

EVALUATION ON THE POTENTIAL OF Aquilaria malaccensis FOR HEAVY METALS PHYTOREMEDIATION IN CONTAMINATED SOIL

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EVALUATION ON THE POTENTIAL OF *Aquilaria malaccensis* FOR HEAVY METALS PHYTOREMEDIATION IN CONTAMINATED SOIL



By

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DEDICATION

Dedicated to my beloved family and the people who are special to me for supporting and inspiring me during my entire study period. 'Thank you for all the supports, helps, kindness and love, I really appreciate it'

ABSTRACT

Phytoremediation is one of the methods that can help reduce pollution by absorbing heavy metals through plant roots. A study was conducted at the nursery of Faculty of Forestry UPM to evaluate the potential of Karas (Aquilaria malaccensis) as a phytoremediator species. The objectives of this study were : (i) to determine the effectiveness of *A. malaccensis* for taking up heavy metals from contaminated soil after harvesting and (ii) to evaluate the potential A. malaccensis as a phytoremediator plant in taking up heavy metals and translocate heavy metal elements in plant parts (leaves, stems, roots) by using an indicator of Translocation Factor (TF) and Bio-concentration Factor (BCF). A. malaccensis saplings were planted in growth media applied with Zn, Cu and Fe. The treatments were T_0 = control, T_1 =100ppm of Zn, T_2 =200ppm of Zn, T₃=300ppm of Zn, T₄=100ppm of Cu, T₅=200ppm of Cu, T₆=300ppm of Cu, T₇=100ppm of Fe, T₈=200ppm of Fe and T₉=300ppm of Fe. Each type of treatments was replicated for four times. The highest growth of A. malaccensis was recorded for the T₉ growth media. The highest concentration of Zn in the roots of the A. malaccensis plant was in the T3 growth media (14.49mg/kg). The highest accumulation of Fe (350.46 mg/kg) was recorded in the roots of A. malaccensis in the T₉ growth media, whereas the stem of the A. malaccensis in T₆ recorded the highest Cu accumulation (0.99mg/kg). The Transfer Factor (TF) values were more than 1 but the Bio-concentration Factor (BCF) values were less than 1 for Zn, Cu and Fe. The roots of A. malaccensis were ideal in uptaking and storing Fe, Cu and Zn. More studies need to be conducted, especially in field conditions, to optimize the potential of the A. malaccensis plant as a phytoremediator.

ABSTRAK

Fitoremediasi adalah salah satu kaedah yang boleh membantu mengurangkan pencemaran dengan menyerap logam berat melalui akar tumbuhan. Satu kajian telah dijalankan di tapak semaian Fakulti Perhutanan UPM untuk menilai potensi Karas (Aquilaria malaccensis) sebagai spesies phytoremediator. Objektif kajian ini adalah: (i) untuk menentukan keberkesanan A. malaccensis dalam menyerap logam berat dari tanah yang tercemar selepas penuaian dan (ii) untuk menilai potensi A. malaccensis sebagai tumbuhan phytoremediator dalam menyerap logam berat dan translokasi unsur logam berat di bahagian tumbuhan (daun, batang, akar) dengan menggunakan penunjuk faktor Translokasi (TF) dan faktor Biokepekatan (BCF). Anak-anak pokok A. malaccensis ditanam dalam media pertumbuhan yang dirawat dengan Zn, Cu dan Fe. Rawatan adalah To = kawalan, $T_1 = 100$ ppm Zn, $T_2 = 200$ ppm Zn, $T_3 = 300$ ppm Zn, $T_4 = 100$ ppm Cu, $T_5 = 200$ ppm Cu, $T_6 = 300$ ppm Cu, $T_7 = 100$ ppm Fe, = 200 ppm Fe dan $T_9 = 100$ 300ppm Fe. Setiap jenis rawatan direplikasi sebanyak empat kali. Pertumbuhan tertinggi A. malaccensis dicatatkan di media pertumbuhan T₉. Zn yang paling tinggi dalam akar tumbuhan A. malaccensis adalah dalam media pertumbuhan T₃ (14.49mg / kg). Pengumpulan tertinggi Fe (350.46 mg / kg) direkodkan dalam akar A. malaccensis dalam media pertumbuhan T₉, sedangkan batang A.malaccensis dalam T₆ mencatatkan pengumpulan Cu tertinggi (0.99mg / kg). Nilai Factor Translokasi (TF) adalah lebih daripada 1 tetapi nilai Faktor Biokepekatan (BCF) kurang dari 1 untuk Zn, Cu dan Fe. Akar A.malaccensis adalah ideal dalam mempercepatkan dan menyimpan Fe, Cu dan Zn. Lebih banyak kajian perlu dijalankan, terutamanya dalam keadaan lapangan, untuk mengoptimumkan potensi tanaman A.malaccensis sebagai phytoremediator.

