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RESEARCH ARTICLE

Relationships of Zn Between *Centella Asiatica* and Geochemical Fractions of the Habitat Topsoils: Implications of Biomonitoring of Zn

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Abstract:

Background:

Present study focused on the relationships of Zn concentrations between *Centella asiatica* (leaves, stems and roots) and their habitat topsoils.

Methods & Materials:

For leaves, it is found that Zn levels in the leaves significantly ($P< 0.05$) correlated with geochemical fractions of easily, freely, leachable or exchangeable (EFLE) ($R= 0.94$), acid-reducible (AR) ($R= 0.63$), oxidisable-organic (OO) ($R= 0.85$), resistant (R) ($R= 0.79$) and summation of all four fractions (SUM) ($R= 0.83$). For stems, it is found that Zn levels in the stems significantly ($P< 0.05$) correlated with AR ($R= 0.73$), R ($R= 0.75$) and SUM ($R= 0.72$). For roots, it is found that Zn levels in the roots significantly ($P< 0.05$) correlated with EFLE ($R= 0.88$), AR ($R= 0.65$), OO ($R= 0.86$), R ($R= 0.77$) and SUM ($R= 0.82$).

Conclusion:

These results indicated that the three parts of *C. asiatica* are able to reflect the Zn concentrations in the habitat topsoils. Based on ecological risk (Er) of the habitat topsoils, all samplings sites were categorized as 'Low potential ecological risk' according to Hakanson classification. Based on the positive significant relationships of Zn concentrations between plant parts and geochemical fractions of their habitat topsoils, present study indicated that *C. asiatica* can be used as biomonitoring plant of Zn polluted topsoils.

Keywords: Zinc, *Centella asiatica*, Topsoils, Relationships between plant and topsoils.

INTRODUCTION

Several routes involved in metal uptake by plants from soils and metal translocation within the plants were studied [1]. These routes include uptake of bioavailable metals, metal chelation and compartmentation in roots, metal translocation from root to shoot, and metal chelation and compartmentation in leaves [2, 3].

Zinc is one of the most common elements in the Earth's crust. High concentration of Zn can cause the phytotoxicity which can inhibit metabolic activities and result in growth retardation and senescence in plant. Excessive Zn can also

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give rise to Fe, Mn and Cu deficiency that reduce the transfer of those micronutrients from roots to shoots [4]. A medicinal plant *Centella asiatica* has been used widely in folk medicine for hundreds of years to treat a wide range of illness [5]. These plants were used to treat various illnesses thus awareness of the toxic effect of the medication due to the presence of excessive Zn accumulation shall be of public concern.

In Malaysia, Zn concentrations have been reported in mussels [6] and sediments [7]. However, Zn concentrations in terrestrial soils in relation to *C. asiatica* are lacking in the literature. Zn contamination of natural soil resources can be easily understand through this study which has emerged as an important issue due to the extension of urbanization and industrialization in Peninsular Malaysia. The objective of this study was to assess the potential of *C. asiatica* as a good biomonitor of Zn pollution based on correlation analysis of Zn between the plant and their topsoils collected from the field.

MATERIALS AND METHODS

In this paper, the Zn data from 9 sampling sites from Peninsular Malaysia (Fig. (**1**), Table **1**) in the leaves, stems and roots of *C. asiatica,* and also their habitat topsoils were cited from [8], in which the data were presented in graphs. Briefly, the plants of 2-4 months maturity were collected and placed in plastic bags. The four geochemical fractions of Zn data in the soils were also cited from [8]. However, Ong *et al*. [8] did not report the relationships of Zn levels between the plant parts and different geochemical fractions of the habitat topsoils specifically.

