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Magnesium in Local Edible Ulam (*Centella asiatica)* and Its Relation to Their Habitat Soils in Peninsular Malaysia

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ABSTRACT

The aim of this study was to determine the Mg levels in *Centella asiatica* and their relationship to the habitat soils. Based on the levels of Mg in soils from the 12 sampling sites, its concentration was found to range from 13080 to 45350 µg/g dw. Although higher than the continental crust and European topsoils baselines, the soils of Peninsular Malaysia were considered 'unpolluted to moderately polluted' based on EF and 'deficiency to minimal enrichment' based on Igeo. As for plants, the highest Mg level was found in roots (3250 ± 815 µg/g dw), followed by leaves (2900 ± 565 µg/g dw) and stems (1660 ± 393 µg/g dw). This is in agreement with the transfer factor (TF). Based on correlation analysis and multiple linear regression analysis, Mg-Soil was found as a significant and the most important factor controlling the Mg uptake from the soils to the three plant parts. The direct relationships between Mg(plant)-Mg(Soil) also indicates that *C. asiatica* roots, leaves and stems are able to reflect the Mg levels of the sampling sites. Thus, the experimental transplantation studies under field and laboratory conditions confirmed the results from the field collected samples and indicated the roots, leaves and stems can be used as good biomonitors of Mg levels in the habitat soils.

Keywords: Biomonitor, *Centella asiatica*, Correlation studies, Magnesium, Transplantation

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INTRODUCTION

Magnesium (Mg) is an alkaline earth metal and the eighth most abundant element in the Earth's crust. Although Mg is naturally present in soil, local sources such as industry, agriculture, sewage sludge, waste incineration and road traffic have increased the concentration of Mg in soil (Celik et al., 2005). Therefore, concern about the Mg concentration in the environment should arise to ensure the safety of human usage. During the mineral weathering process, Mg^{2+} cation, released from minerals in soil solutions, can be taken up by roots and stored in vegetation over shorter (months) or longer (hundreds of years) periods (Bolou-Bi et al., 2012). Mg can be accumulated in crops or plants and may lead to damage and alteration of animal or human physiological functions through the food chain. Biologically, high Mg levels can alter cellular ion balance and activity, especially Ca^{2+} activity, which directly affects neural and muscular functions (Barbare, 2005). In Malaysia, the levels of Mg are not found in the literature, especially in the local edible ulam, *Centella asiatica*. Furthermore, there are no baseline data for Mg in the plants and soils from Malaysia. Although there is no clear Mg pollution in Malaysia, the focus on Mg levels could shed some lights into the possibility of Mg pollution or to confirm that Mg is not an environmental concern in Malaysia at the moment.

The plant *Centella* has been used widely in folk medicine for hundreds of years to treat a wide range of illnesses (Brinkhaus et al., 2000). From the entire genus of *Centella*, only the *asiatica* species is found in commercial drugs today and acknowledged by WHO as one of the important medical plant species to be conserved (Zainol et al., 2003). In addition, locally, they are consumed as ulam. Therefore, public concerns over the potential ecotoxicological hazards posed by the presence of excessive Mg accumulation should be checked when using the plants to treat various illnesses and consuming it as a local vegetable.

Information on Mg levels in terrestrial soils in relation to *C. asiatica* is lacking in the literature. Hence, Mg contamination of natural soil resources due to urbanisation and industrialisation in Peninsular Malaysia can be understood better through this study. For this purpose, a low cost method to determine the extent of Mg contamination in local environments was assessed in this study. Therefore, the objective of this study was to determine the levels of Mg in *C. asiatica* and habitat surface soils, and later to relate the Mg levels in both plants and soils.

MATERIALS AND METHODS

Vegetation and soil sampling

Plant and soil samples were collected from 12 sampling sites from Peninsular Malaysia (Figure 1). Plants of 2-4 months maturity were collected and placed in plastic bags. At the same time, the surface soil of 3-5 cm depth (litters were removed) was collected into a plastic bag using a plastic scoop.

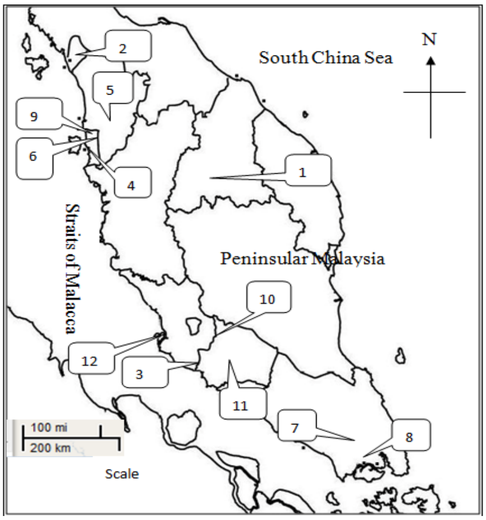

Figure 1. Map showing the sampling sites in Peninsular Malaysia

Note.

