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EFFECT OF MANGANESE AND CADMIUM ON BIOLOGICAL ATTRIBUTES OF WILD WATER SPINACH (Ipomoea aquatica Forssk.)

BILLY GUAN TECK HUAT

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By

BILLY GUAN TECK HUAT

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

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

EFFECT OF MANGANESE AND CADMIUM ON BIOLOGICAL ATTRIBUTES OF WILD WATER SPINACH (*Ipomoea aquatica* Forssk.)

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BILLY GUAN TECK HUAT

September 2017

Chairman : Ferdaus @ Ferdius Mohamat Yusuff, PhD
Faculty : Environmental Studies

Heavy metals are inorganic pollutants that are hazardous and toxic to the environment. Agricultural activities have indirectly introduced heavy metals peculiarly manganese (Mn) and cadmium (Cd) to the ecosystem and eventually have polluted aquatic ecosystem which included the ponds located in Universiti Putra Malaysia. Water pollution caused by the heavy metals can greatly affect the life of the wild water spinach (*Ipomoea aquatica* Forssk.), an edible aquatic plant that is living in the ponds. Consequently, human health can be threatened when the metal-contaminated wild water spinach was foraged for consumption. Hence, the metals effects of Mn and Cd on the health status, growth, anatomy, and DNA quality of the wild water spinach were studied. Furthermore, the metal uptake ability by the wild water spinach was determined. The metal bioavailability and health risk were also assessed upon consumption of the metal-contaminated wild water spinach. The mature wild water spinach was hydroponically cultivated under greenhouse conditions and was subjected to Mn and Cd treatments which included low treatment (0.30 mg/L for Mn and 0.10 mg/L for Cd), high treatment (1.50 mg/L for Mn and 0.50 mg/L for Cd), and the control (distilled water) for seven days. ANOVA analysis indicated that significant reduction was observed for roots length and surface area, shoots length, leaves surface area in the metal-contaminated wild water spinach with the increasing Mn and Cd concentrations ($p < 0.05$). Toxicity symptoms such as chlorosis and necrosis also occurred on the wild water spinach from the metal exposure. In the cellular level, the xylem, phloem, epidermis, parenchyma, sclerenchyma, and cell walls of the cross-sectional and longitudinal roots, stems, and leaves have experienced breaking and changes in size, shape, and arrangement that were induced by the metal accumulation. ANOVA results showed that the leaves' DNA concentrations were significantly reduced ranging from 67.73 to 195.54 ng/µL and 56.10 to 212.05 ng/µL at higher Mn and Cd concentrations; similarly to the changes in DNA purity ($p < 0.05$). The ANOVA statistics showed that the removal efficiency, water-to-shoot bioaccumulation factor (BAF), and root-to-shoot translocation factors (TF) was significantly reduced at higher Mn concentrations ($p < 0.05$). The highest concentration of Mn and Cd was found in the dried (DHS) and raw (RHS) shoots with the highest slope values of 3.75

and 19.50, respectively. Both Mn and Cd had the highest bioaccessibility for absorption in the gastric phase (slope values = 9.68 and 28.28) than intestinal phase (slope values = 0.24 and 17.99). The health risk index showed values > 1, indicated that the raw (RHS) and cooked (CHS) wild water spinach contaminated with Mn and Cd were not safe to be consumed for the studied population in Selangor, Malaysia. As conclusion, impacts of Mn and Cd were clearly seen when changes occurred in the health status, growth, histological structure, and DNA quality of the metal-contaminated wild water spinach. These metals absorbed in the human gastrointestinal tract could eventually cause health hazards when consuming the metal-contaminated wild water spinach as demonstrated in this work. Nevertheless, wild water spinach can serve as an alternative for phytoremediation on metals-contaminated aqueous medium due to its fairly good metal uptake ability.

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

KESAN MANGAN DAN KADMIUM KE ATAS ATRIBUT BIOLOGI KANGKUNG LIAR (*Ipomoea aquatica* Forssk.)

Oleh

BILLY GUAN TECK HUAT

September 2017

Pengerusi : Ferdaus @ Ferdius Mohamat Yusuff, PhD
Fakulti : Pengajian Alam Sekitar

