



POTENTIAL OF AQUATIC PLANT SPECIES AS
PHYTOINDICATORS FOR HEAVY METAL
CONTAMINANTS

BY

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ABSTRACT

This research explores the potential of specific aquatic plant species to be used as water quality indicators for unhealthy aquatic ecosystem. The success in the economic growth and industrialization in Malaysia has led to environmental problems with ever-increasing land, air, and water pollution. Industrial production without adequate regard for environmental impacts has increased water and air pollution, and has led to soil degradation and large-scale global impacts such as acid rain, global warming, and ozone depletion. These kinds of pollutions led to many environmental impacts to living organism especially in human daily life. Aquatic plants are emergent, submerge, or floating. They are beneficial to aquatic ecosystem because they provide shelter for flora and fauna, produce oxygen, which assists with overall lake functioning, and provide food for fish and wildlife. The absence of aquatic plants may also indicate water quality problems as a result of excessive nutrients, herbicides or heavy metals and may interfere with lake functioning. In this study, ten samples of water were collected from four different places where six different aquatic plant species were abundance and dominant. For the determination of metals in water, samples were treated according to (American Public Health Association, American Water Works Association, Water Environment Federation, 1999) method and analysed by atomic absorption spectrometry for six types of heavy metals which are iron (Fe), lead (Pb), copper (Cu), zinc (Zn), and nickel (Ni) and manganese (Mn). Results showed that the abundance of certain aquatic plant species indicate high concentration of certain heavy metal in that particular environment. Some of the concentration of heavy metal are exceeded the limits of recommended range INWQS. All were found as good ecological indicators for heavy metal contaminants either at low or high level. Analysis of heavy metals contaminants from four different locations and six different aquatic plant species which are *Eichhornia crassipes*, *Hydrilla verticillata*, *Cabomba fuscata*, *Salvinia natans*, *Nelumbo nucifera* and *Pistia stratiotes* exhibiting highly significant differences ($P < 0.0001$) between aquatic plant species widespread, locations and the heavy metals content. This clearly demonstrates that freshwater environment with abundance of invasive aquatic plant species can have an important influence and indication on the accumulation of heavy metals content. The importance of the interaction components emphasises that the changes in heavy metals composition are complex and the responses are not consistent across all aquatic plant species. Examination of the summarised data revealed that, of the six species analysed at all different locations, all exhibits as potential ecological indicators for unhealthy aquatic ecosystems or as phytoindicator for heavy metal contaminants either at low or high level contamination. The best phytoindicators for excess iron were *C. fuscata* > *S. natans* > *N. nucifera*. Meanwhile, magnificent phytoindicators for excess Pb were *E. crassipes* > *S. natans* > *N. nucifera*. In addition, good phytoindicators for zinc were *E. crassipes* > *N. nucifera* > *S. natans*. The best phytoindicator for excess copper were *S. natans* > *C. fuscata* > *E. crassipes*. Meanwhile, magnificent phytoindicators for excess manganese were *E. crassipes* > *S. natans* > *N. nucifera*. Lastly, good phytoindicators for nickel were *E. crassipes* > *S. natans* > *N. nucifera*. In conclusion, the most reliable phytoindicators for overall experiment were *E. crassipes*, *S. natans* and *N. nucifera*. Therefore, macrophyte is effective in responding heavy metal in low level environmental contamination that might otherwise be difficult to detect.