No	Sampling sites	Sites Description
1.	Wakaf Baru, Kelantan	Near a housing area.
2.	Arau, Perlis	Near an agriculture area.
3.	Universiti Putra Malaysia (UPM), Selangor	Near an agriculture area.
4.	Butterworth, Penang	Near an industrial and highway.
5.	Karangan, Kedah	Near an oil palm plantation.
6.	Permatang Pauh (PPauh), Penang	Near a housing area and highway.
7.	Pontian, Johore	Near a plant agriculture area.
8.	Kempas, Johore	Near a housing area.
9.	Kepala Batas (K.Batas), Penang	Near housing and agriculture areas.
10	Seremban, Sembilan	Near shop lots and road sides.
11	Senawang, Sembilan	Near an industrial area.
12	Port Klang (P.Klang), Selangor	Near port and industrial areas.

Transplantation Study

The transplantation studies were divided into experimental field and experimental laboratory conditions. All the plants used were obtained from the University Agricultural Park (UAP) at Universiti Putra Malaysia. The plants were planted together in the same condition for two months to reach the maturation stage before they were transferred or transplanted to specified locations. Four sites were selected for this transplantation study. Based on observations and reported studies, UPM was regarded as an unpolluted site, while Seri Kembangan (SK) and Balakong were taken as potentially receiving industrial wastes and Sg. Juru as a highly industrial area in Penang (Yap et al., 2009). Prior to the transplantation study, surface soils were collected from the four sites and their Mg levels were analysed. The results showed that the Mg levels (µg/g dw) in the surface soils were 15130 ± 2920 for UPM, 21650 ± 1110 for SK, 26050 ± 3160 for Balakong, and 32420 ± 1840 for Sg. Juru. Based on the comparison of the continental crust guidelines of Wedepohl (1995) (22000 µg/g dw) and Taylor (1964) (23300 µg/g dw), the four sites could be categorised as ‘low Mg’ at UPM, ‘semi-high Mg’ at both SK and Balakong, and ‘relatively high Mg’ at Sg. Juru.

In the experimental field condition study, the randomly selected plants were transplanted from UPM to SK, Balakong and Sg. Juru for three weeks (week 0 till week 3). After 3 weeks, the plants were back-transplanted to the control site and were left to grow for 3 more weeks (week 3 till week 6). As for the experimental laboratory condition, the method was the same as that of the field condition; the difference was that the plants were planted in trays containing soils taken from the four selected sites (UPM, SK, Balakong and Sg. Juru). The duration of 3 weeks for transplantation was chosen because according to USEPA (1996), obvious effects could be observed on plants after 2 weeks of transplantation. In the transplantation study, three replicates were conducted for each site. The field study consisted of traps with the measurements of 75 cm × 75 cm × 10 cm and the laboratory study consisted of trays with the measurements of 60 cm × 35 cm × 10 cm.

Sample Treatments

In the laboratory, the plants were separated into leaves, stems and roots. The roots of the plants were washed with clean water under running tap so that no soil particles would be adsorbed on the root surface. Later, the leaves, stems and roots were rinsed with distilled water. The separated plant parts and soil samples were dried in an oven (65°C) for 5 days, which resulted in constant dry weight. The dry samples obtained were ground in an electronic agate homogenizer to yield a homogenous powder (± 2mm mesh size) and ensure that the elements within each sample were uniformly distributed. The homogenous samples were shaken manually and stored in polyethylene vials with weights ranging from 0.15-0.20g. The vials were all heat-sealed until further analysis.

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

The TRIGA MARK II reactor at Agensi Nuklear Malaysia (NUKLEAR MALAYSIA) in Bangi, Selangor (Malaysia), was used to perform irradiations on the samples. Briefly, a pneumatic transfer system (PTS) was used and each sample was irradiated for a period of 30-60 seconds on the same position for a short irradiation to enable immediate counting of short-lived isotopes including Mg, Ti, Mn, K and Ca. In particular, the ^{27}Mg determined in this study was a short-lived radioisotope with a half-life of 9.46 minutes (Holden, 2004).

