Pertanika J. Trop. Agric. Sci. 36 (4): 311 - 336 (2013)

PERTANIKA JOURNALS

TROPICAL AGRICULTURAL SCIENCE

Journal homepage: http://www.pertanika.upm.edu.my/

Accumulation of Heavy Metals and Antioxidative Enzymes of *Centella asiatica* in Relation to Metals of the Soils

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ABSTRACT

Antioxidative enzymes have been claimed as being beneficial for enhancing fitness and for preventing disorders in plants due to the production of reactive oxygen species (ROS) caused by heavy metal stresses. *Centella asiatica* plants and soil sediments from nine sampling sites were collected between May and June of 2010. They were tested for their Cd, Cu, Fe, Ni, Pb and Zn contents. The plants were also analyzed for the activities of antioxidative enzymes namely superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX) and guaiacol peroxidase (GPX). This study revealed positive and significant ($P<0.05$) correlations between plants (leaves and roots) and soils for Cd, Zn, Ni and Fe content. It also showed that the significant correlations between Cd, Fe and Pb accumulations did not seem to be a factor for the increase in antioxidative enzyme activities due to their low concentrations in the plant; but the accumulated Cu, Zn and Ni levels were significantly ($P<0.05$) correlated with increases in antioxidative activities.

Keywords: *Centella asiatica,* Heavy metals, antioxidative enzymes

ARTICLE INFO

Article history:
Received: 8 February 2011
Accepted: 28 August 2012

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INTRODUCTION

Recently, toxicities caused by heavy metals have become a major issue to the public due to their capabilities to be transferred and accumulated in plants, animals and humans (Garcia-Rico *et al*., 2007). Moreover, there are concerns on the long term persistence of these metals which might cause hazardous health issues to humans (Gisbert *et al*., 2003;

ISSN: 1511-3701

Stankovic *et al*., 2012). There are several factors influencing the concentrations of these metals in medicinal plants such as the species of the plant, climate, air pollution and other environmental factors (Sovljanski *et al*., 1989).

High concentrations of these metals might cause growth inhibition and even death of the plants (Schutzendubel *et al*., 2001). Heavy metals are involved in many steps in the production of deleterious free radicals/reactive oxygen species (ROS) such as singlet oxygen ($O_{2)}$, superoxide radical ($O^{-}_{2)}$, hydrogen peroxide (H_2O_2), hydroxyl ion ($OH^{-)}$ and free hydroxyl radical (OH) (Halliwell and Gutteridge, 1984). ROS is highly reactive and harmful, but it can also act as a signaling molecule and as an inducer in the antioxidative enzyme system of plants for protecting themselves (Foyer *et al*., 2009; Parra-Lobato *et al*., 2009). The synchronous action of superoxide dismutase (SOD), catalase (CAT), peroxidases (POD), ascorbate peroxidase (APX), dehydroascorbate reductases (GDOR) and glutathione reductase (GR) is part of the system that protects plants against ROS in various compartments of the plant cell (Singh *et al*., 2006).

In this study, we focused on *Centella asiatica* (family: Umbelliferae) which is widely used in folk medicine for hundreds of years to treat a wide range of illnesses (Brinkhaus *et al*., 2000). It is also listed as one of the useful medicinal herbs by WHO (1999). In Malaysia, it is used to rapidly heal small wounds, chaps and scratches, surgical wounds and so on (Ong *et al*., 2011). Of the entire *Centella* genus, only the *asiatica* species is found in commercial drugs today (Zainol *et al*., 2003). Much of the work related to heavy metal biomonitoring featuring plants had been done around the world for example those by Aksoy and Demirezen (2006), Baycu (2003) and Yilmaz *et al.* (2006). Thus , *Centella asiatica* can be chosen as an ideal biomonitor due to it being sedentary, abundant, easy to identify, available for sampling throughout the year, large enough to provide sufficient tissues for (individual) analysis, resistance to handling stress caused by laboratory studies of metal kinetics and/or field transplantations, tolerant to exposure to environmental variations in physico-chemical parameters and most important of all its capability as a net accumulator of the metal with a simple correlation between metal concentration in tissues over a short time period (Rainbow and Phillips, 1993; Wittig, 1993).

In addition, the effects of geochemical fraction of soils on metal concentrations in *C. asiatica* in Malaysia are still unclear. There is very little information available on antioxidative activity in medicinal herbs caused by heavy metals in the recent literature. Therefore, the objective of this study was to determine the relationships between selected metal concentrations and antioxidative activities of *C. asiatica* with the level of these metals in the soil.

MATERIALS AND METHODS

Sample collection

Centella asiatica was collected from nine sampling sites in Peninsular Malaysia

(Fig.1) between May and June, 2010. The plant samples collected from the wild were from Permatang Pauh (PPauh), Karangan, Kluang, Butterworth, Universiti Putra Malaysia (UPM) in Serdang, Kapar, Seremban, Kampung Simpang Renggam (KSR) and Pontian (Table 1). Three replicates were collected per sampling site and around 100 g of fresh weight of samples were collected for each replicate. During collection, surface sediments (top 3-5cm) were also collected to determine the levels of heavy metals. The soil sediment was collected by using a plastic scoop after the litter had been removed and three replicates were collected for each sampling site.

Determination of heavy metal concentrations

Three replicate determinations were done for each sampling site. For determination of metal concentrations, plants were separated into two different parts namely leaves and roots. The separated plant tissues, and the sediments, were then dried in an oven for 72 hours at 60 ºC to constant dry weights. About 0.5g of dried plant tissue parts were placed in a digestion tube and 10 ml of concentrated nitric acid (AnalaR grade, BDH 69%) were added to digest the plant tissues. Then, the digestion tubes were placed in a hot block digester at 40°C for 1 hour and then at 140°C for at least 3 hours

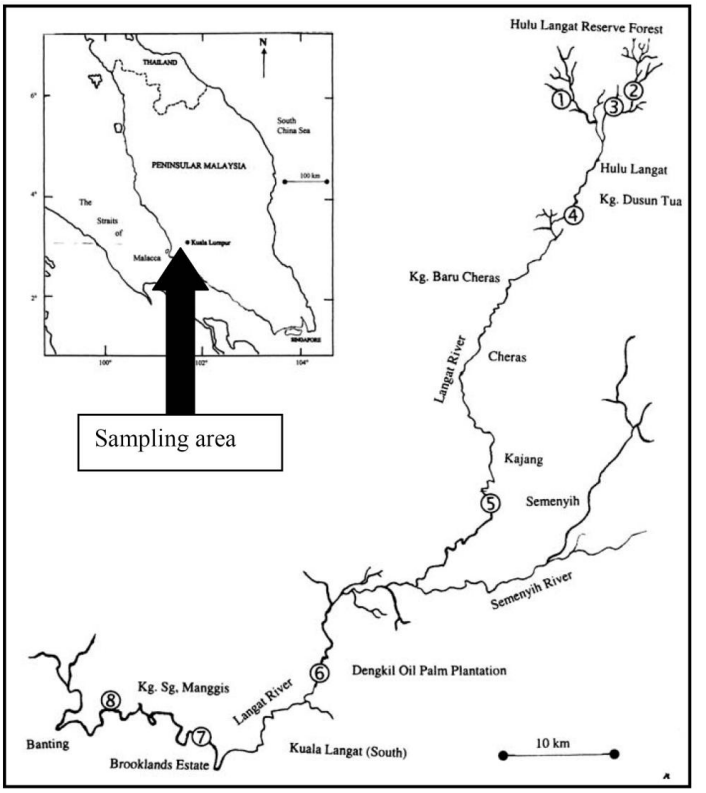

Fig.1: Map showing the sampling sites for *Centella asiatica* in Peninsular Malaysia.

TABLE1
Sampling sites, sampling date and site description of *Centella asiatica*

No	Sampling site	Sampling date	Site description
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.
8.	Karangan, Kedah	12 June 10	Near oil palm plantations.
9.	Permatang Pauh (PPauh), Penang	12 June 10	Near a housing area and highway.

