



UNIVERSITI PUTRA MALAYSIA

***BIOMONITORING, DISTRIBUTION AND RISK ASSESSMENT OF HEAVY
METALS AND POLYCYCLIC AROMATIC HYDROCARBONS IN
Asystasia gangetica (L.) T. ANDERSON AND SURROUNDING TOPSOIL
FROM
PENINSULAR MALAYSIA***

CHEW WEIYUN

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By

CHEW WEIYUN

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December 2016

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

BIOMONITORING, DISTRIBUTION AND RISK ASSESSMENT OF HEAVY METALS AND POLYCYCLIC AROMATIC HYDROCARBONS IN *Asystasia gangetica* (L.) T. ANDERSON AND SURROUNDING TOPSOIL FROM PENINSULAR MALAYSIA.

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December 2016

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In this study, the invasive weed *Asystasia gangetica* with their surrounding topsoil (0 - 10cm) were collected at 23 sites from Peninsular Malaysia. Samples were collected throughout Peninsular Malaysia during the period of 8th June 2011 to 17th January 2012. The plants (leaves, stems, and roots) and topsoil samples were determined for heavy metals (Cd, Cu, Fe, Ni, Pb and Zn) and 16 polycyclic aromatic hydrocarbons (PAHs). The ranges of the heavy metals in the plant ($\mu\text{g/g}$, dry weight) of this study for the respective metals are Cd: 0.01 – 5.11 $\mu\text{g/g}$, Cu: 4.05 – 139.03 $\mu\text{g/g}$, Fe: 18.10 – 2537.01 $\mu\text{g/g}$, Ni: 0.03-8.28 $\mu\text{g/g}$, Pb: 0.01 -31.59 $\mu\text{g/g}$, and Zn: 9.84 – 299.96 $\mu\text{g/g}$, while the ranges of PAHs concentrations (ng/g, wet weight) in the plant were 2 – 3 rings PAHs: 6.64 – 64.91 ng/g, 4 rings PAHs: 3.32 – 27.26 ng/g, and 5 – 6 rings PAHs: 1.42 – 226.37 ng/g. Topsoil from Sg. Kembung (S7), Juru (S18) and Kuala Terengganu (S21) have high concentrations of heavy metals and PAHs. Topsoil from these locations have high enrichment of heavy metals ($\text{EF} > 20$) and posed very strong ecological risk ($\text{ERI} > 600$). Furthermore, topsoil from these locations also can affected human health adversely. Present study determined highest toxicity equivalency factors (TEF) from S7 (34.28 ng/g), S18 (151.57 ng/g), and S21 (67.33 ng/g). The three sites also posed non-carcinogenic risk ($\text{HI} > 1$) and carcinogenic risk ($\text{Total Risk} > 1 \times 10^{-6}$) to adult and children in the area. Chemometric studies revealed that the three sites received significant input of mix sources of anthropogenic sources (heavy metals and PAHs). However, determination on the origin of anthropogenic sources was not possible due to lack of data. As for accumulation of contaminant in *A. gangetica*, plants sampled from Sg. Lembing (S13) and Juru (S18) generally accumulated higher heavy metals concentrations than other sites. Whereas, plant samples from Bidor (S4), Sepang (S6), Sg. Kembung (S7), and Kuala Krai (S11) generally contain higher PAHs than other sites. Accumulation behaviours of heavy metals in plants were generally consistent, however, increase of heavy metals in their habitat will induce changed in

accumulations behaviours. Present study revealed that *A. gangetica* have higher uptake of Cu and Zn from topsoil to roots while having low translocations of Cu, Fe, and Zn. However, it was revealed that leaves and roots of the plant have similar accumulations behaviours of heavy metals. As for accumulations behaviours of PAHs, leaves usually accumulated more PAHs than other parts. Roots accumulated higher concentrations of 2-3 rings PAHs than 5-6 rings PAHs from topsoil. Translocations of PAHs in plants were low. Principal component analysis (PCA) and correlation suggested that there were no relationships of the pollutants between plants and topsoil. Present study suggested that *A. gangetica* can be a potential biomonitor, however, further studies are needed.



