UNIVERSITI PUTRA MALAYSIA

ASSESSMENT OF HEAVY METALS IN Amaranthus viridis L. FROM PENINSULAR MALAYSIA

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FS 2022 6

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By

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Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfillment of the Requirement for the Degree of Master of Science

September 2021

Abstract of the thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the Degree of Master of Science

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September 2021

Chairman : Yap Chee Kong, PhD
Faculty: Sains

Human health risk and phytoremediation of potentially toxic metals (PTMs) in edible vegetables have been widely discussed nowadays. This study aimed to determine the concentrations of six PTMs, namely Cd, Cu, Fe, Ni, Pb, and Zn) in *Amaranthus viridis* (leaves, stems and roots) collected from 11 sampling sites in Peninsular Malaysia and to assess their human health risk (HHR). Fifteen individuals of *A. viridis* were analysed from each 11 sampling sites with three replicates. All metal content in plant and soil samples were determined by the atomic absorption spectrophotometer (AAS) iCE 3000 series at the Department of Chemistry of UPM. In general, the metal levels followed the order: roots> stems> leaves. The metal concentrations (µg/g) in the leaves of *A. viridis* ranged from 0.45 to 2.18 dry weight (dw) (0.05-0.26 wet weight (ww)), from 74.8 to 535 dw (8.97-64.2 ww), 2.02 to 7.45 dw (0.24-0.89 ww), and 65.2 to 521 dw (7.83-62.6 ww), for Cd, Fe, Ni and Zn, respectively. The positive relationships between the metals, the plant parts and the geochemical factions of their habitat topsoils indicated the potential of *A. viridis* as good a biomonitor of Cd, Fe, Ni, and Pb pollution. With most of the values of BCF> 1.0 and TF> 1.0, thus *A. viridis* is a very potential phytoextraction agent of Cu, Ni and Zn. Additionally, with most of the values of BCF> 1.0 and TF< 1.0, *A. viridis* is a very potential phytostabiliser of Cd, Fe, and Pb. The experimental greenhouse findings indicated three points. Firstly, wastewater irrigation could cause the accumulation of Cu and Pb in the different parts of *A. viridis,* although these two metals did not highly contaminate the wastewater. Secondly, the accumulated metal levels in the different parts of *A. viridis* after 35 days of the experimental greenhouse study were mainly within the metal ranges found in the field-collected samples. Thirdly, the findings also indicated that *A. viridis* had the potential to be biomonitors of Cu and Pb. Concerning HHR, the target hazard quotients (THQ) for Cd, Cu, Fe, Pb and Zn in the leaves of *A. viridis* were all below 1.00, indicating there were no non-carcinogenic risks four metals to consumers, including children and adults. Still, routine monitoring of PTMs in *Amaranthus* farms is much needed.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