(Yap *et al*., 2003). After that, the digested samples were left to cool and were topped up (diluted) to 40 ml with double de-ionized water. Lastly, the solution was filtered through a Whatman No. 1 filter paper into an acid-washed (Yap *et al*., 2003) pill box and stored at room temperature until required for metal concentration determinations. Soil sediments were sieved through a 63 µm mesh followed by direct aqua-regia digestion and the sequential extraction technique (SET) described below .

Direct aqua-regia digestion

About 1 g of each dried sample was weighed and placed in an acid washed digestion tube. A combination of concentrated nitric acid (69 %) and perchloric acid (60 %) was prepared in the ratio of 4:1 and added to each digestion tube (Yap *et al*., 2002). The tubes were then placed in a digestion block at 40 ºC for 1 hour and were then fully digested at 140 ºC for 2-3 hours (Yap *et al*., 2002). After they were cooled to room temperature, the digests were topped up (diluted) to 40 ml with double de-ionized water. Each diluted sample was then filtered through a Whatman No. 1 filter paper into an acid-washed pill box. The samples were then stored until used for metal determination .

Sequential Extraction Technique (SET)

Sequential extraction was performed by using a four stage procedure originally recommended by Badri and Aston (1983). Sequential extraction analysis (SET) provides information on heavy metals which are of anthropogenic sources such as easily, freely leachable or exchangeable fraction (EFLE), acid reducible fraction, oxidisable organic fraction and resistant fraction in the sediments.

Extraction of EFLE fraction

For each site, three replicate extractions were done. Ten grams of dried samples were weighed and placed in a 250 ml Erlenmeyer

flask. After that, 50 ml of 1.0 M ammonium acetate at pH 7 was added to the sample and agitated in an orbital shaker model GYROMAX 722 at constant speed (2500 rpm) for 3 hours at room temperature. Then it was filtered through a Whatman No. 1 filter paper into an acid washed pill box. The remainder in the Erlenmeyer flask was washed with 20 ml of double de-ionized water and then filtered through the same filter paper into the same pill box. The residue left on the filter paper was dried in an oven between 40 to 60 ºC until a constant dry weight was obtained. The dried residue was scraped off from the filter paper and placed in the same Erlenmeyer flask. The dry weight was measured and recorded to determine the amount of sediment that dissolved in the extractant.

Extraction of acid reducible fraction

The same procedure as above was followed but 50 ml of 0.25 M hydroxyl ammonium chloride at pH 2 were used instead. The second fraction is known as the 'acid reducible' fraction. The filter paper containing the residue was dried in an oven between 40 to 60 ºC until a constant dry weight was obtained.

Extraction of oxidisable-organic fraction

The dried residue obtained in Section 2.2.2.2 was oxidised with 15 ml 30 % hydrogen peroxide (H_2O_2) in a water bath (90 ºC to 95 ºC). During the experiment, cool water was prepared to slow down the reaction if the reaction was too vigorous. The experiment was carried on until the mixture dried up. After cooling, the metal released from the organic complex was continuously agitated with 50 ml of 1 M ammonium acetate at pH 3.5 at room temperature. The washing, filtration and measurement of dry weight were repeated as described earlier.

Extraction of resistant fraction

The sediments were again dried and scraped off from the filter paper. About 1 g of each sediment sample was weighed from the residue from the extraction of oxidisable–organic fraction and the experiment was carried out based on the direct aqua-regia method which was described previously.

Heavy metal determination

All ~~the~~ stored plant and soil samples were analyzed using an air-acetylene Perkin-Elmer™ flame atomic absorption spectrophotometer model AAnalyst 800. The light used for each metal was different. Each light have its own wavelengths namely 228.8 nm for Cd; 324.8 nm for Cu; 248.3 nm for Fe; 232.0 nm for Ni; 283.3 nm for Pb and 213.9 nm for Zn (Perkin-Emer, 1990). Blank determination was carried out to calibrate the instrument. Standard solutions for Cd, Cu, Fe, Ni, Pb and Zn were prepared from 1000 ppm stock solutions provided by MERCK Titrisol. All data obtained from the ASS w ere presented in µg/g dry weight basis.

Assay of antioxidative enzyme activity

Enzyme extraction

All chemical used for assay of antioxidative activity were freshly prepared to prevent the degradation of its activities. About 0.2g of (leave and root) fresh tissues was homogenized in an ice-cooled mortar with 5 ml of 100 mM potassium phosphate buffer (pH 7.0) containing 0.1 mM EDTA and 1% (w/v) polyvinylpyrrolidone (Mishra *et al*., 2006). The pH of the phosphate buffer was adjusted by adding monopotassium phosphate (KH_2PO_4) and dipotassium phosphate (K_2HPO_4). The homogenate was transferred to a 1.5 ml Eppendorf tube and centrifuged at 15000xg for 15 min at 4°C (Mishra *et al*., 2006). The supernatant was used for enzyme activity determination.

Superoxide dismutase (SOD) activity

The activity of SOD was assayed by measuring the inhibition of the photochemical reduction of nitrobluetetrazolium (NBT) (Beauchamp and Fridovich, 1971). The experiments were carried out in test tubes. Two sets of test tubes were prepared: one set of test tubes was under illumination while another set of test tubes was covered with aluminum foil and used as the control.

The assay mixture contained ~~of~~ 1.5 ml of 50 mM phosphate buffer (pH 7.8), 0.3 ml of 130 mM methionine, 0.3 ml of 750 µM NBT, 0.3 ml of 0.1 mM EDTA, 0.3 ml of 20 µM riboflavin, 0.05 ml of enzyme extract and 0.25 ml of deionized H_2O in a total volume of 3.0 ml. Riboflavin was added last, and the tubes were shaken and then illuminated for 15 min under standard florescent light (10 lamp watts per foot of length). The change in absorbance was recorded at 560 nm.

Catalase (CAT) activity

The assay for CAT activity was done based on the method of Aebi (1984). The assay mixture contained 0.2 ml of tissue extract, 1.5 ml of 50 mM phosphate buffer (pH 7.8), 1.0 ml of deionized H_2O and 0.3 ml of 0.1 M H_2O_2. H_2O_2 was added last and the mixture was shaken before the decrease in absorbance was recorded at 240 nm for 3 min.

Guaiacol peroxidase (GPX) activity

GPX activity was determined following the oxidation of guaiacol using the method described by Hemeda and Klein (1990) with some modifications. The assay mixture contained 2.9 ml of 50 mM phosphate buffer with pH 6.0, 1.0 ml of 2% H_2O_2 and 0.1 ml of the enzyme extract. After the addition of 1.0 ml 50 mM guaiacol, the mixture was shaken before the increase in absorbance as guaiacol was oxidized was measured at 470 nm for 3 min.

Ascorbate peroxidase (APX) activity

APX activity was determined by the method of Nakano and Asada (1981). The assay mixture contained 1.8ml of 50 mM phosphate buffer (pH 7, containing 0.2 mM EDTA-Na_2), 0.1ml of 7.5 mM ascorbic acid, 1 ml of 300 mM H_2O_2 and 0.1 ml of enzyme extract. H_2O_2 was added last and the mixture

was shaken before the change in absorbance was recorded at 290 nm.

Total protein determination

Total soluble protein concentration was determined using the method established by Bradford (1976). Standard Bovine Serum Albumin (BSA) was prepared at different concentrations: 0, 40, 80, 120, 160 and 200 µg/ml. Total protein content was expressed as mg of BSA equivalent by using the equation obtained from the standard curve of BSA.

Statistical analysis

The STATISTICA version 8 software was used to determine the correlation coefficient and for hierarchical cluster analysis. The analysis of variance (ANOVA), Student-Newman-Keuls (SNK) and Post hoc test were done using the SPSS software version 17.0 for Windows to find the differences between the means of heavy metal concentrations in the different parts of the plants from different sites (Zar, 1996).

RESULTS

The study was focused on the accumulation of common non-ionised form of metals in the leaves and roots of *C. asitica*, and soil sediments from selected sampling sites in Malaysia. As the metal concentrations in the stems did not show any significant correlations with those in the soils from all sampling sites, they are not further discussed. Based on the concentrations of heavy metals in the leaves of plants from nine sampling sites as listed in Table 2, there were significantly higher ($P<0.05$) Cu concentrations in leaves collected in Butterworth, Seremban and Pontian . For Cd in leaves, only those collected in Butterworth showed significant difference ($P<0.05$) in concentration. The concentrations of Zn in leaves from PPauh and Butterworth were significantly higher ($P<0.05$) than the rest. For Ni, samples from PPauh and Seremban were significantly higher ($P<0.05$) in concentrations. Leaves samples from Butterworth, Kapar, Seremban and Pontian showed significantly higher ($P<0.05$) concentrations of Pb. Fe concentration of plants from Butterworth and Seremban were significantly higher ($P<0.05$) in leaves than in roots .

