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An investigation of arsenic contamination in Peninsular Malaysia based on *Centella asiatica* and soil samples

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Abstract The first objective of this study was to provide data of arsenic (As) levels in Peninsular Malaysia based on soil samples and accumulation of As in *Centella asiatica* collected from 12 sampling sites in Peninsular Malaysia. The second objective was to assess the accumulation of As in transplanted *C. asiatica* between control and semi-polluted or polluted sites. Four sites were selected which were UPM (clean site), Balakong (semi-polluted site), Seri Kembangan (semi-polluted site) and Juru (polluted site). The As concentrations of plant and soil samples were determined by Instrumental Neutron Activation Analysis. The As levels ranged from 9.38 to 57.05 μg/g dw in soils, 0.21 to 4.33 μg/g dw in leaves, 0.18 to 1.83 μg/g dw in stems and 1.32–20.76 μg/g dw in roots. All sampling sites had As levels exceeding the CCME guideline (12 μg/g dw) except for Kelantan, P. Pauh, and Senawang with P. Klang having the highest As in soil (57.05 μg/g dw). In *C. asiatica*, As accumulation was highest in roots followed by leaves and stems. When the As level in soils were higher, the uptake of As in plants would also be increased. After the transplantation of plants to semi-polluted and polluted sites for 3 weeks, all concentration factors were greater than 50 % of the initial As level. The elimination factor was around 39 % when the plants were transplanted back to the clean sites for 3 weeks. The findings of the present study indicated that the leaves, stems and roots of *C. asiatica* are ideal biomonitors of As contamination. The present data results the most comprehensive data obtained on As levels in Malaysia.

Keywords Arsenic · *Centella asiatica* · Peninsular Malaysia · Neutron activation analysis

Introduction

Arsenic (As) occurs naturally in the environment as a result of the weathering of parent rocks even though rarely in its elemental form (O'Neil 1995; CCME 2001). Historically, As compounds (calcium arsenate,

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lead arsenate, and sodium arsenite) have been used as pesticides and fertilizers (ATSDR 2007). As has also been used as a decolorizer in the manufacture of glass, in various metallurgical processes such as the production of alloys, veterinary and human medicines, and lead-acid batteries (CCME 2001; IPCS 2001; ATSDR 2007; Kabata-Pendias and Mukherjee 2007). Hence, a more efficient and practical approach for assessing As pollution is needed. Inorganic compounds of As are extremely toxic and may cause gastrointestinal symptoms, cardiovascular, and nervous system function disturbances and eventually death (IPCS 2001).

In this study, we used Instrumental Neutron Activation Analysis because it is accepted as an important technique for the analysis of different elements in the local environment. In fact, the application of this technique was initiated by national needs (Hassan 2008). NAA is a well-known reference method being widely used for the determination of the concentrations of many trace elements in environmental materials (Parry 1991). Besides, this method can be used to detect total element content because a neutron has no charge and can pass through most materials without difficulty. NAA is free from laboratory and reagent contamination and it is nondestructive, thus the samples will not be permanently damaged and can be reanalyzed at any time (IAEA-TECDOC-1215 2001). This will reduce human error since no digestion is needed. Hence, high accuracy and precision data can be obtained through NAA.

Centella asiatica (family: Umbelliferae) is the only plant extract from this genus to be found in commercial drugs today (Zainol et al. 2003). Currently, the World Health Organization (WHO) has acknowledged *C. asiatica* as one of the most important medicinal plant species to be conserved. The major route for entering of toxic elements into living organisms is via the food chain which is also considered very serious for the biology and health of man and animals (Kassem et al. 2004). According to Lee et al. (1991), none of the local vegetable samples showed levels greater than 2.00 μg/g dw of As in Malaysia. The As contamination in Malaysia did not reach the level of concern to the public at that time. However, the concentration levels of As of 19 % of well water samples from Sabah in 2010 showed levels exceeding those in the WHO health-based guidelines (Kato et al. 2010). This indicates that the As level in Malaysia had increased throughout the years due to human activities. Therefore, more attention should be placed on the levels of As in Malaysian plants.

In Malaysia, currently, no comprehensive soil reference values are available to establish levels of potentially toxic As for different land uses such as agricultural, residential, industrial, and recreational land (Yap and Pang 2011). Therefore, this study can help in the better assessment of the As contamination of natural soil resources which has emerged as an important issue due to the extension of urbanization and industrialization in Peninsular Malaysia. So far, in the literature review, there is no reported As contamination in *C. asiatica* in Malaysia. The aim of this study was to assess the degree of anthropogenic influence in soils and in the accumulation of As in *C. asiatica*. The second objective was to assess the accumulation of As by transplanted *C. asiatica* between control and semi-polluted or polluted sites.