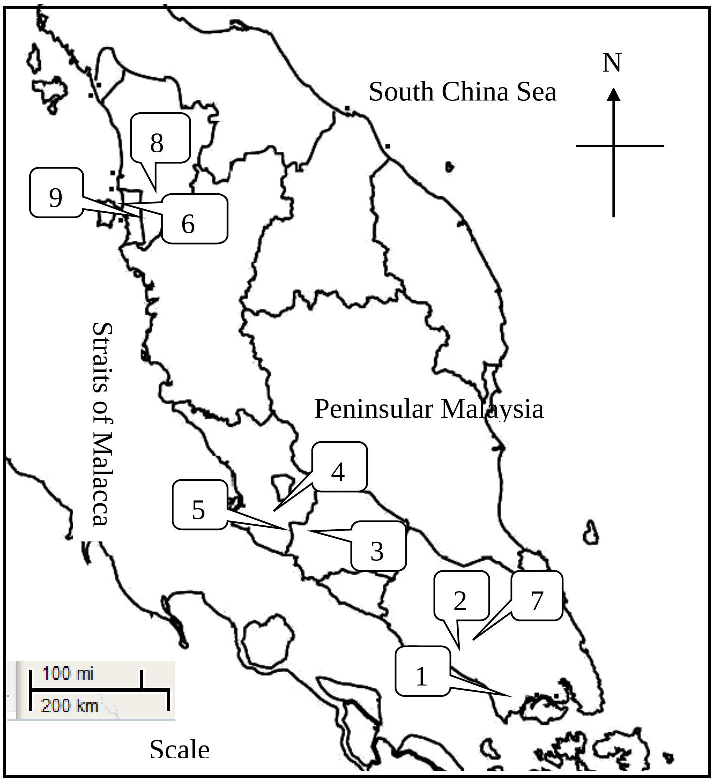

Fig. (1). Map showing the sampling sites for *Centella asiatica* in Peninsular Malaysia [8].

Table 1. Sampling sites, sampling date and sites description of *Centella asiatica* in Peninsular Malaysia [8].

No	Sampling sites	Sampling dates	Sites descriptions
1.	Pontian, Johore	9 May 10	Near a plant agriculture area.
2.	Kampung Simpang Renggam (KSR), Johore	9 May 10	Near a housing area.
3.	Seremban, Sembilan	4 June 10	Near shop lots and road sides.
4.	Kapar, Selangor	5 June 10	Small scale housing area.
5.	Universiti Putra Malaysia (UPM), Selangor	5 June 10	Near agriculture area.
6.	Butterworth, Penang	12 June 10	Near an industrial area and highway.
7.	Kluang, Johore	19 June 10	Near paddy fields.

(Table 1) contd.....

No	Sampling sites	Sampling dates	Sites descriptions
8.	Karangan, Kedah	12 June 10	Near oil palm plantations.
9.	Permatang Pauh (PPauh), Penang	12 June 10	Near a housing area and highway.

Briefly, the sampling and analytical procedures of Zn can be found similarly as [9]. The separated plant parts (leaves, stems and roots) and the sediments were then dried in an oven for 72 hours at 60°C to constant dry weights. About 0.50 g of dried plant tissue parts were weighed using an analytical balance. Ten ml of concentrated nitric acid (AnalaR grade, BDH 69%) were added to a digestion tube to digest the plant tissues. For sediments, they were sieved under 63µm followed by sequential extraction technique (SET) [9]. Three replicates were done for each sampling site. Then, the digestion tubes were placed in a hot block digester at 40°C for 1 hour and 140°C for at least 3 hours [6]. After dilution, the solution was filtered through a Whatman No. 1 filter paper into an acid-washed pill box until Zn analysis by using an air-acetylene Perkin-Elmer™ flame atomic absorption spectrophotometer model AAnalyst 800. Standard solutions for Zn were prepared from 1000 ppm stock solution provided by MERCK Titrisol.

Geochemical fractions of Zn in the sediments were obtained using the modified SET (Sequential Extraction Technique) described by [10]. The four fractions 'easily, freely or leachable or exchangeable (EFLE; F1)', 'acid-reducible (AR; F2)', 'oxidisable-organic (OO; F3)' and resistants (R; F4) were employed [9].

Data Treatment

Geochemical Indices in the Topsoils

There are two geochemical indexes involved in this study which are geoaccumulation (Igeo) and enrichment factor (EF).