ملخص البحث

الهدف من هذا البحث هو لدراسة إمكانية تنوع الاحياء المائية لاستخدامها كمؤشر لبيئة غير صحية مثل المسطحات المائية العذبة الملوثة. فالنباتات المائية التي تنمو في الماء او بالقرب من المياهاما ان تكون ناشئة او مغمورة او عائمة. النباتات المائية مفيدة لنظم الايكولوجية المائية لانها توفر المأوى للاسماك والركيزة للفقريات المائية. كما انها تنتج الاكسجين, التي تساعد في اداء البحيرة بصورة شاملة, وتوفر الغذاء لبعض الاسماك و غيرها من الحيوانات البرية. قلة النباتات المائية في نظام الحياة المائية وهو امر متوقع الحدوث تشير الى انخفاض اعداد الاحياء البرية. بالإضافة لذلك, فعدم وجود النباتات المائية قد تشير ايضا الى مشاكل نوعية المياه نتيجة الإفراط في المواد الغذائية, المبيدات الحشرية اوالمعادن الثقيلة و التي تتداخل مع عمل البحيرات, الأنشطة الترفيهية والانتقاص من الشكل الجمالي للنظام. في هذه الدراسة, تم جمع ثلاث عشرة عينه من المياه من أربعة أماكن مختلفة حيث هيمنة ستة أنواع من النباتات المائية المختلفة على العينة وبشكل وفير. كل عينات المياه تم تحليلها الى ستة أنواع من المعادن الثقيلة والتهيا الحديد, رصاص, نحاس, زنك, نيكل, والمنغنيز. حيث اظهرت النتائج ان وفرة انواع معينة من الاحياء المائية تشير الى ارتفاع تركيز بعض المعادن الثقيلة في البيئة الخاصة تلك. ان تركيز بعض المعادن الثقيلة قد تجاوز حدود النطاق الموصى بها ب معايير نوعية المياه الوطنية المؤقتة. ان جميع ما تم العثور عليه مؤشرات بيئية جيدة لملوثات المعادن الثقيلة سواء في مستوي منخفضاً ومرتفع. تحليل المعادن الثقيلة الملوثة من أربعة مواقع مختلفة و لستة انواع مختلفة من النباتات المائية والتي هي Eichhornia crassipes, Hydrilla verticillata, Cabomba furcata, Salvinia natans, Nelumbo nucifera and Pistia stratiotes. اظهرت فروقات عالية ذات دلالة احصائية ($P < 0.0001$) بين انواع النباتات المائية على نطاق واسع , ومواقع محتوي المعادن الثقيلة. هذا يدل بوضوح على ان بيئة المياه العذبة معروفة بأنواع المايكروبيات الغازية يمكن أن يكون لها تأثيرها مودلالة على تراكم المعادن الثقيلة. أهمية مكونات التفاعلات على أن التغييرات في تكوين المعادن الثقيلة معقدة والاستجابات ليست متسقة عبر كافة أنواع النباتات المائية. فحصولنا لبيانات اظهرت أن, من أنواع المايكروبات الغازية الستة التي حللتها جميع المواقع المختلفة, جميع المعروضات كمؤشر للبيئية المحتملة لنظم الايكولوجية المائية غير صحية او كمؤشر انبثائية لملوثات المعادن الثقيلة سواء في المستويات المنخفضة او العالية التلوث. ان افضل مؤشر نباتي للحديد الزائد كان $C. fuscata > S. natans > N. nucifera$. وفي نفس الوقت, فقد كان المؤشر النباتي لزيادة الرصاص كان في $E. crassipes > S. natans > N. nucifera$. بالإضافة لذلك, فان مؤشر نباتي جيد للزنك كان في $E. crassipes > N. nucifera > S. natans$. وكان افضل مؤشر نباتي للنحاس الزائد كان في $S. natans > C. fuscata > E. crassipes$. في حين ان افضل مؤشر نباتي للمنغنيز الزائد كان في $E. crassipes > S. natans > N. nucifera$. اخيراً فان مؤشراً نباتياً جيداً للنيكل كان في $E. crassipes > S. natans > N. nucifera$. في الختام, فقد كان المؤشر النباتي الاكثروثوقية للتجربة عموماً كان $E. crassipes, S. natans$ and $N. nucifera$. لذلك, فان النباتات المجهرية فعالة في الاستجابة للمعادن الثقيلة في مستويات منخفضة من التلوث البيئي والتي تكون بهذا الامر صعب اكتشافها.