Table 3 shows the concentrations of heavy metals in roots. Samples collected from Butterworth, UPM , Seremban and Pontian. showed significant differences ($P<0.05$) for Cu concentrations. For the concentrations of Fe and Cd, samples from Butterworth was significantly highest ($P<0.05$). For Zn, samples collected from PPauh and Butterwoth showed significantly higher ($P<0.05$) concentrations. Samples from Seremban and PPauh were observed to be significantly higher ($P<0.05$) for Ni concentrations in roots. Lastly, samples from Butterworth, Kapar and Pontian showed significant ($P<0.05$) difference in Pb concentrations.

For soil samples, the percentages of the resistant and non-resistant fractions are presented in Tables 4, 5, 6, 7, 8, and 9. For Cd, only two sampling sites showed more than 40% in the non-resistant fraction

TABLE 2
Concentrations (mean ± SD, µg/g dry weight) of heavy metals in leaves of *Centella asiatica* collected from 9 sampling sites from Peninsular Malaysia. (N=9)

Sampling sites	Cd				Cu				Fe				Ni				Pb				Zn			
PPauh	1.31	±	0.24	b	11.78	±	0.24	a,b	334.45	±	6.12	c,d	11.86	±	2.42	a	21.07	±	3.04	b	315.6	±	4.02	a
Karangan	1.04	±	0.08	c	6.75	±	1.24	d	291.12	±	13.36	c,d	4.24	±	0.58	d,e	13.44	±	2.27	c	172.24	±	25.01	b,c
Kluang	0.91	±	0.28	c,d	9.33	±	0.38	b,c	67.44	±	3.54	f	6.01	±	0.12	c,d	8.68	±	0.80	d	145.15	±	28.15	d,e,f
Butterworth	2.24	±	0.57	a	13.49	±	0.92	a	1244.8	±	144.95	a	9.33	±	1.45	a,b	46.64	±	7.60	a	336.75	±	24.02	a
UPM	0.88	±	0.42	c,d	11.96	±	2.65	a,b	367.36	±	29.76	c,d	2.59	±	0.84	f	7.46	±	0.76	d	144.59	±	12.53	d,e,f
Kapar	0.16	±	0.08	e	8.93	±	2.41	c	257.2	±	106.64	d	2.91	±	0.47	e,f	50.85	±	11.66	a	121.35	±	0.44	f
Seremban	1.87	±	0.66	a,b	13.28	±	1.25	a	887.12	±	138.2	a	11.87	±	0.48	a	42.45	±	5.19	a	182.19	±	10.11	b
KSR	1.25	±	0.24	b,c	5.36	±	0.64	e	470.8	±	17.12	c	6.13	±	0.49	c,d	26.52	±	9.43	b	160.30	±	2.48	b,c,d
Pontian	1.23	±	0.04	b,c	13.28	±	1.25	a	719.76	±	131.37	b	4.64	±	0.98	d	43.12	±	5.75	a	182.40	±	7.80	b

a,b,c : different alphabet in each column shows the different of significant mean (SNK test, P<0.05)

TABLE 3
Concentrations (mean ± SD, µg/g dry weight) of heavy metals in roots of *Centella asiatica* collected from 9 sampling sites from Peninsular Malaysia. (N=9)

Sampling sites	Cd				Cu				Fe				Ni				Pb				Zn			
PPauh	2.00	±	0.45	b	14.08	±	1.06	a	388.88	±	4.12	b	14.20	±	0.08	a	31.71	±	1.77	b	335.92	±	19.07	a
Karangan	1.36	±	0.28	b,c	7.92	±	0.23	b	311.00	±	12.05	b,c	4.80	±	0.22	b,c,d	15.24	±	0.40	c	207.52	±	11.00	b
Kluang	1.17	±	0.26	a,b	12.32	±	2.27	a,b	220.40	±	23.17	c	7.60	±	0.74	a,b,c,d	9.60	±	4.11	d	184.91	±	2.82	b,c
Butterworth	4.00	±	0.24	a	17.36	±	1.58	a	2008.8	±	13.22	a	10.77	±	2.45	a,b	63.52	±	5.88	d	349.20	±	10.99	a
UPM	1.52	±	0.11	b,c	15.76	±	3.00	a	482.16	±	9.05	b	2.99	±	1.70	d	10.91	±	0.40	d	207.76	±	2.26	b
Kapar	0.20	±	0.06	d	13.36	±	1.13	a,b	400.51	±	25.24	b,c	4.27	±	0.53	c,d	64.56	±	6.00	a	134.32	±	2.91	c,d
Seremban	2.00	±	0.06	b	16.00	±	2.54	a	1182.96	±	10.19	a,b	15.28	±	1.24	a	50.00	±	5.81	a,b	229.80	±	0.06	b
KSR	1.86	±	0.28	b	6.00	±	0.64	b	541.16	±	109.46	b	7.20	±	0.23	a,b,c,d	32.80	±	5.08	b	170.92	±	2.72	b,c
Pontian	1.58	±	0.24	b,c	16.00	±	2.54	a	1379.47	±	169.48	a,b	5.60	±	0.22	b,c,d	59.39	±	5.77	a	213.80	±	35.02	b

a,b,c : different alphabet in each column shows the different of significant mean (SNK test, P<0.05)

TABLE 4
Concentrations (mean ± SD, µg/g dry weight) of Cd in geochemical fractions of sediments collected from 9 sampling sites from Peninsular Malaysia. (N=3)

Sampling sites	EFLE	Acid-reducible	Oxidisable-organic	Resistant	Total (100%)	Non-resistant (%)	Resistant (%)
PPauh	0.074 ± 0.033	0.212 ± 0.018	0.210 ± 0.001	1.160 ± 0.013	1.656	29.95	70.05
Karangan	0.119 ± 0.030	0.088 ± 0.021	0.167 ± 0.026	1.660 ± 0.011	2.034	18.39	81.61
Kluang	0.077 ± 0.040	0.094 ± 0.000	0.038 ± 0.013	1.480 ± 0.020	1.689	12.37	87.63
Butterworth	0.221 ± 0.005	0.697 ± 0.010	0.567 ± 0.031	2.300 ± 0.004	3.885	40.80	59.20
UPM	0.044 ± 0.023	0.090 ± 0.015	0.285 ± 0.027	1.627 ± 0.029	2.046	20.50	79.51
Kapar	0.007 ± 0.005	0.096 ± 0.004	0.035 ± 0.018	1.093 ± 0.032	1.231	11.18	88.82
Seremban	0.116 ± 0.054	0.778 ± 0.025	0.410 ± 0.010	1.720 ± 0.047	3.024	43.12	56.88
Pontian	0.119 ± 0.054	0.225 ± 0.026	0.252 ± 0.016	1.720 ± 0.031	2.316	25.73	74.27
KSR	0.147 ± 0.008	0.300 ± 0.021	0.242 ± 0.022	1.004 ± 0.019	1.693	40.70	59.30

TABLE 5
Concentrations (mean ± SD, µg/g dry weight) of Cu in geochemical fractions of sediments collected from 9 sampling sites from Peninsular Malaysia. (N=3)

