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Mercury Distribution in an Invasive Species (*Asystasia gangetica*) from Peninsular Malaysia

(Taburan Merkuri di dalam spesies invasif *(Asystasia gangetica)* dari Semenanjung Malaysia)

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ABSTRACT

In this study, the Hg levels in eight parts of Asystasia gangetica *(L.) T. Anderson collected from 14 sites were determined using a Mercury Atomizer MA-1S and a Mercury Detector MD-1. It was found that the ranges for all the parts were 3.21-18.2 µg/kg dry weight for flowers, 1.29-11.2 µg/kg dry weight for stalks, 0.32- 29.4 µg/kg dry weight for seeds, 0.87-10.2 µg/kg dry weight for pericarps, 1.45-18.1 µg/kg dry weight for remainders, 11.8-68.2 µg/kg dry weight for leaves, 0.73-20.9 µg/kg dry weight for stems, and 3.40-33.1 µg/kg dry weight for roots. The overall Hg accumulation pattern in decreasing concentrations was leaf > root > flower > flower remains > stalk > stem > pericarp > seed. This study provided the background levels of Hg in this non-native invasive weed species in Malaysia. However, further studies are needed to confirm it as a biomonitors of Hg in this region.*

Keywords: A. gangetica*; biomonitor, Hg; Peninsular Malaysia*

ABSTRAK

Kajian ini menentukan kepekatan Hg pada lapan bahagian Asystasia gangetica *(L.) T. Anderson yang dikumpul dari 14 tempat kajian. Pengatom Merkuri MA-1S dan Pengesan Merkuri MD-1 digunakan untuk menentukan kepekatan Hg pada setiap sampel dalam kajian ini. Nilai kepekatan Hg yang terdapat di dalam setiap bahagian ialah 3.21-18.2 µg/kg berat kering (bunga), 1.29-11.2 µg/kg berat kering (tangkai bunga), 0.32-29.4 µg/kg berat kering (biji-benih), 0.87-10.2 µg/kg berat kering (Perikarpa), 1.45-18.1 µg/kg berat kering (sisa-sisa bunga), 11.8-68.2 µg/kg berat kering (daun), 0.73-20.9 µg/kg berat kering (batang), dan 3.40-33.1 µg/kg berat kering (akar). Pola kepekatan Hg keseluruhan dalam kajian ini ialah mengikut urutan daun > akar > bunga > sisa-sisa bunga > tangkai > batang > perikarpa > biji-benih. Kajian ini telah memberikan tahap latar belakang kepekatan Hg di dalam spesies invasif ini. Walau bagaimanapun, kajian lanjutan diperlukan untuk mengesahkan bahawa spesies ini adalah biomonitor yang sesuai di kawasan ini.*

Kata kunci: A. gangetica*; biomonitor; Hg; Semenanjung Malaysia*

INTRODUCTION

Mercury is a prevalent element in modern society. Coal combustion, open burning of waste products, chlor-alkali facilities and mining activities are some of the sources that contribute to Hg contaminant in urban areas (Harris et al. 2007; Neff 2002). Of all the three forms of atmospheric Hg (particulate Hg, reactive gaseous Hg and elemental Hg), elemental Hg has the highest mobility and capable of remaining in the atmosphere for months before being deposited onto the ground or into the watershed (Harris et al. 2007).

Plants are capable of accumulating heavy metals from its surrounding environment (Cheng 2003). There are two main intake pathways of pollutants from the surrounding into the plants namely via intake of heavy metals by leaves and their absorption by root cells (De Nicola ed al. 2008; Hoodaji et al. 2010). Numerous studies showed that heavy metals taken up by plants are able to reflect it's surrounding environment (ambient air and soil), thus making plants a suitable biomonitor of heavy metals in urban area (Cheng 2003; De Nicola et al. 2008; Hoodaji et al. 2010). Accumulated Hg will often cause adverse effects to the plant, when inorganic Hg are converted into the organic Hg, namely methylmercury (Me-Hg) (Gothberg et al. 2002; Richardson et al. 1995). Accumulated Hg in plant will inhibit photosynthesis, stunt seedling growth and root development (Gothberg et al. 2002; Pais & Jones 1997).