To determine the Igeo, the formula was introduced by Muller [11] as a quantitative measure of the degree of metal pollution in aquatic sediments as below:

$$Igeo = \text{Log } 2\left(\frac{\text{Cn}}{K \text{ x } Bn}\right)$$

Where;

C_n = the concentration of examined metal (n) in the soil;

B_n = the background reference values. The background references values for Fe and Zn used in the present study were 3.09% and 52.0 mg/kg, respectively, followed the upper continental crust values reported by Wedepohl [12] and the K represents for the factor of 1.5 value due to lithogenic effects [13].

To determine the EF, the formula below was used:

$$\text{EF} = \frac{\left(\frac{Me}{Fe}\right)Sample}{\left(\frac{Me}{Fe}\right)Background}$$

Where;

Me/Fe sample = the metal to Fe ratio to be analyzed.

Me/Fe background = the metal to Fe ratio which involved the background reference value, as mentioned above.

For the reading of EF, the EF which is less than 2 is the depletion to minimal enrichment. Whereas, the $2 \leq EF < 5$ categorized as moderate enrichment, $5 \leq EF < 20$ categorized as significant enrichment, and $20 \leq EF < 40$ are very high enrichment. For $EF > 40$, they are extremely high enrichment [14].

Ecological Risk Assessment (ERA) on the Topsoils

For the determination of potential ecological risk, the following formulas were used:

Cf= Cs/Cn

Where;

Cf= contamination factor of Zn

Cs = the examined Zn in the samples.

Cn = the background reference values of Zn, as mentioned above.

Er= Tr × Cf

Tr = the factor of toxic response; In the present study, the Tr employed for Zn was 1.00 [15].

Bioconcentration Factor

In this study, the plant metal accumulation efficiency was measured by Zn bioconcentration factor (BCF), which is a ratio of Zn concentration in plant tissue to that in the soil [16 - 18]:

BCF= Plant (Zn)/Soil (Zn)

where Plant (Zn) is the Zn concentration in the plant tissue (leaves, stems or roots), and Soil (Zn) is the Zn concentration in the habitat topsoil.

RESULTS AND DISCUSSION

The Zn concentrations of *C. asiatica* (cited from [8]) and in their habitat topsoils are presented in (Table **2**). Overall, there were two sampling sites (PPauh and Butterworth) that exceeded 300 µg/g dw in the leaves and roots of the plants. For stems, higher levels of Zn were found in three sites (Butterworth, Seremban and Pontian). Therefore, Butterworth was consistently found to have highest levels of Zn in leaves, stems and roots of the plants.

Table 2. Concentrations (mean ± SD, µg/g dw) of Zn in leaves, stems and roots of *Centella asiatica* and four geochemical fractions in the habitat tosoils.

	leaves	Stems	Roots	F1	F2	F3	R	SUM	NR
PPauh	316	127	336	8.45	39.3	63.8	112	224	60.6
Karangan	172	133	208	2.00	3.14	12.9	31.9	49.9	15.9
Kluang	145	124	185	2.43	4.92	10.8	24.4	42.6	76.6
Butterworth	337	160	349	14.7	52.4	69.3	171	308	103
UPM	145	124	208	0.44	5.13	36.3	46.6	88.5	11.4
Kapar	121	91.3	134	1.64	4.36	5.87	21.3	33.1	37.6
Seremban	182	177	230	3.17	67.1	31.6	108	210	102
KSR	160	127	171	1.93	22.3	31.5	77.1	133	77.3
Pontian	182	172	214	2.15	25.4	53.1	131	212	27.5

Note: Zn data in the leaves, stems and roots of *Centella asiatica* and their habitat topsoils were cited from Ong *et al*. [8]. F1= easily, freely or exchangeable fraction (EFLE); F2= acid-reducible (AR); F3= oxidisable-organic (OO); F4= resistant (R) fractions; SUM= summation of F1, F2, F3 and F4; NR= nonresistant fractions (F1 + F2 + F3).

From Table **2**, overall, the plant roots showed the highest Zn accumulation followed by leaves and stems. Those results were supported by [19]. It has been reported that about 30 µg/g dw of Zn is adequate for plant growth and 300 to 500 mg/kg of Zn is considered toxic to the plants [20]. The physiological disorders and metabolic abnormalities in plants could be resulted from excessive exposure of Zn [21]. Therefore, Zn might be accumulated in the roots and be unable to enter the plant by being kept in the root cells where they would be detoxified by forming complexes or sequestered into vacuoles [22]. This action greatly restricted the translocation of metals to the above-ground organs. Moreover, it could protect the leaf tissues and the metabolically active photosynthetic cells from Zn toxicity [23].

