ASSESSING POTENTIAL FOR HEAVY METAL PHYTOREMEDIATION IN Clinacanthus nutans

NURUL AISYAH BINTI MOHD SAPRI

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ASSESSING POTENTIAL FOR HEAVY METAL PHYTOREMEDIATION IN *Clinacanthus nutans*

By

NURUL AISYAH BINTI MOHD SAPRI

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DEDICATION

I would like to dedicate this thesis to my beloved family who always be my greatest inspiration and people who are supporting and encouraging me during this entire study period.

ABSTRACT

Heavy metals pollution on soil derived from population growth and industrialization poses serious threat to the living things. Phytoremediation which is plant based soil remediation is preferable due to effective cost and minimal side effect on the environment. The objectives of this study were to determine the effectiveness of *Clinacanthus nutans* in removing heavy metal in contaminated soil and to evaluate uptake and translocation of heavy metal in plant parts especially in root, stem and leaves. *Clinacanthus nutans* were planted on ten different treatments level of growth media – T_0 control (1 kg soil), T_1 (1 kg soil + 100 ppm $CuSO_4$), T_2 (1 kg soil + 200 ppm $CuSO_4$), T_3 (1 kg soil + 300 ppm $CuSO_4$),T_4 (1 kg soil + 100 ppm $FeSO_4$),T_5 (1 kg soil + 200 ppm $FeSO_4$), T_6 (1 kg soil + 300 ppm $FeSO_4$),T_7 (1 kg soil + 100 ppm $ZnSO_4$),T_8 (1 kg soil + 200 ppm $ZnSO_4$), and T_9 (1 kg soil + 300 ppm $ZnSO_4$). Plant height and number of leaves were recorded every two week. Plant biomass was measured according to plant parts (leaves, stems, roots) and heavy metal concentrations of plant and soil samples were analysed using Atomic Absorption Spectrophotometry (AAS). Heavy metal concentrations of Cu, Fe and Zn in growth medium after harvest were ranged from 0.23 to 2.19, 579.55 to 697.45 and 2.26 to 28.55 mg/kg, respectively. Among the plant parts, roots of *Clinacanthus nutans* were the most efficient plant parts that capable to accumulate Cu, Fe and Zn. The highest Cu concentrations in plant parts found in T_3 with value 3.29 mg/kg, while for Fe and Zn, the highest concentrations was recorded in T_6 and T_9 with value 1008.97 mg/kg and 41.38 mg/kg, respectively. Bioconcentration factor (BCF) and Translocation factor (TF) of Cu, Fe and Zn showed that BCF value was lower than 1 while TF value was greater than 1 indicating that *Clinacanthus nutans* is capable to translocate metals from roots to shoots and this study confirms that this species as a phytoextractor plant.