Methodology

Sampling

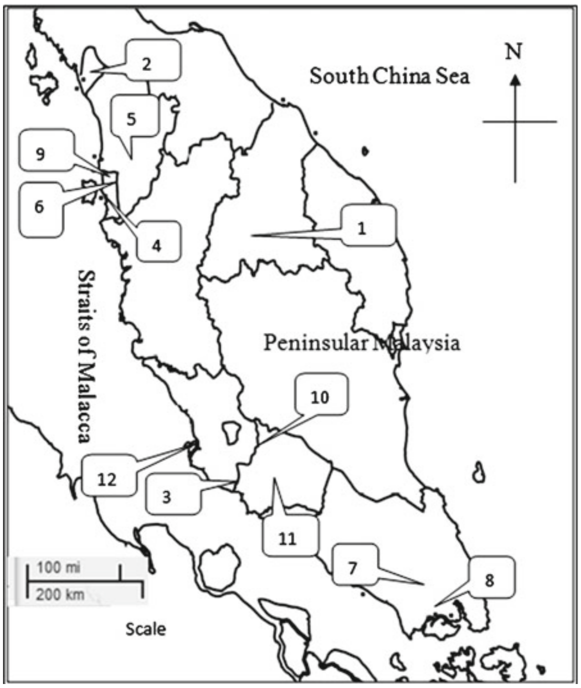
Plant and soil samples were collected from 12 different locations in Peninsular Malaysia (Fig. 1) in 2011. Whole plants (2–4 months maturity) were collected from the sampling sites and put into plastic bags. The plants were separated by hand with clean gloves into three different parts namely leaves, stems and roots. At the same time, surface soil (top 3–5 cm) were also collected by using a plastic scoop after the litter had been removed. The soils were stored in clean plastic bags for transport to the laboratory.

Transplantation study

In the transplantation experiment, the *C. asiatica* was obtained from Taman Pertanian Universiti (TPU), Universiti Putra Malaysia and planted for 2 months to achieve maturity stage. The plants were acclimatized for 1 week before being transferred to the study sites. Four sites were selected, namely UPM's TPU (clean and control site), Balakong (semi-polluted site), Seri Kembangan (SK) (semi-polluted site), and Juru (polluted site) for experimental study.

TPU is selected as the control site because it is an agricultural area whereas Balakong, SK, and Juru

Fig. 1 Map showing the sampling sites in Peninsular Malaysia



No	Sampling sites	Site Description	GPS
1.	Kelantan	Near housing area.	4° 57' 14.9" N, 101° 50' 15.9" E
2.	Arau, Perlis	Near agriculture area.	6° 28' 24.9" N, 100° 15' 0.8" E
3.	Universiti Putra Malaysia (UPM), Selangor	Near agriculture area.	3° 0' 25.5" N, 101° 43' 22.6" E
4.	Butterworth, Penang	Near an industrial area and highway.	5° 25' 41.8" N, 100° 23' 13.2" E
5.	Karangan, Kedah	Near an oil palm plantation.	5° 30' 15.8" N, 100° 37' 45.7" E
6.	Permatang Pauh (P. Pauh), Penang	Near a housing area and highway.	5° 24' 18.0" N, 100° 24' 50.8" E
7.	Pontian, Johore	Near a plant agriculture area.	1° 29' 12.5" N, 103° 23' 55.8" E
8.	Kempas, Johore	Near housing area.	1° 32' 41.3" N, 103° 41' 41.9" E
9.	Kepala Batas (K. Batas), Penang	Near housing and agriculture area.	5° 31' 15.6" N, 100° 26' 12.5" E
10	Seremban, Sembilan	Near shop lots and road sides.	2° 43' 26.3" N, 101° 56' 51.5" E
11	Senawang, Sembilan	Near an industrial area.	2° 42' 55.6" N, 102° 0' 4.7" E
12	Port Klang, Selangor	Near port and industrial area.	3° 0' 15.0" N, 101° 24' 48.1" E

were known as industrial area. Prior to transplantation, soils were collected from UPM, SK, Balakong, and Juru and the soils were determined for As levels. The results showed As in soils were 68.23 μg/g dw for Juru, 53.89 μg/g dw for SK, 48.48 μg/g dw for Balakong and 28.54 μg/g dw for UPM at week 0. Based on the As levels, UPM soil was categorized as clean, SK, and Balakong as semi-polluted sites and Juru as a polluted site. The transplantation studies were carried out under both laboratory and field conditions.

For the experimental field condition, the plants were transferred from UPM (control) to semi-polluted sites

(Balakong, SK) and polluted site (Juru) from week 0 to week 3. For the control, soil was obtained from the top soil in TPU. Afterwards, the plants were back-transplanted from the semi-polluted and the polluted sites to the control site at week 3 and exposed for another 3 weeks (until week 6).

