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CENTELLA ASIATICA: A POTENTIAL CANDIDATE TO ASSESS THE URANIUM CONTAMINATION IN SOIL

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Abstract: Increase of Uranium (U) in the environment has risen the public concern, especially in medicinal plants. Therefore the aim of this study was to determine the potential of *Centella asiatica* (L.) Urban as a good bio indicator of U pollution based on the correlation of U levels between plant and soil and experimental transplantation study. The U in soils and *C. asiatica* was determined by Neutron Activation Analysis (NAA). TheU levels in soils from 12 sampling sites ranged from 1.97µg/g to 10.71µg/g dw. The Enrichment factor ranged from 0.79 to 18.25 and the Igeo values ranged from -1.04 to 2.07. For all sampling sites, the roots (0.98-5.60µg/g dw) showed the highest U accumulation followed by leaves (0.41-1.91µg/g dw) and stems (0.28-1.28µg/g dw). The correlation analysis based on U concentrations between the three parts of the plant and soils were found to be significant ($P< 0.05$) withstems-soils, $R= 0.846$; leaves-soils, $R= 0.775$; roots-soils, $R= 0.786$. Based on the transplantation study under field and laboratory conditions, U concentrations in the leaves, stems and roots of *C. asiatica* were significantly higher ($P< 0.05$) after three weeks of exposure in polluted soils. After three weeks of back transplantation to clean soils, the U levels in the three parts were still higher than the initial U levels even though elimination of U occurred. Based on correlation study from sampling sites and transplantation study, the results suggested that the leaves, stems and roots of *C. asiatica* are good bio indicators of U pollution in soil.

KEYWORDS: Uranium, biomonitor, *Centella asiatica*, transplantation study.

Introduction

Recently, there were reports of the increase of U in the environment due to the nuclear industry and industrial activities which extract and process naturally occurring radioactive materials (Saueia and Mazzilli, 2006). Some sampling sites in the East coast of Peninsular Malaysia show high uranium activities due to several factors from the nearby environment (Mahmood *et al.,* 2008). Normally, U is present in the human body at very low concentration and its property as an essential element is not widely known yet (ATSDR, 2011). However, normal body functions such as kidney, liver, brain, heart and other systems can be affected by exposure to U (Craft *et al.,* 2004).

Centella asiatica is a medicinal plant that has been used widely in folk medicine for hundreds of years to treat a wide range of illnesses (Brinkhaus *et al*., 2000).In addition to it, *C. asiatica* was also found to be an accumulator of heavy metals (Ong *et al.,* 2011). Thus, there shall be awareness about the U concentration in the plant when using it to cure illnesses. This is because the presence of excessive U in the environment will cause its accumulation in the plant which will subsequently cause toxic effects on consumers. Furthermore, information on U levels in terrestrial soils in relation to *C. asiatica* are lacking in the literature. Therefore, it is important to study the levels of natural radionuclides to access the risk to the public's health when exposed to them. The objective of this study was to determine the potential of *C. asictica* as a bio indicator of U pollution based on the correlation of U concentrations between the plant and soils.

Methodology

Sample Collection

A total of 12 sampling sites were randomly selected and determined for plant and soil sample collections from Peninsular Malaysia (Table 1). *Centella asiatica* aged 2-4 months were collected and placed in plastic bags. Top surface soil from depth of 3-5 cm (with litter removed) was also collected into a plastic bag by a plastic scoop. The plants were separated into leaves, stems and roots in the laboratory.

Transplantation Study

For the transplantation experiment, *C. asiatica* was obtained from the University Agricutural Park (UAP), Universiti Putra Malaysia (UPM) and planted for 2 months to achieve the maturity stage. Four sites were selected namely UPM's UAP, Balakong, Seri Kembangan (SK) and Juru for the experimental study. UAP was selected because it is an agricultural area whereas Balakong, SK and Juru are known as industrial areas. The plants were acclimatized at UAP for 1 week before being transferred to the study sites. The transplantation studies were carried out under both field and laboratory conditions.