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 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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LIST OF ABBREVIATIONS

AAS	atomic absorption spectrometer	O ₂	oxygen
Ag	silver	Os	osmium
Al	aluminum	P	phosphorus
As	arsenic	Pb	lead
B	boron	Pd	palladium
Ba	barium	Pt	platinum
BOD	biochemical oxygen demand	Rh	rhodium
Ca	calcium	Ru	ruthenium
Cl	chlorine	Se	selenium
CN	cyanide	Sn	tin
Cd	cadmium	SS	suspended solids
Co	cobalt	U	uranium
COD	chemical oxygen demand	V	vanadium
Cr (IV)	chromium (IV)	WQI	Water Quality Index
Cr (II)	chromium (II)	Zn	zinc
Cu	copper		
DO	dissolved oxygen		
EDTA	Ethylenediaminetetraacetic acid		
F	fluorine		
Fe	iron		
g	gram		
Hg	mercury		
Ir	iridium		
K	kalium		
L	liter		
mg	milligram		
Mg	magnesium		
Mn	manganese		
Mo	molybdenum		
N	nitrogen		
Na	sodium		
Ni	nickel		
NO ₂	nitrogen dioxide		
NWQSM	National water quality for standard Malaysia		

CHAPTER 1

INTRODUCTION

1.1 AQUATIC ECOSYSTEM

Concerns for the environment, especially water quality, is an issue of great concern for Asian countries due to the frightening possibility that freshwater will be a scarce resource in the future. The management of many current and emerging environmental problems will require significant reductions in the emissions of environmentally harmful chemicals, if not total elimination. In this drive to preserve freshwater sources, water quality monitoring programs are necessary. Water quality is directly related to environmental pollution (Jin, 1992). Environment pollutants in aquatic systems include heavy metal and nutrients. Some heavy metals may transform into persistent metallic compounds with high toxicity, which can be bioaccumulated in the organisms and magnified in the food chain thereby, threatening human health (Jin, 1992).

Understanding the patterns and processes related to sustainability represents one of the greatest challenges to theoretical and applied ecology. For example, the number of research programs attempting to define the patterns existing in biodiversity and the mechanisms that explain such patterns are growing rapidly due to greater importance attributed to it in the form of the International Biodiversity Observation Year in 2001. The findings of this research are of particular relevance to aquatic plant ecology, with several articles about biodiversity and aquatic macrophytes published. Furthermore the determinants of aquatic macrophyte richness include some well-known factors such as locality and water chemistry (Amoros and Bornette, 1999).

1.2 RESEARCH BACKGROUND

In recent years, there has been increasing interest in the use of aquatic plants for the removal of pollutants from domestic and industrial sewage effluents. Among several plants studied, water hyacinths (*Eichhornia crassipes*) are the most commonly cited and appear to have the greatest potential for use in water pollution control. Water hyacinths are known to accumulate nutrients and potentially toxic water pollutants (Prasad et al., 2001; 2005; McCutcheon and Schnoor, 2003; Kamal et al., 2004; Peles et al., 2002; Hattink et al., 2000; Sheppard and Motycka, 1997). *Lemna* species are also known to be excellent bioaccumulators of various heavy metals and trace elements. This capacity allows the *Lemna* to treat a variety of waste streams from landfill leachates to highly polluted industrial wastes (Prasad et al., 2001; 2005). The notable environmental contaminants are toxics that include inorganic and organic pollutants that can be traced and remediated (COST action 837, 2003; COST action 859, 2005). Heavy metal and other toxic compounds discharged into the environment from industrial, agricultural, and domestic sources. The surrounding water resources become affected when discharged into the aquatic ecosystem, which is maintaining the surrounding flora and fauna.

In Malaysia, most aquatic plants inhabit fresh water bodies such as ponds, ditches, lakes and rivers which are termed as hydrophytes. Halophytes are those plants that inhabit saline and brackish coastal waters. There are no less than 200 species of aquatics worldwide but only 1% of the angiosperms are classified as hydrophytes (Halijah and Yahya, 2000). There are many kinds of aquatic plants that can be utilized for such purposes as feeds, fuel, building materials, soil improvement and water purification. Therefore, by identifying the aquatic species, characteristic, classification

and functions the problems of water pollution could be overcome. Furthermore, such actions could increase public awareness.

