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Effects of Metal-contaminated Soils on the Accumulation of Heavy Metals in Different Parts of *Centella asiatica*: A Laboratory Study

(Kesan Penimbunan Logam Berat di dalam Pelbagai Bahagian *Centella asiatica* dari Tanah yang Tercemar dengan Logam Berat: Satu Kajian dalam Makmal)

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ABSTRACT

Centella asiatica *is widely used as a medicinal plant in Malaysia and other parts of the world. In the present study, the growth and uptake of heavy metal by* C. asiatica *were determined based on the plant exposure to different treatment of metal-contaminated soils under laboratory conditions. Heavy metals uptake in different parts of the plants namely roots, stems and leaves were determined. In general, it was found that the metal uptake capacity followed the order: roots > stems > leaves. Since a close positive relationship was established between the concentrations of metal accumulated in different parts of the plant and the metal levels in the most contaminated soil,* C. asiatica *has the potential of being used as a biomonitoring plant for heavy metal pollution in the polluted soils.*

Keywords: Centella asiatica; *laboratory study; metal-contaminated soils*

ABSTRAK

Centella asiatica *merupakan tumbuhan yang digunakan sebagai ubat secara meluas di Malaysia dan di seluruh pelusuk dunia. Dalam kajian ini, pertumbuhan dan penyerapan logam berat oleh* C. asiatica *telah ditentukan berdasarkan kepada pendedahan tumbuhan tersebut terhadap pelbagai treatmen dengan tanah yang tercemar dengan logam berat di bawah keadaan laboratori. Penyerapan logam berat dalam bahagian berlainan tumbuhan telah ditentukan di akar, batang dan daun. Secara umumnya, didapati bahawa kapasiti penyerapan logam berat dalam tumbuhan ini adalah mengikut urutan: akar> batang> daun. Berdasarkan kepada hubungan positif yang rapat antara penimbunan logam berat di bahagian-bahagian berlainan tumbuhan ini dengan kandungan logam berat dalam tanah yang paling tercemar,* C. asiatica *amat berpotensi sebagai biopemonitoran bagi pencemaran logam berat di dalam tanah yang tercemar.*

Kata kunci: Centella asiatica; *kajian makmal; tanah tercemar logam berat*

INTRODUCTION

Medicinal herbs are consumed all over the world. The use of medicinal herbs to relieve and treat many human diseases is increasing around the world due to their mild features and low side effects. However, most medicinal herbal products are unlicensed, their efficacy, safety or quality are still undetermined (Ernst et al. 1992). In addition, knowledge on the interesting chemical compositions of medicinal herb products is growing because of the ongoing developments in nutritional and in biochemical surveys and mineral prospecting (Rodushkin et al. 1999). However, many medicinal herbs and their mixtures can present health risks due to the presence of toxic metals such as Pb, Cd, Al and Hg which are hazardous to humans, at various degrees depending on their oxidation states as well as their concentrations (Lekouch et al. 2001).

Moreover, medicinal herbs may be contaminated during growing and processing stages. The environment, pollution, the atmosphere, soil, harvesting and handling are some of the factors, which could play important roles leading to the contamination of medicinal plants by metals (Al Moaruf et al. 2004). In addition, high levels of metals can be found when agricultural aids are used; including Cd-containing fertilizers, organic Hg or Pb-based pesticides, and contaminated irrigation water (Abou-Arab et al. 1999).

An important link in the transfer of trace metals from soil to man is plants. Plants can contain the heavy metals that are found in soil (including contamination of plant material with soil), water or the air (McLaughin et al. 1999). The level of essential metals in plants is conditional, the content being affected by the geochemical characteristics of the soil and by the ability of the plant to selectively accumulate some of these elements (Bin et al. 2001). The bioavailability of these elements depends on the nature of their bonds with the constituents of the soil. Plants readily assimilate metals that dissolved in water and those that are in ionic forms through their roots. A number of minerals essential to human nutrition such as

Cd, Co and Ag are accumulated in different parts of plants (Dushenkov et al. 1995). All of the above have been the reasons why there are a lot of literature regarding studies on heavy metals using medicinal plants (Arpadjan et al. 2008; Barthwal et al. 2008; Khatun et al. 2008; Street et al. 2009).

