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Barium Levels in Soils and *Centella asiatica*

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Abstrak: Dalam kajian ini, *Centella asiatica* dan permukaan tanah telah dikumpul daripada 12 kawasan pensampelan di Semenanjung Malaysia dan ditentukan kepekatan barium (Ba). Julat kepekatan Ba (µg/g berat kering) dalam tanah adalah dari 63.72 ke 382.01 µg/g manakala di dalam *C. asiatica* adalah antara dari 5.05 ke 21.88 µg/g untuk akar, 3.31 ke 11.22 µg/g untuk daun dan 2.37 ke 6.14 µg/g untuk batang. Di dalam *C. asiatica*, pengumpulan Ba didapati tertinggi di akar dan diikuti oleh daun dan batang. Pekali korelasi (r) Ba antara tumbuh-tumbuhan dan tanah didapati mempunyai hubungan yang signifikan dengan yang tertinggi di antara akar-tanah (r+0.922, p<0.05), diikuti oleh daun-tanah (r=0.848, p<0.05) dan batang-tanah (r=0.848, p<0.05). Ini menunjukkan tiga bahagian *C. asiatica* adalah biomonitor yang baik untuk pencemaran Ba. Untuk kajian pemindahan, empat tapak telah dipilih sebagai tidak tercemar [(Universiti Putra Malaysia (UPM)], tapak separuh tercemar (Seri Kembangan dan Balakong) dan tercemar (Juru). Berdasarkan kajian pemindahan di bawah bidang eksperimen dan keadaan makmal, kepekatan Ba di dalam *C. asiatica* adalah lebih tinggi secara signifikan (p<0.05) selepas tiga minggu pendedahan di Seri Kembangan, Balakong dan Juru. Oleh itu, dapatan eksperimen ini mengesahkan bahawa daun, batang dan akar boleh mencerminkan tahap Ba dalam tanah di mana *C. asiatica* ditemui. Selepas tiga minggu dan dipindahkan kembali ke tanah yang bersih, tahap Ba di dalam *C. asiatica* adalah masih lebih tinggi daripada peringkat awal Ba walaupun penghapusan berlaku. Kesimpulannya, daun, batang dan akar *C. asiatica* adalah biomonitor yang baik untuk mengesan pencemaran Ba.

Kata kunci: Barium, *Centella asiatica*, Korelasi Pekali, Pemindahan, Biomonitor

Abstract: In this study, *Centella asiatica* and surface soils were collected from 12 sampling sites in Peninsular Malaysia, and the barium (Ba) concentrations were determined. The Ba concentration [µg/g dry weight (dw)] was 63.72 to 382.01 µg/g in soils while in *C. asiatica*, Ba concentrations ranged from 5.05 to 21.88 µg/g for roots, 3.31 to 11.22 µg/g for leaves and 2.37 to 6.14 µg/g for stems. In *C. asiatica*, Ba accumulation was found to be the highest in roots followed by leaves and stems. The correlation coefficients (r) of Ba between plants and soils were found to be significantly positively correlated, with the highest correlation being between roots-soils (r=0.922, p<005), followed by leaves-soils (r=0.890, p<005) and stems-soils (r=0.848, p<005). This indicates that these three parts of *C. asiatica* are good biomonitors of Ba pollution. For the transplantation study, four sites were selected as unpolluted [(Universiti Putra Malaysia (UPM)], semi-polluted (Seri Kembangan and Balakong) and polluted sites (Juru). Based on the transplantation

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study under experimental field and laboratory conditions, Ba concentrations in *C. asiatica* were significantly (p<0.05) higher after three weeks of exposure at Seri Kembangan, Balakong and Juru. Thus, these experimental findings confirm that the leaves, stems and roots of *C. asiatica* can reflect the Ba levels in the soils where this plant is found. Three weeks after back transplantation to clean soils, the Ba levels in *C. asiatica* were still higher than the initial Ba level even though Ba elimination occurred. In conclusion, the leaves, stems and roots of *C. asiatica* are good biomonitors of Ba pollution.

Keywords: Barium, *Centella asiatica*, Correlation Coefficient, Transplantation, Biomonitor

INTRODUCTION

Barium (Ba) is a silvery-white alkaline earth metal that occurs naturally in different compounds. Ba and its compounds have a variety of uses including as getters in electronic tubes, rodenticide, colourant in paints and x-ray contrast medium (ATSDR 2007). Ba is relatively abundant in the earth's crust, with mean values ranging between 265 and 835 µg/g dry weight (dw) depending on the soil type (Lide 2005). According to a report by the Department of Environment (DOE) in Malaysia, naturally occurring concentrations of Ba in soils range between 5 and 21 µg/g (DOE 2009). Ba is not a very mobile element in most soil systems due to the formation of water-insoluble salts and their partition into soils and sediments (WHO 2001). Ba can be transported in the atmosphere, surface waters, soil runoff and groundwater.

Ba has not been reported as an essential trace element for plants, and it was included in a list of elements that pose a risk to human health and are most commonly found in cases of soil contamination (USEPA 2003; CETESB 2001). Ba absorption by plant species grown in polluted areas has been observed by Abreu *et al.* (2012). Pais *et al.* (1998) found that Ba contents of 200 mg/kg could be moderately toxic and that 500 mg/kg could be considered toxic for plants (Pais *et al.* 1998). Therefore, there is increasing concern regarding Ba in plants, especially in edible plants, because Ba can cause discomfort or damage in the human body. The ingestion of Ba can result in several human health problems: muscular paralysis, gastrointestinal disturbances, heart damage, high blood pressure, and, in some cases, even death (USEPA 2009; Lenntech 2005). Thus, the monitoring of Ba accumulation in soil and plants deserves attention in local and international environmental legislation.

