychlorinated Biphenyls
NH_3-N	Ammoniacal Nitrogen	pH	Acidic or basic substance
NWQS	National Water Quality Standard for Malaysia	POPs	persistent organic pollutants
NWRP	National Water Resources Policy	RegHCM-PM	Regional Hydro-climate model of Penisnular Malaysia
O&G	Oil and Grease	RegHCM-SS	Regional Hydro-climate model of Sabah and Sarawak
PAHs	Polycyclic Aromatic Hydrocarbons	ROS	Reactive oxygen species
RWQM	River Water Quality Monitoring	RUE	Radiation Use Efficiency model
SEM-EDX	elemental X-ray analysis	SLR	Sea level rise
SWAT	Soil and Water Assessment Tool	TCE	Trichloroethylene
TDS	Total Dissolved Solids	TMs	Trace Metals
UN	United Nation	TSS	Total Suspended Solids
UNDP	United Nations Development Programme	WQI	Water Quality Index

UNEP	United Nations Environmental Programme	WWF	World Wide Fund For Nature
UNWWAP	United Nations World Water Assessment Programme	SS	Suspended Solid
USEPA	United States Environmental Policy Agency	TN	Total Nitrogen
UV	ultraviolet	TOC	Total Organic Carbon
VIC	Variable Infiltration Capacity	TP	Total Phosphorus

CHAPTER ONE

INTRODUCTION

1.1 RESEARCH BACKGROUND

A recent assessment by the International Union for the Conservation of Nature (IUCN) produced a red list of threatened species among which is the extinction of mangrove vegetation due to deforestation for coastal development, and aquaculture and timber fuel production, especially in the high intertidal and upstream estuarine zones (Polidoro et al., 2010). Almost 44% of the world population living within 150km of the coastline suffer from an unhealthy exploitation of mangrove forests for resources, and overexploitation for fuel, timber, and agricultural production, among others. Southeast Asia's mangroves presently have significantly declined due to over exploitation of wood and agriculture (Primavera, 2000; Saenger, 2002). On a global scale, between 20% and 35% of mangrove forests have been lost since 1980 (Valiela et al., 2001). The ongoing destruction and degradation of mangrove forests over the past few decades has decreased the function of mangrove forests to serve as a protective zone from natural disasters such as tsunamis (Dahdouh-Guebas et al., 2005).

The direct impact from human activities in coastal zones significantly affects climate changes and leads to a variety of other challenges (Lotze et al., 2006). The major direct impacts include deforestation, coastal development, upland runoff of pollutants, sewage and sediments, petroleum pollution, storm and hurricanes, solid waste, small scale extraction for fuel wood and minor clear cutting, conversion to aquaculture, conversion to landfills and terrestrial agriculture tourism all of which lead to a decline of mangrove forests eventually affecting the aquatic biodiversity (Hamm and Stive, 2002; Jusoff, 2008; Ellison and Farnsworth, 1996).

Global sea levels have risen at 1.7 ± 0.5 mm/yr through to the 20th century, while global mean sea surface temperatures have risen approximately 0.6ºC since 1950 and is associated to atmospheric warming in coastal areas (Bindoff et al., 2007) with a predicted warming between 1.4° - 5.8°C will increase the sea level about 0.009 - 0.88 m by the year 2100 (IPCC, 2001). In addition, every 10°C rise of temperature and sea level may lead to coastal area erosion, tidal inundation, salinity, loss of fisheries, and declines of coral, mangrove forests and infrastructure because the coastal landform is highly sensitive to medium or long term changes in energy inputs by sea level rise (Lee and Teh, 2001; Pethick, 2001). Since 1990, the Intergovernmental United Panel on Climate Changes (IPCC) has reported that the average global temperature has increased between 0.15 and 0.3°C per decade until 2005. This may slowly have a negative impact on the ecosystem structure, species and biodiversity especially along coastline areas which are heavily populated and are at the greatest risk of flooding from sea and river. Furthermore, rising sea levels and human development and settlement are factors directly reducing coastal wetlands and mangrove forests (IPCC, 2007). Despite the real threat, limited studies have explicitly addressed the relationship observed between coastal loss and rate of sea level rise and it remains unclear to what extent these losses are associated with relative sea-level rise due to loss of land and global warming (Nicholls et al., 2007).