Centella asiatica (L.), known in Malaysia as Pegaga, (Family: Apiaceae (Umbelliferae)), is an important medicinal plant. The plant is native to India, China, Indonesia, Sri Lanka, Australia, Medagascar and Southern and Central Africa. The plant possesses antileprotic, antistress, antitumour, antifilarial, antiulcerogenic, antibacterial, antifungal and wound healing (Singh et al. 2000) properties and is used as a tonic in Ayurvedic formulations. Tribals of north-east India use the paste of the leaves with pepper and salt to cool down body heat and they administer the leaf paste on an empty stomach for the cure of dysentery (Kharkongor & Joseph 1981). The plant possesses long, brownish, string shaped stolons that are characterized by long internodes with roots at each node. The plant reproduces both through vegetative and sexual means. Since there is no study on heavy metals in *C. asiatica*, the objective of this study was to determine the accumulation of heavy metals in different parts of *C. asiatica* exposed to different treatment with polluted soil under laboratory conditions.

Materials and Methods

Soil and Plants Samples

The plant was collected from the University Agricultural Garden, (Taman Pertanian Universiti), Universiti Putra Malaysia (UPM). The experiment was carried out in a greenhouse by using healthy plants uniform in size and color which were free of disease symptoms. The plants were placed in polystyrene pots 10 cm × 10 cm with a 2.5 cm diameter drainage hole each. The pots were filled with 4 kg of garden soil mixture (loam soil: fertilizer: sand) in the ratio 3:2:1. The soil treatment followed the modified method of Montse and Joan (2006) (Table 1). The polluted soils were collected from a drainage receiving metal industrial wastes from Sri Serdang (Yap et al. 2008). Three replicates for each treatment were used. The plants were watered once every two days (10 mL per kg) and care was taken to avoid leaching of water from the pots by keeping a plastic tube below each pot to collect the leachates. The collected leachates were again returned to the experimental pots. The plants were harvested after 8 weeks for metal analysis.

Heavy Metals Analytical Procedure for Plant and Soil Samples

The easily, freely, leachable or exchangeable fraction (ELFE) of heavy metal concentrations were determined using the procedure described by Badri and Aston (1983).

TABLE 1. Compositions of soils for all treatments used in this laboratory study

Treatment	Description for the soil treatment
A	100% natural soil (control)
B	75% natural soil plus 25% contaminated soil
C	50% natural soil plus 50% contaminated soil
D	100% contaminated soil

The soil samples were dried at 105°C for at least 16 hours until constant dry weights. The dried samples were then crushed by using a pestle and mortar to a size that can passed through a 63 μm stainless steel sieve under vigorously shaking to produce homogeneous sample. In order to determine the EFLE, 10 of dried samples were continuously shaken at 200 rpm for 3 hours with 50 ml 1.0 M ammonium acetate (NH_4CH_3COO), pH 7.0 at room temperature. Then, it was filtered through a Whatman No 1. filter paper into an acid-washed pill box. Erlenmeyer flask was washed with 20 ml double de-ionized water and filtered with filter paper (Whatman No 1.) into the same pill box.

The plants were washed with distilled water and carefully dissected into roots, stems and leaves. The separated parts were then dried in the oven for 72 hours at 105 °C until constant dry weights (Mo & Neilson 1994). The weighted dried plant parts were placed in digestion tubes and 10 mL of concentrated HNO_3 (AnalaR grade, BDH 69 %) was added. The tubes were put onto a hot block digester at 40 °C for 1 hour and the contents were fully digested at 140 °C for 3 hours and then were filtered through a Whatman No.1 filter paper in a funnel. The filtered solution was collected in an acid-washed pill box (Yap et al. 2003). All the prepared samples were determined for heavy metals by using an air-acetylene flame Atomic Absorption Spectrophotometer (AAS) (Perkin-Elmer AAnalyst 800). The data were presented in μg/g dry weight basis. Certified Reference Materials for Soil (International Atomic Energy Agency, Soil-5, Vienno, Austria) were also run and the recoveries for Cd, Cu, Fe, Ni, Pb and Zn were satisfactory (90 - 105%).