Sampling sites	EFLE	Acid-reducible	Oxidisable-organic	Resistant	Total (100%)	Non-resistant (%)	Resistant (%)
PPauh	0.980 ± 0.022	0.154 ± 0.015	34.497 ± 0.165	38.320 ± 0.128	73.951	48.18	51.82
Karangan	1.022 ± 0.010	0.085 ± 0.027	6.254 ± 0.005	10.020 ± 0.057	17.381	42.35	57.65
Kluang	0.721 ± 0.049	0.400 ± 0.005	8.079 ± 1.085	11.740 ± 0.031	20.940	43.94	56.07
Butterworth	7.063 ± 0.084	1.123 ± 0.020	82.343 ± 6.058	100.780 ± 0.208	191.309	47.32	52.68
UPM	0.467 ± 0.015	0.109 ± 0.015	20.046 ± 2.624	33.947 ± 0.229	54.569	37.79	62.21
Kapar	0.686 ± 0.040	0.214 ± 0.023	8.222 ± 0.629	13.400 ± 0.044	22.522	40.50	59.50
Seremban	2.062 ± 0.129	0.961 ± 0.321	61.016 ± 3.144	65.200 ± 0.013	129.239	49.55	50.45
Pontian	3.980 ± 0.084	0.785 ± 0.057	54.779 ± 4.833	90.740 ± 0.406	150.284	39.62	60.38
KSR	0.707 ± 0.084	0.435 ± 0.090	15.162 ± 2.407	27.408 ± 0.190	43.712	37.30	62.70

TABLE 6
Concentrations (mean ± SD, µg/g dry weight) of Fe in geochemical fractions of sediments collected from 9 sampling sites from Peninsular Malaysia. (N=3)

Sampling sites	EFLE	Acid-reducible	Oxidisable-organic	Resistant	Total (100%)	Non-resistant (%)	Resistant (%)
PPauh	259.70 ± 1.91	856.37 ± 81.78	2253.89 ± 10.34	16080.00 ± 1689.38	19449.96	17.33	82.67
Karangan	1405.37 ± 2.47	2675.32 ± 191.47	1475.78 ± 89.94	10038.00 ± 1258.65	15594.46	35.63	64.37
Kluang	569.80 ± 48.51	1939.38 ± 418.03	1008.58 ± 116.01	9662.00 ± 591.14	13179.77	26.69	73.31
Butterworth	518.70 ± 47.52	896.37 ± 86.55	3917.32 ± 223.69	22940.00 ± 3116.93	28272.39	18.86	81.14
UPM	142.80 ± 14.05	416.33 ± 181.44	685.01 ± 50.31	23880.00 ± 3628.11	25124.14	4.95	95.05
Kapar	182.35 ± 15.84	3904.26 ± 525.75	2000.44 ± 29.41	15905.33 ± 3470.10	21992.39	27.68	72.32
Seremban	248.50 ± 22.77	605.73 ± 430.85	3782.67 ± 235.05	24078.00 ± 3193.29	28714.89	16.15	83.85
Pontian	717.15 ± 70.78	2112.72 ± 67.79	2138.61 ± 98.94	15640.00 ± 2319.31	20608.48	24.11	75.89
KSR	480.90 ± 127.76	831.25 ± 0.58	2015.21 ± 5.47	16120.00 ± 2513.26	19447.36	17.11	82.89

TABLE 7
Concentrations (mean ± SD, µg/g dry weight) of Ni in geochemical fractions of sediments collected from 9 sampling sites from Peninsular Malaysia. (N=3)

Sampling sites	EFLE	Acid-reducible	Oxidisable-organic	Resistant	Total (100%)	Non-resistant (%)	Resistant (%)
PPauh	0.700 ± 0.026	0.710 ± 0.046	3.339 ± 0.010	5.800 ± 0.068	10.549	45.02	54.98
Karangan	0.767 ± 0.114	0.417 ± 0.041	1.250 ± 0.099	4.560 ± 0.115	6.994	34.80	65.20
Kluang	0.760 ± 0.045	0.663 ± 0.041	2.325 ± 0.151	7.440 ± 0.088	11.188	33.50	66.50
Butterworth	1.208 ± 0.084	0.662 ± 0.102	4.067 ± 0.325	8.120 ± 0.042	14.057	42.24	57.77
UPM	0.728 ± 0.068	0.315 ± 0.202	1.713 ± 0.143	4.293 ± 0.092	7.049	39.10	60.90
Kapar	0.350 ± 0.129	0.243 ± 0.084	0.354 ± 0.116	3.213 ± 0.015	4.160	22.76	77.24
Seremban	0.795 ± 0.173	0.774 ± 0.385	5.239 ± 0.196	8.600 ± 0.087	15.408	44.19	55.82
Pontian	0.441 ± 0.059	0.254 ± 0.141	2.610 ± 0.141	8.640 ± 0.008	11.945	27.67	72.33
KSR	0.707 ± 0.074	0.315 ± 0.037	3.008 ± 0.295	7.744 ± 0.010	11.774	34.23	65.77

TABLE 8
Concentrations (mean ± SD, µg/g dry weight) of Pb in geochemical fractions of sediments collected from 9 sampling sites from Peninsular Malaysia. (N=3)

Sampling sites	EFLE	Acid-reducible	Oxidisable-organic	Resistant	Total (100%)	Non-resistant (%)	Resistant (%)
PPauh	2.114 ± 0.008	1.442 ± 0.740	17.640 ± 0.165	84.440 ± 0.128	105.636	20.07	79.94
Karangan	2.664 ± 0.342	8.383 ± 0.269	16.345 ± 0.368	52.060 ± 0.179	79.452	34.48	65.52
Kluang	2.282 ± 0.584	1.424 ± 0.617	3.988 ± 0.876	21.000 ± 0.068	28.694	26.81	73.19
Butterworth	3.269 ± 0.178	2.056 ± 0.799	29.310 ± 0.182	125.820 ± 0.547	160.455	21.59	78.42
UPM	2.996 ± 0.303	2.229 ± 0.139	8.844 ± 0.807	55.133 ± 1.463	69.202	20.33	79.67
Kapar	3.617 ± 0.624	2.958 ± 0.159	10.704 ± 0.839	46.533 ± 0.948	63.812	27.08	72.92
Seremban	4.407 ± 0.351	5.785 ± 1.211	34.818 ± 0.869	132.020 ± 0.010	177.030	25.43	74.58
Pontian	4.032 ± 0.752	7.098 ± 0.391	41.857 ± 2.600	147.980 ± 0.267	200.967	26.37	73.63
KSR	2.625 ± 0.467	1.428 ± 0.026	34.453 ± 1.286	92.160 ± 0.177	130.666	29.47	70.53

TABLE 9
Concentrations (mean ± SD, µg/g dry weight) of Zn in geochemical fractions of sediments collected from 9 sampling sites from Peninsular Malaysia. (N=3)

Sampling sites	EFLE	Acid-reducible	Oxidisable-organic	Resistant	Total (100%)	Non-resistant (%)	Resistant (%)
PPauh	8.449 ± 0.018	39.283 ± 0.550	63.752 ± 0.204	112.040 ± 0.188	223.524	49.88	50.12
Karangan	1.995 ± 0.045	3.136 ± 0.398	12.893 ± 0.104	31.860 ± 0.309	49.884	36.13	63.87
Kluang	2.433 ± 0.064	4.919 ± 2.034	10.813 ± 0.790	24.420 ± 1.246	42.585	42.66	57.34
Butterworth	14.732 ± 0.025	52.364 ± 0.295	69.284 ± 1.042	171.220 ± 1.140	307.600	44.34	55.66
UPM	0.443 ± 0.091	5.127 ± 0.375	36.286 ± 2.244	46.613 ± 0.343	88.469	47.31	52.69
Kapar	1.643 ± 0.163	4.358 ± 1.249	5.869 ± 0.825	21.253 ± 0.269	33.123	35.84	64.16
Seremban	3.171 ± 0.079	67.067 ± 0.162	31.624 ± 0.396	107.920 ± 0.448	209.782	48.56	51.44
Pontian	2.149 ± 0.049	25.382 ± 2.138	53.061 ± 1.433	131.260 ± 0.421	211.852	38.04	61.96
KSR	1.932 ± 0.178	22.299 ± 3.446	31.506 ± 0.557	77.076 ± 0.373	132.813	41.97	58.03

TABLE 10
Overall mean for aqua-regia, SET (mean, µg/g dry weight) and the percentage of similarity of soils collected from 9 sampling sites in Peninsular Malaysia. (N=3)