After irradiation in the reactor, the radioactivity measurement 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 varied from 5-20 minutes for 1st gamma-ray counting for ^{27}Mg determination.

Data Verification

Certified reference material (CRM) IAEA-SOIL-7 was prepared using the same conditions and it was also used as quality control for each batch. The recovery of Mg based on IAEA-SOIL-7 was 96.56% (CRM certified value: 11300.00 ± 565.00µ/g dw; measured value: 10911.59 ± 1050.14µ/g dw).

Transfer Factor

The ratio of the Mg concentration in the plant to that in the soil was defined as the transfer factor (TF). The TF was based on total Mg in the soil, which was formulated by Alexander et al. (2006), as follows:

$$TF = CplantCsoil$$

where C_{plant} is the Mg concentration in the plant and C_{soil} is Mg concentration in the soil. Taking the source of Mg into account, the second formula is as follows:

$$TFadded = Cplant\text{-}Ccontrol\ plantCsoil\text{-}Ccontrolsoil$$

where C_{plant} is the Mg level in the test plant, $C_{control\ plant}$ is the Mg level in the control plant, C_{soil} is the Mg level in the test soil, and $C_{control\ soil}$ is the Mg level in the control soil.

Enrichment Factor

The calculation for differentiating metal origins by human activities or from natural sources is known as enrichment factor (EF). It can also be used to analyse the degree of anthropogenic influence in soil. It was calculated by using the following formula (Buat-Menard & Chesselet, 1979):

$$EF= \left(\frac{Cn\ (sample)/Cref\ (sample)}{Bn\ (baseline)/Bref\ (baseline)}\right)$$

where C_n (sample) is the concentration of the examined metal, C_{ref} (sample) is the concentration of the reference metal, B_n (baseline) is the content of the examined metal, and B_{ref} (baseline) is the content of the reference metal.

In order to normalise Mg, Titanium (Ti) was selected because it is a conservative element that is known to be derived mainly from crustal weathering (Schütz & Rahn, 1982). The baseline values were selected from the element's concentrations in the continental crust (Mg - 22000 µg/g dw and Ti - 4010 µg/g dw by Wedepohl, 1995) (Mg - 23300 µg/g dw and Ti - 3800 µg/g dw by Taylor, 1964). Since Malaysia does not have these baseline values, the values were based on the global average values. The levels of EF are categorised as reported by Han et al. (2006): <2 (deficiency to minimal enrichment), 2-5 (moderate enrichment), 5-20= (significant enrichment), 20-40 (very high enrichment) and >40 (extremely high enrichment).

Geoaccumulation Index

The geoaccumulation index (Igeo) was calculated based on the following equation (Müller, 1969):

$$Igeo= Log2\left(\frac{Cn}{1.5xBn}\right)$$

where C_n is the concentration of the examined metal, and B_n is the content of the reference metal. Factor 1.5 is the background matrix correction factor due to lithogenic effects. Since there are no background Malaysia values of the metals of interest, we adopted the earth crust values as in the EF calculation (Wedepohl, 1995; Taylor, 1964). The following Igeo classifications are given according to Müller (1969): < 0 (practically unpolluted), 0-1 (unpolluted to moderately polluted), 1-2 (moderately polluted), 2-3 (moderately to strongly polluted), 3-4 (strongly polluted), 4-5 (strongly to very strongly polluted) and >5 (very strongly polluted).

Concentration Factor and Accumulation Rate

Concentration factor (CF) is a way to identify the uptake level of Mg in plants for transplantation studies. It was calculated based on the following formula (Yap et al., 2004):

$$\mathrm{CF} = \frac{\mathrm{Mg}_{\text{end of accumulation}}}{\mathrm{Mg}_{\text{initial}}}$$

Where, the end of accumulation was based on the length of time for the accumulation of Mg in the plants after three weeks (21 days). The Mg accumulation rate (AR) was calculated based on the following formula (Yap et al., 2004):

$$\mathrm{AR} = \frac{\mathrm{Mg}_{\text{exposed}} - \mathrm{Mg}_{\text{initial}}}{\text{Day(s) of Mg exposure}}$$

Elimination Factor and Elimination Rate

Elimination factor (EF) was used to determine the elimination rate of Mg in plants for the transplantation study, and it was calculated according to Yap et al. (2004):

$$\mathrm{EF} = \frac{\mathrm{Mg}_{\text{end of elimination}}}{\mathrm{Mg}_{\text{initial}}}$$

Where, the end of elimination was based on the length of time for the elimination of Mg from the plants after three weeks (21 days). The Mg elimination rate (ER) was calculated according to Yap et al. (2004):

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

Statistical Analysis

Correlation analysis (CA) and multiple linear regression analysis (MLRA) were performed based on all mean values. They were log10(mean + 1) before CA and MLRA in order to reduce the variance (Zar, 1996). Both analyses were done by using STATISTICA version 8 software.