For the experimental field conditions, the plants were transferred from UPM (control) to the semi-polluted site (SK) and to the polluted sites (Juru and Balakong) from week 0 to week 3. After that, the plants were back-transplanted from the semi-polluted and the polluted sites to the control site at week 3 and grown for another 3 weeks (until week 6). For the experimental laboratory conditions, top soils from UPM, Balakong, SK and Juru were collected and placed into trays. At week 0 to week 3, plants from the controlled trays were transferred to trays with contaminated soils collected from Balakong, SK and Juru. From week 3 to week 6, the plants from the semi-polluted and the polluted trays were back-transplanted to the controlled trays.

Three replicates were done for each site (three trays of 75cm x 75cm x 10cm for the field study and three trays of 60cm x 35cm x 10cm for the laboratory study). The plants were transplanted every 3 weeks because an obvious effect could be observed after 2 weeks of transplantation (U.S.EPA, 1996). The plants were also harvested every 3 weeks for analysis. Soil samples were collected at week 0 and week 6 for analysis.

Neutron Activation Analysis (NAA)

All the plant and soil samples were dried in an oven at 65°C for around 5 days to obtain constant dry weights. The dried samples were ground by using an electronic agate homogenizer to obtain a homogenous powder (about 2mm mesh size) to ensure the elements within each sample were uniformly distributed. Then, the samples were stored in polyethylene bottles for future analysis. For all samples, the homogenous powder samples were shaken manually and had a weight ranging 0.15-0.20g. Samples were transferred into a polyethylene vial and heat-sealed. Certified reference material (CRM) IAEA-SOIL-7 was prepared using the same conditions and used as quality control for each batch. The recovery of U based on IAEA-SOIL-7 was 114.59% (CRM certified value: 2.60± 0.13μ/g dw; measured value: 2.98 ±1.64μ/g dw).

Irradiations were performed in the TRIGA MARK II reactor at the Agensi Nuklear Malaysia (NUKLEAR MALAYSIA), Bangi, Selangor (Malaysia). Uranium is a short lived radioisotope which has 23.45 minute shalf-life. Hence, a pneumatic transfer system (PTS) was used and each sample was irradiated for a period of 30-60 seconds on the same position for short irradiation to enable immediate counting. After irradiation by thermal neutron flux in the TRIGA MARK II research reactor, the radioactivity measurements of the samples were carried out after a proper cooling time by using various close-end coaxial high purity germanium detectors (Model GC3018 CANBERRA Inc and Model GMX 20180, EG4G ORTEC Nuclear Instrument) and their associated electronics. The cooling time varied from 5-20 minutes for 1st gamma-ray counting for U (U.S.EPA, 2001; IAEA-TECDOC-1360, 2003).

Geochemical Index

Titanium (Ti), aluminum (Al) and iron (Fe) were selected for normalizing U concentrations in the samples because they were conservative elements which were known to be derived mainly from crustal weathering (Schütz and Rahn 1982). The baseline values were selected from the element's concentrations in the continental crust of U - 1.7

ppm, Al - 79600 ppm, Ti - 4010 ppm and Fe - 43200 ppm by Wedepohl (1995) and U- 2.7 ppm, Al - 83200 ppm, Ti - 3800 ppm and Fe - 83200 ppm by Taylor (1964) since Malaysia does not have these baseline values and the reference values were based on the global average values.

The Enrichment Factor (EF) is used to differentiate between metals originating from human activities and those from natural sources. It can also provide information on naturally occurring high concentrations of heavy metals of a region. The value of the EF was calculated by using a modified formula reported by Buat-Menard and Chesselet (1979).The level of EF was categorized according to Han *et al.,* (2006).The geo accumulation index (Igeo) was calculated using the equation found in Yap and Pang (2011). The earth crust values were adopted as baseline values in the EF calculation as we did in the Igeo calculation. The Igeo level was categorized according to Muller (1981).

The concentration factor was used to determine the uptake of U by plants for the transplantation studies (week 0-3). The elimination factor can be used to determine the elimination of U by plants for the transplantation studies (week 3-6). It was calculated according to Yap *et al*., (2004).

$$\text{Concentration Factor} = \frac{U_{\text{end of metal accumulation}}}{U_{\text{initial}}}$$

$$\text{Elimination Factor} = \frac{U_{\text{end of metal elimination}}}{U_{\text{initial}}}$$

Results

Prior to transplantation, soils from UPM, SK, Balakong and Juru were collected and determined for U levels (Juru6.11µg/g dw, SK 5.09µg/g dw, Balakong5.91µg/g dw and UPM 2.45µg/g dw). Therefore, UPM was categorized as being clean, SK as being semi-polluted while Juru and Balakong were polluted sites based on the U concentrations in soils where 1.6µg/gdw was the provisional level for residential areas (U.S.EPA, 2005).