Sampling sites	Cd			Cu			Fe			Ni			Pb			Zn		
	Aqua	SET	%	Aqua	SET	%	Aqua	SET	%	Aqua	SET	%	Aqua	SET	%	Aqua	SET	%
PPauh	1.63	1.66	101.60	62.24	73.95	118.82	21634	19450	89.90	11.72	10.55	90.01	90.97	105.64	116.12	203.43	223.52	109.88
Karangan	1.68	2.03	121.07	24.98	17.38	69.58	13713	15594	113.72	5.37	6.99	130.24	66.18	79.45	120.05	40.50	49.88	123.17
Kluang	1.40	1.69	120.64	25.09	20.94	83.46	12196	13180	108.07	9.03	11.19	123.90	24.72	28.69	116.08	43.68	42.59	97.49
Butterworth	3.72	3.89	104.44	146.99	191.31	130.15	27917	28272	101.27	12.97	14.06	108.38	125.67	160.46	127.68	237.11	307.60	129.73
UPM	1.67	2.05	122.50	50.24	54.57	108.62	25907	25124	96.98	6.95	7.05	101.43	53.73	69.20	128.80	51.92	88.47	170.40
Kapar	1.21	1.23	101.76	22.24	22.52	101.27	23424	21992	93.89	4.01	4.16	103.75	53.64	63.81	118.96	26.12	33.12	126.81
Seremban	2.64	3.02	114.55	102.03	129.24	126.67	27424	28715	104.71	13.49	15.41	114.22	170.43	177.03	103.87	232.10	209.78	90.38
Pontian	1.69	2.32	137.04	120.70	150.28	124.48	24694	20608	83.46	9.27	11.95	128.86	179.03	200.97	112.25	188.79	211.85	112.22
KSR	1.66	1.69	101.99	39.07	43.71	111.88	21832	19447	89.08	10.48	11.77	112.35	117.40	130.67	111.30	150.44	132.81	88.28

namely Butterworth (40.80%) and Seremban (43.12%). Soil samples collected from Seremban, PPauh and Butterworth showed 49.55%, 48.18% and 47.32%, respectively, of Cu in the non-resistant fraction while having 44.19%, 45.02% and 42.24% of Ni there. For Fe and Pb, all soil samples showed less than 40% in the non-resistant fraction. Lastly, soil samples from PPauh and Butterworth showed 49.88% and 44.34% respectively of Zn in the non-resistant fraction.

The heavy metal concentrations in the soils based on the aqua-regia method analysis are presented in Table 10. Generally samples from PPauh, Seremban, Butterworth and Pontian showed higher metals concentration. The Cu levels were higher in the samples from Butterworth, Pontian and Seremban. For Cd and Fe, samples from Seremban and Butterworth showed higher concentrations. Samples collected from Butterworth, Seremban and PPauh had higher levels of Zn and Ni. Samples from Seremban and Pontian were higher in Pb in soils.

Fig.5 shows the levels of antioxidative enzymes in leaves; SOD was present at significant levels ($P<0.05$) in the samples from Seremban and Butterworth samples. In the samples collected from Butterworth and PPauh there were observations of significant ($P<0.05$) activities for GPX. CAT and APX did not show any significant value for all metal concentrations in leaves. For antioxidative levels in roots (Fig.6), samples fromSeremban, Butterworth and PPauh showed significant ($P<0.05$) activities for

GPX. APX showed similar results for the samples collected from Butterworth and PPauh. CAT and SOD did not show any significant result for all metal concentrations in roots.

DISCUSSION

Relationships of metals between roots and soils

In general, sampling sites at Butterwoth, Seremban and PPauh showed higher levels of both metals and antioxidative enzymes in plants and soils. Based on the correlations of metals between the soils and different parts of *C. asiatica* (Table 11), the correlations between roots and soils for Cd, Zn, Ni and Fe were high with R=0.855, R=0.827, R=0.888 and R=0.857, respectively. This showed that when the concentrations of Cd, Zn, Ni and Fe were higher in soils, the levels of metal in the roots would also subsequently be higher. This result is supported by Ratko *et al*. (2011) who showed statistically significant correlations between contents of heavy metals (Zn, Cu, Pb and Cd) in soil and plants. Metals in soils were easily taken up by roots and translocated to different parts of the plant such as leaves because ~~only~~ roots are the only organ that is covered in soil all the time when compared to the other parts of the plant. Besides that, root is a good storage area due to the abundance of root hairs present which increased its surface area for adsorption and absorption (Yap *et al*., 2010; Street *et al*., 2009).

Of the metals mentioned above, Fe showed the highest uptake in roots namely 220.40-2008.80 µg/g. This was due to its ability to form octahedral complexes with various ligands and its redox potential in response to different ligand environments (Hell and Stephan, 2003). The phytotoxicities of the trace metals followed the following trend (from the most to the least toxic): Pb >Cu >Cd >≈Ni≈Zn (Kopittke *et al*., 2009). Hence, the results showed higher uptake of Zn (121.35-336.75µg/g) in roots compared to Cd and Cu with each having a total of 0.2-4.0 µg/g and 6.0-17.36 µg/g, respectively, because roots tended to reduce the uptake of heavy metals that were more toxic. González-Miqueo *et al*. (2010) found that *Hypnum cupressiforme* was able to uptake Zn (277 µg/g) Pb (56.6 µg/g), Ni (26.2 µg/g), Cu (21.7 µg/g) and Cd (0.49 µg/g) in Azkoitia, Spain. These results supported our current findings with a similar trend of heavy metal concentrations in *C. asiatica*.

Overall, metal concentrations were highest in roots followed by leaves because plants developed a mechanism which caused immobilization of certain metals when they were bound to their cell walls (Yap *et al*., 2011). This prevents the metal from being further uptaken by the roots and also inhibits the metal translocation to the shoot. The metals that were accumulated in the roots or were unable to enter the plant were kept in the root cells where they would be detoxified by forming complexes with amino acids, organic acids or metal-binding peptides or sequestered into vacuoles (Hall, 2002). This action greatly restricts the translocation of the metals to the above-ground organs. Moreover, it can protect the leaf tissues and the metabolically active photosynthetic cells

from heavy metal damage (Navari-Izzo *et al*., 1998; Sgherri *et al*., 2003).

Relationship between the levels of metals in leaves and soils

For correlations between leaves and soils, all metals showed significant correlations except Pb, with Cu (R=0.720), Cd (R=0.867), Zn (R=0.784), Ni (R=0.903) and Fe (R=0.899). The results showed that when the concentrations of Cu, Cd, Zn, Ni and Fe were higher in soils, the levels of metal concentrations in leaves would subsequently also be higher. There was no further discussion of Fe concentrations in leaves due to the naturally high concentrations of Fe in soils when compared to the other metals and it rarely causes any toxic effects to plants (Ong *et al*., 2011). According to Chojnacka *et al*. (2005), the metal transfer was decreased in the order of Zn > Cu> Ni > Pb and Cd which showed that higher concentrations of Zn ,Cu and Ni were transferred from soils to plants compared to Pb and Cd.

The strategy of uptake of metals in plants depends on some physiological processes which require the cell to conserve the intracellular heavy metal ions in a non toxic form (Cobbet, 2000). Pb is considered toxic to plants; therefore plants will have a series of mechanisms to reduce the entry of Pb. Besides that, Pb is a non-essential metal for plants; thus the uptake of Pb by plant is unfavorable (Mehra and Tripath, 2000). There is a correlation between Cd concentration in the soils and leaves even though Cd is a non-essential metal for plant. This happens when the translocation of other ions from roots to shoots causes some trace Cd to be translocated along. The net amount of Cd translocated was in the range of 0.16-2.24 µg/g in leaves. Furthermore, Cd is a very mobile element in the environment and plants therefore can easily uptake and transfer it to their other organs (Vaněk *et al*., 2004).