ABSTRAK

Pencemaran logam berat pada tanah yang berasal dari peningkatan populasi dan pengindustrian menimbulkan ancaman serius kepada benda hidup. Fitoremediasi yang merupakan pemulihan tanah berasaskan tumbuhan adalah lebih baik kerana kos efektif dan kesan sampingan yang minimum terhadap alam sekitar. Objektif kajian ini adalah untuk menentukan keberkesanan pokok belalai gajah (*Clinacanthus nutans)* dalam menghilangkan logam berat di tanah yang tercemar dan menilai pengambilan dan pengalihan logam berat di bahagian pokok terutamanya di akar, batang dan daun. *Clinacanthus nutans* ditanam pada sepuluh jenis media berbeza - Kawalan T_0 (1 kg tanah), T_1 (1 kg tanah + 100 ppm CuSO4), T_2 (1 kg tanah + 200 ppm CuSO4), T_3 (1 kg tanah + 300 ppm CuSO4), T_4 (1 kg tanah + 100 ppm FeSO4), T_5 (1 kg tanah + 200 ppm FeSO4), T_6 (1 kg tanah + 300 ppm FeSO4), T_7 (10 kg tanah + 100 ppm ZnSO4), T_8 (1 kg tanah + 200 ppm ZnSO4), dan T_9 (1 kg tanah + 300ppm ZnSO4). Ketinggian tumbuhan dan bilangan daun dicatatkan setiap dua minggu. Biojisim tumbuhan diukur mengikut bahagian tumbuhan (daun, batang, akar) dan kepekatan logam berat pada sampel tumbuhan dan tanah dianalisis dengan menggunakan mesin Atomic Absorption Spectrophotometer (AAS). Kepekatan logam berat Cu, Fe dan Zn dalam medium pertumbuhan selepas menuai adalah berkisar antara 0.23 hingga 2.19, 579.55 hingga 697.45 dan 2.26 hingga 28.55 mg/ kg. Antara bahagian pokok, akar *Clinacanthus nutans* adalah bahagian tumbuhan yang paling cekap yang mampu mengumpul Cu, Fe dan Zn. Kepekatan Cu tertinggi dalam bahagian tumbuhan yang terdapat di T_3 dengan nilai 3.29 mg/kg, manakala untuk Fe dan Zn, kepekatan tertinggi dicatatkan pada T_6 dan T_9 dengan nilai 1008.97 mg/kg dan 41.38 mg/kg, masing-masing. Faktor biokonsentrasi (BCF) dan faktor translokasi (TF) Cu, Fe dan Zn menunjukkan bahawa nilai BCF lebih rendah daripada 1 manakala nilai TF lebih besar daripada 1 menunjukkan bahawa *Clinacanthus nutans* mampu mengalihkan logam dari akar ke pucuk dan kajian ini mengesahkan bahawa spesies ini sebagai tumbuhan pengekstrak**.**

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APPROVAL SHEET

I certify that this research project report entitled “Assessing Potential for Heavy Metal Phytoremediation in *Clinacanthus nutans*” by Nurul Aisyah Binti Mohd Sapri has been examined and approved as a partial fulfilment of the requirements for the Degree of Bachelor of Forestry Science in the Faculty of Forestry, Universiti Putra Malaysia.

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Assoc. Prof. Dr. Arifin Abdu
Faculty of Forestry
Universiti Putra Malaysia
(Supervisor)

_____

Prof. Dr. Mohamed Zakaria Bin Hussin
Dean
Faculty of Forestry
Universiti Putra Malaysia

Date: 5 January 2018

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LIST OF ABBREVIATIONS

AA	AutoAnalyzer
AAS	Atomic Absorption Spectrophotometer
Ag	Silver
ANOVA	Analysis of Variance
As	Arsenic
BCF	Bioconcentration Factor
Cd	Cadmium
Cr	Chromium
Cu	Copper
FAO	Food and Agriculture Organization
Fe	Iron
G	Gram
Hg	Mercury
K	Potassium
Kg	Kilogram
M	Meter
Mg	Milligram
Mn	Manganese
Na	Sodium
Ni	Nickel
P	Phosphorus
Pb	Lead
pH	Acidity in water
Ppm	Parts per million
Se	Selenium
TF	Translocation Factor
TPU	Taman Pertanian Universiti
UPM	Universiti Putra Malaysia
Zn	Zinc

CHAPTER 1

INTRODUCTION

1.1 General Background

Environmental pollution by heavy metals has become a serious threat in the world. Development in industrialization, agriculture and disturbance of natural ecosystem has led to dissemination of these elements into the soil. Heavy metals are different from organic pollution as they are non-biodegradable in which they are unable to be broken down by microorganism and therefore they become persistent in the environment. The chemical degradation of heavy metal is widely known as impossible to be done and these elements can only be physically removed or transformed into nontoxic compounds. The accumulation and exposure of these heavy metals into the environment through various pathways create high risk to environmental and human health. For example, cadmium toxicity problem in Japan that was due to cadmium contaminated irrigation leads to skeletal problem (Jarup, 2003). While soil grows on contaminated soil appear to shows reduction in growth, performance, and yield.

There are studies reported that the accidental release and dumping of heavy metal contribute to the dissemination of the pollutant into nearest area. These pollutants are known to be accumulated in food cycle and contribute to serious health problem to the living organism and environment. Contamination of heavy metal is considered as the one high risk contamination that contributes to the harmful effect to the soil and water

resource as well as human health (Yoon *et al*., 2006). Even though heavy metal can become pollutant to the living organism, but living organism also require heavy metal such as Co, Cu, Fe, Mn, Mo, Ni, V, and Zn in considerable amount. However, excessive amount of these heavy metal can be damaging to the organism. While other heavy metal such as Pb, Cd, Hg, and As are toxic metal that do not have beneficial functional on the organism and the uptake of these metal can cause high risk to the living organism.