Aquatic plants are beneficial to fresh water bodies because they produce oxygen, which assists with the overall functioning of fresh water bodies, and provide food and shelter for aquatic living organisms. A lack of aquatic plants in a freshwater bodies system where they are expected to occur may suggest a reduced population of macro and micro fauna. In addition, the absence of aquatic plants may also indicate water quality problems as a result of excessive turbidity, herbicides, or salinization. However, an overabundance of aquatic plants can result from high nutrient levels and may interfere with the freshwater bodies system. The importances of aquatic plants are eminent; they increase productivity of the aquatic ecosystem and thus help to maintain a balanced ecosystem. Therefore aquatic plants are excellent indicators of unhealthy environments because they respond to nutrients, light, toxic contaminants, metals, herbicides, turbidity, water level change and salt.

Oxygen concentrations in aquatic systems are a key control of water quality and these dissolved oxygen (DO) concentrations vary with primary production, respiration, physical structure and hydrologic regime. Within the aquatic ecosystem such as rivers, estuaries and lakes, oxygen concentration can be particularly dynamic in vegetated habitats that often dominate shallows (Caraco and Cole, 2002; Lauster et al., 2006). In areas of dense vegetation, high respiratory demand can cause low oxygen to occur regularly at dawn, despite the presence of adequate oxygen in afternoons and evenings (Kaenel et al., 2000). Furthermore, oxygen concentrations tend to be on average lower and oxygen dynamics may be more related to seasonal or tidal variation in hydrologic exchange than to diurnal changes in light regime (Hamilton et al., 1997; Caraco and Cole, 2002). For these reasons the abundance of

water chestnut (*Trapa natans*) and water hyacinth (*Eichhornia crassipes*) can be indicators strongly associated with reduced oxygen concentrations whereas *Azolla pinnata* and *Spirodela polyrhiza* can serve as an indicator for the abundance of nitrogen; phosphate and iron respectively in unhealthy water bodies (Caraco and Cole, 2002; Azizur Rahman et al., 2008). Such low oxygen concentration and high nutrient concentration are associated with negative impacts to certain aquatic species as well as changes in metal, nitrogen and phosphorus cycling (Hamilton et al., 1997; Harrison et al., 2005). Therefore, aquatic plant species distribution in general is greatly affected by the quality of water in the environment. Thus, aquatic plants can be excellent phytoindicator of polluted water as well as water purifier.

1.2.1 Green technology

Green technology is a branch of sustainability development. Some emphasize the term of sustainability development as use of green technology and improvements in health care and education (World Resources Institute, 1992). The applications of plants in environmental protection strategies are called Phytotechnologies. Phytotechnologies employ plants to remediate, stabilize or control toxic contaminants from the environment (COST action 837). Each plant is categorized into a certain group according to their function. Phytoremediation involves the utilization of plants to remove, transfer, stabilize or degrade contaminants in soil, sediment and water (Hughes et al., 1997). The term phytoremediation is derived from the combination of word “phyto” meaning plant, and “remedium” meaning to clean or restore. It refers to a various group of plant-based technologies that use either naturally running, or genetically engineered plants to clean contaminated environments (Cunningham et al., 1997; Flathman and Lanza, 1998).

1.3 ISSUES

1.3.1 Environmental pollution

The nation's potable water supply remains at great risk despite decades of work to reduce pollution from industries, sewage plants and runoff. According to the U.S. Geological Survey, the earth's surface is 71% water, but only 2.5% of that volume is potable. Nearly 70% of that supply is ice and almost 30% is subsurface, held both as soil moisture and within underground aquifers. That leaves 0.007-0.009% usable potable water for people (Figure 1.1).