Logam berat adalah bahan pencemar inorganik yang berbahaya dan bertoksik kepada alam sekitar. Aktiviti pertanian secara tidak langsung menyebabkan logam berat khasnya mangan (Mn) dan kadmium (Cd) memasuki ekosistem dan akhirnya telah mencemarkan ekosistem akuatik termasuklah kolam-kolam yang terletak berhampiran di Universiti Putra Malaysia. Percemaran air oleh logam berat tersebut boleh memberi kesan kepada kehidupan kangkung liar (*Ipomoea aquatica* Forssk.), iaitu sejenis tumbuhan akuatik yang boleh dimakan yang hidup di dalam kolam. Oleh demikian, kesihatan manusia terancam apabila kangkung liar yang tercemar oleh logam berat dimakan oleh mereka. Jadi, kesan-kesan Mn and Cd terhadap status kesihatan, pertumbuhan, anatomi, dan kualiti DNA bagi kangkung liar dikaji. Tambahan pula, keupayaan pengambilan logam berat oleh kangkung liar perlu ditentukan. Bioavailabiliti logam berat dan risiko kesihatan juga telah dinilai apabila kangkung liar tercemar oleh logam berat dimakan. Kangkung liar yang matang telah ditanam secara hidroponik di dalam rumah hijau dan diberikan rawatan Mn dan Cd pada kepekatan yang rendah (0.30 mg/L untuk Mn dan 0.10 mg/L untuk Cd), kepekatan yang tinggi (1.50 mg/L untuk Mn dan 0.50 mg/L untuk Cd), dan air suling sebagai kawalan selama tujuh hari. Analisis ANOVA menunjukkan pengurangan yang ketara telah diperhatikan bagi panjang dan kawasan permukaan akar, panjang pucuk, dan kawasan permukaan daun kangkung liar tercemar oleh logam berat dengan peningkatan kepekatan Mn dan Cd ($p < 0.05$). Simptom toksik iaitu klorosis dan nekrosis juga berlaku pada kangkung liar selepas diberikan rawatan logam berat. Kajian histologi menunjukkan sel xilem, floem, epidermis, parenkima, sklerenkima, dan dinding sel bagi keratan rentas dan memanjang akar, batang, dan daun telah mengalami pemecahan dan perubahan saiz, bentuk, dan susunan yang disebabkan oleh pengumpulan logam berat. Keputusan ANOVA menunjukkan bahawa pengurangan yang signifikan pada kepekatan DNA daun di antara 67.73 dan 195.54 ng/µL dan antara 56.10 dan 212.05 ng/µL apabila kepekatan Mn dan Cd semakin meningkat. Pengurangan yang ketara juga berlaku pada ketulenan DNA daun ($p < 0.05$). Statistik ANOVA menunjukkan bahawa *removal efficiency*, faktor biokonsentrasi *water-to-shoot* (BAF), dan faktor translokasi *root-to-shoot* (TF) telah dikurangkan dengan ketara pada kepekatan Mn yang tinggi ($p < 0.05$).

Kandungan Mn and Cd yang tertinggi telah dijumpai di CHS and RHS dengan kecerunan tertinggi iaitu 3.75 dan 19.50. Kedua-dua logam berat ini menunjukkan bioasesibiliti tertinggi dalam proses penyerapan dalam fasa gastrik (Nilai kecerunan = 9.68 dan 28.28) berbanding dengan fasa usus (Nilai kecerunan = 0.24 dan 17.99). Indeks risiko bahaya (HRI) menunjukkan nilai > 1, menunjukkan kangkung liar yang tercemar dengan Mn and Cd adalah tidak selamat untuk dimakan bagi populasi yang telah dikaji di Selangor, Malaysia. Secara kesimpulannya, kesan-kesan toksik Mn dan Cd dapat dilihat dengan jelas apabila perubahan berlaku pada status kesihatan, pertumbuhan, histologi, dan kualiti DNA Logam berat akan diserap dalam saluran percernaan manusia dan berkemungkinan merbahaya kepada kesihatan Namun demikian, kangkung liar boleh digunakan sebagai alternatif untuk fitoremediasi bagi medium akueus yang tercemar dengan logam berat kerana tumbuhan ini mempunyai keupayaan pengambilan logam berat yang agak baik.

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I certify that a Thesis Examination Committee has met on 8 September 2017 to conduct the final examination of Billy Guan Teck Huat on his thesis entitled "Effect of Manganese and Cadmium on Biological Attributes of Wild Water Spinach (*Ipomoea aquatica* Forssk.)" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Doctor of Philosophy.

Members of the These Examination Committee were as follows:

Latifah Abdul Manaf, PhD
Associate professor
Faculty of Environmental Studies
Universiti Putra Malaysia
(Chairman)

Rosimah Binti Nulit, PhD
Associate professor
Faculty of Science
Universiti Putra Malaysia
(Internal Examiner)

Hishamuddin Bin Omar, PhD
Senior lecturer
Faculty of Science
Universiti Putra Malaysia
(Internal Examiner)

Mokhtar Ibrahim Yousef, PhD
Professor
University of Alexandria
Egypt
(External Examiner)

NOR AINI AB. SHUKOR, PhD
Professor and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 30 November 2017

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

Ferdaus @ Ferdius Mohamat Yusuff, PhD
Senior lecturer
Faculty of Environmental Studies
Universiti Putra Malaysia
(Chairman)