In comparison to the geochemical factions, this is well supported by the highest levels if Zn in EFLE (F1), OO (F3) and resistant (R) fractions of the topsoils. This is again supported by the total summation of all fractions and non-resistant fraction. Hence, these comparisons indicated the use of different parts of *C. asiatica* as a good biomonitor of Zn. In order to see the close relationships of Zn levels between the plants ad topsoils, the graphical relationships of Zn between the both parameters are presented in Fig. (**2**) (based on leaves), Fig. (**3**) (based on stems) and Fig. (**4**) (based on roots).

Based on the relationships of plant leaves and geochemical fractions Fig. (**2**), it is found that Zn levels in the leaves significantly ($P< 0.05$) correlated with EFLE ($R= 0.94$), AR ($R= 0.63$), OO ($R= 0.85$), R ($R= 0.79$) and SUM ($R= 0.83$). Based on the relationships of plant stems and geochemical fractions Fig. (**3**), it is found that Zn levels in the stems significantly ($P< 0.05$) correlated with AR ($R= 0.73$), R ($R= 0.75$) and SUM ($R= 0.72$). Based on the relationships of plant roots and geochemical fractions Fig. (**4**), it is found that Zn levels in the roots significantly ($P< 0.05$) correlated with EFLE ($R= 0.88$), AR ($R= 0.65$), OO ($R= 0.86$), R ($R= 0.77$) and SUM ($R= 0.82$). These results indicated the three parts of *C. asiatica* are able to reflect the Zn concentrations in the habitat topsoils. The positive relationships of Zn between soil non-resistant fractions (EFLE, AR and OO) and plant root indicated a close relationship between soil Zn concentration and root metabolism, which should be further examined to understand how soil Zn concentration can impact root Zn accumulation efficiency. According to [24], when soil metal concentration exceeds the plant tolerance, growth and metabolism will be inhibited and eventually the plant species will be excluded from the site vegetation assemblage even though there is a seed existing in the regional pool. Therefore, the roots, leaves and stems of *C. asiatica* are good biomonitors of Zn pollution in the environment.

Table 3. The values of geochemical indices based on the Zn levels in the habitat topsoils of *Centella asiatica*. Fe concentrations are presented in %.

	Zn soil	Fe (%) soil	EF	Igeo	Cf	Er
PPauh	224	2.16	6.14	1.52	4.30	4.30
Karangan	49.9	1.37	0.64	-2.40	0.29	0.29
Kluang	42.6	1.22	0.62	-2.62	0.24	0.24
Butterworth	308	2.79	6.55	1.98	5.92	5.92
UPM	88.5	2.59	2.03	0.18	1.70	1.70
Kapar	33.1	2.34	0.84	-1.24	0.64	0.64
Seremban	210	2.74	4.55	1.43	4.03	4.03
KSR	133	2.18	3.61	0.77	2.55	2.55
Pontian	212	2.47	5.10	1.44	4.07	4.07

Note: EF= enrichment factor; Igeo= geoaccumulation index; Cf= contamination factor; Er= ecological risk. Data of Zn and Fe in the soils were cited from Ong *et al*. [8].