Asystasia gangetica (L.) was introduced into Malaysia from India as ornamental plants in 1876 and 1923 (Kiew & Vollesen 1997). According to Wiart (2000), in Malaysia, this species is used for anti-inflammatory treatments (snakebites, swelling and rheumatism) and as an anthelminthic (expelling intestinal worms). This plant has now become an invasive weed and is found in urban and rural areas in Peninsular Malaysia. It is known that this plant was planted in Peninsular Malaysian plantations as a cover crop in early 1960s (Kiew & Vollesen 1997). This plant was chosen because it is widely distributed in areas where human activities are found. It usually thrives in the untended areas all year round. Based on these characteristics, it is noted that *A. gangetica* is a potential biomonitor. Criteria of selections of a potential good biomonitor are sedentary, abundant in site of interest, easily identified and sampled, able to thrive in different range of environmental conditions and able to cope with stress of man-handling during sampling (Rainbow Phillips 1993).

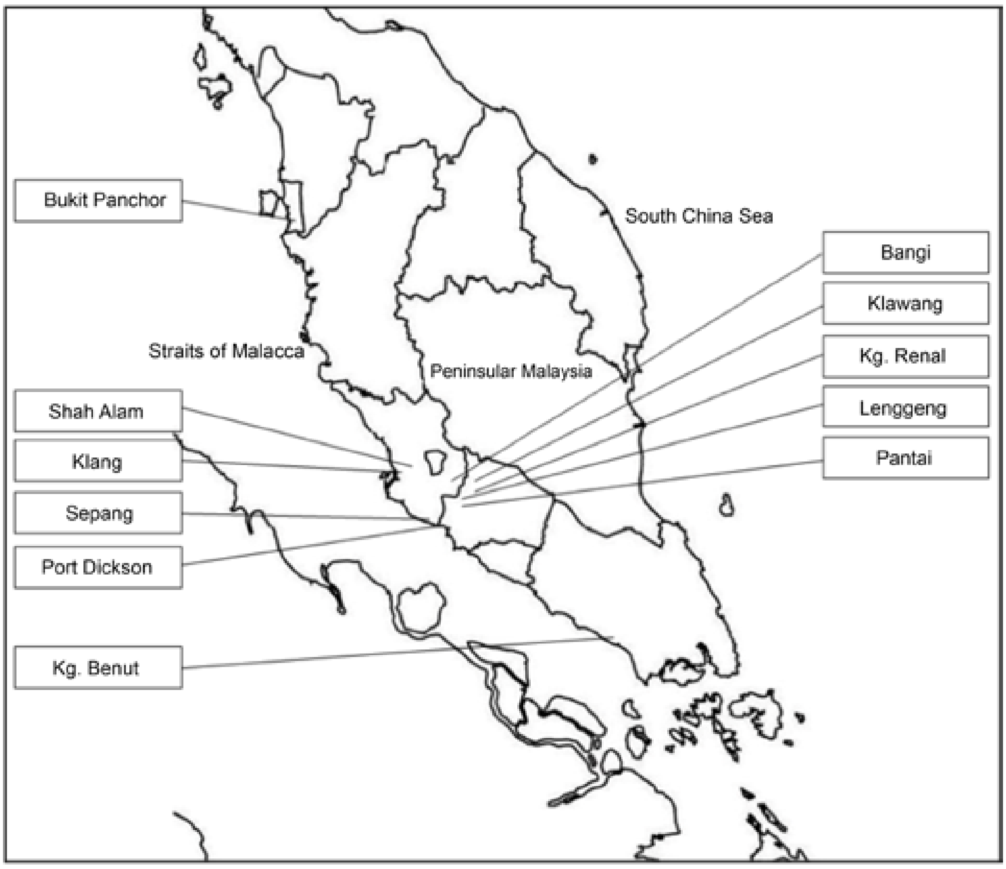
The objective of this study was to determine the Hg levels in different parts of *A. gangetica* collected from Peninsular Malaysia.

MATERIALS AND METHOD

The samples were collected from 9th May 2010 to 30th September 2010 from sampling sites in Peninsular Malaysia (Figure 1). Descriptions of all the sampling sites are given in Table 1. Four to six individuals of *Aystasia gangetica* were taken from sampling location and immediately transported to the laboratory. About 1 to 10 cm of the surface soils were also sampled using a clean stainless steel soil sampler. The surface soils were dried and sieved through 63 μm test sieve in order to collect the clay and silt fraction of the surface soil (Yap et al. 2010). Washed plant samples were divided into different parts namely flowers, stalks, seeds, pericarps, flower's remains, leaves, stems and roots. The plant tissues and the soils were dried at 80°C for 3 - 4 days. The samples were then homogenized before being analyzed by using a Mercury Atomizer, MA-1S and Mercury Dectector, MD-1. Certified Reference Material was used to ensure accuracy of the methods used. The recovery range for the certified values of the soil reference materials, NCS DC 73319 (Approved by China National Analysis Center for Iron and Steel), was within 80.7% - 120% (Certified value, 32 ± 4 μg/kg; measured value, 28.20 ± 1.82 μg/kg). Further statistical analyses such as Student-Newman-Keuls tests were conducted using the SPSS 19 (Statistical Package for the Social Sciences). For the correlation of Hg between plant parts and soils, Pearson correlation analysis was performed based on $\log_{10}$ (mean+1) transformed data using SPSS version 19.