Conventionally, there are many remediation techniques are being recognised that can be used to degrade and decontaminate contaminated soil such as solidification/stabilization, vitrification, soil washing, pyrometallurgical extraction, in situ soil flushing, and electrokinetic treatment (Wuana & Okieimen, 2011). However, these methods have the disadvantages of being very expensive and require intensive workers. These techniques also change and disturb the soil properties and soil structure and having risk of secondary contaminations (Ali *et al*., 2013). Moreover, these techniques applicable at highly contaminated area but not in large area. Therefore, it is necessary for the researchers to identify cost effective and environmental friendly remediation techniques that will help to decontaminate heavy metal polluted soils. Instead of using conventional remediation in removing pollutants from soils, water or air in cleaning up the vast majority of polluted breaking down organic or inorganic substances in the sites, researchers came out with ideas of using plants to overcome this environmental problem.

Among the remediation technique stated above, phytoremediation have been recognised as a green strategy that has potential to help in heavy metal pollution problem. Phytoremediation can be describe as a plant-based technology that use vegetation to clean up the contaminated soil, water and sediments (Tangahu *et al*., 2011). In phytoremediation, plants will be planted on contaminated soil and they will remove the pollutant in contaminated soil by detoxifying the contaminated soil or change physical and chemical characteristics of the toxic in the soil. Thus, the contaminants become invisible to the environment and living things (Cunningham, 1994). This plant-based soil remediation approach is very interesting as it is cost effective and socially accepted compared to conventional remediation technologies. In addition, this approach also helps to reduce the potential of the soil and nutrients to drain away by planting plants on the contaminated site (Chaudhry *et al*., 1998).

1.2 Problem Statement

Malaysia is a developing country with rapid urbanisation and industrialisation among urbanised countries in East Asia. Fast growing of industrialization and rapid increase of population contribute to various environmental problems such as disposal of pollutant in the river and ocean. Shazili *et al.,* (2006) reported that highly polluted rivers in Malaysia that caused by industrialization, effluent, and runoff found to be highly polluted with Pb, Zn, and Cu.

Increasing population derived from industrialization, urbanization and human activities caused serious soil heavy metal pollution. For example, human activities such as excessive use of fertilizers and pesticides, sewage irrigation, and discharge of waste affected soil environment significantly. Soil becomes polluted and excess of heavy metal accumulation in soils is toxic to humans and other animals.

Recent remediation technique to tackle soil pollutant have been investigated but these technique face many limitations such as being in high cost, high number of labour, disturbance to the soil structure and leading to secondary contamination. A promising cost effective technology which is phytoremediation that able to sequester the pollutants from the contaminated soil have been identified and socially accepted by the public. However, success of this approach mainly depends on the species selection that able to accumulate heavy metal from the soil. There are several factors of species selection that has to be taking into consideration to ensure phytoremediation technique success (Chibuike & Obiora, 2014).

In this present study, *Clinacanthus nutans* which was considered also as medicinal plants was selected for evaluation of heavy metal phytoremediation. Nowadays, apart from their traditional uses, the roles of medicinal plants have been discovered in many field including pharmaceuticals, food, cosmetics and ornaments. Despite of human health risk, researchers have developed research and study on medicinal plants as agents towards heavy metal uptake.

1.3 Objective

Numerous studies of phytoremediation on contaminated soils has been done by using shrub, native species and woody tree but phytoremediation using medicinal species is not commonly investigated. Hence, this study was conducted with the aim to investigate phytoremediation of contaminated soil by using medicinal species. The objectives of this study were:

1) To determine the effectiveness of *Clinacanthus nutans* in removing heavy metal in contaminated soil

2) To evaluate uptake and translocation of heavy metal in plant parts especially in root, stem and shoot.