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

PEMONITORAN BIOLOGI, PENILAIAN PENGAGIHAN DAN PENILAIAN RISIKO LOGAM BERAT DAN HIDROKARBON AROMATIK POLISIKLIK PADA *Asystasia gangetica* (L.) T. ANDERSON BERSERTA DENGAN TANAH LAPISAN ATAS PERSEKITARANNYA DARI SEMENANJUNG MALAYSIA.

Oleh

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Di dalam kajian ini, sampel – sampel rumput invasif *Asystasia gangetica* berserta dengan tanah lapisan atas (0 – 10cm) di Semenanjung Malaysia telah dikumpulkan. Cd, Cu Fe, Ni, Pb, and Zn dan 16 jenis hidrokarbon aromatik polisiklik (PAHs) yang terdapat di dalam sampel-sampel berikutnya telah dianalisiskan, termasuk bahagian-bahagian (daun, batang, dan akar) *A. gangetica* dan tanah lapisan atas. Sampel-sampel telah dikumpul daripada 23 lokasi di Semenanjung Malaysia bermula daripada 8 Jun 2011 sehingga 17 Januari 2012. Kepekatan logam berat di dalam sampel tumbuhan (keberatan kering) berjulat di antara Cd: 0.01 – 5.11 µg/g, Cu: 4.05 – 139.03 µg/g, Fe: 18.10 – 2537.01 µg/g, Ni: 0.03-8.28 µg/g, Pb: 0.01 -31.59 µg/g, dan Zn: 9.84 – 299.96 µg/g. Kepekatan PAHs di dalam sampel tumbuhan berjulat di antara 2 – 3 cincin PAHs: 6.64 – 64.91 ng/g, 4 cincin PAHs: 3.32 – 27.26 ng/g, dan 5 – 6 cincin PAHs: 1.42 – 226.37 ng/g. Sampel tanah dari Sg. Kembung, Juru, dan Kuala Terengganu mempunyai kepekatan logam berat dan PAHs yang lebih tinggi apabila dibandingkan dengan lokasi lain-lain. Sampel tanah dari lokasi-lokasi tersebut terdapat kekayaan logam berat yang tinggi (EF > 20) dan dapat menimbulkan risiko ekologi yang tinggi (ERI > 600). Selain itu, lokasi-lokasi tersebut juga memberi kesan-kesan buruk kepada kesihatan manusia. Penyelidikan ini mendapati bahawa S7 (34.28 ng/g), S18 (151.57 ng/g), and S21 (67.33 ng/g) mempunyai ‘Toxicity equivalency factors (TEF)’ yang lebih tinggi apabila dibandingkan dengan lokasi lain-lain. Selain itu, lokasi-lokasi tersebut didapati mempunyai risiko secara bukan karsinogenik (HI >1) dan risiko secara karsinogenik (Jumlah Risiko > 1 x 10⁻⁶) yang tinggi kepada dewasa and kanak-kanak. Kajian ‘chemometric’ mendedahkan bahawa tiga lokasi tersebut menerima input sumber antropogenik tercampur untuk logam berat and PAHs. Kajian ini tidak dapat menentukan sumber asal antropogenik di lokasi-lokasi tersebut kerana kekurangan data yang diperlukan. Secara umumnya, sampel-sampel tumbuhan yang dikumpulkan dari Sg. Lembing (S13) dan Juru (S18) mempunyai kepekatan logam berat yang lebih tinggi

apabila dibandingkan dengan lokasi lain-lain. Sampel-sampel tumbuhan dari Bidor (S4), Sepang (S6), Sg. Kambung (S7), dan Kuala Krai (S11) mempunyai kepekatan PAHs yang lebih tinggi apabila dibandingkan dengan lokasi lain-lain. Secara umumnya, tingkah-laku pengumpulan logam berat di dalam tumbuhan adalah konsisten, akan tetapi, peningkatan logam berat di sekeliling menyebabkan perubahan pada tingkah-laku pengumpulan logam berat. Kajian ini mendapati bahawa *A. gangetica* mempunyai pengumpulan Cu dan Zn yang lebih tinggi dari tanah ke dalam akarnya dan, pada masa yang sama, Cu, Fe, dan Zn mempunyai translokasi yang rendah. Tumbuhan-tumbuhan ini juga mempunyai tingkah-laku pengumpulan logam berat yang serupa pada daun-daun and akar-akar tumbuhan. Bagi tingkah-laku pengumpulan PAHs, daun-daun biasanya mempunyai PAHs lebih tinggi daripada bahagian-bahagian tumbuhan lain. Akar-akar pula terkumpul 2-3 rings PAHs yang lebih tinggi berbanding dengan 5-6 rings PAHs dari tanah. Analisis komponen prinsipal (PCA) menentukan bahawa bahan-bahan pencemar di dalam kedua-dua tanah dan bahagian-bahagian tumbuhan adalah tidak berkaitan antara satu sama lain. Kajian ini mendapati bahawa *A. gangetica* adalah potensi 'biomonitor', tetapi, kajian-kajian perlu dilanjutkan untuk mengesahkan potensi tumbuhan tersebut sebagai 'biomonitor'.

