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

BILLY GUAN TECK HUAT

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

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

September 2017
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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.)

By

BILLY GUAN TECK HUAT

September 2017

Chairman : Ferdaus @ Ferdius Mohamad 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

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September 2017

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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 termasuk kolam-kolam yang terletak berhampiran di Universiti Putra Malaysia. Percemaran air oleh logam berat tersebut boleh memberi kesan kepada kehidupan kangkung liar (Ipomea aquatica Forssk.), iaitu sejenis tumbuhan akuatik yang boleh dimakan yang hidup di dalam kolam. Oleh demikian, kesihatan manusia terancam apabila logam berat dimakan oleh mereka. Jadi, kesan-kesan Mn dan Cd terhadap status kesihatan, pertumbuhan, anatomi, dan kualiti DNA bagi kangkung liar dikaji. Tambah pula, keupayaan pengambilan logam berat oleh kangkung liar perlu ditentukan. Bioavailabiliti logam berat dan risiko kesihatan juga telah dinilai untuk apabila logam berat dimakan. Kangkung liar yang matang telah ditanam secara hidroponik di dalam rumah hijau dan diberikan rawatan Mn dan Cd pada kepekatan 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, sklerenkim, 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 dikiturangkan 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 kesehatan, pertumbuhan, histologi, dan kualiti DNA Logam berat akan diserap dalam saluran percernaan manusia dan berkemungkinan merbahaya kepada kesehatan Namun demikian, kangkung liar boleh digunakan sebagai alternatif untuk fitoremediasi bagi medium akuues 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.

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<td>Cross Section of Wild Water Spinach Stems (Magnification 400×) (a) Mn Experiment; (b) Cd Experiment (n = 3). Abbreviation: Epidermis (ep), Collenchyma (c), Parenchyma (p), Sclerenchyma (scl), Xylem (xyl), and Phloem (phl). Scale: 100 µm. Arrow Indicates the Breaking of Cortex Cells and Changes in Size, Shape, and Arrangement of Vascular Bundles</td>
<td>85</td>
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<tr>
<td>4.4</td>
<td>Cross Section of Wild Water Spinach Leaves (Magnification 100×) (a) Mn Experiment; (b) Cd Experiment (n = 3). Abbreviation: Epidermis (ep), Collenchyma (c), Parenchyma (p), Sclerenchyma (scl), Xylem (xyl), and Phloem (phl). Scale: 100 µm. Arrow Indicates the Breaking of Cortex Cells, Vascular Bundles, Etc.</td>
<td>86</td>
</tr>
<tr>
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<td>88</td>
</tr>
<tr>
<td>4.6</td>
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<td>89</td>
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<tr>
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<td>90</td>
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4.9 Agarose Gel Electrophoresis of the DNA extracted from the Wild Water Spinach (a) Roots; (b) Stems; (c) Leaves with Identical Sample Arrangement. Lanes 1 and 20 = The Lambda Hindlll DNA Marker (fragments from 564 to 2027, 2322, 4361, 6557, 9416, and 23130 bp); Lanes 2 to 7 = The Mn-Control Specimen of 1 to 6; Lanes 8 to 13 = The Mn-T1-Treated Specimen of 1 to 6; Lanes 14 to 19 = The Mn-T2-Treated Specimen of 1 to 6; Lanes 21 to 26 = The Cd-T2-Treated Specimen of 6 to 1; Lanes 27 to 32 = The Cd-T1-Treated Specimen of 6 to 1; Lanes 33 to 38 = The Cd-Control Specimen of 6 to 1