MENILAI LOGAM BERAT DI DALAM *Amaranthus viridis* L. DARI SEMENANJUNG MALAYSIA

Oleh

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September 2021

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Risiko kesihatan manusia dan fitoremediasi terhadap logam berpotensi toksik (PTM) dalam sayur-sayuran yang boleh dimakan telah dibincangkan secara meluas pada hari ini. Kajian ini bertujuan untuk menentukan kepekatan enam PTM iaitu Cd, Cu, Fe, Ni, Pb dan Zn) dalam *Amaranthus viridis* (daun, batang dan akar) yang diperolehi dari 11 tapak persampelan di Semenanjung Malaysia, dan seterusnya membuat penilaian terhadap risiko kesihatan manusia. (HHR). 15 individu *A. viridis* telah dianalisis dari 11 tapak persampelan dengan 3 replika setiapnya. Semua kandungan logam dalam sampel tumbuhan dan tanah ditentukan oleh spektrofotometer serapan atom (AAS) iCE siri 3000 di Jabatan Kimia UPM. Secara umum, tahap logam mengikut urutan: akar> batang> daun. Kepekatan logam (µg/g) dalam daun *A. viridis* adalah antara 0.45 hingga 2.18 berat kering (dw) (0.05-0.26 berat basah (ww)), dari 74.8 hingga 535 dw (8.97-64.2 ww), 2.02 hingga 7.45 dw (0.24-0.89 ww), dan 65.2 hingga 521 dw (7.83-62.6 ww), masing-masing untuk Cd, Fe, Ni dan Zn. Hubungan positif antara logam, bahagian tumbuhan dan lapisan geokimia tanah habitatnya menunjukkan potensi *A. viridis* sebagai biomonitor pencemaran Cd, Fe, Ni dan Pb yang baik. Dengan kebanyakan nilai BCF> 1.0 dan TF> 1.0, maka *A. viridis* dianggap sebagai agen fitoekstraksi yang sangat berpotensi bagi Cu, Ni dan Zn. Manakala nilai BCF> 1.0 dan TF< 1.0, meletakan *A. viridis* sebagai agen fitostabiliti yang sangat berpotensi untuk logam Cd, Fe dan Pb. Hasil eksperimen rumah hijau pula menunjukkan tiga perkara. Pertama, pengunaan air sisa boleh menyebabkan pengumpulan Cu dan Pb di bahagian *A. viridis* yang berbeza walaupun air sisa tidak benar-benar tercemar oleh kedua-dua logam ini. Kedua, paras logam yang terkumpul di bahagian berlainan *A. viridis* selepas 35 hari kajian rumah hijau mendapati kebanyakannya berada dalam julat logam yang terdapat dalam sampel yang dikumpul di lapangan. Ketiga, dapatan juga menunjukkan bahawa *A. viridis* mempunyai potensi untuk menjadi biomonitor Cu dan Pb. Berkenaan dengan HHR, THQ untuk Cd, Cu, Fe, Ni, Pb dan Zn dalam daun *A. viridis* semuanya di bawah 1.00, ini menunjukkan tiada risiko bukan karsinogenik bagi enam logam tersebut kepada pengguna, termasuk kanak-kanak dan orang dewasa. Namun, pemantauan rutin PTM di ladang *Amaranthus* amat diperlukan.

ACKNOWLEDGEMENTS

First of all, I am grateful to Allah S.W.T. for establishing me to live, learn, and experience many things throughout this Master journey. Thank you Allah S.W.T., for giving me the strength and courage to complete this.

I want to take this opportunity to express my gratitude toward my helpful and friendly supervisor, Prof. Dr. Yap Chee Kong, for his outstanding support, patience, and encouragement throughout my studies without him, this thesis would be impossible to be complete. I am also thankful to my co-supervisors, Assoc. Prof. Dr. Rosimah bt Nulit for her willingness to be a part of the committee members and for helping me complete my study.

I wish to thank all the staff in the Department of Biology, En Hidir, En. Izani, and En. Abdullah and from the Department of Chemistry, En. Zainal Zahari for their assistance and cooperation during the studies, especially during my laboratory work and samples analysis.

I dedicate this thesis to my family, especially my wife, Yaneliawati bt Lauhana, my lovely children Adam, Hambali, Uzair and Sarah for their support, love, and patience that has given me the motivation to keep on going until the end.

Not forgotten, thanks to all my friends, especially Wong Koe Wei, Zainurfatin and biology department members, without your help and support, i may face numerous problems along this journey; thank you so much. May Allah bless you all.

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

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This is to confirm that:

- The research conducted and the writing of this thesis was under our supervision;
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LIST OF ABBREVIATIONS

As	Arsenic
BCF	Bioconcentration Factor
Cd	Cadmium
CI	Condition index
Co	Cobalt
CPI	Combined Pollution Index
CRM	Certified Reference Material
Cu	Copper
CV	Coefficient of variation
EDI	Estimated Daily Intakes
EF	Enrichment factor
EFLE	Easily, freely or leachable and exchangeable
Fe	Iron
Mn	Manganese
Ni	Nickel
Pb	Lead
PERI	Potential Ecological Risk Index
PTMs	Potentially Toxic Metals
RfD	Oral reference dose
SET	Sequential Extraction Technique
TF	Translocation Factor
THQ	Target Hazard Quotient
Zn	Zinc

CHAPTER 1

INTRODUCTION

1.1 Background of the study

A public health concern is that contaminated wastewater was used to irrigate *Amaranthus*, a food source for humans in Nigeria (Adekiya et al., 2019; Oguntade et al., 2015). The primary source of contamination by potentially toxic metals (PTMs) in agricultural lands is usually anthropogenic inputs such as sewage sludge (Kabata-Pendias and Pendias, 2001) residues from widespread mining and various industries (Benavides et al., 2005; Yap, 2019). Improper applications of fertilisers or pesticides from atmospheric sources elevated the concentration of PTMs in soil (Zarcinas et al., 2004). In general, the primary sources of PTMs are from the manufacturing industries, urbanisation practices and agro-based industries (Parisa et al., 2010) due to the effects of wastewater used for the irrigation of *Amaranthus* have been reported in the literature (Adekiya et al., 2019; Oguntade et al., 2015; 2018).