For the experimental laboratory conditions, soils from UPM, Balakong, SK, and Juru were collected and placed onto trays. At week 0 to week 3, plants from the control trays were transferred to trays containing soils collected from the semi-polluted sites namely Balakong, SK, and polluted site namely Juru. From week 3 to week 6, the plants from the semi-polluted and the polluted trays were back-transplanted to the control trays.

Three replicates were done for each site (three traps of 75×75 cm for field study and three trays of 60×35×10 cm for laboratory study). The plants were transplanted every 3 weeks because transplantation work normally can have an obvious effect after 2 weeks (U.S.EPA 1996). The plants were harvested at every 3 weeks. Soils samples were also collected at week 0 and week 6.

Neutron Activation Analysis (NAA) (U.S.EPA, 2001; IAEA-TECDOC-1360 2003)

The plant and soil samples were dried in an oven at 65 °C for around 5 days until constant dry weights. The dried samples were ground by using an electronic agate homogenizer to obtain homogenous powder of about 2-mm mesh size to ensure the elements within each sample were uniformly distributed. Then, the samples were stored in polyethylene bottles for future analysis. For all samples, the homogenous powder samples were shaken manually and had a weight ranging 0.15–0.20 g transferred into a polyethylene vial and heat-sealed. Certified reference material (CRM) IAEA-SOIL-7 was prepared under identical conditions and used as quality control for each batch. The recovery of As based on CRM was 89.25 % and the relative (%) standard deviation was 18 % (CRM certified value, 13.40±0.67 μ/g dw; measured value, 11.96±2.16 μ/g dw). The limit of detection of As by NAA was 0.001 mg/g. Therefore, it was highly sensitive, precise, and accurate.

The irradiations were performed in the TRIGA MARK II reactor at the Agensi Nuklear Malaysia (NUKLEAR MALAYSIA), Bangi, Selangor (Malaysia). As is a long-lived radioisotope which has 26.40 h half life. Hence, long irradiation with neutron flux of $4–5\times 10^{12}$ n/cm^2 was used for long-lived isotopes such as As. After irradiation by thermal neutron flux in the TRIGA MARK II research reactor, the radioactivity measurements of the samples were carried out after a proper cooling time by using various close-end coaxial high purity germanium detectors (Model GC3018 CANBERRA Inc and Model GMX 20180, EG4G ORTEC Nuclear Instrument) and their associated electronics. The cooling time for the counting varied between 3–6 days. The live time for the counting of As was 3,600 s.

Geochemical index

Enrichment factor (EF) was utilized to differentiate between metals originating from human activities and those from natural sources. In addition it can also assess the degree of anthropogenic influence. The value of the EF was calculated by a modified formula suggested by Buat-Menard and Chesselet (1979): $\mathrm{EF} = \left(\frac{C_\mathrm{n}(\mathrm{sample})/C_\mathrm{ref}(\mathrm{sample})}{B_\mathrm{n}(\mathrm{baseline})/B_\mathrm{ref}(\mathrm{baseline})}\right)$ C_n (sample) was the concentration of the examined metal, C_ref (sample) was the concentration of the reference metal, B_n (baseline) was the content of the examined metal, B_ref (baseline) was the content of the reference metal.

Titanium (Ti), aluminum (Al), and iron (Fe) were selected for normalizing As concentrations in the samples due to it being a conservative element which is known to be derived mainly from crustal weathering (Schütz and Rahn 1982). The baseline values were selected from the element's concentrations in the continental crust (As—1.7 ppm, Al—79,600 ppm, Ti—4010 ppm and Fe—43,200 ppm by Wedepohl 1995) (As—1.8 ppm, Al—83,200 ppm, Ti—3800 ppm and Fe—83,200 ppm by Taylor 1964; Matini et al. 2001) since Malaysia does not have these baseline values and the reference values are based on the global average values. The level EF was categorized in Table 3 according to Han et al. 2006.

According to Nael et al. (2009), the lithogenic element concentration of a given soil location was estimated from the Ti concentration (in micrograms per gram) of soils for particular location and the C_n (sample)/Ti_ref (baseline) ratio of the baseline as

$$C_{\mathrm{n\,lithogenic}} = \left(\mathrm{Ti} \times \frac{C_{\mathrm{n}}}{\mathrm{Ti}_{\mathrm{ref}}(\mathrm{baseline})}\right)$$

The difference between the measured concentration and the estimated lithogenic concentration of C_{n} (sample) was used to estimate depletion in the soil of interest, i.e.

$$C_{\mathrm{n}}(\mathrm{sample})_{\mathrm{measured}} < C_{\mathrm{n}}(\mathrm{sample})_{\mathrm{lithogenic}}(\mathrm{lithogenic})$$

$$C_{\mathrm{n}}(\mathrm{sample})_{\mathrm{measured}} < C_{\mathrm{n}}(\mathrm{sample})_{\mathrm{lithogenic}}(\mathrm{enrichment})$$

The geoaccumulation index (Igeo) can be calculated by the following equation (Yap and Pang 2011):

$$\mathrm{Igeo} = \mathrm{Log2}\left(\frac{C_{\mathrm{n}}}{1.5 \times B_{\mathrm{n}}}\right)$$

C_{n} was the concentration of the examined metal, B_{n} was the content of the reference metal. Factor 1.5 is the background matrix correction factor due to lithogenic effects. Because we did not have the background values of the metals of interest, just as we did in the EF calculation, we adopt the earth crust values (Wedepohl 1995; Taylor 1964; Matini et al. 2001) in the Igeo calculation.