4.10 Comparisons between the Mean Cd Concentrations and Maximum Permissible Limits (mean ± SE, n = 3)
# LIST OF SYMBOL

<table>
<thead>
<tr>
<th>Symbol</th>
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</thead>
<tbody>
<tr>
<td>%</td>
<td>Percentages</td>
</tr>
<tr>
<td>°C</td>
<td>Celsius</td>
</tr>
<tr>
<td>μmol</td>
<td>Micromols</td>
</tr>
<tr>
<td>rpm</td>
<td>Revolutions per minute</td>
</tr>
<tr>
<td>mBar</td>
<td>Millibars</td>
</tr>
<tr>
<td>H</td>
<td>Hours</td>
</tr>
<tr>
<td>min</td>
<td>Minutes</td>
</tr>
<tr>
<td>ms</td>
<td>Millisiemens</td>
</tr>
<tr>
<td>μS/cm</td>
<td>Microsiemens per centimeter</td>
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<tr>
<td>L</td>
<td>Liters</td>
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<tr>
<td>mL</td>
<td>Milliliters</td>
</tr>
<tr>
<td>μL</td>
<td>Microliters</td>
</tr>
<tr>
<td>cm²</td>
<td>Square centimeters</td>
</tr>
<tr>
<td>cm</td>
<td>Centimeters</td>
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<tr>
<td>mm</td>
<td>Millimeters</td>
</tr>
<tr>
<td>μm</td>
<td>Micrometers</td>
</tr>
<tr>
<td>nm</td>
<td>Nanometers</td>
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<td>kg</td>
<td>Kilograms</td>
</tr>
<tr>
<td>g</td>
<td>Grams</td>
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<tr>
<td>G</td>
<td>Gravity forces</td>
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<tr>
<td>mg</td>
<td>Milligrams</td>
</tr>
<tr>
<td>μg</td>
<td>Micrograms</td>
</tr>
<tr>
<td>mg/kg</td>
<td>Milligrams per kilogram</td>
</tr>
<tr>
<td>mg/g</td>
<td>Milligrams per gram</td>
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</tbody>
</table>
\begin{itemize}
\item \(\mu g/g\) Micrograms per gram
\item \(mg/L\) Milligrams per liter
\item \(\mu g/L\) Micrograms per liter
\item \(mg/mL\) Milligrams per milliliter
\item \(mg/\mu L\) Milligrams per microliter
\item \(ng/\mu L\) Nanograms per microliter
\item \(g/d\) Grams per day
\item \(mg/d\) Milligrams per day
\item \(mg/kg/d\) Milligrams per kilogram per day
\item \(\mu g/d\) Micrograms per day
\item \(kg/d\) Kilograms per day
\item \(g/cm^3\) Grams per cubic centimeter
\item ppm Parts per million
\item \(mg/m^3/year\) Milligrams per square meter per year
\item \(ng/m^3\) Nanograms per cubic meter
\item \(\mu g/m^3\) Micrograms per cubic meter
\item \(gm/Nm^3\) Grams per normal cubic meter
\item mA Microamperes
\item \(g/mL\) Grams per milliliter
\item M Molars
\item mM MilliMolars
\item \(\mu M\) MicroMolars
\item \(\mu M/L\) MicroMolars per liter
\item \(mg/dm^3\) Milligrams per cubic decimeter
\end{itemize}
# LIST OF ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>AAS</td>
<td>Atomic absorption spectroscopy</td>
</tr>
<tr>
<td>ANOVA</td>
<td>Analysis of variance</td>
</tr>
<tr>
<td>ANVISA</td>
<td>National Agency for Sanitary Vigilance</td>
</tr>
<tr>
<td>BAF/BCF</td>
<td>Bioaccumulation factor/bioconcentration factor</td>
</tr>
<tr>
<td>C, T1, and T2</td>
<td>Control, low treatment, and high treatment</td>
</tr>
<tr>
<td>CAC</td>
<td>Codex Alimentarius Commission</td>
</tr>
<tr>
<td>Cd</td>
<td>Cadmium</td>
</tr>
<tr>
<td>CTAB</td>
<td>Cetyltrimethylammonium bromide</td>
</tr>
<tr>
<td>DHS, RHS, and CHS</td>
<td>Dry-harvest shoots, raw-harvest shoots, and cook-harvest shoots</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>DO</td>
<td>Dissolved oxygen</td>
</tr>
<tr>
<td>DOE</td>
<td>Department of Environment of Malaysia</td>
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<tr>
<td>DSM</td>
<td>Department of Statistics Malaysia</td>
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<tr>
<td>EC</td>
<td>Electrical conductivity/ European Commission</td>
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<tr>
<td>EQA</td>
<td>Malaysia Environmental Quality Act</td>
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<tr>
<td>EU</td>
<td>European Union</td>
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<tr>
<td>FAA</td>
<td>Formalin, acetic acid, and alcohol</td>
</tr>
<tr>
<td>FAMA</td>
<td>Federal Agricultural Marketing Authority</td>
</tr>
<tr>
<td>FAO/WHO</td>
<td>Joint Food and Agriculture Organization and World Health Organization</td>
</tr>
<tr>
<td>G1, G2, and G3</td>
<td>Greenhouse 1, greenhouse 2, and greenhouse 3</td>
</tr>
<tr>
<td>GT</td>
<td>Gastrointestinal tract</td>
</tr>
<tr>
<td>HKFEHD CFS</td>
<td>Hong Kong Food and Environmental Hygiene Department, Centre for Food Safety</td>
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<tr>
<td>HMs</td>
<td>Heavy metals</td>
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<td>Abbr.</td>
<td>Full Form</td>
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<tr>
<td>HRI</td>
<td>Health risk index</td>
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<tr>
<td>ICP-OES</td>
<td>Inductively coupled plasma optical emission spectrometry</td>
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<tr>
<td>INWQS</td>
<td>Interim National Water Quality Standards Malaysian</td>
</tr>
<tr>
<td>MHPRC</td>
<td>Ministry of Health of the People’s Republic of China</td>
</tr>
<tr>
<td>MFR</td>
<td>Malaysian Food Regulations</td>
</tr>
<tr>
<td>Mn</td>
<td>Manganese</td>
</tr>
<tr>
<td>MWA</td>
<td>Malaysian Water Association</td>
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<tr>
<td>PFA</td>
<td>Prevention of Food Adulteration Act</td>
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<tr>
<td>ROS</td>
<td>Reactive oxygen species</td>
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<tr>
<td>SRM</td>
<td>Standard reference material</td>
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<tr>
<td>TF</td>
<td>Translocation factor</td>
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<tr>
<td>UK</td>
<td>United Kingdom</td>
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<tr>
<td>USA</td>
<td>United States of America</td>
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<tr>
<td>USDA</td>
<td>United States Department of Agriculture</td>
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<tr>
<td>USDHHS</td>
<td>United Stated Department of Health and Human Services</td>
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<tr>
<td>USEPA</td>
<td>United States Environmental Protection Agency</td>
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<tr>
<td>WEPs</td>
<td>Wild edible plants</td>
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<tr>
<td>WHO</td>
<td>World Health Organization</td>
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<tr>
<td>WHO/EU</td>
<td>World Health Organization Regional Office for Europe</td>
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</tbody>
</table>
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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