REFERENCES

Ahmadpour, P., Ahmadpour, F., Mahmud, T. M. M., Abdu, A., Soleimani, M., & Tayefeh, F. H. (2012). Phytoremediation of heavy metals: A green technology. *African Journal of Biotechnology*, *11*(76), 14036-14043.

Ahmadpour, P., Nawi, A. M., Abdu, A., Abdul-Hamid, H., Karam, D. S., Hassan, A., Majid, N. M., Jusop, S. (2010). Uptake of heavy metals by *Jatropha curcas* L. planted in soils containing sewage sludge. *American Journal of Applied Sciences*, *7*(10), 1291-1299.

Ali, H., Khan, E., & Sajad, M. A. (2013). Phytoremediation of heavy metals—concepts and applications. *Chemosphere*, *91*(7), 869-881.

Baker, A. J. (1981). Accumulators and excluders-strategies in the response of plants to heavy metals. *Journal of Plant Nutrition*, *3*(1-4), 643-654.

Baker, Alan & R. Brooks, R. (1989). Terrestrial Higher Plants which Hyperaccumulate Metallic Elements –A Review of their Distribution, Ecology and Phytochemistry. *Biorecovery*, *1*, 81-126.

Bang, J. (2002). Dissolution of soil heavy metal contaminants as affected by pH and redox potential. North Carolina State University, United States.

Bennett, L. E., Burkhead, J. L., Hale, K. L., Terry, N., Pilon, M., & Pilon-Smits, E. A. (2003). Analysis of transgenic Indian mustard plants for phytoremediation of metal-contaminated mine tailings. *Journal of Environmental Quality*, *32*(2), 432-440.

Chaney, R. L., Malik, M., Li, Y. M., Brown, S. L., Brewer, E. P., Angle, J. S., & Baker, A. J. (1997). Phytoremediation of soil metals. *Current Opinion in Biotechnology*, *8*(3), 279-284.

Chaudhry, T. M., Hayes, W. J., Khan, A. G., & Khoo, C. S. (1998). Phytoremediation- focusing on accumulator plants that remediate metal-contaminated soils. *Australasian Journal of Ecotoxicology*, *4*(1), 37-51.

Chibuike, G. U., & Obiora, S. C. (2014). Heavy metal polluted soils: effect on plants and bioremediation methods. *Applied and Environmental Soil Science*, *2014*(2014), 1-12.

Chuan, M. C., Shu, G. Y., & Liu, J. C. (1996). Solubility of heavy metals in a contaminated soil: effects of redox potential and pH. *Water, Air, & Soil Pollution*, *90*(3), 543-556.

Cobbett, C. (2003). Heavy metals and plants–model systems and hyperaccumulators. *New Phytologist*, *159*(2), 289-293.

Cunningham, S. D., & Ow, D. W. (1996). Promises and prospects of phytoremediation. *Plant Physiology*, *110*(3), 715.

Dayang, S. N., & Fauziah, I. C. (2013). Soil factors influencing heavy metal concentrations in medicinal plants. *Pertanika Journal of Tropical Agricultural Science*, *36*(2), 161-178.

Duruibe, J. O., Ogwuegbu, M. O. C., & Egwurugwu, J. N. (2007). Heavy metal pollution and human biotoxic effects. *International Journal of Physical Sciences*, *2*(5), 112-118.

Eriksson, J. E. (1989). The influence of pH, soil type and time on adsorbtion and uptake by plants of Cd added to the soil. *Water, Air, and Soil Pollution*, *48*(3-4), 317-335.

Evanko, C. R., & Dzombak, D. A. (1997). Remediation of metals-contaminated soils and groundwater. Pittsburg: Ground-Water Remediation Technologies Analysis Center.

Garbisu, C., & Alkorta, I. (2001). Phytoextraction: a cost-effective plant-based technology for the removal of metals from the environment. *Bioresource Technology*, *77*(3), 229-236.

Goldhaber, S. B. (2003). Trace element risk assessment: essentiality vs. toxicity. *Regulatory Toxicology and Pharmacology*, *38*(2), 232-242.

Greaney, K. M. (2005). An assessment of heavy metal contamination in the marine sediments of Las Perlas Archipelago, Gulf of Panama. Edinburgh.School of Life Sciences Heriot-Watt University.