RESULTS

Based on Figure 2, the Mg levels in soils from the 12 sampling sites ranged from 13080 to 45350, with the mean value of 26730 µg/g dw. Meanwhile, the highest level was recorded in Seremban. Based on the data presented in Table 1, the ranges of EF varied from 0.53 to 4.53, with Kalangan being the highest, and the least was from Arau. The Igeo values were ranged from -1.42 to 0.46. For all the sampling sites, the roots (3250 ± 810 µg/g dw) showed

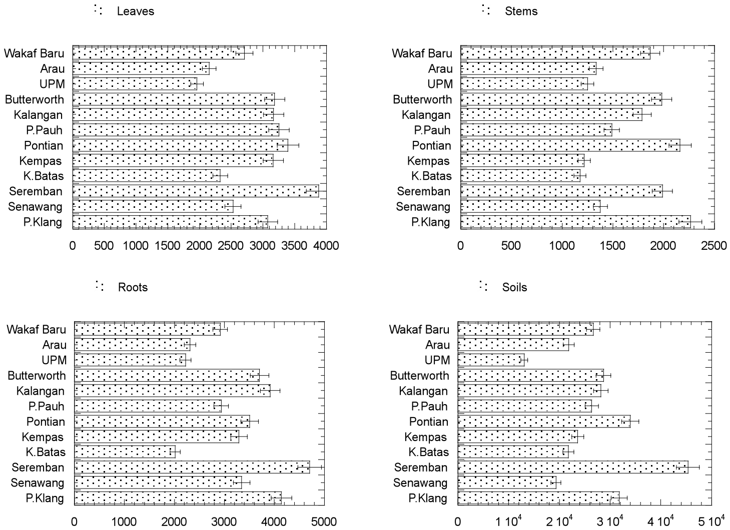

Figure 2. Mg concentrations (mean ± SD, µg/g dry weight) in leaves, stems and roots of *Centella asiatica* and habitat surface soils collected from 12 sampling sites in Peninsular Malaysia

Table 1

Levels of enrichment factor (EF) and geoaccumulation index (Igeo) of Mg from 12 sampling sites in Peninsular Malaysia

	Sites	EF[a]	EF[b]	Igeo[a]	Igeo[b]
1.	P.Klang	1.58	1.41	-0.05	-0.14
2.	Senawang	0.97	0.87	-0.77	-0.85
3.	Seremban	2.46	2.20	0.46	0.38
4.	K.Batas	1.21	1.09	-0.60	-0.68
5.	Kempas	1.03	0.93	-0.48	-0.56
6.	Pontian	0.97	0.87	0.04	-0.04
7.	P.Pauh	2.80	2.51	-0.32	-0.41
8.	Kalangan	1.84	1.64	-0.23	-0.31
9.	Butterworth	1.84	1.65	-0.20	-0.28
10.	UPM	0.60	0.54	-1.33	-1.42
11.	Arau	0.75	0.67	-0.60	-0.68
12.	Wakaf Baru	0.93	0.84	-0.31	-0.39

Note: a: Background values by Wedepohl (1995); b: Background values by Taylor (1964) the highest Mg accumulation, followed by leaves (2900 ± 565 µg/g dw) and stems (1660 ± 393 µg/g dw). In leaves and roots, the samples from Seremban were highest in Mg concentration, while P. Klang showed the highest Mg concentration in stems.

In Table 2, the accumulation of Mg increased for all parts when transplanted from control to semi-polluted and polluted sites under field condition (week 0 to week 3). The increases were highest for Juru, followed by SK and Balakong for roots, leaves and stems. However, the accumulation decreased (week 3 to week 6) after the 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 laboratory conditions, the trend was exactly similar to the transplantation study under field conditions, with lower

Table 2

Concentrations (mean ± SD, µg/g dry weight) of Mg in leaves, stems and roots of Centella asiatica for transplantation studies under field and laboratory conditions