The One-way ANOVA based on Turkey HSD multiple homogenous subset was performed by using SPSS version 12.

Results

From Table 2, it can be seen that the highest levels of Cd, Cu, Fe, Ni, Pb and Zn were found in the soil of Treatment D. As expected, Treatment D (100% contaminated soils) had higher metal levels when compared to Treatment C (50% contaminated soil) and Treatments B (25% contaminated soil). This result indicated that Treatment D with 100% contaminated soil is the most metal-polluted soils among all the treatments investigated.

TABLE 2. Concentrations (mean [μg/g dry weight] ± standard error) of heavy metals in the EFLE fractions in the soils of all treatments.

Metals	T-A	T-B	T-C	T-D
Cd	0.015 ± 0.005	0.006 ± 0.002	0.013 ± 0.003	0.030 ± 0.005
Cu	0.301 ± 0.010	0.448 ± 0.060	0.434 ± 0.050	1.03 ± 0.310
Fe	1.090 ± 0.110	1.12 ± 0.200	1.22 ± 0.150	2.45 ± 0.050
Ni	0.171 ± 0.040	0.205 ± 0.001	0.535 ± 0.030	0.988 ± 0.200
Pb	0.028 ± 0.005	0.072 ± 0.010	0.013 ± 0.006	0.225 ± 0.020
Zn	0.174 ± 0.050	0.774 ± 0.070	2.71 ± 0.640	13.4 ± 1.52

Note: T= Treatment

The heavy metal concentrations in the roots, stems and leaves of *C. aisatica* are given in Table 3, 4 and 5, respectively. In general, Treatment D was found to have the highest levels of Cd, Cu, Fe, Ni, Pb and Zn in the roots, stems and leaves of *C. asiatica,* when compared to the other Treatments.

From Table 6, concentrations of Cu, Fe and Ni are found to be the highest in roots for all the four different treatments while concentrations of Cd and Pb are found to be the highest in roots for two out of four treatments. For Zn concentrations, only treatment C and D are found to be the highest in the roots. Therefore, we can conclude that, in general, roots had higher concentrations of heavy metals when compared to the stems and leaves. Second point is metal redistribution in the polluted treatments. For example, Cd concentrations were redistributed in Treatment D when leaves had higher Cd levels than the roots and stems. For Cu, stem had higher level than leave in Treatment D.

TABLE 3. Mean concentrations (μg/g dry weight) of heavy metals in the roots of *Centella asiatica* for all treatments after 8 weeks

Treatments	Cd	Cu	Fe	Ni	Pb	Zn
A	1.44^{a}	12.4^{a}	1890^{a}	1.44^{a}	7.14^{a}	56.7^{a}
B	1.57^{a}	29.8^{b}	1861^{a}	8.53^{a}	39.7^{b}	209^{b}
C	2.07^{a}	64.2^{c}	1858^{ab}	13.9^{a}	97.0^{c}	350^{b}
D	2.89^{a}	122^{d}	1775^{b}	76.0^{b}	189^{d}	384^{d}

Note: [a] Different letters in the same column indicate significant difference at the 0.05 level according to the protected Tukey HSD Multiple Homogenous Subset

TABLE 4. Mean concentrations (μg/g dry weight) of in the stems of *Centella asiatica* for all treatments after 8 weeks

Treatments	Cd	Cu	Fe	Ni	Pb	Zn
A	0.81^{a}	4.72^{a}	728^{a}	BDL[a]	6.83^{a}	145^{a}
B	0.84^{a}	16.9^{b}	1366^{c}	BDL[a]	16.7^{a}	167^{b}
C	1.19^{b}	13.5^{c}	1514^{a}	BDL[a]	12.9^{a}	217^{c}
D	1.61^{c}	28.9^{d}	1105^{b}	5.22^{b}	29.0^{b}	368^{d}

Note: [a] Different letters in the same column indicate significant difference at the 0.05 level according to the protected Tukey HSD Multiple Homogenous Subset
BDL = Below Detection Limit