Grifferty, A., & Barrington, S. (2000). Zinc uptake by young wheat plants under two transpiration regimes. *Journal of Environmental Quality*, *29*(2), 443-446.

Horton, S., & Ross, J. (2003). The economics of iron deficiency. *Food Policy*, *28*(1), 51-75.

Järup, L. (2003). Hazards of heavy metal contamination. *British Medical Bulletin*, *68*(1), 167-182.

Justin, V., Majid, N. M., Islam, M. M., & Abdu, A. (2011). Assessment of heavy metal uptake and translocation in Acacia mangium for phytoremediation of cadmium contaminated soil. *Journal of Food, Agriculture & Environment* , *9*(2), 588-592.

Khan, S., Cao, Q., Zheng, Y. M., Huang, Y. Z., & Zhu, Y. G. (2008). Health risks of heavy metals in contaminated soils and food crops irrigated with wastewater in Beijing, China. *Environmental Pollution*, *152*(3), 686-692.

Khanif, Y. M., & Salmijah, S. (1996). Contaminants and the soil environment in Malaysia. In *Contaminants and the Soil Environment in the Australasia-Pacific Region* (pp. 563-578).Dordrecht: Springer

Klassen, S. P., McLean, J. E., Grossl, P. R., & Sims, R. C. (2000). Fate and behavior of lead in soils planted with metal-resistant species (river birch and smallwing sedge). *Journal of Environmental Quality*, *29*(6), 1826-1834.

Lin, Z. Q., & Terry, N. (1999). 4 Remediation of Selenium-Polluted Soils and Waters by Phytovolatilization. In N. Terry, & G. S. Banuelos (Ed), Phytoremediation of Contaminated Soil and Water (pp. 61-78). Florida: Lewis Publisher.

Liu, Y., Su, C., Zhang, H., Li, X., & Pei, J. (2014). Interaction of soil heavy metal pollution with industrialisation and the landscape pattern in Taiyuan city, China. *PloS one*, *9*(9), e105798.

Majid, N. M., Islam, M. M., Nap, M. E., Ghafoori, M., & Abdu, A. (2012). Heavy metal uptake and translocation by *Justicia gendarussa* Burm F. from textile sludge contaminated soil. *Acta Agriculturae Scandinavica, Section B-Soil & Plant Science*, *62*(2), 101-108.

Manan, F. A., Tsun-Thai, C., Samad, A. A., & Mamat, D. D. (2015). Evaluation of the phytoremediation potential of two medicinal plants. *Sains Malaysiana*, *44*(4), 503-509.

Muchuweti, M., Birkett, J. W., Chinyanga, E., Zvauya, R., Scrimshaw, M. D., & Lester, J. N. (2006). Heavy metal content of vegetables irrigated with mixtures of wastewater and sewage sludge in Zimbabwe: implications for human health. *Agriculture, Ecosystems & Environment*, *112*(1), 41-48.

Nagajyoti, P. C., Lee, K. D., & Sreekanth, T. V. M. (2010). Heavy metals, occurrence and toxicity for plants: a review. *Environmental Chemistry Letters*, *8*(3), 199-216.

Pickering, K. T., & Owen, L. A. (1997). *Water resources and pollution. An Introduction to Global Environmental,*(2nd ed.). London: Psychology Press
Pilon-Smits, E. (2005). Phytoremediation. *Annual Review Plant Biology*, *56*, 15-39.

Plum, L. M., Rink, L., & Haase, H. (2010). The essential toxin: impact of zinc on human health. *International Journal of Environmental Research and Public Health*, *7*(4), 1342-1365.

Poschenrieder, C., & i Coll, J. B. (2003). Phytoremediation: principles and perspectives. *Contributions to Science*, *2*(3), 333-344.

Rajoo, K. S., Abdu, A., Abdul-Hamid, H., Karam, D. S., Jusop, S., Jamaluddin, A. S., & Zhen, W. W. (2013). Assessment of heavy metals uptake and translocation by *Aquilaria malaccensis* planted in soils containing sewage sludge. *American Journal of Applied Sciences*, *10*(9), 952-964.