The U levels in soils from the 12 sampling sites were 2.92µg/gdw (1.97µg/g to 10.71µg/g dw) (Figure 1). The U level in soil from Seremban was significantly the highest ($P<0.05$) when compared to the other sampling sites. The ranges of EF varied from 0.79 to 18.25 with Kalangan being the highest and the least was from Kelantan (Table 1). The Igeo values ranged from -1.04 to 2.07. For all the sampling sites, the roots (2.51 ± 1.37µg/g dw) showed the highest U accumulation followed by leaves (0.74 ± 0.42µg/g dw) and stems (0.50 ± 0.30µg/g dw) (Figure 1). The samples from Seremban showed the highest U concentrations in all parts.

The accumulation of U increased for all parts when transplanted from control to semi-polluted and polluted sites under field conditions (week 0 to week 3) (Table 2). The increases were highest for Juru followed by Balakong and SK for roots, leaves and stems. However, the accumulation decreased (week 3 to week 6) after transplantation from the semi-polluted and polluted sites back to the control sites. The accumulation was still highest in Juru followed by Balakong and SK. For the transplantation study under laboratory conditions, the trend was exactly the same as the trend of the transplantation study under field conditions. The overall values for the concentration factor were highest for Juru under field and laboratory conditions and the elimination factor varied for different sites (Table 3).

Discussion

U Levels in Soils

U levels in soils from the 12 sampling sites ranged from 1.97µg/g to 10.71µg/g dw. These values were within the range reportedby UNSCEAR (1993) which was from 0.3µg to 11.7µg/g. The provisional levels of U for residential and industrial soils were 1.6µg/g dw and 200µg/g dw respectively (U.S. EPA, 2005). According to the CCME (2007) soil guidelines, concentrations below 23µg/g dw of U were suggested for agricultural and residential purposes and below 33µg/g dw of U for commercial uses. Based on the results of this study, none of the soil samples had exceeded the limits for agricultural, residential and commercial activities.

Garden soil (0-5 cm) from the city of Ottawa, Canada had U concentrations ranged from 0.66µg/g to 2.64µg/g dw (Rasmussen *et al.,* 2001). These U concentrations were low when compared with those of the agricultural sites in the present study because Ottawa is a city

with a low concentration of heavy industries. In the soil of Karu surrounding Abuja Metropolis, Nigeria, the U level was 18.00 ± 4.90µg/g dw (Kogo *et al.,* 2009). This reported concentration was much higher when compared to the present study due to Karu emerging as an industrial base. Bermudez *et al.,* (2010), reported a 3.1 ±0.1µg/g dwof U level in the top soil surrounding a cement plant in Córdoba, Argentina. The activities in the cement plant seemed not to affect the U concentration in the soil. In the West coast of Peninsular Malaysia, there are a few cement plants but such activities did not tend to affect the U concentration in the soil.

The EF values of soils for most sampling sites were greater than 1 based on all the references (Table 1) indicating that the U contamination originated from human activities (Nael et al., 2009).Three sites showed deficient to minimal enrichment,<2; six sites showed moderate enrichment, 2-5 and three sites showed significant enrichment, 5-20 (Han *et al.,* 2006). Based on Muller (1981), most of the samples showed moderate pollution (Igeo class 2) based on Igeo[g] while they were considered unpolluted to moderately polluted (Igeoclass 1) based on Igeo[h]. According to Klos *et al.,* (2011), the EF valuesof U in soils of Bory Stobrawskie Southern Poland (north-east of the city of Opole) were from 0.85 to 1.43. These different EF values might be due to different soil properties showing different enrichment values (ATSDR, 2011). More studies are required to determine the U contamination in Peninsular Malaysia.