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APPROVAL SHEET

I certify that this research project report entitled "Evaluation on the Potential of *Aquilaria malaccensis* for Heavy Metals Phytoremediation in Contaminated Soil" by Nurul Atikah binti Abu Bakar Hamzah 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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LIST OF ABBREVIATIONS



CHAPTER 1

INTRODUCTION

1.1 General Background

Rapid growing of agricultural activities and industrialization have resulted in increasing contamination of heavy metals in areas of land, surface waters and groundwater and gives rise to a serious concerns about the environment. According to Ali *et al.*, (2013), heavy metals are non-biodegradable that will accumulate in the environment and then it will contaminate the food chain. They also claimed that, some heavy metals are mutagenic, carcinogenic, teratogenic, endocrine disruptors and also can cause neurological and behavioral changes especially in children. Thus, this have been proven that heavy metals released into the environment has reached 22,000 tonnes for the cadmium (Cd), 939,000 tonnes of copper (Cu), 1,350,000 tonnes of zinc (Zn) and 738,000 tonnes of lead (Pb) (Singh *et al.*, 2003). Heavy metal contamination of soil is one of the most important environmental problems throughout the world.

Various human activities have drastically changed the heavy metals geochemical cycles and biochemical balance even they are actually natural constituents of the earth's crust. Exposure of heavy metals such as, copper, lead, iron, and zinc in long term can cause malicious wellbeing impacts in people (Singh *et al.*, 2011).

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Since heavy metals can cause bad impact in human health, remediation of heavy metals pollution need to be concerned. There are many physical and chemical remediation technique used but most of it are expensive, time consuming and environmentally destructive. Many government agencies preferred to use the technologies of cleanup of polluted soils with direct reduction of the total content of pollutants in the soil to the permissible level and another strategy is to manage the effects using technologies of containment that reduce the mobility and biological availability of pollutants (Kopstik, 2014). The existing method are vitrification, consolidation, electroremediation, soil washing and many more.

Phytoremediation is one of the technology that using live plants to cleaning up contaminated sites which is cost effective with long term applicability and has aesthetic advantages (Jadia & Fulekar, 2009). Recently, the potential tropical forest species *Aquilaria malaccensis* was identified as a potential phytoremediator because of it its high biomass which the character of phytoremediation species.

1.2 Problem Statement

Rapidly growing of industrial areas, mine tailings, disposal of high metal wastes, land application of fertilizers and many more human activities lead to accumulation of heavy metals that result is soil contaminated since soil are the major sink for heavy metals released into the environment because it cannot be degraded. Heavy metal contamination of soil may risks and

hazards to humans and the ecosystem. Some metals such as manganese, copper, zinc and nickel are important and beneficial to plants, and animals, but high concentrations all these metals have strong toxic effects and pose an environmental threat. Accumulation of heavy metals can reduce soil quality, reduce crop yield and the quality of agricultural products, and thus give negative impacts to the health of human, animals, and the ecosystem. The term heavy metal pollution refers to heavy metal levels that are abnormally high relative to normal background levels and the excessive deposition of toxic heavy metals in the soil caused by human activities. Conventional remedial technologies are highly cost and inhibit the soil fertility that can give negative impacts on the ecosystem. Phytoremediation is one of the organic method that is cost effective and environmental friendly. It is bioremediation process that uses various types of plants to remove, transfer, stabilize or destroy contaminants in the groundwater.