Field	week	Leaves			Stems			Roots		
Juru	0	1120	±	94	930	±	54	1230	±	69
	3	1580	±	20	956	±	47	2412	±	23
	6	1514	±	37	950	±	32	2326	±	45
Balakong	0	1120	±	94	930	±	54	1230	±	69
	3	1390	±	55	950	±	33	2204	±	77
	6	1216	±	45	936	±	21	1873	±	68
SK	0	1120	±	94	930	±	54	1230	±	69
	3	1411	±	66	939	±	47	1687	±	52
	6	1402	±	73	934	±	68	1398	±	43
UPM	0	1120	±	94	930	±	54	1230	±	69
	3	1124	±	45	936	±	46	1230	±	53
	6	1119	±	55	932	±	71	1228	±	46
Laboratory	week	Leaves			Stems			Roots		
Juru	0	1120	±	94	930	±	54	1230	±	69
	3	1547	±	13	950	±	62	2382	±	79
	6	1414	±	47	944	±	48	2226	±	61
Balakong	0	1120	±	94	930	±	54	1230	±	69
	3	1215	±	64	950	±	53	2177	±	68
	6	1192	±	45	941	±	27	1570	±	35
SK	0	1120	±	94	930	±	54	1230	±	69
	3	1410	±	58	944	±	47	1643	±	71
	6	1314	±	45	937	±	13	1314	±	46
UPM	0	1120	±	94	930	±	54	1230	±	69
	3	1124	±	46	936	±	46	1230	±	53
	6	1119	±	54	932	±	71	1228	±	46

concentrations of Mg accumulated (Table 2). In Table 3, the overall values for CF and AR were highest for Juru under field and laboratory conditions. Hence, the EF varied for different sites, and the ER was fastest for Balakong (Table 3).

Table 3
Concentrations (mean ± SD, µg/g dry weight) of Mg in leaves, stems and roots of Centella asiatica for transplantation studies under field and laboratory conditions

Sites	Field conditions			Laboratory conditions		
	Leaves	Stems	Roots	Leaves	Stems	Roots
CF						
Juru	1.41	1.03	1.96	1.38	1.02	1.94
Balakong	1.24	1.02	1.79	1.09	1.02	1.77
SK	1.26	1.01	1.37	1.26	1.01	1.34
AR						
Juru	21.79	1.14	56.38	20.36	0.86	54.95
Balakong	12.92	0.85	46.48	4.57	0.85	45.20
SK	13.92	0.31	21.86	13.86	0.56	19.79
EF						
Juru	0.96	0.99	0.96	0.91	0.99	0.93
Balakong	0.87	0.99	0.85	0.98	0.99	0.72
SK	0.99	1.00	0.83	0.93	0.99	0.80
ER						
Juru	-2.96	-0.28	-4.09	-6.29	-0.28	-7.43
Balakong	-8.32	-0.65	-15.76	-1.08	-0.42	-28.93
SK	-0.44	-0.22	-13.77	-4.58	-0.31	-15.66

The TF values for all the 12 sampling sites are shown in Figure 3. The values ranged from 0.085-0.150 for leave, 0.044-0.096 for stem and 0.093-0.173 for root. Based on the mean values, the TF is highest in the root (0.126), followed by leave (0.113) and stem (0.064).

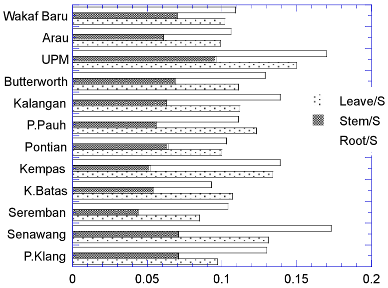

Figure 3. Transfer factors in the different leaves, stems and roots of all the sampling sites. Note: S= soil

The TF values for the three transplantation sites, both under field and laboratory conditions, are shown in Figure 4. The field condition values are higher in the leave (0.009-0.043) and root (0.026-0.064) compared to those (leave: 0.007-0.030; root: 0.013-0.058) under laboratory conditions. Based on the roots under both conditions, Sg. Juru showed the highest TF values, followed by Balakong and SK.

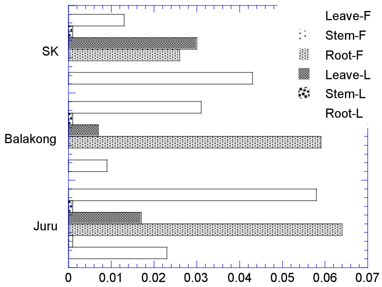

Figure 4. Transfer factors in the different leaves, stems and roots of the transplanted sites ate Seri Kembangan (SK), Balakong and Sg. Juru. Note: S= soil

Based on the leaves under both conditions, SK showed the highest TF values, followed by Sg. Juru and Balakong, while the stems did not show any significant variations of TF values in the sites.