The concentration factor can be used to determine the uptake of As by plants for transplantation studies. It was calculated according to Yap et al. (2003).

$$\text{Concentration factor} = \frac{\mathrm{As}_{\text{end of metal accumulation}}}{\mathrm{As}_{\text{initial}}}$$

The rate of As accumulation was calculated according to a formula (Yap et al. 2003) as follows

$$\text{Rate of As accumulation} = \frac{\mathrm{As}_{\text{exposed}} - \mathrm{As}_{\text{initial}}}{\text{Day(s)of As exposure}}$$

Note: weeks 0–3 was accumulation

The elimination factor used to determine the elimination of As by plants for the transplantation studies was calculated according to Yap et al. (2003).

$$\text{Elimination factor} = \frac{\mathrm{As}_{\text{end of metal elimination}}}{\mathrm{As}_{\text{initial}}}$$

The rate of As elimination was calculated according to the following formula (Yap et al. 2003):

$$\text{Rate of As elimination} = \frac{\mathrm{As}_{\text{exposed}} - \mathrm{As}_{\text{initial}}}{\text{Day(s) of As elimination}}$$

Note: weeks 3–6 was elimination

Results

Based on the levels of As in soils from the 12 sampling sites, the range of As concentration in Peninsular Malaysia was from 9.38 to 57.05 μg/g dw. The As level in soils from P. Klang was significantly ($P<0.05$) highest (57.05 μg/g dw) (Fig. 2) compared to the other sampling sites. According to the data presented in Table 1, all the EF values were greater than 1 with EF from P. Klang being highest and the least was from Kelantan. In Fig. 2, all the sampling sites showed enrichment since all the measured levels of As were higher than the calculated levels. For all the sampling sites, the roots showed the highest As accumulation followed by leaves and stems (Fig. 3). Based on Fig. 3, P. Klang, K. Batas, and Kelantan showed the highest As accumulations in stems and roots. In leaves, K. Batas, Kelantan, Arau, and P. Klang were highest in As levels.

In Fig. 4, the accumulation of As increased for all parts when transplanted from control to semi-polluted and polluted sites under field condition (week 0 to week 3). In roots, the increases were highest for Juru followed by SK and Balakong. For leaves and stems, the increase of As accumulations showed the same trend as in roots. However, the accumulation decreased (weeks 3 to 6) after transplantation from the semi-polluted and polluted sites back to the control sites. The accumulation was still highest in Juru followed by SK and Balakong. For the transplantation study under

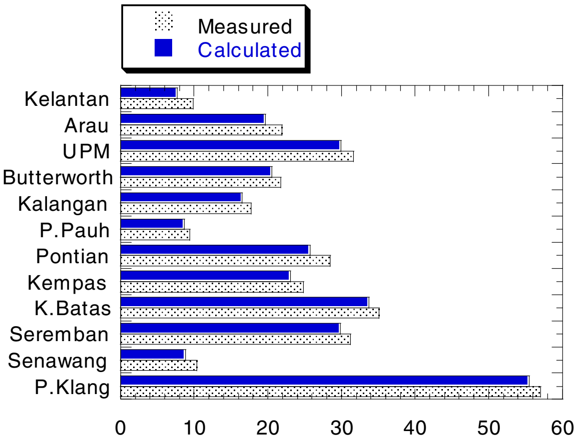


Fig. 2 Measured total and calculated enrichment concentrations (mean ± SD, in micrograms per gram dry weight) of As in soils collected from 12 sampling sites from Peninsular Malaysia

Table 1 Levels of enrichment factor of U from12 sampling sites in Peninsular Malaysia

	Sites	EF[a]	EF[b]	EF[c]	EF[d]	EF[e]	EF[f]	Igeo[g]	Igeo[h]
1.	P. Klang	36.58	32.74	32.64	40.17	31.56	31.16	2.76	3.43
2.	Senawang	6.71	6.00	10.88	13.39	7.79	7.69	3.00	3.67
3.	Seremban	21.92	19.62	32.64	40.18	18.46	18.23	3.60	4.27
4.	K. Batas	25.34	22.68	28.48	35.06	15.08	14.88	3.08	3.75
5.	Kempas	14.06	12.58	17.87	21.99	12.02	11.86	2.89	3.55
6.	Pontian	10.55	9.45	23.20	28.56	10.90	10.76	3.35	4.02
7.	P. Pauh	12.88	11.53	16.44	20.23	7.37	7.28	1.16	1.83
8.	Kalangan	14.95	13.38	25.09	30.89	36.85	36.37	3.31	3.98
9.	Butterworth	18.11	16.21	15.69	19.31	11.57	11.43	3.16	3.83
10.	UPM	18.73	16.76	14.34	17.65	17.73	17.50	1.69	2.36
11.	Arau	9.72	8.70	27.27	33.57	7.00	6.91	2.96	3.62
12.	Kelantan	4.48	4.01	7.67	9.44	5.04	4.98	1.73	2.39