The coastal vegetated wetlands are sensitive to climate change and long-term sea levels as their location is intimately linked to sea level. This is illustrated by the global losses from 2000 to 2080 of 33% to 44% from modelling of all coastal wetlands especially on brackish and freshwater ecosystem as given by a 36 cm and

72 cm rise in sea level respectively (McFadden et al., 2007; Sun et al., 2002). On the other hand, the IUCN stated that 126,000 species depend on freshwater ecosystems as part of their lifecycle. The Convention on Biological Diversity defined biological diversity as the variability among living organisms from all sources including terrestrial, marine and other aquatic ecosystem, while ecological complexes include diversity within species, and between species and ecosystems. Even though the freshwater ecosystem consist of only 1% of the planet's surface, 12% of species live in freshwater and more than 25% of vertebrate species depend on freshwater ecosystems (UNEP, 2010). In the last three decades, freshwater ecosystems have declined resulting in a threat of biodiversity due to water degradation. The population of freshwater species fell almost 50% on average; two-thirds greater than terrestrial and marine species (MA, 2005).

Both human and natural activities would change the physical, chemical and biological characteristics of aquatic ecosystem that may affect human and ecosystem health. Changes in water quality affects nutrients, sedimentation, temperature, pH, heavy metals, non-metallic toxins, persistent organic and pesticides, and biological factors (Carr and Neary, 2008) and these pollutants are globally persistent in the environment and can be transported long ranges to regions where they have never produced (UNEP, 2009). Human activities from agricultural productions, industries, mining, water-system infrastructure and direct disposal of untreated human waste high affect water quality (UNEP, 2008). The United Nation (UN) estimates that by 2050, the world population will surpass 9 billion people with the most growth occurring in developing countries and urban areas (UN, 1999). As the population grows, more domestic waste and sewage can overload streams and treatment systems and lead to more polluted waters.

Mangroves in Malaysia have been declined due to human activities in maximizing the use of land such as aquaculture (7%), agriculture (43%), urban development (20%) and other activities (29%). 22, 299 ha from the overall total mangrove forest and the population along the coastal areas and riverbanks was about 26.8% in 1970 to 60% in 2000 and over exploitation of fishery resources causes environmental challenges through a rise of sea levels (Giri, et al., 2008; Abdul Halim et al., 2011; Zakaria et al., 2001). Mangrove forests continuously suffer by increasing demand in food supply and shrimp farming either for aquatic habitats or human consumption (Asmawi, 2009).

The loss of 1% of mangrove area per year in Malaysia due to development such as overexploitation, pollution and conversion is a conservative estimate of mangrove destruction in the Asia-Pacific region (Duke et al., 2007). The IPCC and United Nations Environmental Programme (UNEP) reported that the current climate changes in Malaysia is 0.7°C and normal daily temperature is 40°C and have increased sea levels in Southeast Malaysia from one to two metres. As long as the temperature does not increase beyond the limitation between 0.7°C until 2°C, this phenomenon will only impact the coastline area in the form of soil erosion and will trigger mass coral bleaching in Malaysia, which will affect the aquatic ecosystem and habitat (WWF, 2010). According to Tanggang (2009), if the temperature increases above 5°C to 6°C the world and life as we know it will be submerged under water.

Since 1951, the temperature has increased approximately 0.18°C per decade with an average annual rise in sea level of approximately 1.25mm. The mean temperature in Malaysia rose from 0.3°C to 4.5°C while the rainfall has changed to ± 30%. These conditions may affect the rising sea levels from 15 to 95cm along the Southern coast in Peninsular Malaysia (Tan et al., 2009; Chong and Mattews, 2001).

The rise in sea levels have resulted in the encroachment of mangrove forests. In mangrove forests, the sediment consist of clay formerly deposited in brackish water (Yakzan and Hassan, 1997). A simulation model by NAHRIM shows the prediction of sea levels in the year 2050 and 2100 in Malaysia (Table 1.1).