The present study investigated the presence of PTMs in *A. viridis* in Malaysia, which is considered a high-biomass non-hyperaccumulating plant (HBNP). Suman et al. (Suman et al., 2018) reported that the metal limits in the dry biomass of plants to be considered as hyperaccumulators are 1.00×10^2 mg/kg for Cd, 1.00×10^3 mg/kg for Ni, and 1.00×10^4 mg/kg for Zn (Baker and Brooks, 1989). These values are up to 100–1,000-fold over non-hyperaccumulating species under comparable conditions (Rascio and Navari-Izzo, 2011; Reeves, 2006).

The use of fast-growing weeds and non-woody species for phytoextraction is a sensible decision, owing to the facts that these taxa i) can provide high biomass in a moderately short time frame, ii) have a high rate of water uptake and a deep root framework, iii) can be adequately and quickly reused through re-growing and, iv) their aboveground parts are not difficult to sample. Medicinal plants like peppermint (*Mentha piperita*) and lavender (*Lavandula angustifolia*) have been demonstrated to accumulate elevated levels of PTMs in their biomass (Zheljazkov and Nielsen, 1996a, 1996b). For example, maize (*Zea mays*) is generally known as HBNP for its quick growth rate, high biomass production, and general Cd resistance. In a field-scale preliminary study on Cd-polluted farmland in China, *Z. mays* accumulated up to 3 mg/kg of dry biomass while keeping grain Cd levels under the Chinese government's breaking point for coarse oats (Xu et al., 2013).

Kanakaraju et al. (Kanakaraju et al., 2007) studied the accumulations of Zn, Cu, Mn, Co, Pb and Fe in leafy vegetables (kale, green mustard and white mustard), as well as the long been gathered from Siburan and Beratok at Kuching, Sarawak, Malaysia. They found marginally higher Pb ranges in the vegetables. Molina et al. (Molina et al., 2011) reported the presence of nine PTMs (including Cd, Fe and Zn) in *A. dubious* used as forage in the diet of sheep, goats, pigs and cattle. Studies on PTMs health risk

assessments and phytoremediation capacity of edible *Amaranthus* have been reported in the literature (Azi et al., 2018; Lukatkin et al., 2021; Oluwatosin et al., 2009; Sahu and Kacholi, 2016), but to our knowledge, any specific trial on *A. viridis* has not been done.

A plant potential as a phytoremediator can be determined by calculating the plant bioconcentration factor (BCF; metal concentration ratio of plant roots to the soil) and translocation factor (TF; metal concentration ratio of plant shoots to roots) values (Nik Majid et al., 2012). The BCF (remainder of the substance of a given metal of the plant to its substance of soil) characterises the plant's capacity to accumulate PTMs. The TF is a proportion of the phytoextraction limit of plants (Pachura et al., 2016). Suppose both the BCF and TF values are above 1. In that case, the plant species has the potential to be a phytoextractor of metals, while a BCF value above 1 but a TF value below 1, indicating that the plant has potential as a phytostabiliser of metals (Yoon et al., 2006).

In this study, *A. viridis* was studied because i) it is among the top ten most popular vegetables, which is easy to grow and can quickly be harvested just after 28 days; ii) it has been used in previous research studies and reported in the literature (Kabata-Pendias and Pendias, 2001), and iii) it is highly recommended by the District Agriculture Department of Hulu Perak (Peninsular Malaysia) as a research vegetable. Therefore, the present study aimed i) to determine the PTMs levels (Cd, Fe, Ni and Zn) in *A. viridis* (leaves, stems and roots) collected from Peninsular Malaysia, ii) to assess the human health risks of PTMs in the edible leafy parts of *A. viridis* from Peninsular Malaysia, and iii) to assess the potentials of *A. viridis* as a phytoremediator of PTMs.