Centella asiatica (L.) is commonly known as pennywort or *pegaga* in Malaysia. It is a creeping plant belonging to the Umbelliferae family. The aerial part of the plant is used for medicinal purposes. Ba concentrations in plant become a main concern because these metals may be transferred and accumulated in the body of animals or human beings through the food chain. Currently, there is no established background level of Ba in soils and in edible *C. asiatica* for Malaysia. Therefore, the objectives of this study were as follows: (1) to determine the background levels of Ba in soils and in *C. asiatica* from Peninsular Malaysia and (2) to assess the potential of *C. asiatica* as a good biomonitor of Ba based on correlation analysis.

MATERIALS AND METHODS

Sample Collection

A total of 12 sampling sites were allocated for plant and soil sample collection in Peninsular Malaysia (Fig. 1). *C. asiatica* individuals with a maturity of 2–4 months were collected and placed in plastic bags. Surface soil (3–5 cm depth) was also collected into plastic bags with a plastic scoop. The plants were separated into three main parts, namely, leaves, stems and roots, in the laboratory.

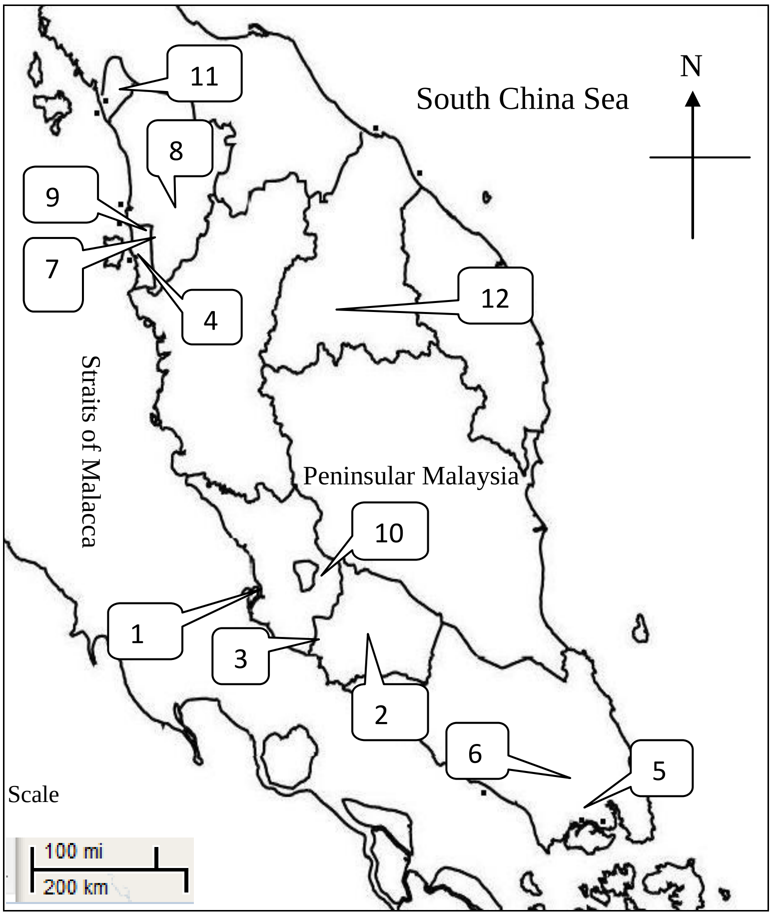


Figure 1: Map showing the sampling sites in Peninsular Malaysia.
Notes: 1-Port Kelang, 2-Senawang, 3-Seremban, 4-Kepala Batas, 5-Kempas, 6-Pontian, 7-Permatang Pauh, 8-Kalangan, 9-Butterworth, 10-Universiti Putra Malaysia (UPM), 11-Arau, 12-Wakaf Baru

Transplantation Study

For the transplantation study, *C. asiatica* was obtained from University Agricultural Park (UAP), Universiti Putra Malaysia (UPM) and planted for 2 months to achieve maturity. The plants were acclimatised for 1 week before being transferred to the study sites. Four sites were selected, namely, UPM's UAP, Balakong and Seri Kembangan in Selangor, and Juru in Pulau Pinang, for experimental study. UAP was selected because it is an agricultural area whereas Balakong, Seri Kembangan and Juru are industrial areas. Prior to transplantation, soils from UPM, Seri Kembangan, Balakong and Juru were collected and analysed for Ba levels. The results show that Ba concentration in the soil was 362.74 µg/g dw for Juru, 209.77 µg/g dw for Seri Kembangan, 201.22 µg/g dw for Balakong and 112.99 µg/g dw for UPM at week 0. Based on the Ba levels, UPM was categorised as a clean site, Seri Kembangan and Balakong as semi-polluted sites and Juru as a polluted site. The transplantation studies were carried out in both laboratory and field conditions.

For the experimental field conditions, the plants were transferred from UPM (control) to the semi-polluted sites (Balakong and Seri Kembangan) and the polluted site (Juru) from week 0 to week 3. For the control, soil was obtained from the top soil in UAP. Afterwards, the plants were back-transplanted from the semi-polluted and 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, Seri Kembangan 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 and the polluted site. 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 performed for each site (3 trays of 75 x 75 cm for the field study and 3 trays of 60 x 35 x 10 cm for the laboratory study). The plants were transplanted every 3 weeks because transplantation work normally has an obvious effect after 2 weeks (USEPA 1996). The plants were harvested every 3 weeks. Soils samples were also collected at week 0 and week 6.