RESULTS AND DISCUSSION

The Hg concentrations in the different parts of *A. gangetica* from all the sampling sites are presented in Table 2. *A. gangetica* in Sepang-2 contained the highest Hg level in flowers (18.00 ± 0.21 μg/kg dry weight), pericarps (7.99 ± 0.16 μg/kg dry weight), and stems (19.70 ± 1.17 μg/kg dry weight) when compared to the other sites. The Hg level in stalks (10.80 ± 0.38 μg/kg dry weight), leaves



RAJAH 1. The sampling sites for *Asystasia gangetica* in Peninsular Malaysia

TABLE 1. Description of the sampling sites in Peninsular Malaysia

No.	Coordinate	Sampling Area	Site descriptions	Time of sampling
1	N 2° 27' 57.40" E101° 51' 0.20"	Port Dickson (COMAS), Negeri Sembilan	Centre of Oceanography and Mariculture Studies, car park place, urban area	11.00 am 9 May 2010
2	N 2° 28' 39.94" E 101° 51' 16.71"	Port Dickson (Fuel station), Negeri Sembilan	Near fuel station, roadside, urban area	1.30 pm, 9 May 2010
3	N 2° 36' 35.16" E101° 41' 10.32"	Sepang, Selangor	Car park, recreation area, near urban area.	12.30 pm, 13 May 2010
4	N 2° 36' 25.98" E101° 41' 20.46"	Sepang – 2, Selangor	Roadside, recreation area, near urban area	2 pm, 13 May 2010
5	N 2° 55' 1.50" E102° 1' 34.30"	Kg. Renal, Negeri Sembilan	Remote prawn farming area, roadside, rural area	11.30 am, 13 May 2010
6	N 2° 58' 44.20" E102° 1' 47.00"	Klawang, Negeri Sembilan	Roadside, rural area	12.30 pm, 13 May 2010
7	N 2° 46' 48.50" E101° 59' 32.90"	Pantai – 1, Negeri Sembilan	Residential area, roadside, rural area	10.15 am, 20 May 2010
8	N 2° 48' 17.60" E101° 59' 21.00"	Pantai – 2, Negeri Sembilan	Secluded prawn farming, rural area	11.30 am, 20 May 2010
9	N 2° 57' 57.96" E101° 47' 16.98"	Bandar Baru Bangi – 1, Selangor	Near construction site, urban area	9 am, 12 Jun 2010
10	N 1° 38' 27.70" E103° 15' 35.10"	Benut, Johor	Car park, residential area, rural area	12 pm, 29 July 2010
11	N 3° 0' 41.88" E101° 29' 36.36"	Taman Perindustrian Amj (3SA), Shah Alam, Selangor	Small illegal dumping sites, roadside, industrial area, urban area	12 pm, 29 July 2010
12	N 2° 59' 58.00" E101° 29' 43.60"	Jalan Kebun (6SA), Shah Alam, Selangor	Rubbish heap, industrial area, roadside, urban area	10.30 am, 30 September 2010
13	N 2° 56' 22.90" E101° 27' 43.90"	Sijangkang (7SA1), Shah Alam, Selangor	Residential area, roadside, near urban area	12 pm, 30 September 2010
14	N 2° 56' 22.90" E101° 27' 43.90"	Sijangkang (7SA2), Shah Alam, Selangor	Residential area, roadside, near urban area	1 pm, 30 September 2010