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I certify that a Thesis Examination Committee has met on 21 December 2016 to conduct the final examination of Chew Weiyun on his thesis entitled "Biomonitoring, Distribution and Risk Assessment of Heavy Metals and Polycyclic Aromatic Hydrocarbons in *Asystasia gangetica* (L.) T. Anderson and Surrounding Topsoil from Peninsular Malaysia" 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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LIST OF ABBREVIATIONS

AC	Accumulation coefficient
ACY	Acenaphthylene
ACP	Acenaphthene
ANT	Anthracene
BaA	Benzo[<i>a</i>]anthracene
BaP	Benzo[<i>a</i>]pyrene
BbF	Benzo[<i>b</i>]fluoranthene
BghiP	Benzo[<i>g,h,i</i>]perylene
BkF	Benzo[<i>k</i>]fluoranthene
Cd	Cadmium
CHR	Chrysene
CRM	Certified reference materials
Cu	Copper
DahA	Dibenzo[<i>a,h</i>]anthracene
ERI	Ecological Risk Index
EF	Enrichment factor
FLT	Fluoranthene
FLR	Fluorene
I _{geo}	Geoaccumulation index
HI	Hazard Index
HMW PAHs	High molecular weight Polycyclic Aromatic Hydrocarbons
IcdP	Indeno[<i>1,2,3-cd</i>]pyrene
Fe	Iron

Pb	Lead
LMW PAHs	Low molecular weight Polycyclic Aromatic Hydrocarbons
NAP	Naphthalene
Ni	Nickel
PHE	Phenanthrene
PCA	Principal Component analysis
PYR	Pyrene
TEF	Toxic Equivalency Factor
TF	Translocation Factor
TOC	Total Organic Carbon
Zn	Zinc

CHAPTER 1

INTRODUCTION

1.1 Background of study

Malaysia is a fast developing country with increasing traffic and industrial activities accompanying the modern lifestyle of today's society (DOE, 2015). However, the fast paced development of Malaysia has increased the output of anthropogenic sources (such as heavy metals and polycyclic aromatic hydrocarbons (PAHs) into its ambient environments. Coal burning, transportation, industrial, mining and agricultural activities and landfill emissions are a few examples which contributes to the anthropogenic heavy metals pollution in urban areas (Zakaria et al., 2005; Yap et al., 2011). In Peninsular Malaysia, numerous studies have already reported the occurrences of heavy metals and PAHs pollutions in coastal areas, estuarine rivers, mangroves, urban areas, lakes and etc. due to increasing urban activities (Zakaria et al., 2000; Zakaria et al., 2002; Yap et al., 2006; Omar et al., 2007; Maimon et al., 2009; Yap et al., 2009; Ahmad et al., 2010; Ashraf et al., 2011). However, limited studies have been focussed on terrestrial pollution in Malaysia.

It has been reported that heavy metals and polycyclic aromatic hydrocarbons (PAHs) are capable of disrupting the ecosystems of a living environment (Wolfe et al., 2007). Furthermore, long term exposure to these pollutants is toxic to human health (Akagi et al., 1995; Xue and Warshawsky, 2004).

Monitoring these anthropogenic sources is crucial to avoid unwanted toxicity on human and the environment. Generally, chemicals in the environment were measured using a few techniques, namely biological, exposure, emission, environmental, and process monitoring (Becher and Bjorseth, 1987). Each monitoring system has its own usage and target. As the amount of anthropogenic substances is rising in Malaysia, suitable biomonitorings are required to monitor the changes of anthropogenic substances. Monitoring antropogenic substances is imperative to not only ensure a healthy environment but also public health throughout time. Biomonitor which utilizes an organism as a chemical monitoring agent, allows study of contaminants within an organism. It enables the measurement of a long term effects of pollutants/xenobiotics in an organism (Siddig et al., 2016).