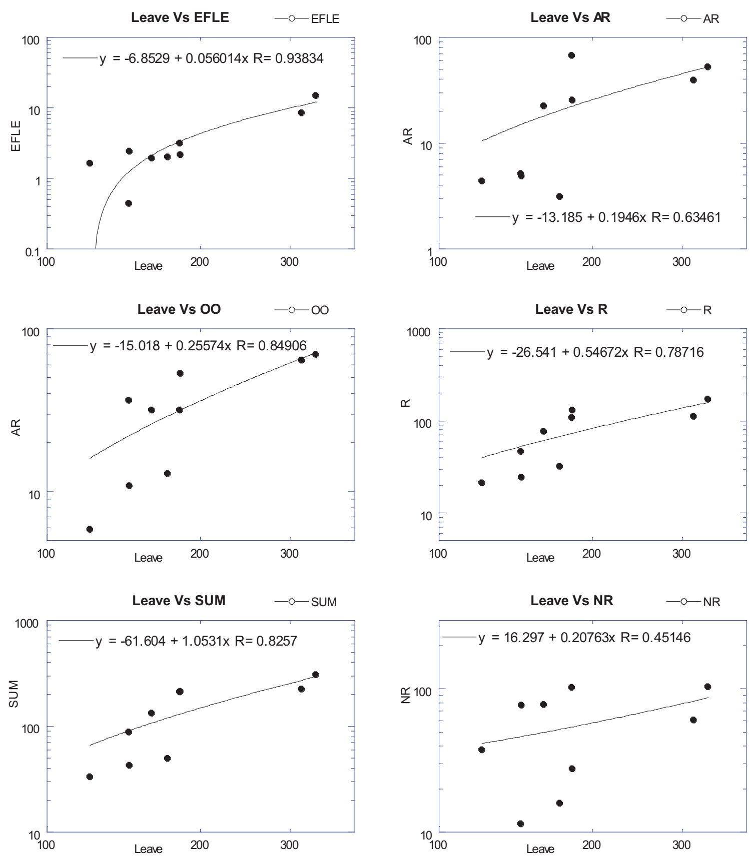

Fig. (2). Relationships of Zn between leaves of *Centella asiatica* and different geochemical fractions of habitat topsoils, based on log10 axes of x and y.

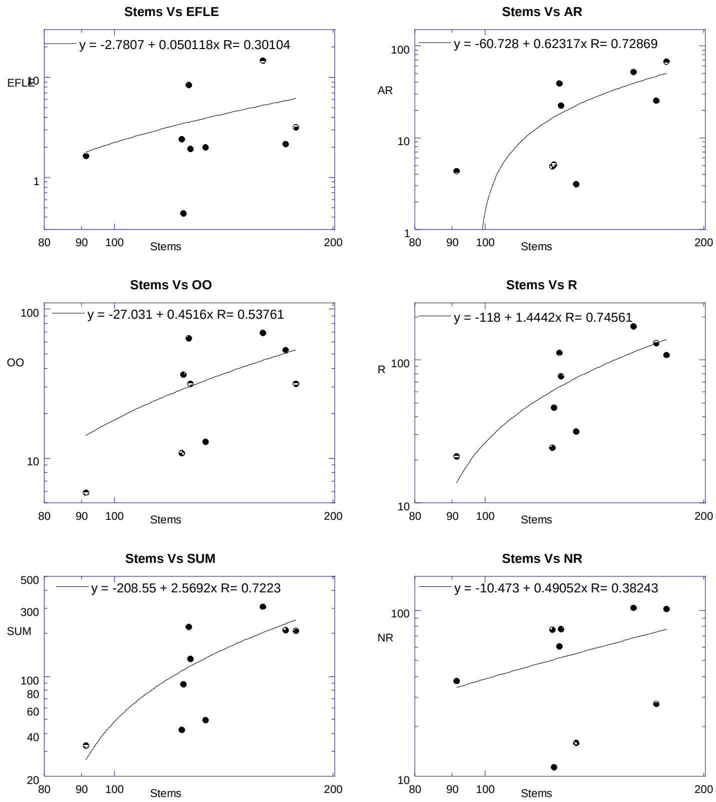

Fig. (3). Relationships of Zn between stems of *Centella asiatica* and different geochemical fractions of habitat topsoils, based on log10 axes of x and y.