DISCUSSION

Mg in the soil samples

When compared to the continental crust guidelines of Wedepohl (1995) (22000 µg/g dw) and Taylor (1964) (23300 µg/g dw), the eight samplings sites were higher than both the guidelines, particularly for the Seremban soils which was significantly ($P<0.05$) highest (Figure 2). Also, all the soil samples from 12 sampling sites were higher than the European topsoils baseline 11,800 ± 11,730 (<100–24,600) µg/g dry weight (Salminen et al., 2005). Therefore, these sites could be categorised as 'higher than background Mg level'; however, the interpretation of EF and Igeo values is needed to determine whether or not these sites were polluted by Mg.

The present average Mg level (26730 ± 8150 µg/g dw) in the soils from Peninsular Malaysia was higher than some reported data in the literature. For example, Jodral-Segado et al. (2006) reported the average Mg levels (µg/g dw) in agricultural soils of Southeastern Spain as 14230 ± 2250, sewage sludge as 15340 ± 1880, industrialised zone as 14360 ± 1890 and non-industrialised zone as 14160 ± 2250. They explained that the lower Mg levels could be attributed to natural sources. De Temmerman et al. (2003) also reported low Mg level in the soils (1380 µg/g dw) from Belgium. However, the soils from Turkey were reported to contain a high Mg level of 75200 µg/g dw (Hooda et al., 2004), and this elevation might be due to several factors, including geographic location, type of rock, pH, nature of drainage water, clay content, cation exchange capacity, weathering and climatic conditions, and type of plants grown (Jo & Koh 2004; Scheuner et al., 2004; Yanai et al., 2004).

The present EF values ranged from 0.53 and 4.53 (Table 1). Eight sites showed EF below 2, indicating 'deficiency in minimal enrichment', while only four sites showed EF more than 2, which indicated that the EF was in 'moderate enrichment'. This is in agreement with most reported studies, where data were usually below 5. Hernández-Mena et al. (2011) reported that the EF of Mg level in the city of Guadalajara, Mexico, was less than 5 (> 5; very strongly polluted), which was attributable to geological origins. This result is similar with the present study due to the different activities from different sampling sites. This indicated that the EF of Mg in Peninsular Malaysia was not significant. Most of the Igeo values were less than 0 (unpolluted) and only two sites showed less than 1 (unpolluted to moderately polluted). The present Igeo values (-1.42 to 0.46) indicate 'deficiency to minimal enrichment'. Therefore, the Igeo values indicate a similar conclusion about the Mg status in Peninsular Malaysia as the EF. Hence, we can conclude the Mg level in the soils of Peninsular Malaysia did not indicate pollution by this metal.

Mg in the Plant Samples

The higher Mg levels found in roots than leaves from this study could be due to the large surface area of roots because root hairs elevate the adsorption and absorption of metals and facilitate nutrient uptake (Yap et al., 2010). This is supported by the TF values found in the present study, in which the Mg uptake in the different plant parts showed highest in the roots, followed by leave and stem. Roots adhere to the soil all the time to facilitate the absorption of water and nutrients. The uptake of metals must pass through the roots before reaching the other parts of plants (Ong et al., 2011). Therefore, the exposure of roots towards Mg in soil is higher, increasing the chances of Mg accumulation in roots. Hence, less amount of Mg was able to be transported to the upper parts of plants. This was supported by Shtangeeva et al. (2011), who showed that the roots of wheat had highest Mg levels (1650 ± 1000 µg/g dw), followed by the leave (1560 ± 544 µg/g); and higher in the root of rye (1870 ± 420 µg/g dw) than in the leave (1720 ± 295 µg/g dw). The TF values found in the present study also indicated variations of Mg uptake in the different sampling sites. The highest TF values in the root from Sg. Juru could explain it being an industrial polluted site (Yap et al., 2009) and the Mg level in the soils was also the highest. Therefore, more Mg was being transferred to the plant root.

The present Mg levels of the leaves were comparable to some reported studies in the literature. For example, Oladipo et al. (2012) reported Mg concentrations (µg/g dw) in the leaves of some medicinal plants from Northern Nigeria (*Boerhavia diffusa*; 6640; *Euphoria hirta*, 1960; *Senna occidentalis*, 4730; *Senna obtusofolia*, 2510; *Cyprus dilatatus*, 3550; *Mitracarpus villosus*, 2670). Łozak et al. (2002) reported the Mg concentration (5778 µg/g) in mints leaves. This indicated that Mg concentration in *C. asiatica* from Peninsular Malaysia was within the average range as reported in the literature.

