UNIVERSITI PUTRA MALAYSIA

EFFECTS OF HEAVY METAL ACCUMULATION ON BIOLOGICAL ACTIVITIES AND GENETIC VARIATION OF *CENTELLA ASIATICA* (L.) URBAN

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By

ONG GHIM HOCK

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Doctor of Philosophy

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EFFECTS OF HEAVY METAL ACCUMULATION ON BIOLOGICAL ACTIVITIES AND GENETIC VARIATION OF *CENTELLA ASIATICA* (L.) URBAN

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Chairman : Associate Professor Yap Chee Kong, PhD

Faculty : Science

Trace metals have become a main concern nowadays because these metals may be transferred and accumulated in the body of animals or human beings through the food chain. Trace metals are toxic to the biota at high bioavailabilities, hence trace metal contamination in medicinal plants should be monitored to ensure the safety of consumers. *Centella asiatica* is sedentary, abundant, easily to be identified, large enough for analysis, has the potential to reflect bioavailability and tolerant to the raised of metal bioavailability in the environment. Hence, it is suitable to be used as a biomonitor. The first objective was to determine trace metals accumulation of *C. asiatica* collected from 16 sampling sites from Peninsular Malaysia. The metals determined included As, Al, Cd, Co, Cr, Zn, Ba, Br, Ca, Ce, Cs, Cu, Dy, Eu, Fe, Ga, Hf, K, La, Lu, Mg, Mn, Na, Ni, Pb, Rb, Sc, Sm, Ta, Tb, Th, Ti, U, V, Yb, Zn, Zr by

air-acetylene flame Atomic Absorption Spectrophotometer (AAS) and Instrument Neutron Activation Analysis (INAA). The second objective was to determine the relationship between metal accumulations and antioxidative activities in *C. asiatica* from 16 sampling sites from Peninsular Malaysia. The third objective was to determine the tolerance and toxicities of Pb, Cu and Zn to *C. asiatica* and their metal effects to the antioxidative levels under laboratory conditions. The fourth objective was to assess the accumulation of trace metals by using transplanted *C. asiatica* between control and semi-polluted or polluted sites. Lastly, the objective was to determine the relationships between genetic variations and metal concentrations based on polluted and clean populations of *C. asiatica* from the transplantation study. According to the results, the metal accumulations were highest in roots followed by leaves and stems. This study revealed positive and significant correlations ($P<0.05$) between plant (leaves, stems and roots) and soil for such as Ba, Br, Ca, Ce, Cs, Ga, Hf, K, La, Mg, Mn, Na, Rb, Sb, Ta, Th, U, V, Yb, Zn and Zr. It was found that only a few metals showed significant correlation between metals and antioxidative enzymes in leaves of *C. asiatica* including Ca, Cd, Ce, Cu, Hf, K, La, Mg, Mn, Ni, Pb, Rb, Ta and Zn. For the toxicity studies, the leaves, stems and roots were significantly correlated with one another for Cu, Pb and Zn. It was found that Zn correlated significantly with all antioxidative enzymes in leaves. Pb was correlated significantly with Catalases (CAT) but Cu did not show any correlation with antioxidative enzymes in leaves. In roots, Zn showed significant correlations with Guaiacol peroxidise (GPX) and Ascorbate peroxidise (APX) while Cu showed significant

correlations with Superoxide dismutase (SOD) and GPX. For the transplantation study, the accumulation of metals increased for all parts when transplanted from control to semi-polluted or polluted sites under field conditions (week 0 to week 3). However, the accumulation decreased (week 3 to week 6) after transplantation from the semi-polluted and polluted sites back to the control sites. Higher levels of metals were found in the back-transplanted plant in week 6 than in week 0 in that they were far from reaching the initial metals concentration (week 0). The findings of the present study indicated that the leaves, stems and roots of *C. asiatica* are good biomonitors of trace metal contaminations. For the transplantation study under field and laboratory conditions, the dendrogram divided the populations into two major groups. UPM week 3 and UPM week 6 were grouped in a major cluster, whereas Juru week 3, Juru week 6, Balakong week 3, Balakong week 6, SK week 3 and SK week 6 were grouped in another major cluster. From the present results, it was found that correlations of metal accumulations between *C. asiatica* and genetic variations occurred. The findings of the present study indicated that the leaves, stems and roots of *C. asiatica* are good biomonitors of metal contaminations. The present data are the results of the most comprehensive study done on terrestrial metal levels in Malaysia.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

KESAN PENGUMPULAN LOGAM BERAT TERHADAP AKTIVITI BIOLOGI DAN VARIASI GENETIK KE ATAS *CENTELLA ASIATICA* (L.) URBAN

Oleh

ONG GHIM HOCK

Disember 2012

Pengerusi : Profesor Madya Yap Chee Kong, PhD

Faculti : Sains

Logam surih telah menjadi kebimbangan utama pada masa kini kerana logam ini boleh dipindahkan dan terkumpul di dalam badan haiwan atau manusia melalui rantai makanan. Logam surih toksik kepada biota pada ketersediaan yang tinggi, oleh itu pencemaran logam surih dalam tumbuhan perubatan perlu dipantau untuk memastikan keselamatan pengguna. *Centella asiatica* adalah sedentari, mudah didapati, mudah untuk dikenal pasti, cukup besar untuk analisis, mempunyai potensi untuk mencerminkan bioavailabiliti dan toleran kepada bioavailabiliti logam dalam persekitaran yang meningkat. Oleh itu, ia adalah sesuai digunakan sebagai biomonitor. Objektif pertama adalah untuk menentukan pengumpulan logam surih dalam *C. asiatica* yang dikumpul dari 16 kawasan persampelan dari Semenanjung Malaysia. Logam surih termasuk As, Al, Cd, Co, Cr, Zn, Ba, Br, Ca, Ce, Cs, Cu, Dy, Eu, Fe, Ga, Hf, K, La, Lu, Mg, Mn, Na, Ni, Pb, Rb, Sc, Sm, Ta, TB, Th, Ti, U, V, Yb,