1.2 Problem statement

The rapid growth of industrial areas in Malaysia significantly contributes to public health issues. It is believed the total amount of heavy metals in the air persistently increases. However, the biomonitoring programme in Malaysia still has no best choice to reduce or overcome this problem. Currently, green mussels *Perna viridis* (Yap and Al-Barwani, 2012), tree bark (Ameran et al., 2014), mud crab (*Scylla Serrata*) (Ong et al., 2015) were suggested as living agents for heavy metals biomonitoring programmes. *Perna viridis* of bivalve species seems to be a great biomonitor in the aquatienvironmentsnt due to its ability tote various chemicals. However, the mussels are limited to aq aquatic biomonitoring. Meanwhile, tree bark utilisation as a biomonitor never appeared as a uniform result because different species have different capabilities in metal accumulation. While *Scylla serrata* can accumulate anthropogenic substances in situ as they are localized. Since the monitoring pollutants with single proposed species are deficiently, and lichen is the only living agent for terrestrial conditions, it is crucial to find out holistic terrestrial biomonitors to guarantee that the heavy metals monitoring in Malaysia may be possible done effectively.

Therefore, the present study investigated the potentials of *Amaranthus viridis* in assessing heavy metals pollution in Peninsular Malaysia. This species is proposed as a monitbecauseit is distributed abundantly in this country and its healthy growth due to

suitable temperature, pH soils, and environment humidity. In certain farming areas, *A. viridis* has been planted in open areas such as roadside, palm oil plantation and riverbanks. Being in abundantly distributed areas, the species will be a good indicator and provide information on pollutants for various farming sites in Malaysia. In addition, besides the plant being consumed as a delicious vegetable, it also offers different types of nutrients and vitamins by the locals. Therefore, it is essential to study this plant thoroughly if it accumulates a high concentration of metals, it could influence public health by consuming it.

The findings of this research soon would give new information and a better understanding. Efficiently plan and manage farming sites toward the accumulation of heavy metals in the environment and frame future strategies in solid waste management. This research would also serve as a foundation to evaluate the potential threat of heavy metals in Malaysia. Develop better strategies to prevent heavy metal pollution only when the ecological characteristics of heavy metals are understood.

1.3 The objectives of this study were:

1. To determine the heavy metal levels in *Amaranthus viridis* (leaves, stems and roots) collected from Peninsular Malaysia.

2. To determine the human health risk assessment of heavy metals in the edible leafy parts of *A. viridis* based on target hazard quotient (THQ) values from Peninsular Malaysia.

3. To assess the potentials of *A. viridis* as a phytoremediator of the heavy metals. Its potential as a phytoremediator of the six heavy metals was evaluated based on the transfer factor (TF) and bioconcentration factor (BCF) values.

4. To assess the potentials of *A. viridis* as a biomonitor of heavy metals. Evaluating its potential as a biomonitor of heavy metals was based on the correlation analysis of the metals in the different plant parts (leaves, stems, and roots) and its habitat topsoil. In addition, its potential as a biomonitor of Cu and Pb was conducted under a greenhouse experimental accumulation study.

1.4 Scope and limitations

The present study analysed only 6 elements; Cadmium (Cd), Copper (Cu), Iron (Fe), Lead (Pb), Nickel (Ni) and Zinc (Zn) in species of *Amaranthus viridis. A. viridis* is a farm species, not wild species, so permission was needed before sample collection was conducted in this study. Samples were collected from 11 following farming areas of Peninsular Malaysia ; Ara Kuda (Penang), Kg Sitiawan (Perak), Felda Taib Andak (Johor), Sikamat (Negeri Sembilan), Kuala Pegang (Kedah), Kg Raja (Terengganu),

Kelaboran (Kelantan), Bt 12 Gombak (Selangor), Simpang Ampat (Melaka), Kg Tawar (Perak) and Benta (Pahang). The parts of the plant investigated in this study were leaves, stems and roots. However, for health risk assessments, the roots of the plants were not analysed for the element because humans do not consume them. The levels of metals content were analysed from 15 matured individuals that readily for consumptions while soils were studied in only necessary packaging.

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