Normala Halimoon, PhD
Senior lecturer
Faculty of Environmental Studies
Universiti Putra Malaysia
(Member)

Christina Yong Seok Yien, PhD
Senior lecturer
Faculty of Science
Universiti Putra Malaysia
(Member)

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Supervisory
Committee: ________________________

Signature: ________________________
Name of
Member of
Supervisory
Committee: ________________________

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%	Percentages
℃	Celsius
µmol	Micromols
rpm	Revolutions per minute
mBar	Millibars
H	Hours
min	Minutes
ms	Millisiemens
µS/cm	Microsiemens per centimeter
L	Liters
mL	Milliliters
µL	Microliters
cm^2	Square centimeters
cm	Centimeters
mm	Millimeters
µm	Micrometers
nm	Nanometers
kg	Kilograms
g	Grams
G	Gravity forces
mg	Milligrams
µg	Micrograms
mg/kg	Milligrams per kilogram
mg/g	Milligrams per gram

μg/g	Micrograms per gram
mg/L	Milligrams per liter
μg/L	Micrograms per liter
mg/mL	Milligrams per milliliter
mg/ μL	Milligrams per microliter
ng/ μL	Nanograms per microliter
g/d	Grams per day
mg/d	Milligrams per day
mg/kg/d	Milligrams per kilogram per day
μg/d	Micrograms per day
kg/d	Kilograms per day
g/cm^3	Grams per cubic centimeter
ppm	Parts per million
$mg/m^2/year$	Milligrams per square meter per year
ng/m^3	Nanograms per cubic meter
$\mu g/m^3$	Micrograms per cubic meter
gm/Nm^3	Grams per normal cubic meter
mA	Microamperes
g/mL	Grams per milliliter
M	Molars
mM	MilliMolars
μM	MicroMolars
μM/L	MicroMolars per liter
mg/dm^3	Milligrams per cubic decimeter

LIST OF ABBREVIATIONS

AAS	Atomic absorption spectroscopy
ANOVA	Analysis of variance
ANVISA	National Agency for Sanitary Vigilance
BAF/BCF	Bioaccumulation factor/bioconcentration factor
C, T1, and T2	Control, low treatment, and high treatment
CAC	Codex Alimentarius Commission
Cd	Cadmium
CTAB	Cetyltrimethylammonium bromide
DHS, RHS, and CHS	Dry-harvest shoots, raw-harvest shoots, and cook-harvest shoots
DNA	Deoxyribonucleic acid
DO	Dissolved oxygen
DOE	Department of Environment of Malaysia
DSM	Department of Statistics Malaysia
EC	Electrical conductivity/ European Commission
EQA	Malaysia Environmental Quality Act
EU	European Union
FAA	Formalin, acetic acid, and alcohol
FAMA	Federal Agricultural Marketing Authority
FAO/WHO	Joint Food and Agriculture Organization and World Health Organization
G1, G2, and G3	Greenhouse 1, greenhouse 2, and greenhouse 3
GT	Gastrointestinal tract
HKFEHD CFS	Hong Kong Food and Environmental Hygiene Department, Centre for Food Safety
HMs	Heavy metals

HRI	Health risk index
ICP-OES	Inductively coupled plasma optical emission spectrometry
INWQS	Interim National Water Quality Standards Malaysian
MHPRC	Ministry of Health of the People's Republic of China
MFR	Malaysian Food Regulations
Mn	Manganese
MWA	Malaysian Water Association
PFA	Prevention of Food Adulteration Act
ROS	Reactive oxygen species
SRM	Standard reference material
TF	Translocation factor
UK	United Kingdom
USA	United States of America
USDA	United States Department of Agriculture
USDHHS	United Stated Department of Health and Human Services
USEPA	United States Environmental Protection Agency
WEPs	Wild edible plants
WHO	World Health Organization
WHO/EU	World Health Organization Regional Office for Europe

CHAPTER 1

INTRODUCTION

Surface water serves as the breeding habitat for aquatic life. However, the quality of surface water is deteriorating due to the increasing of anthropogenic activities. Huang *et al*. (2015) have reported that the number of clean rivers in Malaysia was reduced from 338 to 278 when compared to year 2005 with 2012. Surface water pollution occurs when there is excessive of organic or inorganic pollutant present in the water. Heavy metals (HMs) such as chromium (Cr), copper (Cu), zinc (Zn), manganese (Mn), iron (Fe), magnesium (Mg), nickel (Ni), and cobalt (Co), mercury (Hg), arsenic (As), cadmium (Cd), and lead (Pb) are examples of inorganic pollutants. Agriculture activity is one of the anthropogenic sources for heavy metals particularly Mn and Cd. Many of the agrochemicals used in the agriculture contain Mn and Cd (Zhao *et al*., 2015). Thus the uncontrollable usage of fertilizers and pesticides can indirectly pollute the surface waters like lakes, ponds, and streams that are located near to the agricultural land through surface runoff (Parris, 2011; Wang *et al*., 2016).