Neutron Activation Analysis (NAA) (IAEA 2003; USEPA 2001).

The plant and soil samples were dried in an oven for 72 hours at 60°C to obtain constant dry weights. The dried samples were ground using an electronic agate homogeniser to obtain homogenous powder of approximately 2 mm mesh size to ensure the elements within each sample were uniformly distributed. Then, these samples were stored in polyethylene bottles for future analysis.

For irradiation, all the homogenous powder samples were shaken manually, had weights ranging between 0.15–0.20 g and were transferred into polyethylene vials and heat-sealed. The irradiations were performed in the TRIGA MARK II reactor at the Agensi Nuklear Malaysia (NUKLEAR MALAYSIA), Bangi, Selangor (Malaysia). Ba is a long-lived radioisotope that has a 12-day half-life. Hence, long irradiation with neutron flux of 4–5 x 10^{12} n/cm^2 was used. After irradiation, the radioactivity measurement of the samples was carried out after an appropriate cooling time 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 Ba was 3600 seconds (IAEA 2003; USEPA 2001).

IAEA-SOIL-7 Certified Reference Material (CRM), SRM 1573a Tomato Leaves and 1575a Pine Needles were prepared in identical conditions and used as quality controls for each patch. The recovery of Ba based on CRM was 79.43% for IAEA-SOIL-7 (CRM certified value: 159.00±7.95 µ/g dw; measured value: 126.30±3.15 µ/g dw), 129.71% for SRM 1573a Tomato Leaves (CRM certified value: 63.00±7.95 µ/g dw; measured value: 81.72±32.66 µ/g dw) and 111.14% for 1575a Pine Needles (CRM certified value: 6.00±0.20 µ/g dw; measured value: 5.00±0.09 µ/g dw). According to Barney (2011), the acceptable recovery ranges for NAA are 70% to 120%; therefore, Ba recovery was accepted.

Geochemical Index

The Enrichment Factor (EF) was utilised to differentiate between metals originating from human activities and those from natural sources. Additionally, 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):

$$EF = \left(\frac{C_n(\text{sample}) / C_{ref}(\text{sample})}{B_n(\text{baseline}) / B_{ref}(\text{baseline})}\right)$$

where

C_n (sample) = the concentration of the examined metal
C_{ref} (sample) = the concentration of the reference metal
B_n (baseline) = the content of the examined metal
B_{ref} (baseline) = the content of the reference metal.

Titanium (Ti), Aluminum (Al) and Iron (Fe) were selected for normalising Ba concentrations in the samples due to Ba being a conservative element that is known to be derived mainly from crustal weathering (Schütz & Rahn 1982). The baseline values were selected from elemental concentrations in the continental crust [(Ba – 584 ppm, Al – 79600 ppm, Ti – 4010 ppm and Fe – 43200 ppm; Wedepohl 1995) (Ba – 425 ppm, Al – 83200 ppm, Ti – 3800 ppm and Fe – 83200 ppm; Taylor 1964)] because baseline values have not been established for Malaysia; thus, the reference values are based on the global average values. EF values are categorised in Table 1 according to Han *et al.* (2006).

Table 1: Contamination categories based on EF (Han *et al*. 2006).

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

$$\mathrm{Igeo} = \mathrm{Log2}\left(\frac{C_n}{1.5 \times B_n}\right)$$

where
C_n = the concentration of the examined metal
B_n = 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, we adopted the earth crust values in the Igeo calculation, as we did in the EF calculation (Wedepohl 1995; Taylor 1964). Igeo values are categorised in Table 2 according to Muller (1981).

The concentration factor can be used to determine the uptake of Ba by plants for transplantation studies (week 0–3). It was calculated according to Yap *et al.* (2004):

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

The rate of Ba accumulation was calculated according to the following formula (Yap *et al.* 2004):

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

The elimination factor can be used to determine the elimination of Ba by plants for the transplantation studies (week 3–6). It was calculated according to Yap *et al.* (2004):

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

The rate of Ba elimination was calculated according to the following formula (Yap *et al.* 2004):

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

Statistical analyses were performed using the statistical software SPSS software version 17.0 for Windows. An analysis of variance (ANOVA), the Student Newman Keuls (SNK) test and the Post hoc test were applied.

Additionally, the STATISTICA (StatSoft Inc., USA) version 8 software was also used to determine the correlation coefficient.

