UNIVERSITI PUTRA MALAYSIA

EVALUATION OF THE POTENTIAL OF Melastoma malabathricum L. AND Ricinus communis L. FOR HEAVY METALS PHYTOREMEDIATION ON SOIL CONTAMINATED WITH SEWAGE SLUDGE

NUR NAZIRAH BINTI PATEK MOHD

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By

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

July 2016

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the Degree of Master of Science

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The increasing human population every year has resulted in an enormous volume of sewage sludge throughout the world. Disposing of sewage sludge is a major concern due to its high concentrations of heavy metals. These heavy metals need to be removed before they are applied on soils as an amendment. Phytoremediation is a biological treatment by which plants are used to remove pollutants from the soil environment. The objectives of this study were to evaluate heavy metals uptake and translocation in plant parts; to quantify the heavy metals concentrations of sewage sludge in the growth medium before the planting and after the harvesting period; and to quantify the percentage of reduction of heavy metals from the initial metals input and leaching studies. Seedlings were planted on six different growth media – a control (100% soil), T_1 (80% soil + 20% sewage sludge), T_2 (60% soil and 40% sewage sludge), T_3 (40% soil + 60% sewage sludge), T_4 (20% soil + 80% sewage sludge) and T_5 (100% sludge) for 6 months. In this study, a Randomized Complete Block Design (RCBD) was used. Plant heights and number of leaves were measured every month during the study period. Plant biomass was measured using plant parts (leaves, stems and roots) and total concentrations of heavy metals were determined by Atomic absorption spectrophotometry (AAS). For *Melastoma malabathricum*, the best plant height was in T_2 (40% sewage sludge + 60% soil), with a value of 104 cm, and the highest number of leaves was in T_3 (60% sewage sludge + 40% soil), with a value 975. The best growth performance for *Ricinus communis* was 114 cm in T_1 (100% soil) for plant height, and 11 in T_1 (100% soil), for number of leaves. In these experiments, both species were able to reduce heavy metals in the soil.The highest concentration of Cu after harvest was detected in T_5 (4.93 mg/kg) for *Ricinus communis*. The highest total Cu concentration in plant parts, with value of 0.45 mg/kg, was treatment T_0 for *Melastoma malabathricum*. The highest Fe concentration in growth media

after the harvest was 1602.13 mg/kg in T_5 (*Ricinus communis*), while all species stored the highest Fe concentrations in T_5. *Ricinus communis* was not suitable as an accumulator plant for Fe because its BF and TF values were below 1 in T_5 (9.73 mg/kg). Both species can be considered accumulators of Mn due to their high TF values, and both were able to translocate and accumulate Mn from their roots to their shoots after taking it from the soil. The highest total Pb concentration was in T_5, with a value of 10.35 mg/kg, for *Melastoma malabathricum* and *Ricinus communis*, which both can be considered accumulators of Pb, having BF and TF values above 1. The highest concentration of Zn in the growth medium was in T_5, with a value of 47.75 mg/kg, for *Melastoma malabathricum*. With TF values of more than 1, both species were suitable as accumulators of Zn. There is lack information about removal heavy metals from soil contaminated with sewage sludge through leaching during phytoremediation process. The results demonstrate Fe concentrations in leachate was highest compared to Cu, Mn, Pb and Zn. The highest leachate was Fe with value 74.56 mg/L in T_0 and *Ricinus communis* leached out more Fe elements compared *Melastoma malabahtricum*. The highest percentage reduction of heavy metals was recorded for *Melastoma malabathricum* in T_2 (Cu), with values of 53.17%, while the highest for *Ricinus communis* was recorded in T_1 (Zn), with values of 54.89%. This research found that *Melastoma malabathricum* and *Ricinus communis* can be used as phytoremediators of Cu, Mn, Pb and Zn due to their ability to accumulate the elements in the roots and translocate them to the shoots. None of the species were capable of being accumulators of Fe because their BF and TF values were lower than 1.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Master Sains

PENILAIAN LOGAM BERAT KE ATAS POTENSI FITOREMEDASI OLEH *Melastoma malabathricum* DAN *Ricinus communis* KE ATAS TANAH YANG TERCEMAR DENGAN ENAP CEMAR KUMBAHAN