(61.40 ± 3.61 μg/kg dry weight), and roots (32.30 ± 0.79 μg/kg dry weight) collected in Sepang were found to be the highest when compared with plant samples from the other sites. The highest Hg level in seeds (10.7 ± 9.33 μg/kg dry weight) in *A. gangetica* was found in Pantai – 2. The highest Hg level in surface soils (545 ± 45.48 μg/kg dry weight) was found in Jalan Kebun, Klang. The overall ranges of the Hg levels in the different parts of *A. gangetica* are given in Table 3. The ranges of Hg were 1.59 - 12.7 μg/kg dry weight for flowers, 1.76 - 9.33 μg/kg dry weight for stalks, 10.6 - 57.8 μg/kg dry weight for leaves, 1.95 - 6.14 μg/kg dry weight for stems, and 2.86 - 32.5 μg/kg dry weight for roots. In this study, rural areas (Kg. renal, Klawang, Pantai-1, Pantai-2 and Benut), in general, have lower hg concentration in leaves. Places at close proximity to urban areas (such as Port Dickson, Sepang, Bandar Baru Bangi, Shah Alam and Kelang) generally have higher Hg concentration in leaves. Based on the correlation analysis in Table 5, the Hg concentration of the surface soil was not significantly correlated with the other parts of the plant ($p>0.05$). However, plant leaves were found to have positive correlations with plant stems ($r = 0.664$, $p<0.01$).

The order of Hg distributions in the different parts of *A. gangetica* are given in Table 4. In general, the order of Hg concentrations were leaf > root > flower > flower remains > stalk > stem > pericarp > seed. Generally, leaves accumulated higher Hg concentrations compared with other parts of the plant. Seeds exhibited the lowest Hg concentrations when compared with other parts of the plants.

The toxicity testing of Hg uptake from the roots of *Vigna mungo* based on a experimental hydroponic study by Hussain et al. (2010), showed the accumulation in decreasing order of roots > stems > leaves. Their results revealed and confirmed that the translocation of the Hg from roots to leaves were limited. In this study, the high Hg accumulation in the leaves might not be due to the uptake of Hg from the soil. Hence, there is the possibility that the Hg accumulation on *A. gangetica* leaves was the result of the atmospheric Hg deposition. This also helps explain the reason of higher Hg concentration in

TABLE 2. Hg concentrations (mean ± SE, μg/kg dry weight) in different parts of *Asystasia gangetica* collected from 15 sampling sites