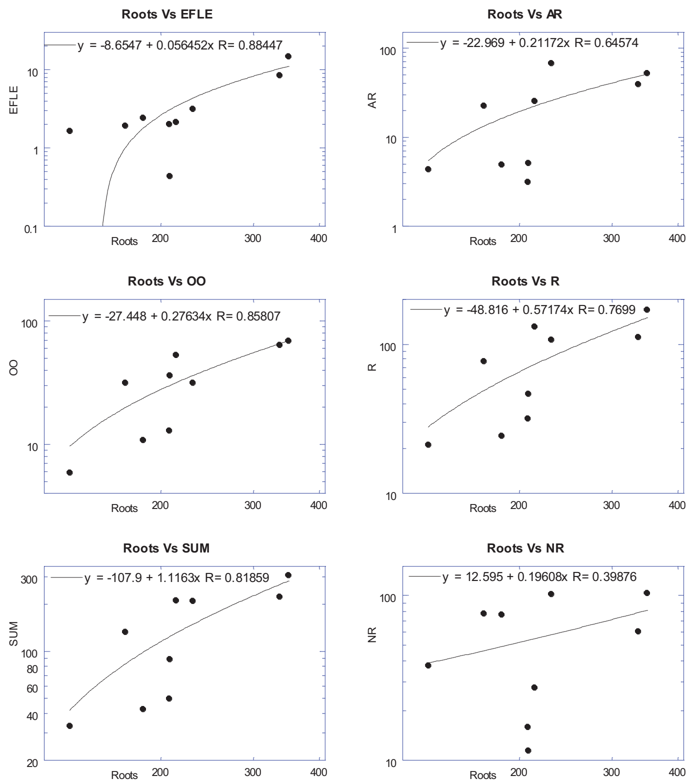

Fig. (4). Relationships of Zn between roots of *Centella asiatica* and different geochemical fractions of habitat topsoils, based on log10 axes of x and y.

The geochemical indices for EF, Igeo, CF and ER of the topsoils are presented in (Table **3**). For EF, there are three sites (PPauh, Butterworth and Pontian) with classification of 'moderate severe enrichment; 5-10' (Table **4**). Also, there are two sites with 'moderate enrichment', one site with minor enrichment and others with 'no enrichment', according to Taylor [25] classification.

Table 4. Grading standards for enrichment factor (EF; Taylor [25]), geoaccumulation index (I_{geo}; Muller [11]), contamination factor (Cf; Hakanson [15]) and potential risk index for individual metal (Er; Hakanson [15]).

A. Enrichment factor (EF)		
EF ranges		Degree of enrichment
<1		No enrichment
1-3		Minor enrichment
3-5		Moderate enrichment
5-10		Moderately severe enrichment
10-25		Severe enrichment
25-50		Very severe enrichment
50		Extremely severe enrichment
B. Geoaccumulation index (I_{geo})		
I_{geo} values	I_{geo} Classes	Pollution intensity
0	<0	Unpolluted
1	0-1	Unpolluted to moderately polluted
2	1-Feb	Moderately polluted
3	2-Mar	Moderately to strongly polluted
4	3-Apr	Strongly polluted
5	4-May	Strongly to very strongly polluted
6	>5	Very strongly polluted
C. Contamination factor (C*f*)		
Value rangse of C_f		Description
Cf< 1		Low contamination factor
1≤ Cf < 3		Moderate contamination factor
3 ≤ Cf < 6		Considerable contamination factor
Cf ≥ 6		Very high contamination factor
D. Potential risk index for individual metal (Er)		
≤ Value ranges of Er		Description
Er < 40		Low potential ecological risk
40 ≤ Er < 80		Moderate potential ecological risk
80 ≤ Er < 160		Considerable potential ecological risk
160 ≤ Er < 320		High potential ecological risk
Er ≥ 320		Very high potential ecological risk at hand for the substance in question

For Igeo, there are four sites (PPauh, Butterworth, Seremban and Pontian) with classification of 'moderate polluted; 1-2' (Table **4**). Also, there are two sites with 'Unpolluted to moderately polluted' while others with 'unpolluted', according to Muller [11] classification. For Cf, similarly there are four sites (PPauh, Butterworth, Seremban and Pontian) with classification of 'Considerable contamination factor; 3-6 (Table **4**). Also, there is one site with 'Moderate contamination factor' while others with 'Low contamination factor', according to Hakanson [15] classification. For Er, all sites were below 40 with 'Low potential ecological risk' according to Hakanson [15] classification.

Ratios of nonresistant/resistant (NR/R) are given in (Table **5**). Based on NR/R, the topsoils from two sites (Kluang and Kapar) are found to have been dominated (> 1.00) by nonresistant fraction of the soils while the rests are mostly originated from natural sources (< 1.00).

Table 5. Ratios values calculated from the present study.