Relationship between metal concentrations and antioxidative enzymes

As is shown in Table 12, the correlations between Zn in leaves and the levels of the antioxidative enzymes (CAT, GPX and APX) were significant with R=0.732, R=0.738 and R=0.710, respectively, but SOD did not show any significant correlation. In roots, there were similar significant ($P< 0.05$) correlations between Zn concentration and CAT (R=0.856), GPX (R=0.726) and APX (R=0.794) activities while SOD activity remained insignificant ($P> 0.05$). These results were supported by those of Candan and Tarhan (2003) who reported that GPX and APX activities increased in the presence of Zn^{2+} from roots to leaves. For Ni, the correlations were GPX (R=0.796) and APX (R=0.719) for leaves and CAT (R=0.838), GPX (R=0.734) and APX (R=0.696) for roots. The main response of plants towards increases in the levels of Zn and Ni was to generate SOD to accelerate the dismutation of O_2^- to H_2O_2. For each of these reactions, two molecules of H_2O_2 were produced from one O_2^- causing high concentration of it in the plant. Thus, higher activities of CAT,

TABLE 11
The correlation coefficients between different parts of *Centella asiatica* (Log_{10}mean+1) and aqua-regia concentrations (Cd, Cu, Fe, Ni, Pb and Zn) (N=9).

	Cu	Cd	Zn	Ni	Pb	Fe
Leaves-sediment						
Cu	**0.720**	0.474	0.653	0.528	0.273	0.411
Cd	**0.785**	**0.867**	**0.800**	**0.801**	0.651	0.606
Zn	**0.669**	**0.677**	**0.784**	**0.695**	0.464	0.413
Ni	0.539	0.595	**0.799**	**0.903**	0.397	0.334
Pb	0.443	0.374	0.240	0.242	0.573	0.519
Fe	**0.816**	**0.756**	0.534	0.339	**0.885**	**0.899**
Roots- sediment						
Cu	0.574	0.368	0.495	0.390	0.099	0.295
Cd	**0.767**	**0.855**	**0.779**	**0.726**	0.557	0.616
Zn	**0.712**	**0.704**	**0.827**	**0.710**	0.435	0.405
Ni	0.496	0.548	**0.762**	**0.888**	0.351	0.301
Pb	0.506	0.380	0.305	0.257	0.593	0.577
Fe	**0.886**	**0.794**	0.590	0.488	**0.783**	**0.857**

Note: Bold is significant at the level P< 0.05(two-tailed)

TABLE 12
The correlation coefficients between different parts of *Centella asiatica* (Log_{10}X+1) based on the antioxidant level (SOD, CAT, GPX and APX) (N=9).

	SOD	CAT	GPX	APX
Leave-Antioxidant				
Cu	**0.922**	**0.723**	**0.694**	**0.797**
Cd	0.524	0.451	0.576	0.640
Zn	0.566	**0.732**	**0.738**	**0.710**
Ni	0.554	0.665	**0.796**	**0.719**
Pb	0.425	0.384	0.636	0.543
Fe	0.520	0.279	0.553	0.617
Root-Antioxidant				
Cu	**0.811**	0.561	**0.714**	**0.758**
Cd	0.341	0.640	0.459	0.497
Zn	0.592	**0.856**	**0.726**	**0.794**
Ni	0.616	**0.838**	**0.734**	**0.696**
Pb	0.416	0.399	0.579	0.459
Fe	0.491	0.509	0.594	0.522

Note: Bold is significant at the level P< 0.05(two-tailed)

APX and GPX were required to overcome the mass production of H_2O_2.

Finally, for Cd, Pb and Fe, there were no significant correlations in leaves and roots with the antioxidative enzymes (Table 12). In fact, plants with enhanced activities of antioxidative enzymes had been shown to be tolerant to oxidative stress (Mittler *et al*., 2004). This was due to the uptake and translocation of the metals. Cd and Fe did not show any significant correlation in antioxidative activities because their concentrations in leaves and roots were considered low being 0.16-2.24 µg/g and 0.20-4.0 µg/g in leaves and roots for Cd and only 67.44-1244.95 µg/g (leaves) and 220.40-2008.20 µg/g (roots) for Fe. The levels of both the metals were too low to activate any obvious antioxidative activity. In addition, Cd ions were unable to catalyze the Fenton-Haber-Weiss reaction (Cho and Seo, 2005) which generate ROS. Even though Fe concentration in leaves was high for particular sites, but it did not play a role in causing a high level of SOD. This was supported by the data in Table 12, showed no significant correlation between Fe concentration and SOD. This was due to the soils naturally containing high levels of Fe. For Pb, its translocation in plants was limited and normally it was bounded to leaf surfaces and roots. This was supported by data presented Table 11 where there were no significant correlations between Pb in soils and Pb in leaves or roots. Besides that, data from most experimental studies on Pb toxicity showed that high Pb concentrations in the range of 100 to 1,000 mg/kg soil were needed to cause visible toxic effects on photosynthesis, growth, or other parameters (WHO, 1989; 1995). Thus, Pb can only affect plants in sites with very high environmental concentrations of it.

Usually physiological disorders and metabolic abnormalities in plants were caused by ROS production during normal metabolism when exposed to stresses (Marschner, 1995; Singh, 2007). When free radical production is excessive, or when the antioxidative system is insufficient to overcome ROS, it might damage the plant. Decrease of enzymatic and non-enzymatic free radical scavengers, caused by heavy metal toxicities (De Vos *et al*., 1993), might also contribute to a shift in the balance of free-radical metabolism towards H_2O_2 accumulation.

Hence, an increase in antioxidative enzymes can be expected so as to reduce the oxidative stress caused by heavy metals. Our results showed the differences in antioxidative enzyme activities for different metals in the leaves and roots of *C. asiatica*. Data in Table 12 show the correlations between metals levels in leaves and antioxidative levels. Cu showed significant correlations with all the enzymesnamely SOD (R=0.922), CAT (R=0.723), GPX (R=0.694) and APX (R=0.797). For correlations between metals levels in roots and antioxidative levels, Cu also showed high correlation for SOD (R=0.811), GPX (R=0.714) and APX (R=0.758). This shows that uptake of Cu triggers antioxidative enzyme activities in leaves and roots due to the sensitivity of plants

towards Cu toxicity. These was supported by the results of Candan and Tarhan (2003) who found that all antioxidative enzyme activities correlated positively with increasing Cu^{2+} concentrations in all *M. pulegium* organs**.**

In addition, high levels of Cu in plants might lead to metabolic disturbances and growth inhibition; even in quantities slightly higher than the normal level (Fernandes and Henriques, 1991). These excessive concentrations will cause oxidative stress which in turn increases the reactive oxygen species (ROS) within the subcellular compartments (Mittler *et al*., 2004). As a component of the plant's defense mechanism towards metal uptake, SOD was activated as long as the stress was not too strong for the plant's defense capacity (Siedlecka and Krupa, 2002). SOD is the most effective intracellular enzymatic antioxidative which dismutates O_2^- to H_2O_2 (Sarvajeet and Narendra, 2010). In soil, the usual Cu consists of between 2 and 250ppm whereas healthy plant tissues contain Cu in the range of 20–30µg/g dry weight.

Similarities and differences of metals and antioxidative enzymes

From Fig.2, Cd in all parts of the plant from all the sampling sites except Kapar were shown to be in the same cluster which indicated that all the sampling sites except Kapar accumulated a similar amount of Cd while Kapar accumulated the least amount. For Cu, all the sampling sites were grouped into the same cluster except for Karangan and KSR due to lesser amounts of Cu being accumulated at both these sites. Kluang was grouped in a different cluster compared to the other sampling sites which showed that only Kluang accumulated the least amount of Fe. UPM and Kapar accumulated the least amount of Ni compared to the other sampling sites; therefore they were grouped in the same cluster compared to the others. For Pb, Kluang and UPM were grouped in the same cluster compared to the other sampling sites due to their similarity in Pb concentration in plants. Zn concentrations for all the sampling sites were considered similar except for PPauh and Butterworth.

Fig.3 shows the hierarchical cluster analysis for metals concentrations in soils (aqua-regia and SET). Karangan, Kapar and Kluang which were the least contaminated group were in the same cluster for Cu and Fe when compared to the other sampling sites. For Cd, only Butterworth was grouped in a different cluster due to its high concentration in the soil. UPM was grouped in a different cluster for Fe while Kluang formed its own cluster for Pb. This showed that both sites accumulated less amounts of Fe and Pb respectively in the soils. For Ni, UPM and Kapar were considered as having low concentrations because they were grouped in the same cluster when compared to the other sampling sites.