Zn dan Zr ditentukan dengan "Air-acetylene flame atomic absorption spectrophotometer" (AAS) dan "Instrument Neutron Activation Analysis" (INAA). Objektif kedua adalah untuk menentukan hubungan antara pengumpulan logam dan aktiviti antioksida dalam *C. asiatica* daripada 16 tapak pensampelan dari Semenanjung Malaysia. Objektif ketiga ialah untuk menentukan toleransi dan tahap toksik Pb, Cu dan Zn kepada *C. asiatica* dan kesan logam tersebut kepada tahap antioksida di dalam keadaan makmal. Objektif keempat adalah untuk menilai pengumpulan logam surih dengan menggunakan pemindahan *C. asiatica* antara kawalan dan lapangan separa tercemar atau tercemar. Objektif terakhir adalah untuk menentukan hubungan antara variasi genetik dan kepekatan logam berdasarkan lokasi tercemar dan bersih dalam *C. asiatica* daripada kajian pemindahan. Menurut keputusan kajian, pengumpulan logam yang tertinggi di dalam akar dan ini diikuti oleh daun dan batang. Kajian ini menunjukkan hubungan yang positif dan signifikan ($P < 0.05$) antara tumbuhan (daun, batang dan akar) dan tanah bagi logam seperti Ba, Br, Ca, Ce, Cs, Ga, Hf, K, La, Mg, Mn, Na, Rb, Sb, Ta, Th, U, V, Yb, Zn dan Zr. Ia didapati bahawa hanya beberapa logam yang menunjukkan hubungan korelasi yang signifikan antara logam dan enzim antioksida dalam daun *C. asiatica* termasuk Ca, Cd, Ce, Cu, Hf, K, La, Mg, Mn, Ni, Pb, Rb, Ta dan Zn . Bagi kajian ketoksikan, daun, batang dan akar menunjukkan korelasi ketara antara satu sama lain bagi Cu, Pb dan Zn. Ini mendapati bahawa Zn kolerasi dengan semua enzim antioksida dalam daun. Pb telah dikaitkan ketara dengan Catalases (CAT) tetapi Cu tidak menunjukkan apa-apa kaitan dengan enzim antioksida dalam daun. Di dalam akar, Zn telah

menunjukkan korelasi yang signifikan dengan Guaiacol peroxidise (GPX) dan Ascorbate peroxidise (APX) manakala Cu menunjukkan hubungan yang signifikan dengan Superoxide dismutase (SOD) dan GPX. Untuk kajian pemindahan, pengumpulan logam meningkat untuk semua bahagian apabila dipindahkan daripada lokasi kawalan ke lokasi separa tercemar atau tercemar di bawah keadaan lapangan (minggu 0 ke 3 minggu). Walau bagaimanapun, pengumpulan menurun (minggu 3 ke 6 minggu) selepas pemindahan dari lokasi yang separa tercemar atau tercemar kembali ke lokasi kawalan. Tahap logam yang lebih tinggi telah ditemui di dalam tumbuhan untuk pemindahan balik pada minggu 6 berbanding dengan minggu 0 dan mereka adalah jauh daripada mencapai kepekatan logam awal (minggu 0). Dapatan kajian ini menunjukkan bahawa daun, batang dan akar *C. asiatica* adalah biomonitors yang baik bagi pencemaran logam surih. Untuk kajian pemindahan di bawah keadaan lapangan dan keadaan makmal, dendrogram membahagikan lokasi kepada dua kumpulan utama. UPM minggu 3 dan UPM minggu 6 telah dikumpulkan dalam kelompok utama, manakala Juru minggu 3, Juru minggu 6, Balakong minggu 3, Balakong minggu 6, SK minggu 3 dan SK minggu 6 telah dikumpulkan dalam kelompok utama yang lain. Dari keputusan keseluruhan, ia telah didapati bahawa korelasi pengumpulan logam antara *C. asiatica* dan perubahan genetik berlaku. Dapatan kajian ini menunjukkan bahawa daun, batang dan akar *C. asiatica* adalah biomonitors yang baik bagi pencemaran logam. Data pada masa ini adalah hasil kajian yang paling menyeluruh dilakukan ke atas tahap logam daratan di Malaysia.

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I certify that a Thesis Examination Committee has met on 13 December 2012 to conduct the final examination of Ong Ghim Hock on his thesis entitled "Effects of Heavy Metal Accumulation on Biological Activities and Genetic Variation of *Centella asiatica* (L.) Urban" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Doctor of Philosophy degree.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

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DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.

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ONG GHIM HOCK

Date: 13 December 2012

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