U Levels in Plants

For all sampling sites, roots showed the highest U accumulation followed by leaves and stems (Figure 1). Those results were in line with the research findings reported by Ong *et al.,* (2011) that roots were able to uptake the highest concentration of metals followed by leaves and stems. The mobility of U in plant tissues is limited, as it tends to be absorbed into the cell wall materials. According to Shahandeh and Hossner (2002), U concentrations were typically higher in tissues found lower in the plant and were highest in the roots (30-50 times greater than in the shoots). The U concentrations ranged from 89 to 810µg/g dw in roots compared to shoots from 3.2 to 24µg/g dw.

Uranium uptake was evaluated for plants (lettuce, tomato, squash, and radish) grown in control soil and irrigated with well water from the Nambe region of northern New Mexico, USA (Hakonson-Hayes *et al.,* 2002). It was found that 2.3±3.0µg/g dw of Uwas accumulated in the plants where the value was similar to that found in the present study. According to the report from Karu analyzed by Kogo *et al.,* (2009), 9.668 ± 0.217µg/g dw of U in leaves was found in the location at Aso Radio and 65.32 ± 2.87µg/g dw of U inthe location at the Airport Road. These were higher concentrations of U in plants compared to the present study due to the location of Karu as an industrial base where U was released into the soils in Karu during the industrial activities.

The correlation coefficients of U between plants and soils (Figure 3) were found to be the highest between stems-soils ($R= 0.846$, $P< 0.05$), followed by roots-soils ($R= 0.786$, $P< 0.05$) and leaves-soils ($R= 0.775$, $P< 0.05$). The above results indicated that the three parts of *C. asiatica* were able to reflect the U levels in the soils. Therefore, the roots, leaves and stems of *C. asiatica* serve as good bio indicator for U contamination.

U in Transplantation Studies

The accumulation of U from the 12 sampling sites showed a similar trend of accumulation with root having the highest accumulation followed by leaves and stems. The accumulation of U (Table 2) increased in all parts of the plants when they were transplanted from the controlled site to the semi-polluted and polluted sites in the field study (week 0 to 3). All the calculated concentration factors were higher than 1 which indicated that the plants were able to uptake high levels ofU. In addition, the higher values of the concentration factor showed that the plants were able to uptake at least 100% more U than the initial value. This also shows that the U contamination of soil can be reflected through its accumulation in the plants. Hence, *C. asiatica* is an ideal choice as a bio indicator because it has the capability to be a net accumulator of the metal over a short time period.

The U concentration in the plants decreased from week 3 to week 6 when they were transplanted back to the control site but the U

levels in the plants remained higher than the content of U in the control site. The situation was similar for the plants under laboratory conditions (Table 2). The elimination factor values for the field and laboratory conditions were at least 60% (Table 3) which indicated that U from all plant parts could be removed when the condition of the site was less contaminated then that of the previous site but the rate was slower. Thus it required a longer time which was more than 3 weeks for U to be eliminated from the plants. In addition to this, the accumulation and elimination of metals in plants were also dependent on the transplantation period (Hedouin *et al.,* 2011).

Conclusion

Uranium accumulation was the highest in *C. asiatica* roots followed by its leaves and stems. The significant correlation of U between *C. asiatica* and the soils used for its growth and development indicated that the roots, leaves and stems are able to reflect the U pollution of the sampling sites. The results based on our experimental studies under field and laboratory conditions confirmed that the use of roots, leaves, and stems of *C. asiatica* as good bio indicator of U in soil pollution. Hence, *C. asiatica* is a potential candidate to assess the U contamination in soil.

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Table 1: Values of Enrichment Factor (EF) and Igeo of U from 12 Sampling sites in Peninsular Malaysia.

	Sites	EF[a]	EF[b]	EF[c]	EF[d]	EF[e]	EF[f]	Igeo[g]	Igeo[h]
1.	P.Klang	3.32	3.84	3.43	2.18	2.29	2.81	1.23	0.57
2.	Senawang	5.31	4.57	7.41	3.49	2.73	6.08	1.47	0.80
3.	Seremban	6.32	7.51	11.18	4.16	4.48	9.17	2.07	1.40
4.	K.Batas	3.21	5.40	6.07	2.11	3.22	4.98	1.55	0.89
5.	Kempas	3.16	3.70	4.70	2.08	2.21	3.85	1.35	0.69
6.	Pontian	3.45	3.34	7.34	2.27	1.99	6.02	1.82	1.15
7.	P.Pauh	1.55	2.71	3.46	1.02	1.62	2.84	-0.37	-1.04
8.	Kalangan	18.25	7.40	12.43	12.01	4.42	10.20	1.78	1.12
9.	Butterworth	4.18	6.54	5.67	2.75	3.90	4.65	1.63	0.96
10.	UPM	1.60	1.69	1.29	1.05	1.01	1.06	0.16	-0.51
11.	Arau	2.18	3.03	8.51	1.44	1.81	6.98	1.43	0.76
12.	Kelantan	1.49	1.32	2.26	0.98	0.79	1.86	0.19	-0.47

Note:

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

Table 2: Concentrations (mean ± SD, µg/g dry weight) of U in Leaves, Stems and Roots of *Centella asiatica* for the Transplantation Study Under Field and Laboratory Conditions.

Field condition	week	Leaves	Stems	Roots
Juru (polluted)	0	0.43 ± 0.16	0.38 ± 0.14	0.58 ± 0.21
	3	0.79 ± 0.04	0.71 ± 0.03	1.07 ± 0.02
	6	0.57 ± 0.04	0.50 ± 0.05	0.92 ± 0.05
Balakong (polluted)	0	0.43 ± 0.16	0.38 ± 0.14	0.58 ± 0.21
	3	0.76 ± 0.14	0.54 ± 0.10	0.94 ± 0.30
	6	0.48 ± 0.03	0.47 ± 0.04	0.81 ± 0.22
Sk (semi-polluted)	0	0.43 ± 0.16	0.38 ± 0.14	0.58 ± 0.21
	3	0.52 ± 0.15	0.50 ± 0.13	0.77 ± 0.09
	6	0.45 ± 0.02	0.43 ± 0.03	0.63 ± 0.04
UPM (clean)	0	0.43 ± 0.16	0.38 ± 0.14	0.58 ± 0.21
	3	0.44 ± 0.15	0.40 ± 0.17	0.62 ± 0.20
	6	0.43 ± 0.11	0.38 ± 0.02	0.58 ± 0.22
Laboratory condition	**week**	**Leaves**	**Stems**	**Roots**
Juru (polluted)	0	0.43 ± 0.16	0.38 ± 0.14	0.58 ± 0.21
	3	0.76 ± 0.16	0.69 ± 0.06	1.11 ± 0.24
	6	0.51 ± 0.13	0.47 ± 0.11	0.92 ± 0.18
Balakong (polluted)	0	0.43 ± 0.16	0.38 ± 0.14	0.58 ± 0.21
	3	0.65 ± 0.22	0.64 ± 0.23	0.87 ± 0.39
	6	0.47 ± 0.05	0.45 ± 0.04	0.80 ± 0.06
Sk (semi-polluted)	0	0.43 ± 0.16	0.38 ± 0.14	0.58 ± 0.21
	3	0.51 ± 0.20	0.56 ± 0.11	0.65 ± 0.25
	6	0.49 ± 0.18	0.43 ± 0.13	0.61 ± 0.26
UPM (clean)	0	0.43 ± 0.16	0.38 ± 0.14	0.58 ± 0.21
	3	0.44 ± 0.15	0.40 ± 0.17	0.62 ± 0.20
	6	0.43 ± 0.11	0.38 ± 0.02	0.58 ± 0.22

Table 3: Concentration Factor and Elimination Factorof U in the Transplantation Study under Field and Laboratory Conditions.

Sites	Field condition			Laboratory condition		
	Leaves	Stems	Roots	Leaves	Stems	Roots
Concentration factor						
Juru	1.84	1.86	2.36	1.75	1.80	1.91
Balakong	1.75	1.41	1.61	1.50	1.67	1.50
SK	1.20	1.32	1.33	1.19	1.48	1.11
Elimination factor						
Juru	0.72	0.70	0.75	0.68	0.68	0.83
Balakong	0.64	0.87	0.86	0.72	0.71	0.92
SK	0.86	0.85	0.82	0.95	0.77	0.95

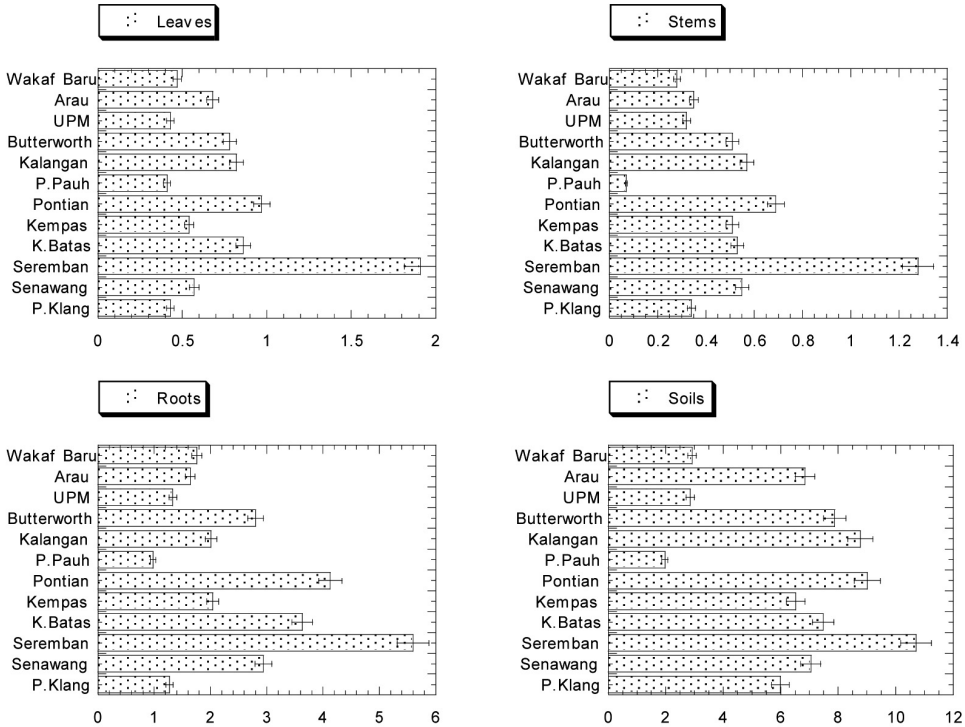

Figure 1: Uranium (U) Concentrations (mean ± SD, µg/g dry weight) in Leaves, Stems and Roots of *Centella asiatica* and Soils Collected from 12 Sampling Sites in Peninsular Malaysia.

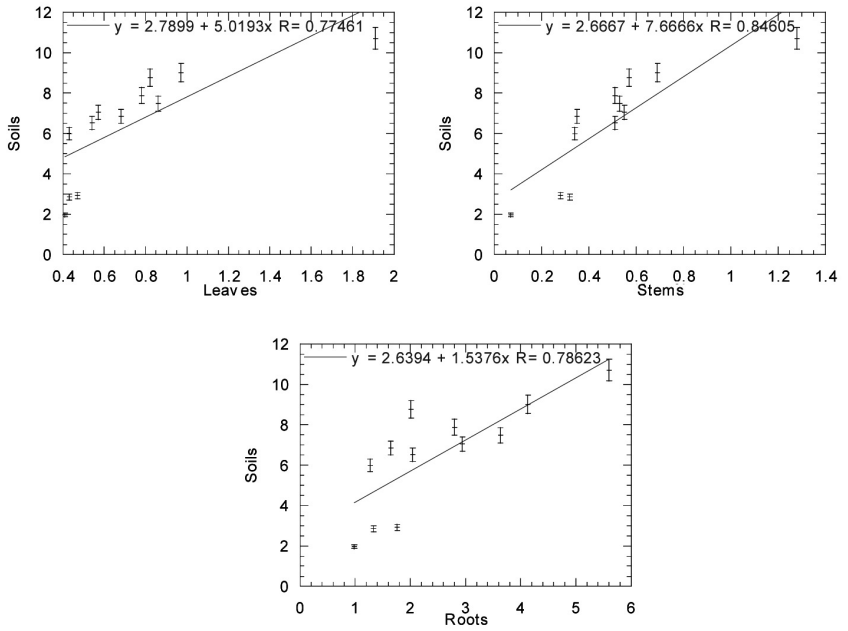

Figure 2: The Correlation Coefficients between Different Parts of *Centella asiatica* and Soil Concentrations of Uranium (U).

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