For this study, I selected *A. malaccensis* and tested its potential as phytoremediator as this species is highly tolerant to highly acidic soils with high concentration of heavy metals and its characteristic which have large biomass.

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1.3 Objectives

The objectives of this study were:

- a) To examine the heavy metal concentrations in the growth media after harvesting periods.
- b) To evaluate heavy metal uptake and translocation in plant parts especially roots, stems and leaves by using an indicator of Translocation factor (TF) and Bio-concentration factor (BCF).



REFERENCES

Abdu, A., Aderis, N., Abdul-Hamid, H., Majid, N. M., Jusop, S., Karam, D. S., & Ahmad, K. (2011). Using Orthosiphon stamineus B. for phytoremediation of heavy metals in soils amended with sewage sludge. *American Journal of Applied Sciences*, *8*(4), 323.

Abdullahi, M. (2015). Soil contamination, remediation and plants: Prospects and challenges. In Khalid, RH, Muhammad, S., Munir, O., Ahmet, RM, (Eds), Soil Remediation and Plants (pp. 629-659). Mersin University of Academic Press.

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

Alkorta, I., Hernández-Allica, J., Becerril, J. M., Amezaga, I., Albizu, I., & Garbisu, C. (2004). Recent findings on the phytoremediation of soils contaminated with environmentally toxic heavy metals and metalloids such as zinc, cadmium, lead, and arsenic. *Reviews in Environmental Science and Biotechnology*, *3*(1), 71-90.

Benavides, M. P., Gallego, S. M., & Tomaro, M. L. (2005). Cadmium toxicity in plants. *Brazilian Journal of Plant Physiology*, *17*(1), 21-34.

Chen, H., Teng, Y., Lu, S., Wang, Y., & Wang, J. (2015). Contamination features and health risk of soil heavy metals in China. *Science of the Total Environment*, *512*, 143-153.

Davies, D. J. A., Thornton, I., Watt, J. M., Culbard, E. B., Harvey, P. G., Delves, H. T., & Quinn, M. J. (1990). Lead intake and blood lead in two-year-old UK urban children. *Science of the Total Environment*, *90*, 13-29

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

Deng, H., Ye, Z. H., & Wong, M. H. (2004). Accumulation of lead, zinc, copper and cadmium by 12 wetland plant species thriving in metalcontaminated sites in China. *Environmental Pollution*, *132*(1), 29-40.

Ebbs, S. D., & Kochian, L. V. (1997). Toxicity of zinc and copper to Brassica species: implications for phytoremediation. *Journal of Environmental Quality*, *26*(3), 776-781.

Fitz, W. J., & Wenzel, W. W. (2002). Arsenic transformations in the soil– rhizosphere–plant system: fundamentals and potential application to phytoremediation. *Journal of Biotechnology*, *99*(3), 259-278.

Fontes, R. L. F., & Cox, F. R. (1998). Zinc toxicity in soybean grown at high iron concentration in nutrient solution. *Journal of Plant Nutrition*, *21*(8), 1723-1730.

Jadia, C. D., & Fulekar, M. H. (2009). Phytoremediation of heavy metals: recent techniques. *African Journal of Biotechnology*, *8*(6)

 \bigcirc

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

angronsveld, J., Herzig, R., Weyens, N., Boulet, J., Adriaensen, K., Ruttens, A., ... & van der Lelie, D. (2009). Phytoremediation of contaminated soils and groundwater: lessons from the field. *Environmental Science and Pollution Research*, *16*(7), 765-794.