Heavy metal contamination in surface water can endanger the aquatic life that is living in the water. Aquatic plants absorb nutrients from the water through roots that are essential for photosynthesis. Meanwhile, heavy metals that are existed in the water are being absorbed by the aquatic plants as well. Consequently, the continuous accumulation of heavy metals can disrupt the plant growth and trigger photo-oxidative stress (Lambert and Davy, 2011). Heavy metals contaminated aquatic plants in the water become a human health concern because some species of aquatic plants are edible. Examples of edible aquatic plants are wild water spinach, wild taro, cattails, wild rice, etc. The edible aquatic plants mentioned previously are actually being harvested or foraged for consumption by the locals in some countries including Malaysia. The heavy metals that were bioaccumulated in the edible aquatic plants can be absorbed, transferred, and stored in the human bodies from ingestion; in the long-term, the central nervous system, liver, kidneys, heart, lungs, skin, reproduction can be damaged due to the carcinogenicity of heavy metals (Panagos *et al*., 2013). One of the most serious cases of heavy metal poisoning was happened in Toyama, Japan in the early 1950s where the locals suffered a disease called as *itai-itai* disease that was caused by acute cadmium toxicity (Bhattacharya, 2009; Yang *et al*., 2012). The outbreak of the disease was due to the consumption of cadmium contaminated rice.

Different countries have different mitigation approaches to overcome the water pollution issues. In Malaysia, legislations such as Environmental Quality Act (EQA) 1974, National Water Quality Standards (NWQS), Malaysian Water Association's (MWA) raw water quality criteria, and water quality index (WQI) are adopted to control the water pollution; besides that, swale, infiltration facility, bioretention, gross pollutant traps (GPTs), sediment ponds, wet ponds, wetlands, and wastewater treatment plant were implemented which were proposed in the Urban Stormwater Management Manual for Malaysia (MSMA) to improve the water quality (Mamum and Zainudin, 2013). On the other hand, a hands-on approach is applied in China to deal with the water pollution which includes water diversions, dredging, and wetland construction

(Yang *et al*., 2010). In addition, physical, chemical, and biological methods, for example membrane filtration, ion exchange, electrodialysis, and biosorption can be carried out to solve the water pollution problems (Gunatilake, 2015). These techniques are effective but also expensive, labor and energy intensive, hazardous, and complicated (Barakat, 2011).

Phytoremediation is a promising method that is relatively low cost, safe, and easy to remove unwanted heavy metals from the contaminated water. Phytoremediation is the use of plants to remediate contamination. In order to effectively remove heavy metals from the water, it is crucial to select suitable plant species that able to adapt well in the aqueous environment. Aquatic plants are ideal choices because of their free-floating and submerge capability in water. Water hyacinth, water lettuce, and duckweed are examples of heavy metal hyperaccumulating aquatic plants. Generally, heavy metals is taken, accumulated, translocated, and stored in plant organs. The metal uptake mechanisms by a plant can be through adsorption, accumulation, and absorption. Phytoremediation is becoming increasingly popular, trendy, and fast growing especially in the United States and Europe (Lelie *et al*., 2001). Nevertheless, phytoremediation is still not well-known in the Asian countries and thus it is deserved to be further explored.

This research has proposed an edible aquatic plant that is commonly found in the ponds or lakes to be added into the existing list of potential plants for phytoremediation. Wild water spinach or *Kangkung* is one of the native plants in Malaysia and it is merely considered as a type of vegetable; despite that, this underrated plant can be exploited for the application of phytoremediation to clean the heavy metals contaminated surface water. It will be beneficial to promote the establishment of many research and development (R & D) companies to focus in phytoremediation technology in the future. Since wild water spinach is easily available and abundant but most importantly it is effective in eliminating heavy metals, therefore it will certainly be an attractive addition to other aquatic plants species such as water hyacinth and duckweed that were hugely studied for remediating heavy metal polluted water. Furthermore, this research will help to promote public awareness in regards to food safety. Wild water spinach is able to uptake heavy metals from its surrounding and it will be a public health concern when eating the metal-contaminated wild water spinach. So far it is yet to discover any casualty involved due to the consumption of metal-contaminated wild water spinach.

The objectives of this research are listed as follows:

1. To examine the health status and growth of the metal-contaminated wild water spinach.

2. To identify and investigate the changes on the microscopic cell structure and DNA quality of the metal-contaminated wild water spinach.

3. To determine the effectiveness of Mn and Cd uptake by wild water spinach.

4. To assess the bioavailability of metals for absorption from the *in vitro* gastrointestinal digestion of wild water spinach.

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