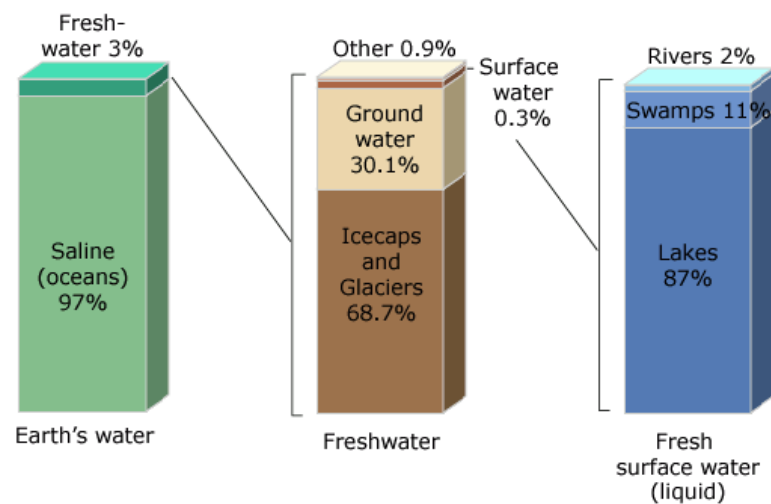


Figure 1.1: Distribution of earth's water

For some time now, Malaysia has faced a number of environmental issues. Rapid development of tin mining which is a traditional industry started at the turn of the century about 100 years ago, caused major river pollution (Shuhaimi-Othman et al., 2007). This industry was followed by the expansion of other industries such as natural rubber and palm oil production from which wastewater from the factories caused further pollution of rivers and seas (Sari and Wan Omar, 2008). The effect of

industrialization was environmental pollution, caused by industrial wastewater and other wastes, which became obvious from the 1970s. According to the Malaysian Environmental Quality Report (2002), the estimated number of sources of water pollution in Malaysia for 2002 was 13,540 comprised predominantly by especially of sewage treatment plants, agro-based industries, manufacturing industries and animal farms. Approximately 53 percent of the total number of sources were domestic sewage facilities (7,126 sources), followed by manufacturing industries (5, 137 or 38 percent), pig farms (807 sources or 6 percent) and agrobased industries (470 sources or 3 percent). Of the total number of effluent sources identified, Johor had the highest number (1 675, 29.9 percent), followed by Selangor (1 485, 26.5 percent), Perak (573, 10.2 percent) and Perlis had the least number (14, 0.25 percent).

1.3.2 Water quality of freshwater bodies

The use of indicators and indices for the evaluation and assessment of the environmental status of diverse ecosystems is becoming a widespread procedure to analyse the various and often complex components of a system. Indicators can be handled as information tools, as they represent an objective system of information and evaluation. Indicators are also key tools for linking to policy objectives and targets, for communicating to countries data priorities and reporting complexity in simple ways that policy makers and public can understand. It is important to clarify and share the definitions of indicators and indices. According to the Organization for Economic Cooperation and Development (OECD) an 'indicator' is defined as a parameter, or a value resulting from parameters, which provides information about the condition of a phenomenon or environment or area, with a significance extending beyond that

directly associated with the parameter value. Index is referred to as a set of aggregated or weighted parameters or indicators (Anon, 1993).

Malaysia does not really have exact values of river water quality measurements for individual monitoring sites. Instead, water quality status is evaluated under three rankings (clean, slightly polluted, and polluted) using a Water Quality Index (WQI) based on six parameters: pH, dissolved oxygen (DO), biochemical oxygen demand (BOD), chemical oxygen demand (COD), ammonia nitrogen, and suspended solids (SS) (Department of Environment, 2003; Shuhaimi-Othman et al., 2007; Sari and Wan Omar, 2008). The WQI serves as the basis for environment assessment of a watercourse in relation to categorization of pollution and classification of beneficial uses as provided under the National Water Quality Standards for Malaysia (NWQS).

Table 1.1
General Quality Indicator for the coastal waters habitat (MCSD 1998-1999)

Indicator	Group	Definition	Unit
General quality of coastal waters	State	This indicator aims at describing the quality. Quality class of coastal waters in accordance with three variables: (1) the bacteriological quality of seawater; quantity or volume (2) the concentration of pollutants in the seawater and in sediments (3) the concentration of pollutants in living organisms	Quality class or quantity or volume
Quality of the biophysical environment	State	This indicator is defined by two sub-indicators: (1) ratio of the area of marine phanerogamous beds and the total infra-coastal area (0-50 m) (2) part occupied by <i>Posidonia oceanica</i> of the total area of water plant communities	Percentage

Thus, there is no accurate information about concentrations of river, lake and fresh water bodies'. Thus, this tool is not responsible to assess all the water quality parameters in checking the water quality status comprehensively. For example, major contribution of nutrients (Phosphorus and Nitrogen) have both direct and indirect effects including increased phytoplankton biomass and production, reduction in water clarity, toxic algal blooms, fish kills, and loss of biodiversity (Boynton et al., 1995; Howarth et al., 2000; Cloern, 2001) (Table 1.2).