Table 2: 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

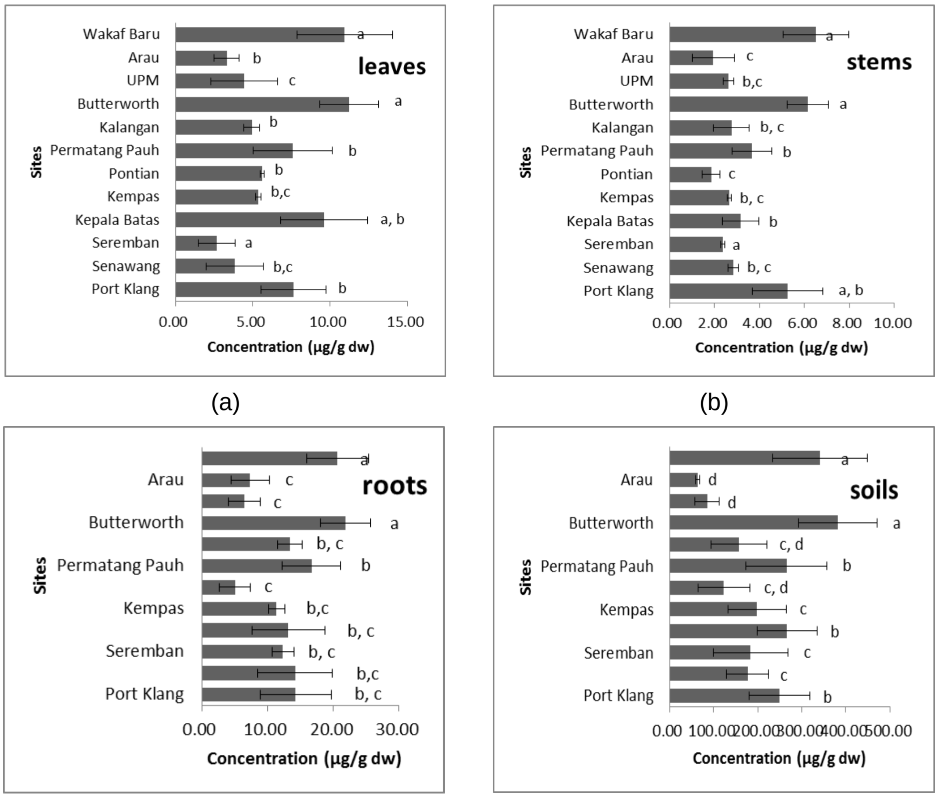
RESULTS

Based on Figure 2, the level of Ba in soils from the 12 sampling sites, i.e., the range of Ba concentration in Peninsular Malaysia, was from 63.72 to 382.01 µg/g dw. The Ba levels in soils from Wakaf Baru and Butterworth were significantly (p<0.05) higher (Fig. 2) compared to the other sampling sites. According to the data from Table 3, the EF for all sites ranged from 0.06 to 2.42. Most of the EF values were less than 1, with the EF from Permatang Pauh being the highest and the EF from UPM and Arau being the lowest. The Igeo of soils ranged from 14.14 to 17.18 for all sampling sites, with Butterworth showing the highest Igeo value (Table 3).

For all the sampling sites, the roots showed the highest Ba accumulation followed by leaves and stems (Fig. 2). The Ba accumulations in *C. asiatica* ranged from 5.05 to 21.88 µg/g dw for roots, 3.31 to 11.22 µg/g dw for leaves and 2.37 to 6.14 µg/g dw for stems. Based on Figure 2, Butterworth and Wakaf Baru showed the highest Ba accumulations in leaves and roots while only Butterworth showed the highest Ba accumulation in stems.

In Table 4, Ba accumulation increased for all plant parts when transplanted from control to semi-polluted and polluted sites under field conditions (week 0 to week 3). For leaves, stems and roots, the increases were highest for Juru followed by Seri Kembangan and Balakong. However, Ba accumulation decreased (week 3 to week 6) after transplantation from the semi-polluted and polluted sites back to the control sites. Ba accumulation was still highest in Juru followed by Seri Kembangan and Balakong (Table 4).

For the transplantation study under laboratory conditions, the trend was similar to the transplantation study under field conditions, with lower concentrations of Ba being accumulated (Table 4). In Table 5, 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 Juru, and the rate of elimination was fastest for Juru (Table 5).



(a) (b)

(c) (d)

Figure 2: Ba concentrations (mean±SD, µg/g dry weight) in leaves (a), stems (b) and roots (c) of *C. asiatica* and soils (d) collected from 12 sampling sites in Peninsular Malaysia.

DISCUSSION

Ba in Soil Samples

Based on Figure 2, the average Ba concentration in soils for all sampling sites was 207.86 µg/g dw. According to Xue *et al.* (2005), the average Ba concentration in urban soils of Xuzhou was 485±54.1 µg/g dw (425 to 628 µg/g). The lower Ba concentration observed in the present study might be due to the differences in nearby human activities between Xuzhou and Peninsular Malaysia. This assumption is supported by Pichtel *et al.* (2000), who found an average of 1130 µg/g dw Ba at dump sites but only an average of 132 µg/g dw Ba at Superfund sites. According to Lowe and Day (2002), the mean concentration of Ba in sediments taken from 16 sampling sites along the southern shore of Lake Ontario and the southeastern shore of Lake Erie ranged from 6.0 to 143.6 µg/g.

Table 3: Levels of EF of Ba from 12 sampling sites in Peninsular Malaysia.

No.	Sites	State	EF^a	EF^b	EF^c	EF^d	EF^e	EF^f	$Igeo^g$	$Igeo^h$
1.	Port Klang	Selangor	0.40	0.47	0.42	0.58	0.61	0.74	–1.81	–1.35
2.	Senawang	Negeri Sembilan	0.39	0.33	0.54	0.56	0.44	0.97	–2.31	–1.85
3.	Seremban	Negeri Sembilan	0.32	0.38	0.56	0.45	0.49	1.00	–2.25	–1.79
4.	Kepala Batas	Pulau Pinang	0.33	0.56	0.63	0.48	0.73	1.12	–1.72	–1.26
5.	Kempas	Johor	0.28	0.33	0.42	0.40	0.43	0.75	–2.14	–1.68
6.	Pontian	Johor	0.14	0.13	0.29	0.20	0.17	0.53	–2.82	–2.37
7.	Permatang Pauh	Pulau Pinang	0.61	1.06	1.35	0.87	1.38	2.42	–1.72	–1.26
8.	Kalangan	Kedah	0.95	0.39	0.65	1.37	0.50	1.16	–2.48	–2.02
9.	Butterworth	Pulau Pinang	0.59	0.92	0.80	0.85	1.20	1.43	–1.20	–0.74
10.	UPM	Selangor	0.14	0.15	0.11	0.20	0.19	0.20	–3.36	–2.90
11.	Arau	Perlis	0.06	0.08	0.23	0.08	0.11	0.41	–3.78	–3.32
12.	Wakaf Baru	Kelantan	0.51	0.45	0.77	0.73	0.59	1.38	–1.36	–0.90