[a] (with Ti) Wedepohl (1995)

[b] (with Ti) Taylor (1964) and (Matini et al. 2001)

[c] (with Fe) Wedepohl (1995)

[d] (with Fe) Taylor (1964) and (Matini et al. 2001)

[e] (with Al) Wedepohl (1995)

[f] (with Al) Taylor (1964) and (Matini et al. 2001)

[g] Wedepohl (1995)

[h] Taylor (1964)

laboratory conditions, the trend was exactly similar to the transplantation study under field conditions but with lower concentrations of As accumulated (Fig. 4). In Table 2, the overall values for the concentration factor and the rate of accumulation were highest for Juru under field and laboratory conditions. The elimination factor was highest for Balakong and the rate of elimination was fastest for Juru (Table 2).

Discussion

As in soil samples

Based on Fig. 2, As contamination in all sampling sites ranged from 9.38 to 57.05 μg/g dw in soils. The soils from all sites contained less than 40 μg/g dw of As except for P. Klang (57.05 μg/g dw). Typical As concentrations reported in uncontaminated soils ranged from 1 to 40 μg/g (ATSDR 2007). Therefore, all sampling sites were considered below the threshold soil guideline values (micrograms per gram dry weight) for As for residential (32), allotment (43), and commercial (640) land use (Environment Agency 2009). The levels of As in Seremban, K. Batas and UPM were within the range for residential guideline while P. Klang was within the range for allotment (allocated for certain uses). The other sampling sites were below the residential values. However, all samplings from Peninsular Malaysia were below the commercial guideline. According to CCME (2001), the guideline for As is 12 μg/g dw. All sampling sites exceed the guideline values except for Kelantan, P. Pauh, and Senawang. This indicated that As levels in Peninsular Malaysia were enriched with As. Therefore, more concern about As contamination should be shown by the public in Malaysia especially for agricultural purpose.

Around 39.8 μg/g dw of As in urban soils was reported at XuZhou, China (Xue et al. 2005). In Peninsular Malaysia, an average of 24.96 μg/g dw of As in soils was found indicating that As contamination in Peninsular Malaysia could still be considered low on the average. Chen et al. (1999) reported that As concentrations in soils ranged from 0.01 to 50.6 μg/g dw in Florida surface soils. The range of As in soils was