Based on the geochemical fraction of soils, more than 50% of heavy metals (Cu, Cd, Ni, Fe, Pb and Zn) were accumulated in the 'resistant' fraction. This showed that the mobility of these metals were quite low because the soils from the sampling sites consisted of a higher percentage of

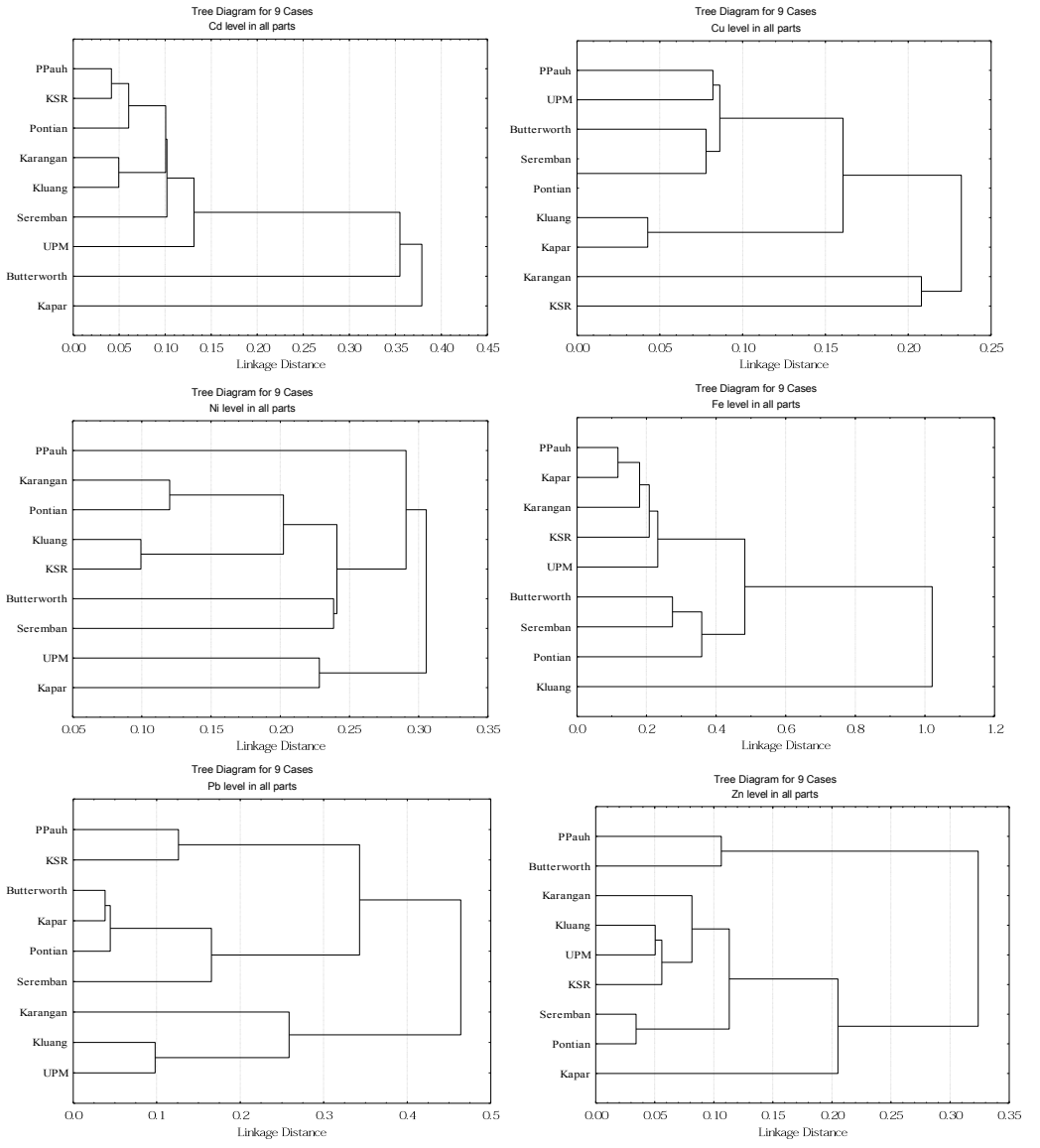

Fig.2: Hierarchical cluster analysis of *Centella asiatica* based on heavy metals (Cd, Cu, Fe, Ni, Pb and Zn) concentrations (Log_{10}mean+1) in all parts (leaves and roots) for all 9 sampling sites.

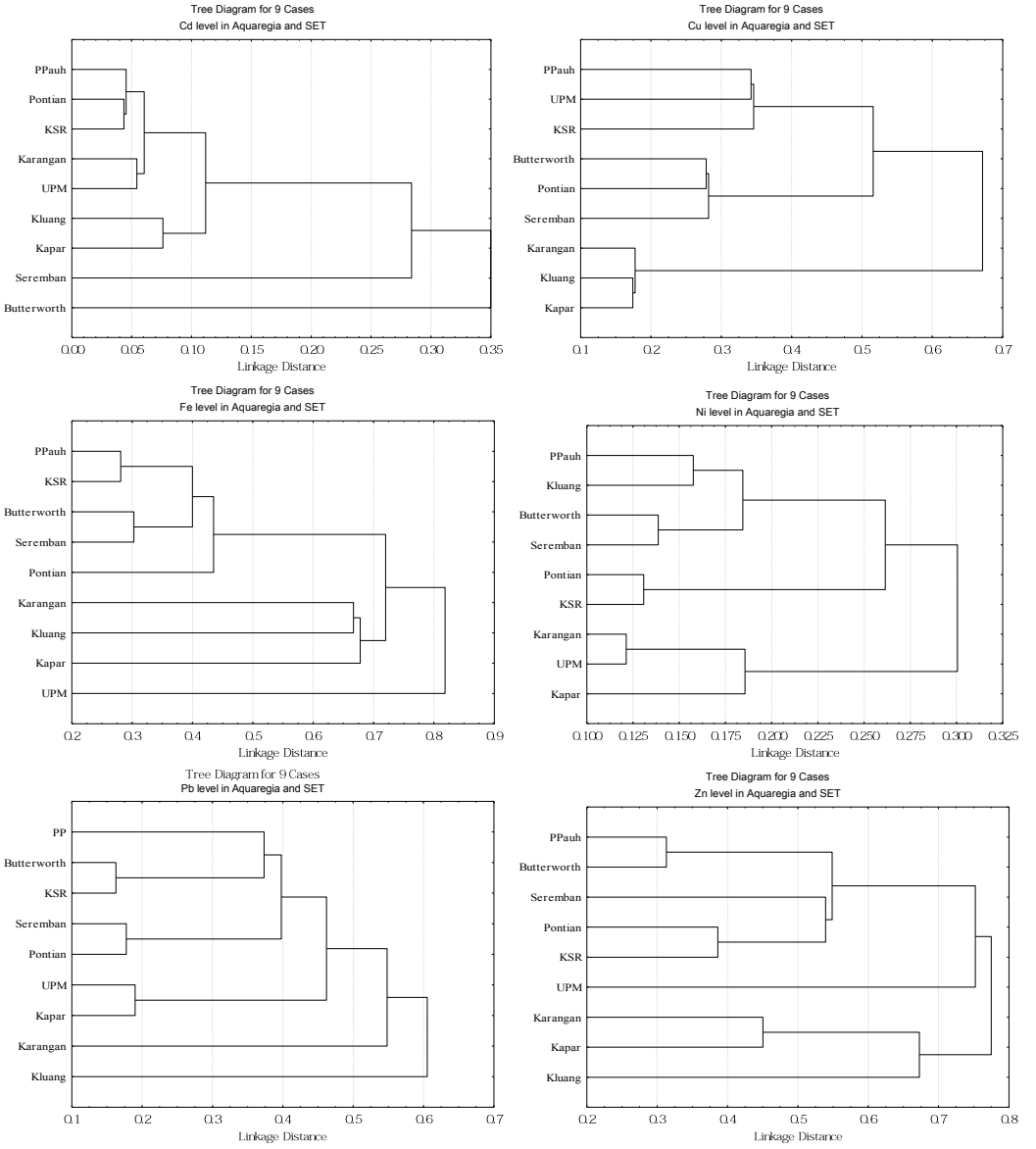

Fig.3: Hierarchical cluster analysis of *Centella asiatica* based on heavy metal concentrations (Log_{10}mean+1) in soils (Aqua-regia and SET) for all 9 sampling sites.