Karami, A., & Shamsuddin, Z. H. (2010). Phytoremediation of heavy metals with several efficiency enhancer methods. *African Journal of Biotechnology*, *9*(25), 3689-3698

Koptsik, G. N. (2014). Modern approaches to remediation of heavy metal polluted soils: a review. *Eurasian Soil Science*, *47*(7), 707-722

Lee, J. H. (2013). An overview of phytoremediation as a potentially promising technology for environmental pollution control. *Biotechnology and Bioprocess Engineering*, *18*(3), 431-439.

Lenntech Water Treatment and Air Purification (2004) Water Treatment. Lenntech, Rotterdamseweg, Netherlands.

Leguizamo, M. A. O., Gómez, W. D. F., & Sarmiento, M. C. G. (2017). Native herbaceous plant species with potential use in phytoremediation of heavy metals, spotlight on wetlands—a review. *Chemosphere*, *168*, 1230-1247.

Maiti, S. K., & Jaiswal, S. (2008). Bioaccumulation and translocation of metals in the natural vegetation growing on fly ash lagoons: a field study from Santaldih thermal power plant, West Bengal, India. *Environmental Monitoring and Assessment*, *136*(1), 355-370

Majid, N. M., Islam, M. M., Yumarnis, R., & Arifin, A. (2012). Assessment of heavy metal uptake and translocation by Pluchea indica L. from sawdust sludge contaminated soil. *Journal of Food, Agriculture & Environment, 10*(2 part 2), 849-855.

Mohd, S. N., Majid, N. M., Shazili, N. A. M., & Abdu, A. (2013). Growth performance, biomass and phytoextraction efficiency of Acacia mangium and Melaleuca cajuputi in remediating heavy metal contaminated soil. *American Journal of Environmental Sciences*, *9*(4), 310.

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.

Ghosh, M., & Singh, S. (2005). A review on phytoremediation of heavy metals and utilization of it's by products. *Asian Journal Energy Environment*, *6*(4), 18.

Ghafoori, M., Majid, N. M., Islam, M. M., & Luhat, S. (2011). Bioaccumulation of heavy metals by Dyera costulata cultivated in sewage sludge contaminated soil. *African Journal of Biotechnology*, *10*(52), 10674-10682.

 \bigcirc

Graziano, M., & Lamattina, L. (2005). Nitric oxide and iron in plants: an emerging and converging story. *Trends in Plant Science*, *10*(1), 4-8.

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

Greger, M. (1999). Metal availability and bioconcentration in plants. In Heavy metal stress in plants. Springer Berlin Heidelberg.

Greipsson, S. (2011). Phytoremediation. *Nature Education Knowledge*, *3*(10), 7.

Gurzau, E. S., Neagu, C., & Gurzau, A. E. (2003). Essential metals—case study on iron. *Ecotoxicology and Environmental Safety*, *56*(1), 190-200.

HO, W. M., ANG, L. H., & LEE, D. K. (2008). Assessment of Pb uptake, translocation and immobilization in kenaf (Hibiscus cannabinus L.) for phytoremediation of sand tailings. *Journal of Environmental Sciences*, *20*(11), 1341-1347.

Kvesitadze, G., Khatisashvili, G., Sadunishvili, T., & Ramsden, J. J. (2006). Biochemical mechanisms of detoxification in higher plants: basis of phytoremediation. Springer Science & Business Media, Heidelberg.

Mahmood, T., & Islam, K. R. (2006). Response of rice seedlings to copper toxicity and acidity. *Journal of Plant Nutrition*, *29*(5), 943-957.

Maiti, S. K., & Jaiswal, S. (2008). Bioaccumulation and translocation of metals in the natural vegetation growing on fly ash lagoons: a field study from Santaldih thermal power plant, West Bengal, India. *Environmental Monitoring and Assessment*, *136*(1), 355-370.

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.

Nouri, J., Khorasani, N., Lorestani, B., Karami, M., Hassani, A. H., & Yousefi, N. (2009). Accumulation of heavy metals in soil and uptake by plant species with phytoremediation potential. *Environmental Earth Sciences*, *59*(2), 315-323.