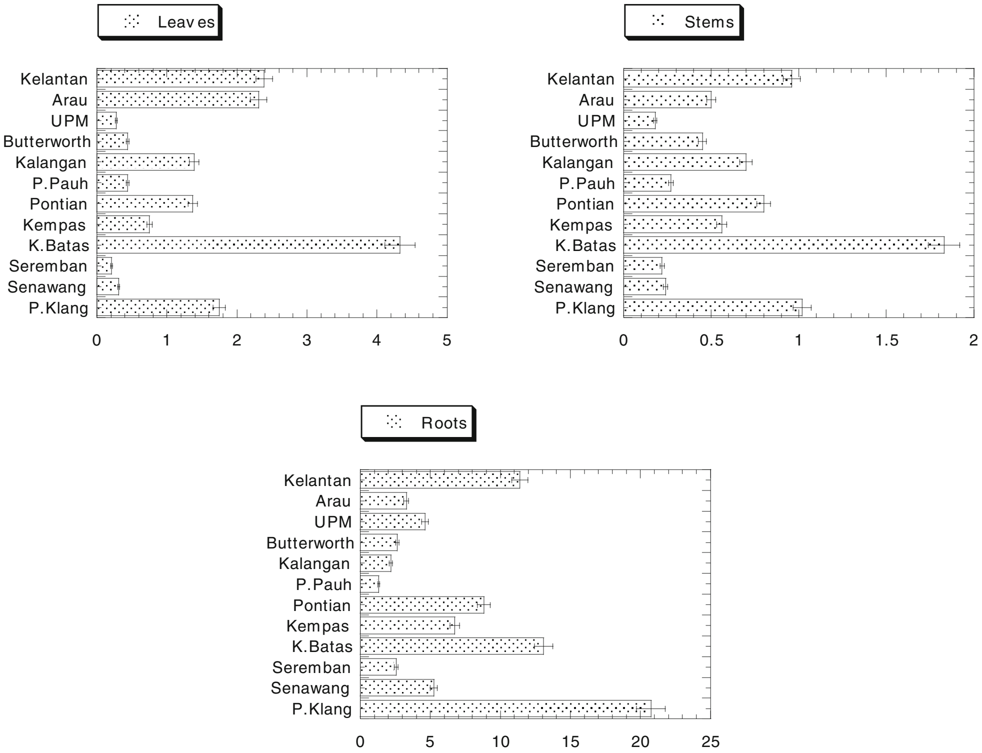


Fig. 3 As concentrations (mean ± SD, in micrograms per gram dry weight) in leaves, stems and roots of *Centella asiatica* collected from 12 sampling sites in Peninsular Malaysia

wide and this could be due to anthropogenic activities causing high As contamination on certain sites. However, 8.7 µg/g dw of As in soils worldwide (guideline values) was reported by Pendias and Pendias (1985). This shows that the As level worldwide has increased day by day due to human activities. Therefore, more attention is required to control the amount of As being released into the environment.

The EF values of soils for all sampling sites were greater than 1 based on both references (Table 1) which indicated that As contamination originated from human activities (Nael et al. 2009). Based on Table 3, all the soil samples showed either significant enrichment or very high enrichment. According to Table 4, three sites showed Igeo class 4 (strongly polluted), four sites showed Igeo class 5 (strongly to very strongly polluted) and five sites showed Igeo class 6 (very strongly polluted). Most natural soils contain low levels of As, but industrial wastes and pesticide applications may increase the concentrations (IPCS 2001). P. Klang was highest in enrichment due to its location as one of the largest and busiest ports in the country and nearby industrial activities. In Fig. 2, the measured concentrations of As were higher than the calculated levels indicating that the concentrations of As in Peninsular Malaysia were enriched with As. EF of soils ranging from 0.6 to 31.2 had been reported in the Orontes River basin of Syria (Kassem et al. 2004). The trend was similar with Peninsular Malaysia in that different nearby activities affected the level of enrichment.

As in plant samples

Based on Fig. 3, As accumulation was highest in roots followed by leaves and stems because plants developed certain mechanisms that could immobilize certain metals when they were bound to their cell walls. Therefore, the metal uptake by roots was reduced and metal translocation to the shoot could be inhibited. Our results were supported by those of Soares et al. (2001); Singh and Sinha (2005) and Tang et al. (2009). The general tolerance level of As is considered to be around 2 mg/kg in plant tissues. Excessive uptake of As will disrupt enzyme function and impair phosphate flow in the plant system (Kabata-Pendias and Mukherjee 2007). Hence, As might be accumulated in the roots and be unable to

Fig. 4 Concentrations (mean ± SD, in micrograms per gram dry weight) of As in leaves, stems and roots of *Centella asiatica* from transplantation studies under field and laboratory conditions