TABLE 5. Mean concentrations (μg/g dry weight) of heavy metals in the leaves of *Centella asiatica* for all treatments after 8 weeks

Treatments	Cd	Cu	Fe	Ni	Pb	Zn
A	0.79[a]	4.88[a]	509.3[a]	BDL[a]	7.21[a]	144[a]
B	1.25[a]	16.2[b]	1282[d]	BDL[a]	17.7[b]	263[b]
C	1.24[a]	13.5[b]	915[c]	BDL[a]	11.0[c]	284[c]
D	8.01[a]	19.9[c]	674[b]	BDL[a]	20.9[d]	383[d]

Note: [a] Different letters in the same column indicate significant difference at the 0.05 level according to the protected Tukey HSD Multiple Homogenous Subset

BDL = Below Detection Limit

TABLE 6. Order of different parts based on metal concentrations, of *Centella asiatica* for all treatments after 8 weeks

Treatments	Cd	Cu	Fe
A	Roots> Stems ≥ Leaves	Roots> Leaves ≥ Stems	Roots> Stems > Leaves
B	Roots≥ Leaves > Stems	Roots> Leaves ≥ Stems	Roots> Stems≥ Leaves
C	Roots≥ Leaves > Stems	Roots> Leaves ≥ Stems	Roots> Stems > Leaves
D	Leaves> Roots≥ Stems	Roots> Stems> Leaves	Roots> Stems > Leaves
Treatments	Ni	Pb	Zn
A	Roots> Stems = Leaves	Leaves≥ Roots> Stems	Stems≥ Leaves> Roots
B	Roots> Stems = Leaves	Roots> Leaves≥ Stems	Leaves> Roots> Stems
C	Roots> Stems = Leaves	Roots> Stems≥ Leaves	Roots> Leaves≥ Stems
D	Roots> Stems ≥ Leaves	Roots> Stems≥ Leaves	Roots> Leaves≥ Stems

Note: > indicates significantly ($P < 0.05$) higher

≥ indicates not significantly ($P > 0.05$) higher

Discussion

The close positive relationships of heavy metals between the different plant parts and EFLE fraction of the soil treatments indicated that *C. asiatica* can be used as a biomonitoring plant of heavy metal pollution in the polluted soils. Unlike animals, plants cannot avoid pollutants from affecting their physiological balances. The ultimate sink for heavy metals is atmospheric deposition and burial in soils (Khan et al. 2000). They often accumulate in the top layer therefore are accessible for uptake by plant roots, which are the principal entry points of heavy metals that eventually affect different physiological processes. This was the major reason why only the EFLE fraction was determined in the surface soil of all the experimental treatments. Depending on the plant species, heavy metal tolerance or adaptations that enable them to grow in heavy metal contaminated soils are the results of two basic strategies, exclusion and accumulation (De Vas et al. 1991). The accumulation strategy involves physiological processes that require the cells to maintain the intra cellular heavy metal ions in non toxic forms (Cobbet 2000) and the stored heavy metal ion complexes may be removed by leaf fall (Ernst et al. 1992). From the present study we noted that the essential metals like Cu, Fe and Zn were highly accumulated in the roots, followed by stems and leaves of *C. asiatica*.

For all the Treatments, noted that the stems accumulated a high level of Fe, (2096.49 µg/g dw) which is not significantly ($p > 0.05$) different from that of the roots 2067.74 µg/g dw. This is because Fe is easily soluble and plants may take up a very large amount of Fe (Kabata-Pendias & Pendias 1984). The metabolic functions of Fe in green plants are relatively well understood, and Fe is considered to be the key metal in the energy transformation needed for biosynthesis and other life processes of the cells. An appropriate content of Fe in plants is essential not only for the health of the plant but also for the nutrient supply to man and animals. The variations among plants in their abilities to absorb Fe are not always consistent and may be affected by the changing conditions of soil and climate and by the stages of plant growth (Kabata-Pendias & Pendias 1984).