Khorasani, N., Hasani, A. H., Seif, F., & Cheraghi, M. (2011). Phytoremediation potential of native plants grown in the vicinity of Ahangaran lead–zinc mine (Hamedan, Iran). *Environmental Earth Sciences*, *6*2(3), 639-644

Rajoo, K. S., Karam, D. S., Arifin, A., & Muharam, F. M. (2016). Phytoremediation potential of Dipterocarpus chataceus planted on sewage sludge contaminated soil. *Middle-East Journal of Scientific Research*, *24*(4), 1169-1177.



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.

Siedlecka, A., Tukendorf, A., Skórzynska-Polit, E., Maksymiec, W., Wojcik, M., Baszynski, T., & Krupa, Z. (2001). Angiosperms (Asteraceae, Convolvulaceae, Fabaceae and Poaceae; other than Brassicaceae). Metals in the Environment. Analysis by Biodiversity. Marcel Dekker, Inc., New York.

Singh, A., & Prasad, S. M. (2015). Remediation of heavy metal contaminated ecosystem: an overview on technology advancement. *International Journal of Environmental Science and Technology*, *12*(1), 353-366.

Singh, A., & Prasad, S. M. (2011). Reduction of heavy metal load in food chain: technology assessment. *Reviews in Environmental Science and Biotechnology*, *10*(3), 199.

Singh, O. V., Labana, S., Pandey, G., Budhiraja, R., & Jain, R. K. (2003). Phytoremediation: an overview of metallic ion decontamination from soil. *Applied Microbiology and Biotechnology*, *61*(5-6), 405-412.

Singh, R., Gautam, N., Mishra, A., & Gupta, R. (2011). Heavy metals and living systems: An overview. *Indian Journal of Pharmacology*, *43*(3), 246–253 Sarma, H. (2011). Metal hyperaccumulation in plants: a review focusing on phytoremediation technology. *Journal of Environmental Science and Technology*, *4*(2), 118-138.

Shukla, K. P., Singh, N. K., & Sharma, S. (2010). Bioremediation: developments, current practices and perspectives. *Genetic Engineering Biotechnology*, *3*(8), 1-20

Solgi, E., Esmaili-Sari, A., Riyahi-Bakhtiari, A., & Hadipour, M. (2012). Soil contamination of metals in the three industrial estates, Arak, Iran. *Bulletin of environmental contamination and toxicology*, *88*(4), 634-638.

Subhashini, V., Swamy, A. V. V. S., & Krishna, R. H. (2017). Pot Experiments to Study the Uptake of Zinc by Weed Species, Flowering Plants and Grass Species in Artificially Contaminated Soils: Phytoremediation-Green Technology. *World Journal of Applied Environmental Chemistry*, *2*(2), 61-71.

 \bigcirc

Thomas, J. C., Malick, F. K., Endreszl, C., Davies, E. C., & Murray, K. S. (1998). Distinct responses to copper stress in the halophyte Mesembryanthemum crystallinum. *Physiologia Plantarum*, *102*(3), 360-368. United Nations Environmental Protection/Global Program of Action, UNEP/GPA, 2004. Why the Marine Environment Needs Protection from Heavy Metals. UNEP/GPA Coordination Office.

United States Protection Agency Reports (2000): Introduction to Phytoremediation. EPA600/R-99/107. UNEP/GPA Coordination Office.

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.

Wintz H, Fox T, Vulpe C (2002) Responses of plants to iron, zinc and copper deficiencies. Biochemical Society Transactions 30(4), 766–768.

Wong, M. H. (2003). Ecological restoration of mine degraded soils, with emphasis on metal contaminated soils. *Chemosphere*, *50*(6), 775-780. 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.

Yruela, I. (2005). Copper in plants. *Brazilian Journal of Plant Physiology*, *17*(1), 145-156.

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.