Notes: a: with Al; Wedepohl (1995); b: with Ti; Wedepohl (1995); c: with Fe; Wedepohl (1995); d: with Al; Taylor (1964); e: with Ti; Taylor (1964); f: with Fe; Taylor (1964); g: Wedepohl (1995); h: Taylor (1964)

This reading is much lower compared to the present study. This difference might be due to the composition of clay particles in the sediments that cause less Ba to be attached. This assumption is supported by Xue *et al.* (2005), who show that Ba is highly correlated with clay.

Ba is a common and quite ubiquitous element, with a mean concentration in the Earth's crust up to 425 µg/g dw (ranging from 550 to 668 µg/g in the upper continental crust) (Kabata-Pendias & Mukherjee 2007). Ba concentration in natural soils is approximately 100 to 3000 µg/g dw (CETESB 2001). Ba concentrations (63.72 to 382.01 µg/g dw) from the 12 sampling sites in this study are within the range reported in natural soils and indicate that the contamination of Ba is not significant in Peninsular Malaysia. According to soil quality guidelines for agriculture, all sampling sites are below the guideline values (750 µg/g dw) [Jaritz 2004; CCME 1999]. This shows that the soils from Peninsular Malaysia contain low amounts of Ba, and these soils are suitable for public uses, especially for agriculture. However, according to a report by the DOE in Malaysia, the natural Ba concentration in soils ranged between 5 and 21 µg/g (DOE 2009). This suggests that the Ba concentration in soils has increased year by year due to increases in human activities that release Ba into the soils.

Table 4: Concentrations (mean±SD, µg/g dw) of Ba in leaves, stems and roots of *C. asiatica* for transplantation study under field and laboratory conditions.

Sites	Week	Leaves	Stems	Roots
Field condition				
	0	11.25±2.73b	7.54±4.47c	27.3±2.46b
Juru	3	35.58±4.34a	28.98±3.38a	54.27±2.62a
	6	29.65±1.49a	14.37±1.39b	42.66±1.3a
	0	11.25±2.73b	7.54±4.47b	27.3±2.46b
Balakong	3	21.74±1.26a	17.01±1.56a	40.81±13.6a
	6	16.82±2.83b	10.34±2b	29.69±1.38b
	0	11.25±2.73b	7.54±4.47b	22.3±2.46b
Seri Kembangan	3	23.61±3.21a	20.68±2.2a	38.51±2.03a
	6	18.43±1.38a	12.79±2.94b	30.68±4.63a
	0	11.25±2.73a	7.54±4.47a	22.3±2.46a
UPM	3	10.97±1.42a	6.99±1.28a	23.17±1.39a
	6	11.05±1.45a	7.14±1.38a	22.3±2.38a
Laboratory condition				
	0	11.25±2.73b	7.54±4.47b	22.3±2.46c
Juru	3	32.4±2.49a	26.48±4.2a	50.82±6.39a
	6	26.94±4.2a	13.76±4.22b	39.05±8.3b
	0	11.25±2.73b	7.54±4.47b	22.3±2.46b
Balakong	3	19.42±3.03a	17.13±5.32a	42.38±4.91a
	6	14.13±2.48b	11.15±4.29b	26.17±5.39b
	0	11.25±2.73b	7.54±4.47b	22.3±2.46b
Seri Kembangan	3	19.91±3.99a	17.45±2.17a	33.81±3.79a
	6	16.12±1.49a	10.94±4.53b	28.04±3.39a
	0	11.25±2.73a	7.54±4.47a	22.3±2.46a
UPM	3	10.97±1.42a	6.99±1.28a	23.17±1.39a
	6	11.05±1.45a	7.14±1.38a	22.3±2.38a

Note: a, b, c: different alphabets in each column show the different significant means (SNK test, $p<0.05$)

The EF values of soils for most sampling sites are less than 2 based on all references (Table 3). Based on Table 1, all the soil samples show a deficiency in the minimal enrichment of Ba except Permatang Pauh, which shows a moderate enrichment of Ba. Compared to the reported studies by Wang and Qin (2007), the EF of Ba in Xuzhou ranges from 0.68 to 1.21. This result is similar to

Table 5: Concentration factor, rate of accumulation (µg/g per day), elimination factor, rate of elimination (µg/g per day) of Ba in the transplantation studies under field and laboratory conditions.