Zn was found to be highly accumulated in roots (383.68 µg/g dw) but the value was not significantly different with those of the stems (368.33 µg/g dw) and leaves (382.90 µg/g dw). Zinc also plays essential metabolic roles in the plant, of which the most significant is its activity as a component of a variety of enzymes, such as dehydrogenase, proteinases, peptidases and phospohydrolases. Shkolnik and Leringrad (1974) indicated that the basic Zn functions in plants are related to the metabolisms of carbohydrates, proteins and phosphate and also to auxin, RNA and ribosome synthesis.

Pb and Cd also appears to be the non essential metals that were highly accumulated in this plant. Lead was high in the roots supporting a previous study by Kabata-Pendias and Pendias (1991). Although Pb occurs naturally in all plants, it has not been shown to play any essential roles in their metabolism. Broyer et al. (1972) reviewed this topic and concluded that if Pb is necessary for plants its concentration at the level of 2 to 6 ppb should be sufficient. Lead has recently received much attention as a major chemical pollutant of the environment. Among the roots, stems and leaves, Ni was only accumulated in the roots for all treatments with the highest Ni concentrations found in the Treatment D, followed by Treatment C, B and A.

Cu is another metal that was highly accumulated in *C. asiatica*. The distribution of Cu within plants is highly variable. Within roots, Cu is associated mainly with the cell walls and is largely immobile. The highest concentrations of Cu in the shoots are always found during phases of intensive growth and at the luxury Cu supply level (Scheffer et al. 1978). By comparing to the permissible limit of Cu in medicinal herbs, the Cu concentration in the roots of *C. asiatica* was found to be in the toxic limit (121.98 µg/g dw) in the treatment with 100% contaminated soil.

The highest levels of heavy metal generally found in the roots of *C. asiatica*. *This* can be explained from three points. First, root hairs contain higher surface area for adsorption and absorption of heavy metals when compared to stems and roots. Thus, it is not surprising to note that the highest levels of heavy metal found in this plant organ. Second, roots are the first organ to take up all the heavy metals from the soils before distributing to other parts of the plant. It is not worthy that roots were found to have significantly higher levels of metals planted in polluted soils. Third, root is the only organ covered with soils when compared to stems and leaves. Therefore, its major function in the uptake of nutrients and pollutant are all manifest due to its physiological feature of this organ. Moreover, Street et al. (2009) found the highest levels of Cd in the roots when compared to bulbs and leaves after being exposed to 2.5, 5 and 10 mg Cd/L. Thus, our finding is supported by Street et al. (2009)'s finding.

The redistribution of Cd and Cu in the Treatment D is very interesting from ecotoxicological point of view. These results indicated that when *C. asiatica* was planted in metal-polluted soils and this could redistribute the metals among roots, stem and leaves of *C. asiatica*. Similar phenomenon was found for the mussel *Perna viridis* collected from metal-polluted sites (Yap et al. 2006). Thus, this preliminary study should merit further studies since metal redistribution could be potentially established as an indicator of heavy metal pollution.

The absorption and accumulation of heavy metals in plant tissues depend upon many factors, such as temperature, moisture, organic matter, pH, and nutrient availability. The treatments with contaminated soil serve as carriers of micronutrients. These contaminated soils might contain organic complexing molecules of low molecular weights and they have been shown to increase heavy metal uptake (Chen & Aviad 1990), whereas the presence of organic matter has been reported to increase the uptake of Zn in the wheat plant (Rupa et al. 2003).

The high potential of metal accumulation by *C. asiatica* in the polluted soils indicated that the pegaga is a good source for bioaccumulation of heavy metals and therefore it is good for phytoremediation (Barthwal et al. 2008), besides being a good biomonitoring plant. On the other hand, since it is a medicinal plant, this preliminary finding should arise a great concern about heavy metal contamination of heavy metal contamination of herbal raw material using *C. asiatica*.

CONCLUSION

It can be concluded that the roots of *C. asiatica* tend to bind more heavy metals followed by the stems and leaves. The close relationships of metal concentration in the contaminated soils with the three different parts of *C. asiatica* indicated that the roots, stems and leaves of *C. asiatica* are capable of accumulating heavy metals. This study using *C. asiatica* as a biomonitor provides important baseline information which can be used to monitor changes in heavy metal contamination and bioavailability. Further studies by using the plant parts and their growth for biomonitoring work are useful and should be conducted regularly.

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