No.	Sampling sites	Flowers	Stalks	Seeds	Pericarps	Remains	Leaves	Stems	Roots	Soils
1	Port Dickson (COMAS), Negeri Sembilan	NA	5.18 ± 0.40^{ab}	1.78 ± 0.18^{a}	2.92 ± 0.65^{abc}	7.13 ± 0.16^{a}	34.6 ± 3.02^{bcd}	3.57 ± 0.36^{bc}	17.5 ± 5.03^{abc}	304 ± 15.9^{c}
2	Port Dickson (Fuel station), Negeri Sembilan	5.82 ± 0.48^{a}	4.31 ± 0.14^{ab}	1.38 ± 0.07^{a}	1.79 ± 0.08^{ab}	4.31 ± 0.08^{a}	16.8 ± 1.59^{a}	3.03 ± 0.19^{bc}	21.4 ± 0.60^{bc}	139 ± 20.6^{abc}
3	Sepang, Selangor	NA	10.8 ± 0.38^{c}	4.22 ± 0.16^{a}	5.68 ±0.12^{cd}	14.0 ± 1.56^{b}	61.4 ± 3.61^{f}	4.78 ± 0.50^{cd}	32.3 ± 0.79^{c}	30 ± 3.36^{a}
4	Sepang – 2, Selangor	18.0 ± 0.21^{b}	5.54 ± 0.03^{ab}	5.54^{a}	7.99 ± 0.16^{d}	16.2 ± 1.89^{b}	48.6 ± 0.52^{e}	19.7 ± 1.17^{f}	12.3 ± 3.51^{ab}	115 ± 0.18^{abc}
5	Kg. Renal, Negeri Sembilan	NA	4.10 ± 2.32^{ab}	0.87 ± 0.12^{a}	1.21 ± 0.15^{a}	1.92 ± 0.26^{a}	21.8 ± 1.66^{bc}	2.15 ± 0.41^{ab}	11.9 ± 1.81^{ab}	124 ± 1.33^{abc}
6	Klawang, Negeri Sembilan	3.38 ± 0.17^{a}	2.90 ± 0.38^{ab}	0.99 ± 0.06^{a}	1.68 ± 0.18^{ab}	3.32 ± 0.42^{a}	14.0 ± 1.17^{a}	2.40 ± 0.08^{ab}	19.1	122 ± 7.55^{abc}
7	Pantai – 1, Negeri Sembilan	NA	3.93 ± 1.04^{ab}	0.82 ± 0.11^{a}	2.03 ± 0.29^{ab}	7.91 ± 2.38^{a}	26.5 ± 0.89^{abc}	1.89 ± 0.13^{ab}	11.8 ± 3.67^{ab}	252 ± 8.00^{abc}
8	Pantai – 2, Negeri Sembilan	NA	5.56 ± 0.88^{ab}	10.7 ± 9.33^{a}	5.09 ± 2.56^{bcd}	7.95 ± 1.38^{a}	25.0 ± 2.29^{abc}	4.08 ± 0.13^{bc}	NA	34.4 ± 15.2^{a}
9	Bandar Baru Bangi – 1, Selangor	NA	3.17	0.35 ± 0.03^{a}	0.91 ± 0.04^{a}	3.67 ± 0.19^{a}	35.9 ± 2.28^{cd}	3.36 ± 0.25^{bc}	4.28 ± 0.37	61.0 ± 1.43^{ab}
10	Benut, Johor	NA	1.32 ± 0.03^{a}	0.88	8.08 ± 0.63^{d}	1.60 ± 0.15^{a}	19.2 ± 0.18^{a}	0.81 ± 0.08^{a}	NA	NA
11	Taman Perindustrian Amj (3SA), Shah Alam, Selangor	NA	4.50 ± 0.54^{ab}	2.96 ± 0.19^{a}	1.97 ± 0.03^{ab}	4.24 ± 0.19^{a}	26.1 ± 3.13^{abc}	1.78 ± 0.12^{ab}	3.54 ± 0.14^{a}	96.2 ± 17.6^{abc}
12	Jalan Kebun (6SA), Shah Alam, Selangor	6.93 ± 2.35^{a}	7.09 ± 0.54^{bc}	2.28 ± 0.10^{a}	3.41 ± 0.43^{abc}	NA	40.9 ± 6.56^{de}	4.86 ± 0.13^{cd}	24.1 ± 2.10^{bc}	545 ± 145^{d}
13	Sijangkang (7SA1), Shah Alam, Selangor	7.77 ± 0.62^{a}	NA	2.78 ± 0.26^{a}	4.68 ± 0.09^{abc}	7.86 ± 0.04^{a}	34.7 ± 2.18^{bcd}	7.87 ± 0.36^{e}	19.4 ± 5.18^{abc}	94.7 ± 7.92^{abc}
14	Sijangkang (7SA2), Shah Alam, Selangor	6.95 ± 0.55^{a}	7.36 ± 0.89^{bc}	2.35 ± 0.27^{a}	4.24 ± 0.26^{abc}	7.37 ± 0.05^{a}	59.1 ± 3.29^{f}	6.45 ± 0.86^{de}	30.8 ± 5.08^{c}	270 ± 0.30^{bc}

Metal concentrations of different sampling sites sharing common letter are not significantly different. ($P > 0.05$), based on the Student-Newman-Keuls tests of one-way ANOVA

'NA' indicates that data on the sample is not available.

TABLE 3. Overall concentrations (μg/kg dry weight) of mean (in brackets) and minimum and maximum concentrations of Hg in *Asystasia gangetica*

Sample		Min – Max Hg concentrations, μg/kg
A. gangetica	Flower	3.21 - 18.2 (7.91)
	Stalk	1.29 - 11.2 (5.14)
	Seed	0.32 - 29.4 (2.54)
	Pericarp	0.87 - 10.2 (3.32)
	Remains	1.45 - 18.1 (6.61)
	Unwashed leaf	13.0 - 46.2 (20.4)
	Washed leaf	11.8 - 68.2 (33.1)
	Stem	0.73 - 20.9 (4.76)
	Root	3.40 - 33.1 (16.4)
Surface Soil (1 - 10 cm)		61.0 - 545 (156)
Herbal drugs permissible limits established by WHO/FDA		1000
Non-toxic concentration range for plants reported by Alloway (1990)		5 – 170
Dutch target value		300
Australia Ecological investigation levels for soil		1000
Investigation level for soil determined by Zarcinas et al. (2004)		350

TABLE 4. Order of Hg distributions in the different parts of *Asystasia gangetica*