Sites	Field conditions			Laboratory conditions		
	Leaves	Stems	Roots	Leaves	Stems	Roots
Concentration factor						
Juru	3.16	3.84	1.99	2.88	3.51	2.28
Balakong	1.93	2.26	1.49	1.73	2.27	1.90
Seri Kembangan	2.10	2.74	1.73	1.77	2.31	1.52
Rate of accumulation						
Juru	1.16	1.02	1.28	1.01	0.90	1.36
Balakong	0.50	0.45	0.64	0.39	0.46	0.96
Seri Kembangan	0.59	0.63	0.77	0.41	0.47	0.55
Elimination factor						
Juru	0.83	0.50	0.79	0.83	0.52	0.77
Balakong	0.77	0.61	0.73	1.24	0.65	0.62
Seri Kembangan	0.78	0.62	0.80	0.81	0.63	0.83
Rate of elimination						
Juru	–0.28	–0.70	–0.55	–0.26	–0.61	–0.56
Balakong	–0.23	–0.32	–0.53	0.22	–0.28	–0.77
Seri Kembangan	–0.25	–0.38	–0.37	–0.18	–0.31	–0.27

the present study (0.06 to 2.42), with bigger ranges due to different activities at different sampling sites. This indicates that the EF of Ba in Peninsular Malaysia is not significant. However, the Igeo value (14.14 to 17.18) is in Igeo class 6 (very strongly polluted). The Igeo suggests the opposite interpretation regarding the Ba contamination in Peninsular Malaysia compared to EF. Hence, we cannot conclude that the soils of Peninsular Malaysia are polluted with Ba. More studies are required to confirm the level of Ba contamination.

Ba in Plant Samples

Based on Figure 2, Ba accumulation is highest in roots followed by leaves and stems because the roots are the first organ to be in contact with the metals and adhere to the soil at all times (Ong *et al.* 2011). Therefore, the exposure of roots to Ba in soil is higher relative to other parts of the plant and increases the chances of Ba accumulation in roots. Moreover, the large surface area of roots due to the root hairs elevates the adsorption and absorption rate of metals (Yap *et al*. 2010). Ba is known to be rather immobile in soils; released Ba^{2+} can be immobilised by the formation of water-insoluble salts and their partition into soils and sediments (WHO 2001). Hence, less Ba can be transported to the upper parts of plants.

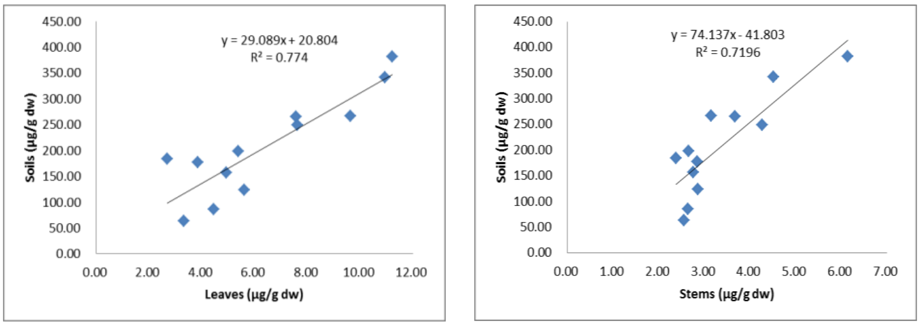
Ba is found in most plants despite not being reported as essential. Ba concentrations in most plants range from 2 to 13 µg/g (Kabata-Pendias & Mukherjee 2007). Mean values of 13.09 µg/g Ba in roots, 6.43 µg/g Ba in leaves and 3.37 µg/g Ba in stems were found in *C. asiatica* in the present study. This indicates that Ba concentration in *C. asiatica* from Peninsular Malaysia is within the average range as reported (Kabata-Pendias & Mukherjee 2007). According to Łozak *et al*. (2002), 31.2±0.6 µg/g Ba was found in herbs peppermint (*Mentha piperitae folium*) and nettle (*Urticae folium*) from Herbapol, Wrocław. That result is slightly higher compared to the present study, which might be due to the lower concentration of Ba in the soils. Mean Ba concentrations of 12.9±9.0 µg/g in roots, 9.80±11.5 µg/g in leaves and 5.66±1.13 µg/g in seeds of wheat (*Triticum aestivum* L.) were measured while 8.89±3.65 µg/g in roots, 2.05±1.14 µg/g in leaves and 1.98±0.29 µg/g in seeds of rye (*Secale cereale* L.) were found (Shtangeeva *et al.* 2011). Those findings are similar to the present study because Ba concentration in all plant parts were within the average range.

However, some plants can accumulate high concentrations of Ba. According to Kabata-Pendias and Mukherjee (2007), high concentrations of 3000 to 4000 µg/g Ba were found in Brazil nut trees (bioaccumulating species). Ba concentrations of 96 to 1990 µg/g in nuts from Brazil, Bolivia, Peru and northern South America were measured, with the greatest concentrations of the elements measured in nuts from Bolivia (Parekha *et al.* 2008). Additionally, legumes, grain stalks, forage plants, trees (red ash, black walnut, hickory, Brazil nut and Douglas fir) and plants of the genus *Astragallu* were reported that show active and strong uptake of Ba. Generally, Ba is not significantly toxic to plants at low concentrations. In plants, 200 µg/g Ba has been found to be moderately toxic while 500 µg/g Ba is considered to be toxic (Pais *et al.* 1998). Growth and antioxidant responses of soybean plants exposed to contrasting soils (Oxisol and Entisol, which were artificially contaminated) showed Ba only affected plant growth at 600 µg/g (Melo *et al.* 2011). Based on the EPA's guidelines (20 to 60 µg/g), none of the plant samples from Peninsular Malaysia exceeded the safety threshold (EPA 1995).

Based on Figure 3, the correlation coefficients of Ba between plant parts and soils were found to be the highest between roots-soils (r=0.922, p<005), followed by leaves-soils (r=0.890, p<005) and stems-soils (r=0.848, p<005). These results are supported by Bermudez *et al.* (2010), who showed that Ba has a good correlation between wheat grains (*T. aestivum*) and soils. Generally, little Ba is bioconcentrated by terrestrial plants relative to the amount found in soils. Additionally, significant correlations of Ba concentration between leaves and roots of 0.63 for wheat (*T. aestivum*) and 0.91 for rye (*S. cereale*) were also reported by Shtangeeva *et al*. (2011). The above results indicate the three parts of *C. asiatica* are able to reflect the Ba levels in soils. Therefore, the roots, leaves and stems of *C. asiatica* are good biomonitors of Ba contamination.