Oleh

NUR NAZIRAH BINTI PATEK MOHD

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Peningkatan populasi manusia setiap tahun telah menyebabkan peningkatan enap cemar kumbahan di dunia. Pelupusan enap cemar kumbahan telah menjadi perhatian utama kerana kandungan kepekatan tinggi logam berat di dalam enap cemar kumbahan. Logam berat perlu dikeluarkan sebelum digunakan sebagai pemindah tanah di tanah pertanian. Fitoremedasi adalah rawatan biologi di mana tumbuh-tumbuhan digunakan untuk membuang bahan pencemar seperti logam berat dari persekitaran tanah. Objektif kajian ini adalah menentukan keberkesanan pokok senduduk (*Melastoma malabathricum*) dan pokok jarak (*Ricinus communis*) dalam mengeluarkan logam berat di dalam tanah tercemar oleh enap cemar kumbahan, menilai pengambilan dan translokasi logam berat di dalam medium pertumbuhan sebelum menanam dan selepas tempoh penuaian. Anak benih ditanam di dalam enam media pertumbuhan yang berbeza: Kawalan (100% tanah), T_1 (80% tanah + 20% enap cemar kumbahan, T_2 (60% tanah dan 40% enap cemar kumbahan), T_3 (40% tanah + 60% enap cemar kumbahan), T_4 (20% tanah + 80% enap cemar kumbahan) dan T_5 (100% enap cemar kumbahan) bagi tempoh 6 bulan. Kaedah rekabentuk blok penuh rawatan (RCBD) telah digunakan dalam kajian ini. Bilangan daun dan ketinggian tumbuhan diukur setiap bulan dalam tempoh kajian. Kepekatan logam berat di dalam pokok di ukur mengikut bahagian tumbuhan (daun, batang dan akar). Jumlah kepekatan logam berat telah ditentukan dengan menggunakan mesin spektrofotometri penyerapan atom (AAS). Bagi *Melastoma malabathricum*, ketinggian tumbuhan yang terbaik adalah di T_2 (40% sisakumbahan + 60% tanah) dengan nilai 104 cm dan bilangan daun tertinggi adalah di dalam T_3 (60% sisakumbahan + 40% tanah) dengan nilai 975. Prestasi pertumbuhan terbaik pada *Ricinus communis* adalah 114 cm T_1 (100% tanah) bagi ketinggian tumbuhan dan 11 helai daun dalam T_1 (100% tanah) untuk jumlah daun. Dalam eksperimen ini, kedua-dua spesies berkeupayaan mengurangkanlogam berat di dalam tanah. Kepekatan tertinggi Cu selepas menuai di kesan pada T_5 (4.93 mg/kg) oleh *Ricinus communis*. Jumlah kepekatan Cu tertinggi di bahagian tumbuhan adalah pada rawatan T_0 dengan nilai 0.45 mg/kg oleh *Melastoma malabathricum*. Kedua-dua spesies ini

boleh di anggap sebagai tumbuhan pengumpul bagi Cu berbanding *Ricinus communis* kerana BF dan TF adalah lebih daripada 1. Kepekatan Fe tertinggi di dalam media pertumbuhan selepas penuaian adalah 1602.13 mg/kg untuk T_5 oleh *Ricinus communis*. Semua spesies menyimpan kepekatan Fe tertinggi padaT_5. *Ricinus communis* tidak sesuai menjadi tumbuhan pengumpul Fe kerana BF dan TF nilai adalah lebih rendah daripada 1. Kepekatan Mn tertinggi selepas tuaian dalam medium pertumbuhan ini adalah T5 (9.73 mg/kg) oleh *Ricinus communis*. Kedua-dua spesies ini boleh menjadi tumbuhan pengumpul Mn kerana nilai TF melebihi daripada 1. Kedua-dua spesies dapat memindahkan dan mengumpul Mn dari tanah bermula dari akar ke pucuk. Jumlah kepekatan Pb tertinggi ialah di T_5 dengan nilai 10.35 mg/kg oleh *Ricinus communis*. *Melastoma malabathricum* dan *Ricinus communis* boleh dianggap sebagai tumbuhan pengumpul Pb kerana nilai BF dan TF adalah lebih daripada 1. Kepekatan tertinggi Zn dalam medium pertumbuhan ini ialah T_5 dengan nilai 47.75 mg/kg oleh *Melastoma malabathricum*. Kedua-dua spesies ini sesuai untuk bertindak sebagai tumbuhan pengumpul Zn kerana nilai TF lebih daripada 1. Keputusan menunjukkan kepekatan Fe dalam larut lesap adalah yang tertinggi berbanding Cu, Mn, Pb dan Zn dengan nilai 74.56 mg / L dalam T_0 dan *Ricinus communis* terlarut lesap lebih unsur Fe berbanding *Melastoma malabahtricum*. Pengurangan peratus yang tinggi logam berat untuk *Melastoma malabathricum* dicatatkan pada T_2 (Cu) dengan nilai 53.17% manakala bagi *Ricinus communis* dicatatkan pada T_1 (Zn) dengan nilai 54.89%. Kajian ini mengesahkan bahawa, *Melastoma malabathricum* dan *Ricinus communis* boleh digunakan sebagai tumbuhan remediasi Cu, Mn, Pb dan Zn kerana kemampuannya untuk mengumpul elemen-elemen logam berat di dalam akar dan translokasi kepada pucuk. Tidak ada spesies boleh menjadi tumbuhan pengekstrak untuk Fe kerana nilai BF dan TF yang diperolehi <1.