Mg in Transplantation Studies

In Table 2, samples from the four sites showed a similar trend as the samples from the 12 sampling sites (Figure 2). Mg concentration was highest in the roots, followed by the leaves and stems. This result is also supported by the TF values in which root showed the highest value. The accumulation of Mg increased in all the plant parts when they were transplanted from the 'low Mg' at UPM to 'semi-high Mg' area in SK and Balakong and 'relatively high Mg' at Sg. Juru under the field conditions from week 0 to week 3 (Table 2). In Table

3, all the CF values were higher than 1, indicating that the plants were able to uptake high concentration of Mg. Within 3 weeks, the plants were able to uptake at least 100% more Mg than the initial value. The range of AR was high starting from 0.31 to 56.38 µg/g dw per day, with the uptake of Mg being the highest in roots, followed by leaves and stems. This shows that C. asiatica can reflect the Mg levels in the environment through the uptake of Mg into the plants. Therefore, C. asiatica can be an ideal biomonitor due to its tolerance to a wide range of Mg concentration and its capability as a net accumulator over a short period of time (Rainbow & Phillips, 1993).

The accumulation of Mg in the plants decreased from week 3 to 6 due to its transplantation back to the control site. As shown in Table 3, the EF under field and laboratory conditions was at least 70%, indicating that Mg could be removed from all plant parts when the condition of the site was less contaminated than its previous site. The Mg was eliminated from plants at a rate of at least 0.22µg/g dw per day. However, the Mg concentration was still higher compared to the initial concentration at week 0, with roots having the highest Mg concentration, followed by leaves and stems. This showed that the elimination of Mg was not completed during the three weeks of transplantation due to the slower elimination rate compared to the accumulation rate. The accumulation and elimination of metals in plants were dependent upon the transplantation period (Hedouin et al., 2011). Hence, a longer time was required for Mg to be eliminated from the plants.

In order to understand the major controlling factor for the Mg uptake, the relationships of Mg levels with other major (Ca and K) and minor (Mn and Ti) metals between the plant and soils were investigated. Based on the data in Table 4, the correlation coefficients of Mg between plants and soils were found to be the highest between leaves-soils (R= 0.89, P< 005), followed by roots-soils (R= 0.77, P< 005) and stems-soils (R= 0.76, P< 005). It is interesting to note that positive significant correlations are also found for Leave-K(Soil), Stem-K(Soil), Root-K(Soil), Leave-Mg/Ti(Soil) and Root-Mg/Ti(Soil) (Table 4). In order to investigate other soils' metal, factors that could control the Mg uptake from the soils to the plant parts, regression analysis with inclusion of other soil factors are shown in Table 5. The inclusion of soil factors could

Table 4
The correlation coefficients of Mg concentrations between different parts of Centella asiatica and major cation ratios in the soils

Soil	Leave	Stem	Root
MgSoil	0.89	0.77	0.76
CaSoil	0.35	0.41	0.34
KSoil	0.68	0.59	0.63
MnSoil	0.32	0.06	0.13
TiSoil	-0.26	0.09	-0.14
Mg/Ca	0.25	0.11	0.18
Mg/K	0.52	0.46	0.41
Mg/Mn	0.04	0.26	0.19
Mg/Ti	0.76	0.43	0.59

Note: Values in bold are significantly correlated at P< 0.05

Table 5
Prediction equations for Mg levels (log10) transfer from soils to the plant parts based on multiple linear regression analysis

Prediction equations	R	R2	P
Mg(Leave)= 0.88+ 0.886 (Mg-Soil)	0.89	0.79	0.001
Mg(Leave)= 2.02 + 1.04(Mg-Soil)- 0.13(Ca-Soil)- 0.14(K-Soil)- 0.10(Mn-Soil)- 0.37(Ti-Soil)	0.92	0.85	0.019
Mg(Leave)= 1.83 + 0.43(Mg-Soil) + 0.11(Mg/Ca-Soil) + 0.12(Mg/K-Soil) + 0.09(Mg/Mn-Soil) + 0.49(Mg/Ti-Soil)	0.92	0.84	0.019
Mg(Stem)= 0.60 + 0.77(Mg-Soil)	0.77	0.59	0.003
Mg(Stem)= -0.90 + 0.52(Mg-Soil) + 0.27(Ca-Soil) + 0.22(K-Soil) + 0.01(Mn-Soil) + 0.32(Ti-Soil)	0.82	0.68	0.147
Mg(Stem)= -0.57 + 1.24(Mg-Soil) - 0.25(Mg/Ca-Soil) - 0.19(Mg/K-Soil) + 0.01(Mg/Mn-Soil) - 0.41(Mg/Ti-Soil)	0.82	0.67	0.153
Mg(Root)= 0.65 + 0.77(Mg-Soil)	0.77	0.59	0.003
Mg(Root)= 1.38 = 0.83(Mg-Soil) + 0.02(Ca-Soil) - 0.04(K-Soil) - 0.22(Mn-Soil) - 0.23(Ti-Soil)	0.79	0.62	0.213
Mg(Root)= 1.26 + 0.51(Mg-Soil) - 0.02(Mg/Ca-Soil) + 0.03(Mg/K-Soil) + 0.21(Mg/Mn-Soil) + 0.30(Mg/Ti-Soil)	0.79	0.62	0.216