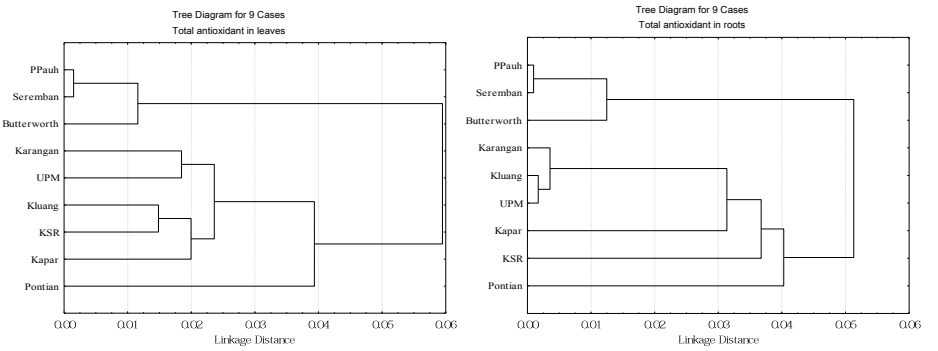

Fig.4: Hierarchical cluster analysis of *Centella asiatica* based on antioxidant enzymes (SOD, CAT, GPX and APX) in leaves and roots (Log_{10}mean+1) for all 9 sampling sites.

non-resistant fraction whereas Butterworth, Seremban, and PPauh were sampling sites suggestive of having more anthropogenic heavy metal inputs other than resistant soils. It was shown that the similarities and differences in the concentrations of metals within plants depended on the concentrations of the metals in the soil when that correlation and cluster analysis were conducted (Miranda *et al*., 1996; Diaz *et al*., 2002; Yongming *et al*., 2006).

It was observed that the uptake of metals into the roots and the translocation of metals to the shoots were highly proportional to the concentration of metals in the soil. In this study Cu did not show any significant correlation between roots and metal concentrations in the soil because generally Cu levels in soils were considered low. The uptake of Cu by osmosis was restricted when competing with other metals. Moreover, it had been postulated (Hill and Matrone, 1970) that elements with similar properties will act antagonistically to one another biologically, as a result of their competition for binding sites on proteins that require metals as cofactors.

For the cluster analysis of the total antioxidative enzymes in leaves and roots (Fig.4), Butterwoth, Seremban and PPauh were shown to be in the same cluster while the other sampling sites were in a different cluster. For the comparison of sites with heavy metal concentrations, Butterworth, Seremban and PPauh were shown to be the sites with the highest metal contamination. As a result of this, a high level of ROS was produced. This would rapidly attack all types of biomolecules such as nucleic acids, proteins, lipids and amino acids (De Vos and Schat, 1991; Mehta *et al*., 1992; Luna *et al*., 1994). Therefore, increased activity of the antioxidative system was required to protect plants from the harmful ROS (Foyer *et al*., 2009; Parra-Lobato *et al*., 2009). The synchronous actions of SOD, CAT, POD and APX were activated to work against ROS in various compartments of the plant cell (Singh *et al*., 2006).

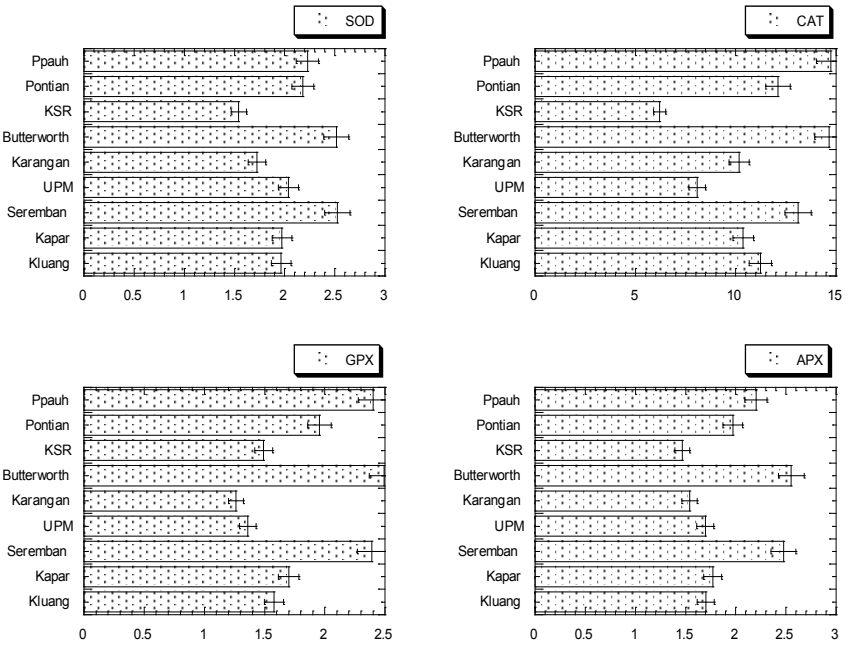

Fig.5: Concentrations (mean ± SD, nmol/mg/g) of antioxidant enzymes (SOD, CAT, GPX and APX) in leaves of *Centella asiatica* collected from nine sampling sites in Peninsular Malaysia. Note: For the activities of SOD, CAT and APX, their actual values are multiplied with 1000.

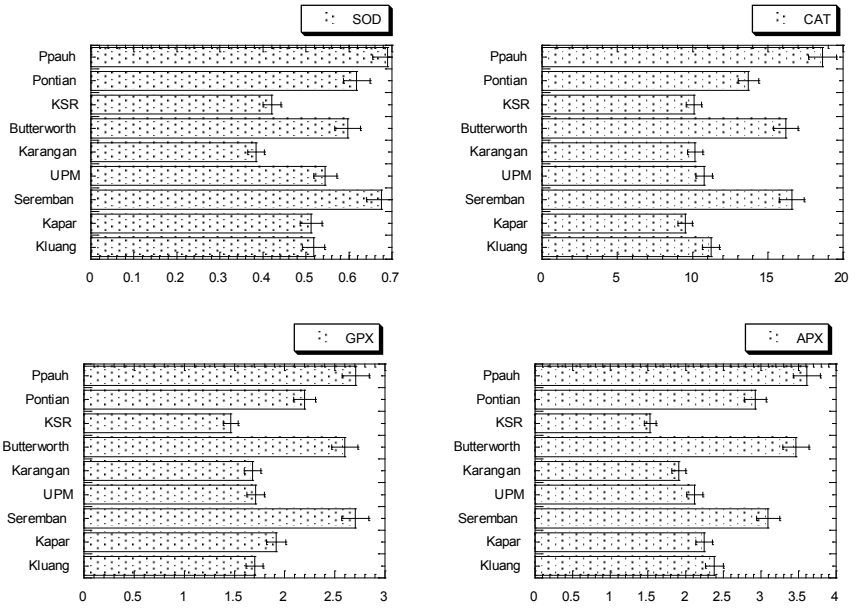

Fig.6: Concentrations (mean ± SD, nmol/mg/g) of antioxidant enzymes (SOD, CAT, GPX and APX) in roots of *Centella asiatica* collected from nine sampling sites in Peninsular Malaysia. Note: For the activities of SOD, CAT and APX, their actual values are multiplied with 1000.

CONCLUSION

The uptake of heavy metals into plant by roots and their translocations to leaves will induce stress in *Centella asiatica*. The main response towards the uptake of heavy metals and their concentrations was an increase in antioxidative activity to counteract the ROS production. Uptake of Cd, Fe and Pb did not seem to be a factor for the increase of antioxidative enzymes due to their low concentrations in plants. But Cu, Zn and Ni which are essential metals, showed obvious increases in the antioxidative activities of SOD, CAT, APX and GPX due to the higher concentrations of these metals. Thus, monitoring the concentration of these metals was essential to ensure the survival and well being of *C. asiatica*. Overall, antioxidative activities were significant in samples from Seremban, Butterworth and P.Pauh due to higher movement of heavy metals from the soils into *C. asiatica.*

ACKNOWLEDGEMENTS

The authors wish to acknowledge the financial support provided through the Research University Grant Scheme (RUGS) [Vote no. 9322400] by Universiti Putra Malaysia.

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