No.	Sampling sites	Order of Hg concentrations by parts
1	Port Dickson (COMAS), Negeri Sembilan	Leaf > Root > Flower remains > Stalk > Stem > Pericarp > Seed
2	Port Dickson (Fuel station), Negeri Sembilan	Root > Leaf > Flower > Stalk = Flower Remains > Stem > Pericarp > Seed
3	Sepang, Selangor	Leaf > Root > Remains > Stalk > Pericarp > Stem > Seed
4	Sepang – 2, Selangor	Leaf > Stem >Flower > Flower Remains > Root > Pericarp > Seed
5	Kg. Renal, Negeri Sembilan	Leaf > Root > Stalk > Stem > Flower remains > Pericarp > Seed
6	Klawang, Negeri Sembilan	Root > Leaf > Flower > Flower remains > Stalk > Stem > Pericarp > Seed
7	Pantai – 1, Negeri Sembilan	Leaf > Root > Flower remains > Stalk > Pericarp >Stem > Seed
8	Pantai – 2, Negeri Sembilan	Leaf > Seed > Flower remains > Stalk > Pericarp > Stem
9	Bandar Baru Bangi – 1, Selangor	Leaf > Root > Flower remains > Stem > Stalk > Pericarp > Seed
10	Benut, Johor	Leaf > Pericarp > Flower remains > Stalk > Seed > Stem
11	Taman Perindustrian Amj (3SA), Selangor	Leaf > Stalk > Flower remains > Root > Seed > Pericarp > Stem
12	Jalan Kebun (6SA), Selangor	Leaf > Root > Stalk > Flower > Stem > Pericarp > Seed
13	Sijangkang (7SA1), Selangor	Leaf > Root > Stem > Flower remains > Flower > Pericarp > Seed
14	Sijangkang (7SA2), Selangor	Leaf > Root > Flower remains > Stalk > Flower > Stem > Pericarp > Seed
	Overall	Leaf > Root > Flower > Flower remains > Stalk > Stem > Pericarp > Seed

TABLE 5. Pearson's correlation matrix of Hg concentrations in plant parts and soils (N=12-14)

	Leaves	Stems	Roots
Stems	0.664**		
Roots	0.255	0.318	
Soils	0.000	-0.034	0.232

**. Correlation is significant at the 0.01 level (2-tailed)

plant leaves that sampled from the places nearer to urban areas. Plants in general are capable of taking up heavy metals from their surroundings via uptake from stomata opening or absorption from roots (Junior et al. 2009). In Table 5, the correlation between leaves and stems has a significant positive result ($r = 0.664$, $p<0.01$). This result shows the transport of accumulated Hg from leaves to stems via translocation. Therefore, it is likely that the Hg accumulation of *A. gangetica* leaves was the result of the atmospheric Hg deposition on leaves. It also indicated that leaves of this species could be a potential atmospheric Hg biomonitor. Due to dissimilarities between *A. gangetica* and *V. mungo*, these two species might have different Hg distributions in different parts of the plants. Hence, more studies are needed to confirm this finding.

Nevertheless, the roots of *A. gangetica* were relatively higher in Hg than the other parts of the plant. It is most likely that the uptake of Hg in roots was from the surface soil. However, the positive correlation between roots and surface soils was not significant. Certain sites such as Port Dickson (COMAS), Negeri Sembilan and Jalan Kebun, Selangor were found to have higher Hg concentrations in soils. However, the Hg distributions in plants of these sites did not reflect the high Hg concentrations of the surroundings. We speculate that the soils total Hg concentrations analysed in this study might not be able to reflect the Hg bioavailability for plant uptake. Furthermore, several abiotic factors and biotic factors often play major roles in altering the Hg bioavailability in soils (Cataldo & Wildung 1978; Yang et al. 2007; Yin et al. 1997). However, it is also possible that the majority of the Hg particles was bound with soils and thus were not entire mobilized for the plant uptake via roots (Raskin et al. 1994).

As shown in Table 3, the Hg concentrations of the surface soils in this study did not exceed those of the Australia Ecological Investigation value (1000 μg/kg). However, it was noted that the Hg concentrations in the soils of Port Dickson (COMAS) and Jalan Kebun in this study were close to or exceeded the Dutch target value (300 μg/kg). All the Hg concentrations in the different parts of *A. gangetica* did not exceed the permissible limit of 1 μg/kg set by the WHO/FDA.

Conclusion

This study provided data of Hg distribution in different parts of *A. gangetica*. Future studies such as plant toxicity testing, determine the relationships with various soil conditions (pH, soil texture), studies on the mobility of Hg in the soil and plant and genetic diversity on this species are required for further understanding and to confirm this species as being a good biomonitor of Hg contamination in Malaysia based on the recommended criteria for a good biomonitor.

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