usually improve the correlation performance between Mg level in the plant and in the soil compared to those based on only the Mg level in the soil (Liang et al., 2013). This is well indicated in the present study, as in Table 5. For example, R2 between log[Mg(Leave)] and log[Mg(Soil)] was 0.79. When Ca-Soil, K-Soil, Mn-Soil and Ti-Soil were combined, the controlled variance was improved to R2= 0.85, and when Mg/Ca-Soil, Mg/K-Soil, Mg/Mn-Soil and Mg/Ti-Soil were introduced in a separate equation, the regression coefficient rose to R2= 0.84. Similar patterns of R2 increments were also observed for Mg(Stem) and Mg (Root). However, these soil factor inclusions in the regression equations did not significantly increase the P values and the significant values. Therefore, this indicated that other factors could also control the Mg uptake; however, the direct correlations between the Mg(plant)- Mg(Soil) are still higher, indicating that the Mg level in the soils is the major controlling factor for the transfer of Mg to the three plant parts, as also evidently seen in Table 4. However, it is undeniable that the Mg contents in plants (oats, maize, yellow lupine and radish) were generally correlated with the accumulation of other macroelements and some microelements (Ciećko et al., 2005), which is affected by the presence of other cations such as ammonium, potassium, manganese and sulphur (Phillips & Chiy, 2002). Although it has been shown that the soil composition can be a factor in determining the growth of *C. asiatica* (Devkota & Jha, 2009), how the soil composition can influence major elemental uptake such as Mg is still unknown to our knowledge. In this study, it is assumed that the major controlling factors on the Mg bioaccumulation in *C.*

asiatica are the anthropogenic input and the environmental natural sources. However, other factors that can potentially affect the Mg uptake in the root, stem and leave of *C. asiatica* should not be ruled out.

According to a Freundlich-type function relationship, a strong relationship could still be expected between plant metal concentration and soil metal concentration (Efroymson et al., 2001). Furthermore, the Freundlich-type function is often used to describe metal transfer from the soil to plants (Krauss et al., 2002). Therefore, the significant and strong correlations of Mg levels between the three plant parts and soils indicated the Mg transfer from the soil to the plant. Mg is one of the secondary macronutrients, which are usually available in soils (Barker & Pilbeam, 2007). Mg is found in chlorophyll molecules and it is essential for photosynthesis. It also helps in the activation of many plant enzymes that are needed for growth and maintenance of cell ionic balance (Gums, 2004). Therefore, a significant correlation was found between soils and *C. asiatica*. The above results indicated that the three parts of *C. asiatica* are able to reflect the Mg levels in the soils. Therefore, the roots, leaves and stems of *C. asiatica* are good biomonitors of Mg enrichment.

CONCLUSION

Although higher than the continental crust and European topsoils baselines, the soils of Peninsular Malaysia are considered as ‘unpolluted to moderately polluted’ based on EF and ‘deficiency to minimal enrichment’ based on Igeo. The Mg concentrations in *C. asiatica* from Peninsular Malaysia were within the average range when compared to the reported data. The highest Mg level was found in the roots, followed by leaves and stems. Based on CA and MLRA, Mg-Soil was found as the significant and most important factor controlling the Mg uptake from the soils to the three plant parts. The direct relationships between Mg(plant)-Mg(Soil) also indicated that *C. asiatica* roots, leaves and stems are able to reflect the Mg levels of the sampling sites. The experimental transplantation studies, under field and laboratory conditions, confirmed the results from the field collected samples and indicated that the roots, leaves and stems could be used as good biomonitors of Mg levels in the habitat soils.

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