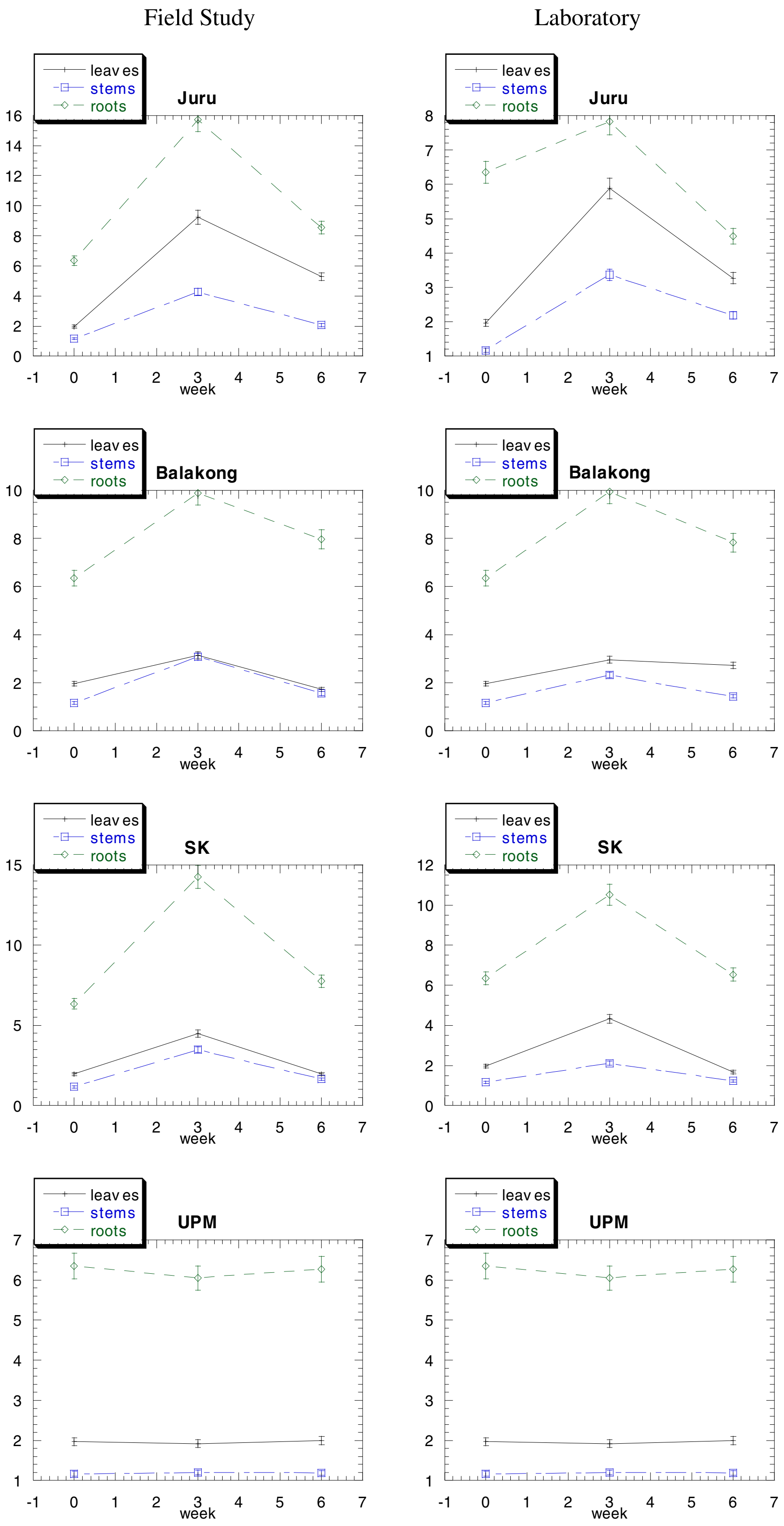


Table 2 Concentration factor, rates of accumulation (µg/g per day), elimination factor, rates of elimination (µg/g per day) of As in transplantation study under field and laboratory conditions

Sites	Field condition			Laboratory condition		
	Leaves	Stems	Roots	Leaves	Stems	Roots
Concentration factor						
Juru	4.69	3.66	2.48	2.98	2.88	1.23
Balakong	1.59	2.64	1.56	1.50	1.98	1.57
SK	2.28	2.97	2.24	2.20	1.80	1.66
Rate of accumulation						
Juru	0.35	0.15	0.45	0.19	0.10	0.07
Balakong	0.06	0.09	0.17	0.05	0.05	0.17
SK	0.12	0.11	0.38	0.11	0.04	0.20
Elimination factor						
Juru	0.57	0.48	0.54	0.56	0.65	0.57
Balakong	0.55	0.50	0.80	0.92	0.62	0.79
SK	0.44	0.47	0.54	0.39	0.58	0.62
Rate of elimination						
Juru	0.19	0.11	0.34	0.12	0.06	0.16
Balakong	0.07	0.07	0.09	0.01	0.04	0.10
SK	0.12	0.09	0.31	0.13	0.04	0.19

enter the plant by being kept in the root cells where they would be detoxified by forming complexes or sequestered into vacuoles (Hall 2002). This action greatly restricted the translocation of metals to the aboveground organs. Moreover, it could protect the leaf tissues and the metabolically active photosynthetic cells from heavy metal damage (Navari-Izzo et al. 2003; Sgherri et al. 2003).

As expected, As concentrations were highest in *C. asiatica* from P. Klang for leaves, stems and roots. Burning of coal and the smelting of non-ferrous metals including copper will release As to the environment (O'Neil 1995). The plants from K. Batas and Kelantan also showed high As in all parts due to their being located nearby agricultural sites. Soil on agricultural land treated with arsenical pesticides may retain substantial amounts of As (Kabata-Pendias and Mukherjee 2007). Mean total As concentrations of 50–60 µg/g dw had been recorded for agricultural soils treated with arsenical pesticides (Sanok et al. 1995). Hence, higher level of As can be taken up by plants.

Table 3 Contamination categories based on enrichment factor (EF) (Han et al. 2006)

Enrichment factor (EF)	Degree of contaminations
<2	Deficiency to minimal enrichment
2–5	Moderate enrichment
5–20	Significant enrichment
20–40	Very high enrichment
>40	Extremely high enrichment

The As levels in Arau were higher in leaves mostly due to the attachment of dust or fly ash on the leaves since the soil content of As was not significantly ($P < 0.05$) high. As is volatile and can be generated in combustion. Fly ash contains environmental toxins in significant amounts including As (43.4 µg/g dw) (U.S.EPA 2007). Ingestion of As from soil and dust may not be a significant source of As intake for adults but it may be significant for children, particularly in locations

Table 4 Geoaccumulation index (Igeo) in relation to pollution extent according to Müller (1981)

Igeo values	Igeo class	Pollution intensity
>5	6	Very strongly polluted
4–5	5	Strongly to very strongly polluted
3–4	4	Strongly polluted
2–3	3	Moderately to strongly polluted
1–2	2	Moderately polluted
0–1	1	Unpolluted to moderately polluted
<0	0	Unpolluted

near industrial and hazardous waste sites (IPCS 2001). Several countries currently use a 1 μg/g limit for As in food and this is cited as the safety level (Duxbury and Zavala 2005). Therefore, we should be aware of the As levels in the plants that we eat.