Table 1.2
Types of Water Quality Index in different countries (Department of Environment, 2003)

Pollutants or parameters	National recommended Water quality Criteria (NRWQC)				Canadian Water Quality Standard (CCME)			USEPA		ASEAN River Classification and Long Term Water Quality Goals				Malaysia
	Freshwater (ug/l)		Human health (ug/l)		Drinking (ug/l)	Aquatic (ug/l)	Agriculture (ug/l)	maximum concentration (CMC) (ug/l)	Criteria continuous concentration (CCC) (ug/l)	Class I Potable water (mg/l)	Class II recreation (mg/l)	Class III commercial fisheries (mg/l)	Class IV irrigation (mg/l)	
	CMC	CCC	Water+ Organism	Organism only										
Metal														
Copper (Cu)			1,300		1000	2-4	1000	13	20	-	-	-	-	
Lead (Pb)	65	2.5			10	1-7	200	2.5	50	-	-	-	-	
Zinc (Zn)	120	120	7,400	26,000	5000	30	5000	120	5000	-	-	-	-	
Iron (Fe)	-	-	-	-	300	300	5000	1000	1000	-	-	-	-	
Cadmium (Cd)	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Mercury (Hg)	1.4	0.77	-	-	1	1	3	-	-	-	-	-	-	-
Nickel (Ni)	470	52	610	4,600	200 ^{BC}	25	2	-	-	-	-	-	-	-
Manganese (Mn)					50	100 ^{BC}	200	-	-	-	-	-	-	-
Aluminium (Al)					0.2 ^{BC}	0.005	5							
Phosphorus					0.1 ^a	0.1 ^a	-	0.05 (point source) 0.10 (non-point source)						0.10 – 0.20 (P) 0.3 (TAN)
N	NH ₄ + -N					2.2, at pH 6.5 1.37 at pH 8.0								0.3 (TAN)
														0.3 (TAN)
														7.0
							0.06							
Physico-chemical														
pH	-	-	-	-	6.5-8.5	6.5-9	-	-	-	6-9	6-9	5-9	5-9	-
BOD	-	-	-	-	-	-	-	-	-	5	5	10	10	-
COD	-	-	-	-	-	-	-	-	-	30	30	100	100	-
Amu-N	-	-	-	-	-	-	-	-	-	0.3	0.3	1	3	-
TSS	-	-	-	-	-	-	-	<10%	-	50	50	150	300	25 - 150
DO	-	-	-	-	-	5 ^{BC}	-	-	-	5	5	3-5	3	3.0 – 7.0
References	USEPA, 2009				Khan et al. 2003			EPA		Second ASEAN State of the Environment Report 2000, AMEQC, 1999, ANWQC, 1999				

Footnotes

^{BC} British Columbia Provincial Water Quality Guidelines
^a Recommended as maximum desirable concentration

1.4 PROBLEM STATEMENT

Heavy metals are the most abundant and persistent environmental inorganic pollutants. Unlike other pollutants, metals cannot be degraded and clean-up is required for their removal. Once released in the aquatic environment, heavy metals like copper, lead, zinc and nickel undergo transformation and most become associated with suspended particulate matter, which accumulate in the bottom sediment. Aquatic plant species adapted to survive in fresh water ecosystem can remove the heavy metals from contaminated water. Thus, ecological indicator offers a simple, cheap and energy efficient method of indicating polluted water and wastewater including heavy metals.

1.5 RESEARCH QUESTIONS

- i) What types of macrophytes species can be used as phytoindicators for heavy metals contaminant either in low and high level concentration?
- ii) What are the specific macrophytes that can be used as phytoindicators for specific heavy metal (iron, lead, copper, mangan, nickel and zinc) contaminants?
- iii) How do the locality or geographical factors influence the macrophyte species as phytoindicators for heavy metal contaminants?

1.6 RESEARCH AIM AND OBJECTIVES

The aim of the research is to study the potential of specific aquatic plant species to be used as water quality indicator for unhealthy environment. In order to achieve this, the following objectives are formulated: