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Anthropogenic Influence of Sb in Tropical Soil and Effects to the Accumulation in *Centella asiatica*

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ABSTRACT

Background: Large amount of plants in contaminated soils were found to accumulate high levels of antimony (Sb) in their edible parts, thereby causing potential risks to human health. In this study, the objectives were to assess the degree of anthropogenic influence of Sb in the soil and its accumulation in *Centella asiatica* based on the relationship of Sb concentrations in plant and soil. The soil and plant samples were collected from 12 sampling sites in Peninsular Malaysia. In order to confirm the effect of Sb concentrations in soil towards the plant, transplantation study was conducted under laboratory condition within one control site (UPM) and two potentially polluted site (Juru and Balakong). The levels of Sb present in soil and *C. asiatica* were analysed with Instrumental Neutron activation analysis (INAA). Sb concentration in soils from the 12 sampling sites in Peninsular Malaysia were ranged from 2.21 µg/g to 5.10 µg/g. Based on enrichment factor (EF) and index of geoaccumulation (Igeo), Sb levels in Peninsular Malaysia were in mild polluted level. The range of Sb in roots were from 0.64 µg/g to 1.61µg/g while 0.22 µg/g to 0.49 µg/g in shoots. Sb levels were found to be highest in roots followed by shoots in *C. asiatica*. Based on the results from the transplantation study, high translocation factor (TF) values indicated high Sb level been translocated from roots to shoots but low BCF values showed low Sb uptake from soil to roots. Overall, Sb pollution in Peninsular Malaysia was considered mild based on Ef and Igeo values. Hence, we can conclude that Sb levels in *C. asiatica* from Peninsular Malaysia were not harmful for human consumption.

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INTRODUCTION

Antimony (Sb) is an important trace element in the world's economy. It is use in different products such as ceramics, glasses, plastics, various alloys, and synthetic fabrics. Due to its usage in industrial items, the anthropogenic release of Sb into the environment is highly significant. According to a study by Zhou *et al.* [1], global antimony emissions projected to be increased by a factor of 2 between 2010 and 2050 based on the projection scenarios from the comprehensive global antimony emission inventory for the period of 1995–2010. According to the Contaminated Land Management and Control Guidelines for Malaysia, 31µg/g of Sb was suggested for residential area while 42µg/g was for industrial area [2]. Although Sb is a potentially toxic element with unknown biological function, yet it is one of the least studied toxic elements.

In nature, Sb is present in more mobile forms in the soil, hence it can be accumulated in plants and affect their growth [3]. Although it is not an essential element for plants, numerous reports have demonstrated that plants residing in a Sb polluted environment can take up a large amount of Sb [4]. According to Tschan *et al.* [5], it was stated that plants can take up large amount of Sb and still look healthy. Therefore when man and animals consume the contaminated plants without realizing it, the toxic elements will enter the body. In addition, the major pathway for toxic elements to enter living organisms is via the food chain [6]. Sb pollution in soil can be estimated by two methods namely enrichment factor (EF) by Buat-Menar and Chesselt [7] and index of geoaccumulation (Igeo) by Muller [8]. These two methods are commonly used to assess the degree of anthropogenic influence. The estimation of plant's potential in Sb uptake can be evaluated by bioconcentration factors (BCF) and translocation factors (TF) [9].

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Centella asiatica (family: Umbelliferae) is the only plant extract from this genus to be found in commercial drugs today [10]. World Health Organization [11] has acknowledged *C. asiatica* as one of the most important medicinal plant species to be conserved. Based on the current literature review, there are still no reported cases of Sb contamination in *C. asiatica* in Malaysia. The objectives of the study were to assess the degree of anthropogenic influence in the soils and the accumulation of antimony (Sb) in *Centella asiatica* based on the relationship of Sb concentration in plant and soil.

Overview, this study provides the range of Sb levels in soil and *C. asiatica* found around Peninsular Malaysia. Based on the plant's potential uptake from the samples of the 12 sampling sites and transplantation study; the potential uptake of Sb into *C. asiatica* can be stipulated. Furthermore, this study provides a general idea regarding the safety and potential health risks on human due to consumption of *C. asiatica* and recommendation for human consumption.

MATERIALS AND METHODS

Sampling:

A total of 12 sampling sites were allocated for plant and soil samples collection around Peninsular Malaysia (Figure 1). Plants with maturity of 2-4 months were collected and placed in plastic bags. A 3-5 cm depth of surface soil (litters in the soil were removed) was also collected into a plastic bag by using a plastic scoop. Then, the plants were separated to shoots and roots in the laboratory.

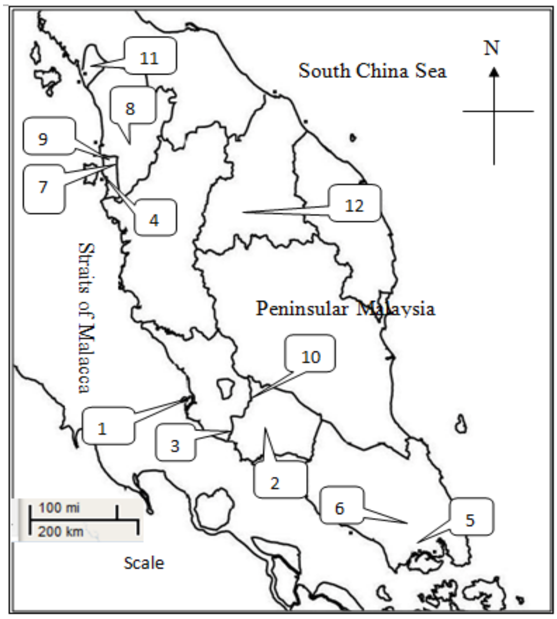

Fig. 1: Map showing the sampling sites in Peninsular Malaysia (Note: Name of location follows those in Table 1).

Transplantation study:

For the transplantation study, *C. asiatica* obtained from the University Agricultural Park (UAP), Universiti Putra Malaysia were planted for two months to reach its maturated stage. There were three study sites been chosen namely UPM's UAP, Balakong, and Juru; UAP was consider as an agricultural site while Balakong and Juru were the industrial site. Prior to transplantation, soils were collected to determine Sb levels and used for transplantation study. The results showed that at week 0, the Sb level for soils in Juru were 6.61 µg/g dw, Balakong (5.24 µg/g dw) and UPM (3.52 µg/g dw). Based on the Sb level from the UPM soil, it was categorized as a control site following the National Background concentration of Sb by the Dutch Environmental Standards [12]. Balakong and Juru were categorized as potentially polluted site. The transplantation studies were carried out under laboratory conditions and triplicate for each site. The collected soils from UPM, Balakong and Juru were placed into trays. At week 0 to week 3, plants from the control trays (UAP soil) were transferred to trays containing soils collected from Balakong and Juru. From week 3 to week 6, the plants from the potentially polluted trays were back-transplanted to the control trays. The plants were transplanted every 3 weeks because obvious effects takes place after 2 weeks in transplantation work [13]. The plants were harvested at week 0, 3 and 6 while the soil samples were collected only at week 0 and 6 to prevent harming the plants growth.

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Sb level determination:

The plant and soil samples were dried in an oven at 65°C for around 5 days to obtain constant dry weight. The dried samples were grind with an electronic agate homogenizer to obtain homogenous powder (about 2mm mesh size) to ensure that the elements within each sample were uniformly distributed. For all samples, the homogenous powder samples were shaken manually and the sample's powder weight ranging 0.15-0.20g was filled into the respective polyethylene vial and heat-sealed. The samples were stored in polyethylene bottles for further analysis. Certified reference material (CRM) IAEA-SOIL-7 was prepared in identical condition and used as quality control for each batch. The recovery of Sb based on CRM was 73.58 % for IAEA-SOIL-7 (CRM certified value: 1.70 ± 0.09µ/g dw; measured value: 1.25 ± 0.27µ/g dw).

The irradiations of the samples were performed in the TRIGA MARK II reactor at the Malaysia Nuclear Agency, Bangi, Selangor (Malaysia). Sb is a long lived radioisotope which has 60.90 days half life. Hence, neutron flux of 4-5 x 10^{12} n/cm^2 was used for long irradiation. After irradiation, the samples undergoes a proper cooling time under various close-end coaxial high purity germanium detectors (Model GC3018 CANBERRA Inc and Model GMX 20180, EG4G ORTEC Nuclear Instrument) and their associated electronics. The cooling time for the counting varied between 3-6 days and the live time for the counting of Sb was 3600 seconds [14].

Enrichment factor (EF) and Geoaccumulation index (Igeo):

The Enrichment Factor (EF) was utilized to differentiate between metals originating from human activities and those from natural sources. It can also assess the degree of anthropogenic influence. The value of EF was calculated by a modified formula suggested by Buat-Menard and Chesselet [7]:

$$EF = \left(\frac{Sb_{(sample)}/Ti_{(sample)}}{Sb_{(baseline)}/Ti_{baseline)}} \right)$$

Titanium (Ti) as a conservative element was selected for normalizing its concentration in the sample. The baseline values were selected from the element concentration in the continental crust based on Wedepohl [15] (Sb: 0.3µg/g; Ti: 4010µg/g). Since Malaysia does not have the baseline values therefore the reference values are based on the global rock average values.

The geoaccumulation index (Igeo) can be calculated by the following as stated by Muller [8]:

$$Igeo = \log 2\left(\frac{Sb_{(sample)}}{1.5 \times Sb_{(baseline)}} \right)$$

The baseline values were selected from the element concentration in the continental crust based on Wedepohl [15] (Sb: 0.3µg/g). Factor 1.5 is the background matrix correction factor due to lithogenic effects. Since Malaysia does not have the background values of Sb, we adopted the earth crust values [15] in the Igeo calculation.

Bioconcentration factor (BCF) and Transfer factor (TF):

Both BCF and TF can be used to assess the potential of a species to uptake metals. BCF is suited to evaluate the ability of plants to accumulate metals from the soil whereas TF is used to estimate the plant's ability to translocate metals from the roots to the shoots. BCF was defined as the ratio of metal concentrations in roots to that in soils while TF was defined as the ratio of metal concentrations in shoots to the roots [16].

$$BCF = \left(\frac{Sb_{(roots)}}{Sb_{(soils)}} \right) \qquad TF = \left(\frac{Sb_{(shoots)}}{Sb_{(roots)}} \right)$$

Statistical analysis:

Statistical analyses were carried out using SPSS version 17.0 for Windows for analysis of variance (ANOVA), SNK, and Post hoc test. STATISTICA version 8 was used to determine the correlation coefficient in this research [17].

RESULTS AND DISCUSSION

Sb level in soils:

As presented in Table 1, the Sb concentrations in soil from the 12 sampling sites in Peninsular Malaysia were ranged from 2.21µg/g to 5.10µg/g. The soil samples from Seremban (5.00µg/g), K.Batas (5.10µg/g) and Butterworth (5.00µg/g) were significantly ($P<0.05$) higher compared to the other sampling sites (Table 1). The Sb concentrations were considered low in soil from the 12 sampling sites when compared to the report from Agency for Toxic Substances and Disease Registry [18] which had a value of 9µg/g. Based on the guideline, all sites reading were below the reported critical value. According to the Contaminated Land Management and Control Guidelines for Malaysia, 31µg/g of Sb was suggested for residential area while 42µg/g was for industrial area [2]. None of the sampling sites in this study exceeded the guidelines for residential and industrial areas.

Table 1: Sb concentrations (mean±SD, µg/g dry weight) in C. asiatica and soil, enrichment factor (EF) and geoaccumulation indices (Igeo) from 12 sites in Peninsular Malaysia

No.	Sites	Soils			Shoots			Roots			EF[a]	Igeo[a]
1.	P.Klang	4.40	±	0.17	0.38	±	0.20	1.04	±	0.58	16.01	-0.18
2.	Senawang	2.50	±	0.10	0.24	±	0.21	0.74	±	0.89	9.20	-1.00
3.	Seremban	5.00	±	0.20	0.43	±	0.22	1.36	±	0.78	19.88	0.00
4.	K.Batas	5.10	±	0.19	0.49	±	0.40	1.61	±	0.20	20.84	0.03
5.	Kempas	3.71	±	0.14	0.35	±	0.38	0.95	±	0.82	11.91	-0.43
6.	Pontian	2.21	±	0.09	0.22	±	0.36	0.64	±	0.18	4.63	-1.18
7.	P.Pauh	2.21	±	0.08	0.22	±	0.53	0.65	±	0.71	17.17	-1.18
8.	Kalangan	4.21	±	0.16	0.37	±	0.21	1.36	±	0.32	20.12	-0.25
9.	Butterworth	5.00	±	0.19	0.48	±	0.46	1.26	±	0.44	23.54	0.00
10.	UPM	3.51	±	0.05	0.29	±	0.16	0.83	±	0.48	11.77	-0.51
11.	Arau	2.30	±	0.09	0.32	±	0.27	0.65	±	0.33	5.78	-1.12
12.	Wakaf Baru	4.40	±	0.16	0.43	±	0.51	1.03	±	0.14	11.31	-0.18

(Note: a: Wedepohl, 1995 was used as baseline values in the continental crust)

Soil usually contains very low concentrations of Sb. However, higher concentrations have been detected at hazardous waste sites and at antimony-processing sites [19]. In several parts of China especially in the Guangxi, Hunan, Yunnan and Guizhou area, large quantities of Sb have been released resulting in serious Sb contamination of the local environments due to Sb mining and smelting processes. Furthermore, coal combustion and Sb products consumed by the domestic market also contributed as the source of Sb contamination [20]. According to He *et al.* [20], Sb concentration in sediments was found to be up to 1163 mg/kg in the proximity of mining and smelting areas compared to the faraway places. Another study conducted by Li *et al.* [21] in Anhua County showed that the total Sb content in soils varied from 185.6 mg/kg to 2081.3 mg/kg, which substantially exceeded the background level. The higher level of Sb concentrations in the soil from China compared to the ones in Peninsular Malaysia was mainly due to the number of mines found in China was higher. He *et al.* [20] reported that there are a total of 114 Sb mines in China. The largest contribution of Sb to the environment was from coal combustion (61.8%) while 26.7% of Sb was emitted from nonferrous metals smelting [22]. In addition to it, Sb and its compounds have long-range transport characteristics [23]; therefore Sb concentration was found high in nearby area due to the dust emitted during industrial processing settling down from the air. Besides that, waste from Sb processing and other Sb using industries is usually dumped into the soil causing the increase in Sb concentration. Thus, the industries involving the production of various alloys, ceramics, glasses, plastics, and synthetic fabrics should have safety precautions in dumping the waste to limit contamination of soil and maintain Sb concentrations in the soil within a safe level. According to a study by Niu *et al.* [24], 1.23 ± 0.61 µg/g of Sb was found in farmland soils across China. There were some sites from Peninsular Malaysia showing similar level of Sb as the farmland. This indicates that contamination of Sb in Malaysia was still considered mild.

Sb levels in soil for all sampling sites were greater than reference values (0.3µg/g by Wedepohl [15] in Table 1 which indicated that Sb contamination was due to human activities [25]. The range of EF was from 4.63 to 33.46 in 12 sampling sites around Peninsular Malaysia. According to enrichment categories by Han *et al.* [26], soils from all sites falls under significant enrichment category (EF: 5-20) except for Pontian which was under moderate enrichment category (EF: 2-5) while K.Batas, Kalangan, Butterworth were under very high enrichment category (EF: 20-40). Based on the study by Yaylalı-Abanuz [27] in Turhal (northern Turkey), element enrichment of Sb was the highest when compared to the other metals. The Igeo values can be categorized in relation to degree of pollution according to Muller [8]. All Igeo values of the current study were less than 1 indicating that all sampling sites were under the unpolluted category. Hence, we can conclude that Sb level in Peninsular Malaysia is greater than the average occurrence of the mineral in the Earth's crust but still do not achieve the polluted level. Therefore public should be aware of the current condition as we are heading towards the polluted category if wastes are continuously added anthropogenically.

Sb level in plant:

The range of Sb in roots was from 0.64µg/g to 1.61µg/g while the range for shoots was from 0.22µg/g to 0.49µg/g (Table 1). For all sampling sites, the roots showed the highest Sb accumulation followed by shoots. Some authors asserted that the root uptake of Sb might be significant and occurred via passive transport [28]; while others suggested that the translocation of Sb from roots to the upper plant parts might be limited by its precipitation on the root membrane barriers that controlled the transport of metal solutions to the leaves. The result was supported by Wilson *et al.* [29], where metalloid concentrations in plant roots were significantly greater than concentrations in above ground plant parts.

According to He *et al*. [20], 5µg/g of Sb in plant tissues were reported as phytotoxic and exceeded the tolerable concentration. Sb can damage plants in many ways such as growth retardation, inhibition of photosynthesis, decreases in the uptake of certain essential elements and decreases in the synthesis of certain metabolites. But plants often have defence mechanisms to alleviate Sb toxicity; e.g., a highly efficient antioxidative system and the ability to immobilise Sb in the cell wall or compartmentalize Sb in the cytosol. These mechanisms had been widely reported in Sb-tolerant and Sb-accumulating plants [19]. Compared to current study, Sb concentrations in shoots and roots in *C. asiatica* were lower than the toxic level. By comparison, the reported background Sb concentrations in terrestrial plants ranged between 0.2µg/g and 50µg/g [28]. These showed that the Sb concentrations in all parts of *C. asiatica* in Peninsular Malaysia were within the reported range of concentrations. The uptake of Sb had been proposed to occur mainly through the passive pathway; however, it was possible that an active pathway exists as well [19]. A literature study found that the uptake of Sb by plants was proportional to the soluble Sb concentration in the soil and more than 1000 ug/g of Sb was found in plants adjacent to a Sb mining area [28]. At present, there is still question regarding the presence of Sb in plants and its role in plant. Some plant was able to take up large amounts of Sb and still look healthy [28]. A recent study on Cretan brake fern found that the plant can accumulate up to 1516 ug/g of Sb in shoots [30].

Transplantation study:

Table 2 showed an increase of Sb level in shoots and roots when the plants were transplanted from the control site to potentially polluted sites (week 0 to week 3). Sample from Juru had the highest Sb level in shoots and roots followed by Balakong and the least in UPM. It was due to the soils from Juru contains highest Sb level. The level of Sb in *C. asiatica* decreased after the plants were transplanted back to the control site (week 3 to week 6) but the level of accumulation was still higher when compared to the initial concentration (Table 2). By comparing the results of week 0 and week 6, the accumulation of Sb was higher in the back-transplanted plant (week 6) when compared to the plants in the initial stage (week 0). They were far from reaching the initial Sb concentration. This could be due to the rate of accumulation and elimination dependent on the transplantation period. This supports the current finding where the elimination of Sb from the plants (week 6) was not completed during the three weeks of back transplantation thus, a higher level of accumulation was been observed. Hence, a longer time was required for the elimination of Sb to take place in the plant.

Table 2: Concentrations (mean ± SD, µg/g dry weight) of Sb in shoots and roots of Centella asiatica, Biocentration factor (BCF) and Transfer factor (TF) for transplantation study

	Week	Shoots			Roots			Soils			BCF	TF
Juru	0	0.43	±	0.15	0.80	±	0.16	6.61	±	4.18	0.12	1.86
	3	0.74	±	0.06	1.35	±	0.12					1.82
	6	0.65	±	0.15	1.13	±	0.21	6.28	±	2.12	0.18	1.74
Balakong	0	0.43	±	0.15	0.80	±	0.16	5.24	±	1.47	0.15	1.86
	3	0.67	±	0.10	1.12	±	0.15					1.67
	6	0.61	±	0.29	1.07	±	0.13	5.13	±	2.27	0.21	1.77
UPM	0	0.43	±	0.15	0.80	±	0.16	3.52	±	2.40	0.23	1.86
	3	0.42	±	0.15	0.81	±	0.23					1.95
	6	0.43	±	0.21	0.80	±	0.17	3.44	±	1.62	0.23	1.86

Based on BCF and TF values, we can estimate the ability of *C. asiatica* in taking up Sb from soil and translocating it to the shoots. Tolerant plants show low accumulation in their biomass, whereas hyperaccumulators actively take up and translocate metals into their aboveground biomass [31]. According to Fitz and Wenzel [31], plants exhibiting TF and BCF values higher than one are suitable for phytoextraction due the ability of plant to uptake high level of metals from soils to roots and eventually to shoots. Based on the result from Table 2, BCF values for all transplantation study were ranged from 0.12 to 0.23 while TF values range from 1.74 to 1.95. This shows the plant had low uptake from soil to roots but great translocation of Sb from roots to shoots. The translocation of Sb from roots to the upper part of the plant might be limited by its precipitation on the root membrane barriers that controlled the transport of metal solutions to the shoots. The result was supported by Wilson et al. [29], it shows that the metalloid transfer to above ground plant parts on all native Australian plants species was low (ratio of leaf concentration/soil concentration lesser than 1).

Conclusion:

Overall, Sb pollution in Peninsular Malaysia was considered mild based on EF and Igeo values. According to the results from the transplantation study, high Sb level shown in translocation from roots to shoots based on the TF values but low Sb uptake from roots to shoots based on the BCF values. Hence, we can conclude that Sb levels in *C. asiatica* were not harmful to human consumption. However, further studies on the other components present in *C. asiatica* are needed in the future in order to confirm the safety consumption.

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