Ba in Transplantation Studies

The samples from 12 sampling sites (Fig. 2) showed a similar trend in Ba accumulation, with the roots having the highest accumulation followed by the leaves and the stems.



(a) (b)

Figure 3: Correlation coefficients between different parts of *C. asiatica* and soil concentrations of Ba.

The accumulation of Ba (Table 4) increases in all the plants parts when they were transplanted from the control site to the semi-polluted and polluted sites in the field study from week 0 to 3. Furthermore, all the calculated concentration factors were higher than 1, which indicates that the plants were able to take up a significant amount of Ba. This also indicates that within 3 weeks, the plants were able to take up at least 100% more Ba than the initial value. The range of the accumulation rate was high, starting from 0.50 to 0.83 µg/g dw per day. This shows that Ba contamination can be reflected through the Ba accumulation level in plants. Therefore, *C. asiatica* was an ideal choice as a biomonitor due to its capability as a net accumulator of the metal, with a simple positive correlation between metal concentrations in tissues and average ambient bioavailable metal concentrations over a short time period.

The accumulation of Ba in the plants decreased from week 3 to week 6 due to the transplantation of plants back to the control site, even though the accumulation levels were higher than the content of Ba in the control site. This was also the same for the plants in laboratory conditions (Table 4). According to Table 5, the elimination factor for field and laboratory conditions was at least 60%, which indicates that *C. asiatica* can remove Ba from all plant parts when the new site is less contaminated than the previous site. Additionally, Ba serves no important role in normal metabolic activities; thus the plant will try to eliminate excess Ba from its system to prevent phytotoxicity caused by high Ba levels.

Ba was found to be higher in the back-transplanted plants in week 6 when compared to the ones in week 0, where they showed high fluctuations from the initial Ba concentration (week 0). This suggests that the elimination of Ba was not completed during the three weeks of transplantation. This also shows that the elimination rate was slower when compared to the accumulation rate. Hence, a longer time was required for the Ba to be eliminated from the plants. Additionally, the accumulation and elimination of metals in plants were dependent on the transplantation period (Hedouin *et al.* 2011).

CONCLUSION

Based on the results of this study, Ba concentration was highest in roots followed by leaves and stems. A significant correlation of Ba between *C. asiatica* and soils was also found. This indicates that the roots, leaves and stems of *C. asiatica* can be used to monitor Ba pollution of sampling sites. The positive results based on experimental studies under field and laboratory conditions confirm the use of roots, leaves and stems as good biomonitors of Ba pollution.

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REFERENCES

Abreu C A, Cantoni M, Coscione A R and Paz-Ferreiro J. (2012). Organic matter and barium absorption by plant species grown in an area polluted with scrap metal residue. *Applied and Environmental Soil Science* 123: 1–7.

Agency for Toxic Substances and Disease Registry (ATSDR). (2007). *Toxicological profile for barium and barium compounds.* Atlanta, GA: Division of Toxicology and Environmental Medicine, US Department of Health and Human Services.

Barney W P. (2011). *NAA: Magnitude of the residue on pomegranate. IR-4 National Pesticide Clearance Protocol NAA/Pomegranate*. Princeton, NJ: IR-4 Project Headquarters, 1–13.

Bermudez G M A, Moreno M, Invernizzi R, Pla R and Pignata M L. (2010). Heavy metal pollution in topsoils near a cement plant: The role of organic matter and distance to the source to predict total and HCl-extracted heavy metal concentrations. *Chemosphere* 78(4): 375–381.

Buat-Menard P and Chesselet R. (1979). Variable influence of the atmospheric flux on the trace metal chemistry of oceanic suspended matter. *Earth and Planetary Science Letters* 42(3): 398–411.

Canadian Council of Ministers of the Environment (CCME). (1999). *Canadian Environmental Quality Guidelines.* Winnipeg: Canadian Council of Ministers of the Environment.

Environmental Company of the State of Sao Paulo (CETESB). (2001). *Report establishment of guiding values for soils and groundwater of the State of Sao Paulo.* Sao Paulo, Brazil: Environmental Agency of the State of Sao Paulo.

Department of Environment (DOE). (2009). *Contaminated land management and control guidelines no. 1: Malaysian recommended site screening levels for contaminated land.* Putrajaya, Malaysia: Department of Environment, Ministry of Natural Resources and Environment.

Environmental Protection Agency (EPA). (1995). *SOP 102: Determination of barium, copper, manganese, vanadium, and zinc in NHEXAS food or beverage composites by graphite furnace atomic absorption spectrometry. Compendium of methods for analysis of trace metals in dietary samples using Total Diet Study procedures. National Human Exposure Assessment Survey (NHEXAS). Arizona study: Quality systems and implementation plan for human exposure assessment.* Tucson, AZ: US Environmental Protection Agency.

Han Y, Du P, Cao J and Eric S P. (2006). Multivariate analysis of heavy metal contamination in urban dusts of Xi'an, Central China. *Science of the Total Environment* 355(1–3): 176–186.

Hedouin L, Pringault L, Bustamante P, Fichez R and Warnau M. (2011). Validation of two tropical marine bivalves as bioindicators of mining contamination in the New Caledonia lagoon: Field transplantation experiments. *Water Research* 45(2): 483–496.