As in transplantation studies

In Fig. 4, all the samples showed a similar trend as for all the wild samples from the 12 sampling sites (Fig. 3), the roots had the highest As accumulation followed by leaves and stems. This is because the roots are the first organ to be in contact with metals and roots adhere to the soil all the time. In Fig. 4, the accumulation of As increased for all parts when transplanted from control to semi-polluted and polluted sites in field conditions (weeks 0 to 3). Based on Table 2, the concentration factor was highest for Juru mostly due to it being more contaminated with As. The As there originated from the repair and maintenance operations in the shipyard located further up the estuary in Juru (Din and Jamaliah 1994; Ramachandran 1997).

All concentration factors were higher than 1, indicating that the plants were able to uptake high As. In 3 weeks' time, the plants were able to uptake at least 50 % higher than the initial value. The rate of accumulation was high ranging 0.04–0.45 μg/g dw per day. Therefore, the plants can reflect the As contamination by their accumulation levels. *C. asiatica* can be chosen as an ideal biomonitor due to its tolerance to exposure to environmental variations in physicochemical parameters. The most important of all is its capability as net accumulators of the metal with a simple correlation between metal concentrations in tissues and average ambient bioavailable metal concentrations over a short time period (Rainbow and Phillips 1993; Wittig 1993).

However, the accumulation decreased (weeks 3 to 6) after transplantation back to the control site even though the accumulation was higher than at the control site. For the transplantation under laboratory conditions, the trend was exactly the same as the transplantation under field conditions with lower concentration of As accumulated (Fig. 4). As was found in most plants, its biochemical role is unclear (Farago et al. 2003; Kabata-Pendias and Mukherjee 2007).

Based on Table 2, the elimination factor for field and laboratory conditions were at least 39 % for all parts. This indicated that As could be eliminated from plants when transplanted to sites less contaminated by As. As does not play an important role in normal metabolic activities. Hence, the plant will try to eliminate excess As from it to prevent phytotoxicity caused by high As levels. Studies on As toxicity have shown that plants will suffer considerable stress upon exposure, with symptoms ranging from inhibition of root growth through to death (Macnair and Cumbes 1987; Meharg and Macnair 1991; Paliouris and Hutchinson 1991; Barrachina et al. 1995). Besides, on exposure to As species, reactive oxygen species will be generated in response to metal stress (Hartley-Whitaker et al. 2001).

By comparing the accumulation and the depuration of As as shown in Fig. 4 for weeks 0, 3, and 6, the accumulation in the laboratory was lower than those in transplanted under field conditions especially for Juru even though the soils were obtained from the same sites. This was due to the continuous supply of As contamination from nearby activities in Juru. Therefore, the accumulation was higher than under laboratory conditions where the As level in the soil decreased with time during the experiment. However, the levels of As in plants were not significantly different ($P<0.05$) between the experimental field and laboratory studies for Balakong and SK. This might be due to As not being significantly added from the source into soils for Balakong and SK.

When comparing between week 0 and week 6, higher As was found in the back-transplanted plant in week 6 than in week 0 in that they were far from reaching the initial As concentration (week 0). This could be due to the accumulation being dependent on the transplantation period (Hedouin et al. 2011). This indicated that the eliminations of As was not complete during the 3 weeks of transplantation for *C. asiatica*. The elimination rate was slower compared to the accumulation rate. Therefore, a longer time is required for the elimination of As in plants.

Conclusion

As levels ranged from 9.38 to 57.05 μg/g dw for soils from 12 sampling sites in Peninsular Malaysia. All sites were considered not contaminated except for P. Klang (57.05 μg/g dw). As accumulation was highest in roots followed by leaves and stems in *C. asiatica*. For the transplantation study, all concentration factors were greater than 50 % and the rate of accumulation ranged from 0.04 to 0.45 μg/g dw per day. The elimination factor was around 39 % and the elimination rate ranged from 0.01 to 0.34 μg/g dw per day. The elimination rate was slower when compared to the accumulation rate. Therefore, a longer time was required to eliminate As from plants. The findings of this study indicated that the leaves, stems, and roots of *C. asiatica* are potential biomonitors of As concentrations. However, further studies on the genetic structure on this species are needed in the future in order to confirm it being an ideal biomonitor.

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