ACKNOWLEDGEMENTS

My gratitude goes to Allah Almighty who let me finish this journey. Thanks for His gifts of patience and wisdom to pursue my dream of attaining academic excellence.

My sincerest gratitude goes to Associate Professor Dr. Arifin Abdu, for I am only able to complete this study through his support and knowledge. Without his encouragement and effort, this thesis would not have been completed or written. I am also thankful to Professor Shamshuddin Jusop for the time and advice throughout my work. I am also grateful to Dr Rezaul Kareem for his help and advice during this project.

I am deeply indebted to Miss Aiza Shaliha, Miss Nur Lyana Farhan, Mr Nazrin, Mr Hadi, Mr Lakarim and Mrs Zarina for their kindness, support, love and invaluable assistance throughout this study. Not to forget Aishah for hardships and good times during our projects.

Special thanks to my Sepakat and Majunas friends who helped me regain some sort of fitness and motivation. Finally, I would like to thank my mother, Hasnah Binti Ibrahim who always support me and comfort me during my hard times. Not to forget my siblings for their enduring support and love. There are no words to describe my appreciation toward them. I hope the accomplishment of this dissertation represent small token of my appreciation.

I certify that a Thesis Examination Committee has met on 22 July 2016 to conduct the final examination of Nur Nazirah binti Patek Mohd on her thesis entitled "Evaluation of the Potential of *Melastoma malabathricum* L. and *Ricinus communis* L. for Heavy Metals Phytoremediation on Soil Contaminated with Sewage Sludge" 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 Master of Science.

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

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LIST OF ABBREVIATIONS

AAS	Atomic Absorption Spectrometer
AA	AutoAnalyzer
ANOVA	Analysis of Variance
Al	Aluminum
BF	Bioaccumulation Factor
Ca	Calcium
CEC	Cation Exchange Capacity
CHNS	Carbon, Hydrogen, Nitrogen and Sulphur Analyzer
Cu	Copper
Exch.	Exchangeable
FAO	Food and Agriculture Organization
Fe	Iron
G	Gram
Kg	Kilogram
K	Potassium
M	Meter
Mg	Milligram
Mn	Manganese
N	Sodium
OM	Organic matter
P	Phosphorus
Pb	Lead
pH	Acidity in water
ppm	Parts per million

r^2	R-squared
TC	Total carbon
TF	Translocation Factor
TN	Total Nitrogen
UPM	Universiti Putra Malaysia

CHAPTER 1

INTRODUCTION

1.1 General Background

The increase in human population every year has resulted in enormous growth of solid waste around the world. Disposing of solid waste from domestic industries has become a concern in developing nations, especially in India, Pakistan, Bangladesh and Sri Lanka (Asian Productivity Organization Tokyo, 2007). If dumped on large areas of ground, domestic and industrial solid waste releases contaminants that lead to soil pollution. This causes the accumulation of organic and inorganic pollutants for ages, and these pollutants are released into our environment in many ways. Solid waste, in this context, includes sewage sludge from wastewater treatment, mining residue and agricultural waste (Parisa *et al*., 2010). There are a few ways to dispose of sewage sludge, such as to dispose of it on the land, at sea or (through incineration) into the air (Odegaard *et al*., 2002). Sewage sludge contains transition metals, lanthanoids, actinoids and mettaloids, however, which belong to the heavy metals group. Heavy metals are not biodegradable, so they persist in the environment for a long time. They also manifest as a large group of trace elements with atomic densities over 6 g cm^{-3} (Alloway and Jackson, 1991). The toxicity of heavy metals has tangible effects on plants, humans, animals, agricultural production and the environment overall. For example, human consumption of heavy metals such as cadmium causes respiratory and gastro intestinal (GI) disorders; in plants, they cause carcinogenic disorders. High concentrations of heavy metals in crops lead to poor yields and stunted growth, so the application of sewage sludge containing pollutants for cultivation increases the concentrations of heavy metals such as iron (Fe), manganese (Mn), zinc (Zn), lead (Pb) and copper (Cu) in crops.