	NR/R	**BCF-1 (Leave/Soil)**	**BCF-2 (Stem/Soil)**	**BCF-3 (Root/Soil)**
PPauh	0.54	1.41	0.57	1.50
Karangan	0.50	3.45	2.68	4.16
Kluang	3.14	3.41	2.90	4.34

(Table 5) contd.....

	NR/R	BCF-1 (Leave/Soil)	BCF-2 (Stem/Soil)	BCF-3 (Root/Soil)
Butterworth	0.60	1.09	0.52	1.14
UPM	0.25	1.63	1.40	2.35
Kapar	1.77	3.66	2.76	4.06
Seremban	0.94	0.87	0.85	1.10
KSR	1.00	1.21	0.96	1.29
Pontian	0.21	0.86	0.81	1.01

Note: NR/R= ratios of nonresistant to resistant fractions of the topsoils.

The three values of accumulation efficiency (BCF) are presented in (Table **5**). The ratios of leave/soil (BCF-1), stem/soil (BCF-2) and root/soil (BCF-3) varied from 0.87-3.66, 0.52-2.90, and 1.01-4.34, respectively. Based on six dominant plant species, Qian *et al*. [18] reported the ratios of BCF (root/soils) varied from 0.56 to 10.8. Higher Zn concentration in plant parts demonstrated that the amount of Zn accumulated in the plant parts is positively related to Zn concentrations in the topsoils. This was consistent with the results from other studies with other metals such as Cd, Ta and V [33] and V [18]. Hence, present three values of BCF indicated accumulation efficiency of Zn in the leaves, stems and roots of *C. asiatica*.

Based on some established soil sediment guidelines for Zn, four sampling sites (PPauh, Butterworth, Seremban and Pontian) had exceeded Canadian soil quality for agricultural use (200 µg/g dw) [26] while Butterworth exceeded the Chinese soil quality standard (250 µg/g dw) . The rest of the sampling sites were found to be below Target value (140 µg/g dw, the baseline concentration value that considered not affecting the natural properties of the soil) according to Dutch soil guideline [27]. Based on the present data, all sampling sites were found to be lower than middle value (430 µg/g dw, a threshold value for further investigation) and intervention value (720 µg/g dw, the maximum tolerable concentration that remediation is required), according to Dutch soil guideline [27].

In Malaysia, industries in Malaysia such as electronics, textiles, food processing and rubber based industry contribute the Zn contamination to the environment [28]. The use of fungicides and fertilizers containing organo-zinc could have caused the excess to leach into the soil [29]. Soils near highways and smelters contained high Zn concentrations as a result of deposition of Zn released in tire abrasion and stack emissions [30]. Zn level in soils was reported decreased with distance from the point source of pollution (CCME, 1999). Therefore, higher level of Zn can be found in nearby of the Zn contamination source.

Previously, Warne *et al*. [31] reported the Zn concentrations are found in the ranges of Zn between 150-300 µg/g dw in the polluted soils. Wang and Qin [32, 33] reported Zn ranges 53-380 µg/g dw in urban topsoil of Xuzhou (China) while Yap and Pang [7] reported the Zn ranges as 88.7-484 µg/g dw in river drainage surface sediments collected from the north western aquatic area of Peninsular Malaysia. Yap *et al.* [34, 35] reported the Zn ranges as 50–336 µg/g dw and 330-484 µg/g dw for intertidal and drainages in Selangor, respectively. All the above studies explained the higher or elevated levels of Zn were related to anthropogenic inputs that had increased the levels of Zn in soils. Therefore, present findings are comparable to the above reported data. This has given a strong indication of Zn contamination in Malaysian topsoils that should be given be concern from environmental management point of view [36].

CONCLUSION

Present study investigated the relationships of Zn concentrations between three parts (leaves, stems and roots) of *Centella asiatica* and their habitat topsoils. Based on Er of the habitat topsoils, all samplings sites were categorized as 'Low potential ecological risk' according to Hakanson [15] classification. Based on the positive significant relationships of Zn concentrations between plant parts and geochemical fractions of their habitat topsoils, present study indicated that *C. asiatica* can be used as biomonitoring plant of Zn polluted topsoils.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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