International Atomic Energy Agency (IAEA). (2003). *Collection and preparation of bottom sediment samples for analysis of radionuclides and trace elements,* IAEA-TECDOC-1360. Vienna: IAEA.

Jaritz M. (2004). Barium. In: E Merian, M Anke, M Ihnat and M Stoeppler (eds.). *Elements and their compounds in the environment*. Weinheim, Germany: Wiley-VCH, 627–634.

Kabata-Pendias A and Mukherjee A B. (2007). *Trace elements from soil to human*. Berlin: Springer.

Lenntech. (2005). *Barium-Ba.* Delft, The Netherlands: Lenntech. http://www.lenntech.com/periodic/elements/ba.htm (accessed on 12 May 2012).

Lide D R. (2005). *CRC handbook of chemistry and physics.* New York: CRC Press, 4–51.

Lowe T P and Day D D. (2002). Metal concentrations in zebra mussels and sediments from embayments and riverine environments of eastern Lake Erie, southern Lake Ontario, and the Niagara River. *Archives of Environmental Contamination and Toxicology* 43(3): 301–308.

Łozak A, Sołtyk K, Ostapczuk P and Fijałek Z. (2002). Determination of selected trace elements in herbs and their infusions. *The Science of the Total Environment* 289(1–3): 33–40.

Melo L C A, Alleoni L R F, Carvalho G and Azevedo R A. (2011). Cadmium and barium-toxicity effects on growth and antioxidant capacity of soybean (*Glycine max* L.) plants, grown in two soil types with different physicochemical properties. *Journal of Plant Nutrition and Soil Science* 174(5): 847–859.

Muller G. (1981) The heavy metal pollution of the sediment of Neckars and its tributary: A stocktaking. *Chemistry in Our Time* 105:157–164.

Ong G H, Yap C K, Maziah M and Tan S G. (2011). Heavy metal accumulation in a medicinal plant *Centella asiatica* from Peninsular Malaysia. *Journal of Biological Science* 11(2): 146–155.

Pais I, Benton J and Jones Jr. (1998). *The handbook of trace elements.* Boca Raton: St Lucie Press.

Parekha P P, Khana A R, Torresa M A and Kittoa M E. (2008). Concentrations of selenium, barium, and radium in Brazil nuts. *Journal of Food Composition and Analysis* 21(4): 332–335.

Pichtel J, Kuroiwa K and Sawyerr H T. (2000). Distribution of Pb, Cd and Ba in soils and plants of two contaminated sites. *Environmental Pollution* 110(1): 171–178.

Schütz L and Rahn K A. (1982). Trace-element concentrations in erodible soils. *Atmospheric Environment* 16(1): 171–176.

Shtangeeva I, Steinnes E and Lierhagen S. (2011). Macronutrients and trace elements in rye and wheat: Similarities and differences in uptake and relationships between elements. *Environmental and Experimental Botany* 70(2–3): 259–265.

Taylor S R. (1964). Abundances of chemicals elements in the continental crust: A new table. *Geochimica et Cosmochimica Acta* 28(8): 1273–1285.

US Environmental Protection Agency (USEPA). (2009). *Ground water and drinking water. Consumer fact sheet on: Barium.* Washington DC: US Environmental Protection Agency. http://www.chemtrails911.com/docs/%202007/EPA%20Ground%20Water%20&%20Drinking%20Water%20%20breadcrumb%20%20Consumer%20Factsheet%20on%20BARIUM.htm (accessed on 5 April 2012).

———. (2003). *Standards for the use disposal of sewage sludge: Final agency response to the National Research Council Report on biosolids applied to land and the results of EPA's review of existing sewage sludge regulations.* 68(250): 75531–75552. Washington DC: US Environmental Protection Agency. http://www.epa.gov/ (accessed on 3 April 2012).

———. (2001). *Methods for collection, storage and manipulation of sediments for chemical and toxicology,* EPA-823-B-01-002. Washington DC: Office of Water, US Environmental Protection Agency.

———. (1996). *Ecological effects test guidelines* (public draft), EPA 712–C–96–163. Washington DC: US Environmental Protection Agency.

Wang X S and Qin Y. (2007). Some characteristics of the distribution of heavy metals in urban topsoil of Xuzhou, China. *Environmental Geochemistry and Health* 29(1): 11–19.

Wedepohl K H. (1995). The composition of the continential crust. *Geochimica et Cosmochimica Acta* 59(7): 1217–1232.

World Health Organization (WHO). (2001). *Barium and barium compounds*. Geneva: World Health Organization. http://www.inchem.org/documents/ehc/ehc221.htm (accessed on 1 April 2005).

Xue S W, Yong Q and Shu X S. (2005). Accumulation and sources of heavy metals in urban topsoils: A case study from the city of Xuzhou, China. *Environmental Geology* 48(1): 101–107.

Yap C K and Pang B H. (2011). Assessment of Cu, Pb, and Zn contamination in sediment of north western Peninsular Malaysia by using sediment quality values and different geochemical indices. *Environmental Monitoring and Assessments* 183(1–4): 23–39.

Yap C K, Ismail A, Tan S G and Ismail A R. (2004). Assessment of different soft tissues of the Green-lipped Mussel *Perna viridis* (Linnaeus) as biomonitoring agents of Pb: Field and laboratory studies. *Water, Air & Soil Pollution* 153(1–4): 253–268.

Yap C K, Mohd Fitri M R, Mazyhar Y and Tan S G. (2010). Effect of metal-contaminated soils on the accumulation of heavy metal in different parts of *Centella asiatica*: A laboratory study. *Sains Malaysiana* 39(3): 347–352.