Thus, if there is no water tidal, the mangrove forest will submerge and gradually disappear. North-east, eastern central and north-western central of Peninsular Malaysia would experience small increases in precipitation while central and southern regions would expect a slight decrease in precipitation between 2041 - 2050 as simulated by Kavvas et al. (2006).

Table 1.1 Projects on climate change and sea level rise in Malaysia (NAHRIM, 2010)

Climate Parameter	Peninsular Malaysia (RegHCM-PM)	Sabah (RegHCM-SS)	Sarawak (RegHCM-SS)
Annual mean surface temp.	1.0-1.5ºC (2050)	1.3-1.7ºC (2050) 2.9-3.5ºC (2100)	1.0-1.5ºC (2050) 3.0-3.3ºC (2100)
Max. monthly rainfall	+113mm (12%) (2050)	+59mm (5.1%) (2050) +111mm (9%) (2100)	+150mm (8%) (2050) +282mm (32%) (2100)
Sea level rise (2100)	2.5-5.2mm/yr	4.3-10.6mm/yr	

The climate change scenario affects water quantity and the water quality of river flows. Changes in water quantity with extreme rainfall may increase floods and soil erosion in addition to sedimentation in river estuaries which may later reduce the inflows to reservoir, stream flows and a recharge of groundwater. Secondly, change in water quality due to water excess may increase pollution such as nutrients and sediments that are highly concentrated in rivers and water bodies (NAHRIM. 2010). The sea level rise (SLR) in coastal areas is affected increasing erosion and sedimentation, flood, infrastructure along the coastal, salt water interference and shifting of ecosystem such as mangroves and marine habitat (NAHRIM, 2010; Ong, 1995). As such, climate change such as the rise of sea levels and high temperatures will affect mangrove ecosystem. This situation may destroy the aquatic ecosystem particularly mangrove and marine habitats.

1.2 PROBLEM STATEMENT

There are two major factors identified in the degradation of aquatic ecosystems in mangroves and estuaries, namely manmade activities and natural impacts. Manmade activities can be divided into the following four categories:

i. Urbanization, population growth and development

ii. Uncontrolled disposal of human waste

iii. Agriculture and aquaculture activities

iv. Industry and energy production

Meanwhile, natural impact can be classified into the following three groups:

i. Climate changes and global warming

ii. Rise of sea level

iii. Tsunami

Based on the above factors of human development and activities as well as climate changes, the issue of waste or pollution load into the aquatic ecosystem arises. For instance, the discharge of untreated wastewater directly into the environment is a certain source of heavy metals which may later diminish these ecosystems' biodiversity and endanger human health.

1.3 ISSUES

The rivers in Peninsular Malaysia are mainly polluted by point (monitored by Department of Environment) and nonpoint sources (untreated sewage and storm runoff). Based on a report from DOE in Ministry of Natural Resource and Environment (DEMNRE, 2009), from a total of 1,063 monitored water quality stations located at 577 rivers, 578 (54%) were found to be clean, 378 (36%) slightly polluted and 107 (10%) polluted. The decreasing number of clean rivers from 306 in 2009 compared to 334 in 2008 is due to the increased number of polluting sources such as sewage treatment plants, manufacturing industries and palm oil mills. The major pollutant parameters detected were Biochemical Oxygen Demand (BOD), Ammoniacal Nitrogen (NH_3-N) and Suspended Solid (SS) contributed from untreated sewage and discharges from agro-based manufacturing industries, livestock farming, domestic sewage, and earthwork and land clearing activities. Meanwhile an analysis of heavy metals of 5637 water samples established that almost all samples complied with Class III of the National Water Quality Standard for arsenic (As), mercury (Hg), cadmium (Cd), chromium (Cr), lead (Pb) and zinc (Zn) except iron (Fe) where the compliance was 97% (DEMNRE, 2009). The 2009 Environmental Quality Report showed that 46% of river water in Malaysia is polluted which is higher than previous years (DOE, 2011). Based on the National Water Resources Study 2000 - 2050, the parameters which have exceeded Class III limits include NH3-N, as the main pollutants result in low Water Quality Index (WQI), organic carbon, heavy metals, oil and grease (Al-Mamun and Zainuddin, 2013). For the chronology of marine pollution, refer to Figures 1.1 and 1.2.