As Malaysia moves into achieving an industrialized nation in 2020, the blooming of industries is significantly causing a major public health issue in our country. It is inevitable that the cumulative amount of heavy metals and PAHs in the air is increasing. Currently in Malaysia, lichens (Samdudin et al., 2013), tree bark (Ameran et al., 2014), mud crab (*Scylla serrata*) (Ong et al., 2015) and green mussels *Perna viridis* (Shahbazi et al., 2010; Yap and Al-Barwani, 2012) were proposed as a few biomonitors for heavy metals and PAHs. One of the suggested agent, (*Scylla serrata*) capable of accumulating anthropogenic substances *in situ* as they are localized. Mussels (*Perna viridis*) are well

known great biomonitor in aquatic environment and capable of accumulating various chemicals. However, mussels are limited to aquatic biomonitoring. Meanwhile, using tree bark as a biomonitor does not give a uniform result as accumulation of compounds varies with species. Lichens however, are geographically diverse, abundance and can accumulate high levels of pollutants without dying; making them a great biomonitor (Bargagli, 2005). Monitoring pollutants with only proposed species is definitely insufficient and with lichen being the only feasible agent for terrestrial conditions. Hence, it is crucial to search for more holistic terrestrial biomonitors to ensure that the monitoring of pollutants in Malaysia could be done efficiently.

Therefore, present study investigated the potentials of *Asystasia gangetica* as a biomonitor of heavy metals and PAHs pollution in Malaysia. This species is proposed as a biomonitor due to the fact that it is vastly distributed and abundance (high specificity and fidelity) in unattended open areas such as roadside, palm oil plantations, and riverbank (Wiert, 2000). Being in an abundance and vastly distributed, the plant is a good ecological indicator and is able to provide information on pollutants for various site locations in Malaysia. In addition, the plant is a herbaceous plant which is consumed by the locals as a traditional medicine. Therefore, it is even more important to study this plant thoroughly which in case if the plant stores a high concentration of pollutant, it could affect the public health by consuming it. In the early 1960s, *A. gangetica* is used as a cover crop in plantation sites, especially in oil palm plantations (Samedani et al., 2015).

As the amount of pollutants is undoubtedly increasing in Malaysia, the current study attempts to determine the accumulation of heavy metals and PAHs in *A. gangetica* collected from various sites and locations in Peninsula Malaysia. Areas with high traffic, industries, and agriculture and horticulture areas are known to accumulate high anthropogenic sources (Wuana and Okieimen, 2011). However, this does not mean that rural and non-highly agricultural areas have low anthropogenic sources (Davis et al., 2009). Hence, in the current study, various sampling sites including landfill, industrial, residential, rubbish heap, ex-mining, and plantation were investigated in the study area. In the present study, *A. gangetica* plant (divided into leaves, stems and roots) and the habitat topsoil were collected from these sites. Anthropogenic sources were determined from the different parts of the plant and topsoil using established methods such as sequential extraction technique (SET) and atomic absorption spectroscopy (AAS) for heavy metal and gas chromatography-mass spectrum (GCMS) for PAHs.

At present, no plant species that respond to a wide range of heavy metals as biomonitors are known (Mertens et al., 2005). Various biomonitors are required so that pollution monitoring in this developing country could be well monitored. Since *A. gangetica* fulfill the criteria as a potential biomonitor, this plant is chosen as the subject in the present study.

1.2 Objectives of the study

- 1) To study the concentrations of the heavy metals (Cd, Cu, Hg, Fe, Ni, Pb, and Zn) in *A. gangetica* (leaves, stems, and roots) and their habitat topsoil.
- 2) To study the concentrations of the polycyclic aromatic hydrocarons (PAHs) in *A. gangetica* (leaves, stems, and roots) and their habitat topsoil.
- 3) To study the relationships of *A. gangetica* and their habitat topsoil.
- 4) To assess the human health risk (non-cancerous and cancerous) posed by the heavy metals and PAHs pollutants found in the habitat topsoil of *A. gangetica*.



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