Reichman, S. M. (2002). *The Responses of Plants to Metal Toxicity: A Review Forusing on Copper, Manganese & Zinc*(pp. 22-26). Melbourne: Australian Minerals & Energy Environment Foundation.

Sahrawat, K. L. (2005). Iron toxicity in wetland rice and the role of other nutrients. *Journal of Plant Nutrition*, *27*(8), 1471-1504.

Salem, H. M., Eweida, E. A., & Farag, A. (2000). Heavy metals in drinking water and their environmental impact on human health. *ICEHM, 2000,* 542-556.

Salt, D. E., Smith, R. D., & Raskin, I. (1998). Phytoremediation. *Annual Review of Plant Biology*, *49*(1), 643-668.

Sarma, H. (2011). Metal hyperaccumulation in plants: a review focusing on phytoremediation technology. *Journal of Environmental Science and Technology*, *4*(2), 118-138.

Sarwar, N., Imran, M., Shaheen, M. R., Ishaq, W., Kamran, A., Matloob, A., Rehim, A., & Hussain, S. (2016). Phytoremediation strategies for soils contaminated with heavy metals: Modifications and future perspectives. *Chemosphere*, *171* (2017), 710-721.

Seth, C. S. (2012). A review on mechanisms of plant tolerance and role of transgenic plants in environmental clean-up. *The Botanical Review*, *78*(1), 32-62.

Shabani, N., & Sayadi, M. H. (2012). Evaluation of heavy metals accumulation by two emergent macrophytes from the polluted soil: an experimental study. *The Environmentalist*, *32*(1), 91-98.

Shazili, N. A. M., Yunus, K., Ahmad, A. S., Abdullah, N., & Rashid, M. K. A. (2006). Heavy metal pollution status in the Malaysian aquatic environment. *Aquatic Ecosystem Health & Management*, *9*(2), 137-145.

Shim, S. Y., Aziana, I., & Khoo, B. Y. (2013). Perspective and insight on Clinacanthus nutans Lindau in traditional medicine. *International Journal of Integrative Biology*, *14*(1), 7-9.

Tangahu, B. V., Sheikh Abdullah, S. R., Basri, H., Idris, M., Anuar, N., & Mukhlisin, M. (2011). A Review on heavy metals (As, Pb, and Hg) uptake by plants through phytoremediation. *International Journal of Chemical Engineering*, *2011(*2011), 31.

Van Aken, B. (2009). Transgenic plants for enhanced phytoremediation of toxic explosives. *Current Opinion in Biotechnology*, *20*(2), 231-236.

Wanikiat, P., Panthong, A., Sujayanon, P., Yoosook, C., Rossi, A. G., & Reutrakul, V. (2008). The anti-inflammatory effects and the inhibition of neutrophil responsiveness by Barleria lupulina and Clinacanthus nutans extracts. *Journal of Ethnopharmacology*, *116*(2), 234-244.

Wei, S., da Silva, J. A. T., & Zhou, Q. (2008). Agro-improving method of phytoextracting heavy metal contaminated soil. *Journal of Hazardous Materials*, *150*(3), 662-668.

World Health Organization. (1996). *Trace elements in human nutrition and health*. Geneva: World Health Organization.

Wuana, R. A., & Okieimen, F. E. (2011). Heavy metals in contaminated soils: a review of sources, chemistry, risks and best available strategies for remediation. *International Scholarly Research Network Ecology*, *2011*, 1-20.

Yap, C. K., Fitri, M., Mazyhar, Y., & Tan, S. G. (2010). Effects of Metal contaminated soils on the accumulation of heavy metals in different parts of *Centella asiatica*: A Laboratory Study. *Sains Malaysiana*, *39*(3), 347-352.

Yoon, J., Cao, X., Zhou, Q., & Ma, L. Q. (2006). Accumulation of Pb, Cu, and Zn in native plants growing on a contaminated Florida site. *Science of the Total Environment*, *368*(2), 456-464.

Zarcinas, B. A., Ishak, C. F., McLaughlin, M. J., & Cozens, G. (2004). Heavy metals in soils and crops in Southeast Asia. *Environmental Geochemistry and Health*, *26*(3), 343-357.