In the previous literature, there are few physical, chemical or biological procedures to restore such contaminated soil to a natural state. Among these are electromediation, soil flushing, soil vapour extraction, soil washing and biopiles. However, these physical and chemical methods have negative impacts, such as inducing secondary damage to ecosystems, high costs and inapplicability to developed countries. An alternative is phytoremediation technologies, which are categorized into five types: phytoextraction, rhizofiltration, phytostabilization, phytodegradation and phytovolatilization. Phytoremediation is environmentally friendly because the process helps to control soil erosion from heavy rain, provides wildlife habitats and acts as a carbon sink (Orooj *et al*., 2015). Various plant species are used to facilitate soil regeneration, and they react to the presence of soil contaminants in many ways. These methods are more efficient and economical, and they have industrial and commercial benefits for soil reclamation purposes when the proper plant species are used in the field. This process is economical because it requires little manpower and the cost of equipment is affordable (Campos *et* *al.,* 2008). Besides, by using plants to remove pollutants, phytoremediation applies a natural process.

1.2 Problem statement

For the past decade, soil pollution has become a serious problem, not only posing a health threat to humans but also leading to environmental problems that affect plant growth since heavy metal contamination contains a variety of inorganic and organic pollutants. In Malaysia, meanwhile, the accumulation of sewage sludge is proportional to the population. This sludge contains heavy metals such as iron (Fe), zinc (Zn), manganese (Mn), copper (Cu) and lead (Pb), and these metals pose possible harm to humans from the presence of heavy metals in the food chain through uptake by natural vegetation and cultivated crops. Treating the sludge is not only expensive but time consuming. Since sewage sludge contains some nutrients, it can be used as a soil conditioner and probable solution as it is also environmental friendly. Researchers are seeking new plant species that are suitable to be used in removing heavy metals from soil contaminated by sewage sludge.

In this present study, two species – *Melastoma malabathricum* and *Ricinus communis* – were selected due to their rapid growth, high biomass, ability to grow in polluted soils, tolerance to acidic conditions, adaptability to various conditions and multiple economic uses (Adhikari and Kumar, 2012). According to Huang *et al*. (2011), *Ricinus communis* is an industrial cash crop because of its quality and quantity of oil for plant-based industries, especially for making eco-friendly paints. This species has attracted attention because it is able to grow in heavily polluted soil and because of its capacity for metal ion accumulation and fast growth rate under tropical weather conditions. For this purpose, *Ricinus communis* has potential as a phytoremediator plants in contaminated sites with additional benefits of multipurpose oilseed production (Pandey, 2013). Some authors have reported that *Ricinus communis* are able to grow in heavily polluted soils which can remediate metal mine tailing (such as Pb, Cd and Zn) and the results showed that this plant had low metals shoot concentrations, high root metal concentrations, low metal translocation factors and in consequence this plant is participating in metal stabilization process (Ruiz *et al*., 2013)

Melastoma malabathricum was found to be very tolerant to the humid tropics of Asia. While this plant indicated a high uptake ability and internal mobility (Watanabe *et al*., 1998), there are four hundred plants in the world – including trees, vegetable crops, weeds (unwanted plants) and grasses – which have been identified as hyperaccumulators of metals. Based on Ashraf *et al*. (2011), *Melastoma malabathricum* have adopted an exclusion strategy which allows them to form metal stable complexes in their root cells. It results in a limited metal translocation to above-ground parts and identitified as a dominants hypertolerants species in tailings area. Studies have revealed that *Melastoma malabathricum* are able to adapt to very poor nutrient environments such as acid sulphate soil and the report describe this plant are suitable as an aluminium accumulator (Atsuya *et al*., 2011). Previous research also stated that, *Melastoma malabathricum* are good candidates for phytoextraction of lead and zinc in Hoagland's solution concentration by using hydroponic experiments (Yeo and Tan, 2011). Besides that, *Melastoma malabathricum* are suited for phytoremediation based on several characteristics such as wide distribution, high above-ground biomass, high bioaccumulation factors (hyperaccumulators), short life cycles, high propagation rates and high arsenic tolerant (Visoottiviseth *et al*., 2002).

1.3 Objectives

Ample studies have been conducted on plant species as phytoremediators of contaminated soils using leafy wild vegetables and ornamental plants. However, research focusing on the potential of noxious weeds to remediate polluted soils is still lacking. Hence, in this study, we evaluate the phytoremediation efficiency for Cu, Fe, Mn, Pb and Zn using *Melastoma malabathricum* and *Ricinus communis* in soil contaminated with sewage sludge.

The objectives of this study were the following:

a) To evaluate heavy metals uptake and translocation in plant parts, especially their roots, stems and leaves.
b) To quantify the heavy metal concentrations of sewage sludge in the growth medium before the planting and after the harvesting period.
c) To quantify the percentage of reduction of heavy metals from the initial metals input and leaching studies.
d) To assess the potential of *Melastoma malabathricum* and *Ricinus communis* as phytoremediatr using remediation indices (BF and TF).

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